Abstract—In anaesthetised rats, long-term potentiation (LTP) was induced unilaterally in the dentate gyrus by tetanic stimulation of the perforant path. Animals were killed 6 h after LTP induction and dendritic spines and synapses in tetanised and untetanised (contralateral) hippocampal tissue from the middle molecular layer (MML) were examined in the electron microscope using stereological analysis. Three-dimensional reconstructions were also used for the first time in LTP studies in vivo, with up to 130 ultrathin serial sections analysed per MML dendritic segment. A volume sampling procedure revealed no significant changes in hippocampal volume after LTP induction and dendritic spines and synapses in tetanised and control tissue.

In the potentiated hemisphere, there were changes in the proportion of different spine types and their synaptic contacts. We found an increase in the percentage of synapses on thin dendritic spines, a decrease in synapses on both stubby spines and dendritic shafts, but no change in the proportion of synapses on mushroom spines. Analysis of three-dimensional reconstructions of thin and mushroom spines following LTP induction revealed a significant increase in their volumes and area. We also found an increase in volume and area of unperforated (macular) and perforated (segmented) postsynaptic densities.

Our data demonstrate that whilst there is no change in synapse density 6 h after the induction of LTP in vivo, there is a considerable restructuring of pre-existing synapses, with shaft and stubby spines transforming to thin dendritic spines, and mushroom spines changing only in shape and volume. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: long-term potentiation, postsynaptic densities, thin-sections, three-dimensional reconstructions.

Long-term potentiation (LTP) of the perforant path in the hippocampus has been extensively studied as an easily elicited form of synaptic plasticity (Bliss and Lømo, 1973; Bliss and Collingridge, 1993), and has become the dominant model of the synaptic basis of memory formation (Abraham et al., 2002). However, its structural basis, as with that of another form of synaptic plasticity, long-term depression, remains the subject of debate (Muller et al., 2000; Sorra and Harris, 1998; Rusakov et al., 1997; Dhantajan et al., 2003; Mezey et al., 2004).

There is evidence for LTP remodelling both at pre- and post-synaptic levels. Malenka and Nicoll (1999) have summarised the importance of the role played by changes in the number and/or properties of postsynaptic receptors. Changes in presynaptic function during long term synaptic plasticity were visualised by Zakharenko et al. (2001) using 2-photon microscopy and Empage et al. (2003) applied optical quantal analysis to reveal a presynaptic component of LTP at hippocampal Schaffer-associational synapses.

Postsynaptic mechanisms of LTP are reflected morphologically in dendritic spine remodelling (Kirov and Harris, 1999; Toni et al., 1999, 2001; Yuste and Bonhoeffer, 2001). Alterations in synapse number or in structure of dendritic spines occur as early as 30 min post LTP induction (Desmond and Levy, 1986a,b, 1988, 1990) and last for hours to days (Geinisman et al., 1991, 1994; Stewart et al., 2000).

Confocal microscopy has provided three-dimensional (3D) representations of dendritic spines and presynaptic boutons (Engert and Bonhoeffer, 1999; Segal, 2001) but there are limitations in using confocal microscopy alone, especially in the study of presynaptic elements (Harris, 1994; Moser et al., 1997). Single-section analysis of electron microscope sections can also be problematical because variability in synapse density and morphology substantially influences the probability of viewing structures on random single sections (Coggeshall and Lekan, 1996; Fiala and Harris, 2001a).

Sorra and Harris (1998) used 3D synaptic reconstructions to show that LTP induced in vitro did not result in formation overall of new synapses at 2 h post-tetanus in hippocampal area CA1. Their study supported the hypothesis that early phase LTP involved a redistribution of synaptic weights amongst pre-existing synapses,
in contrast to electron microscopic observations which suggested the splitting of existing spines contacting the same pre-synaptic bouton (Toni et al., 2001), and confocal studies which point to the formation of new spines after tetanic hippocampal stimulation (Engert and Bonhoeffer, 1999; Korkotian and Segal, 2001).

Here, following LTP induction in vivo we have examined morphological changes in spines and synapses in another hippocampal pathway, the perforant path projection to granule cells of the dentate gyrus. A detailed quantitative examination has been undertaken using stereological analysis and, for the first time in an in vivo LTP study, 3D reconstructions of serial ultrathin sections have been made (Harris, 1994; Fiala and Harris, 2001a; Harris et al., 2003). The perforant path in the left hemisphere was subjected to tetanic electrical stimulation whilst the right hemisphere served as an unstimulated control. In an additional control, unilateral electrical stimulation of the perforant path was employed in a pattern that did not induce LTP. Animals were killed 6 h after the induction of LTP, an interval long enough to ensure that any changes reflected the late, protein-dependent phase of LTP. Our results reveal no change in synaptic density, but a considerable remodelling of existing synapses, with thin dendritic spines (Tsp) replacing shaft and stubby spines, and mushroom spines (Msp) increasing in volume.

**EXPERIMENTAL METHODS**

**Induction of LTP in vivo**

Male Sprague–Dawley rats weighing 300–400 g were anaesthetised with urethane (1.8 g/kg i.p.), and held in a semi-stereotaxic apparatus. A glass recording pipette, filled with artificial cerebrospinal fluid containing Pontamine Sky Blue was placed 4.1 mm posterior and 2.5 mm lateral to bregma and advanced into the dentate gyrus (DG). A bipolar stimulating electrode (Rhodes SNE 100) was inserted on the same side 4.4 mm lateral to lambda and lowered into the angular bundle to activate fibres of the perforant path. The depths of the two electrodes were adjusted to produce maximal responses in the cell body layer. Constant current stimuli (60 μs duration, intensity in the range 70–120 μA) were delivered at intervals of 30 s, and intensity adjusted to produce a population spike with an amplitude of 1–2 mV. Test stimuli were delivered at 30-s intervals for 30 min prior to induction of LTP and for 6 h afterward. LTP was produced by three trains of 50 pulses at 250 Hz, with an inter train period of 30 s. A control animal received no tetanic stimulation but was given the same extracellular stimuli, corresponding to the number of stimuli in the tetanus protocol, spread at equal intervals over the final 6 h of the experiment; the stimulus strength in this animal was also raised for the final 6 h to mirror the increased evoked responses produced during LTP in the potentiated animals. The collection of evoked potentials and the timing and intensity of stimulation, were under computer control (software written by Dr R. M. Douglas, University of British Columbia). All animal experimentation was carried out under UK Home Office licence, and care was taken to minimise the number of animals used and their suffering throughout all stages of the procedures.

**Fixation by perfusion**

Following physiological stimulation, male rats (three animals for LTP and one for electrical stimulation without LTP) were perfused intracardially. Primary fixation occurred via a two-stage process. Firstly, the thorax was opened and the left cardiac ventricle cannulated. The right cardiac auricle was then opened, and 100 ml of phosphate-buffered physiological saline was perfused through the animal. Subsequently, 100 ml of 3% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M Na cacodylate buffer (pH 7.2–7.4) was perfused transcardially at room temperature. In the second stage the hippocampus was removed, and cut into 300 μm slices and fixed in a higher concentration of glutaraldehyde, as described below.

**Hippocampal volume**

The volume of the left and right hippocampi were estimated using the Cavalieri method (Pakkenberg and Gundersen, 1997; Stuart and Oorschot, 1995). Three rats were tetanized exactly as above with a recording electrode in the left, tetanised hemisphere, and another recording electrode in the DG of the right unstimulated hemisphere. After 6 h, the animals were transcardially perfused with aldehyde fixative (as described above) and the brains carefully removed and stored in 0.1 M PB (pH 7.4). The fixed brains were then serially sectioned in the coronal plane at 100 μm (section thickness, t) on a vibrating microtome (Leica). Tissue sections were mounted in order on glass slides, air dried and subsequently stained with a solution of 0.1% Toluidine Blue in 0.1 M PB buffer (pH 7.4) for 2 min. Sections were then washed, dehydrated in an ascending series of alcohols, passed through xylene and finally embedded in DPX. From the complete rostrocaudal set of sections in each animal a 1.3 series was selected for analysis. Sections were viewed and analysed at low magnification in a Nikon E600 digital photomicroscope. Using previously published cytoarchitectonic criteria (Swanson, 1998), the boundaries of the DG (cell body layer and molecular layer) and of the total hippocampus (including CA1, CA2, CA3, DG, polymorph cell and hilar regions, but excluding the subiculum) were defined bilaterally in each rostrocaudal section (Fig. 1A). Bilateral digital images including the hippocampus and surrounding structures were captured electronically and displayed on a computer screen (Fig. 1A). No gross cytoarchitectural differences were detected between control (right) and tetanised (left) hemispheres. The surface areas of the dentate gyr and of the whole hippocampi occurring in each section of the 1.3 series were then calculated using a calibrated measuring programme (Lucia Version 4.8; Laboratory Imaging Ltd., Prague, Czechoslovakia; Fig. 1B). Total hippocampal and total dentate gyral volumes for each hemisphere were subsequently derived by multiplying the total surface areas of each structure in each analysed section by the number of sections in the series (n=3) and by the section thickness (t). Data are presented as mean±S.D. Statistical comparisons in volume data between regions, hemispheres and animals were performed using multiple t-tests.

**Electron microscopy**

After perfusion with fixatives as described above hippocampi were dissected out and slices approximately 300 μm thick were cut transverse to the long axis of the dorsal hippocampus. Four slices were taken from each animal 1–2 mm on either side of the electrode track in the tetanized hemisphere and four slices from a similar location in the contralateral hemisphere. The slices were fixed further by immersion in 0.1 M Na cacodylate buffer (pH 7.2–7.3) containing 2.5% glutaraldehyde for 1–2 h at room temperature, followed by three washes in cacodylate buffer. The tissue was post-fixed with 1% osmium tetroxide and 0.01% potassium dichromate in cacodylate buffer for 1–1.5 h at room temperature.

**Processing for microscopy**

Tissue was dehydrated in aqueous solutions of ethanol at 40, 50, 60, 70, 80 and 96% (each for 10 min) and 100% acetone (three changes,
Stereological analysis was performed according to Harris (1994; of synapses (identified via PSDs), per 100 biased data obtained. Synaptic densities were expressed as number as large dendrites on the grounds that to do so would have potentially within these areas irrespective of the presence of components such approximately 500–800 cubic micrometers. Synaptic number was counted (d/H11005 combined dentate granule cell layer (gcl, dark grey) and the molecular layer (mol, light grey). Scale bar = 1 mm (A and B).

\[ \frac{d}{H11005} = 0.463 \, \text{m} \]

\[ \frac{0.463}{H9262} = 3.8 \, \text{mm from Bregma.} \]

\[ \text{(B) Line drawing of section shown in A indicating the perimeter boundary (black arrow-thick black outline)} \]

\[ \text{defining the whole hippocampus at this RC level. Also shown is the region designated as the DG. DGCL is defined as the} \]

\[ \text{rostrocaudal (RC) axis at approximately } -3.8 \, \text{mm from Bregma.} \]

Each synapse was identified primarily on the basis of the presence of a post-synaptic density (PSD) with vesicles in close proximity to the pre-synaptic zone. There are two main categories of PSDs: macular (with a continuous PSD) and those with a perforation in the PSD (perforated). Segmented PSDs are a subset of perforated PSDs (Sorra and Harris,

\[ \text{each for 10 min.} \]

Specimens were infiltrated with a mixture of 50% epoxy resin, 20% pure water, and 30% macromer (Technovit Epoxy resin) (Technovit Epoxy resin, see Materials and Methods). Each slice was placed on a Teflon support and covered with a capsule containing pure epoxy resin (Epon 812/AralditeM epoxy resin) for 1 h at 60 °C and polymerised overnight at 80 °C. Slices in blocks were coded and all further analyses were carried out with the investigator blind to the experimental status of the tissue. The embedded slices on the block surface were trimmed with a glass knife along the entire surface of the hippocampal slice and 1–2 μm-thick sections cut. The sections were stained with Toluidine Blue and examined in a light microscope. Using a glass knife a trapezoid area was prepared, with one side of 20–25 μm in length, and including area CA1, fimbria, DG and area CA3/CA4. Serial sections were cut with a Diatome diamond knife and allowed to form a ribbon collected on the surface of a water/ethanol solution (2–5% ethanol in water) in the knife bath. A ribbon of serial sections was collected on pioloform-coated slot grids and counterstained with saturated ethanolic uranyl acetate, followed by Reynolds lead citrate (15–20 min each). Fig. 2 illustrates the trapezoid area as cut from the block. Grids were placed in a rotating grid holder to obtain uniform orientation of sections on adjacent grids. Sections were examined with a JEOl 1010 electron microscope and photographed at 6000× magnification. A grating replica (d=0.463 μm; Electron Microscopy Sciences Inc., Fort Washington, PA, USA) was used for calibration of electron microscope magnifications. A cross-sectioned myelinated axon or dendrite spanning all sections provided a fiduciary reference for initial alignment of serial sections. Section thickness was determined using the approach of Fiala and Harris (2001b) and was normally of 60–70 nm-thickness (grey-white colour); approximately 90–130 serial sections were collected per series from each block.

**Stereology of synapses**

Stereological estimates were made of synapse density. We selected for analyses the middle molecular layer (MML) which is the main target area of perforant path fibres; the regions analysed were 60–100 μm (MML), from the proximal edge of the granule cell layer. Stereological analysis was performed according to Harris (1994; Fiala et al., 1998; Sorra et al., 1998), with tissue volumes of approximately 500–800 cubic micrometers. Synaptic number was counted within these areas irrespective of the presence of components such as large dendrites on the grounds that to do so would have potentially biased data obtained. Synaptic densities were expressed as number of synapses (identified via PSDs), per 100 μm³ of tissue.

**Digital reconstructive analysis**

Digitally scanned electron micrograph (EM) negatives with a resolution of 900 dpi were aligned as JPEG images (software available from Drs. Fiala and Harris: http://synapses.bu.edu). Alignments were made with full-field images. Contours of individual dendrites, axons, dendritic spines, PSDs, and mitochondria were traced digitally and volumes, areas, and total numbers of structures, were computed.

**Statistical analysis**

Microcal Origin software was used to plot graphs, to obtain means and S.D.s, and to perform tests of significance, as described in the results. ANOVAs followed by Tukey’s unequal N honest significant differences tests. Data are presented as a mean±S.D.). Significance levels were set at \( P<0.05 \).

**RESULTS**

**Volume measurements**

In order to interpret changes in synaptic density, derived from stereological analysis of EM sections, in terms of changes in synaptic number, we first determined whether the induction of LTP causes any change in volume of the DG.

All rats stimulated exhibited potentiation of the synaptic response that was more than 20% above the pre-tetanus level. The slope of the field excitatory postsynaptic potential (fEPSP) was normalised to the mean value before the tetanus for each animal, and group means are shown for the two groups of animals (three used for EM, and three for estimates of volume changes associated with LTP; Fig. 3).

The volumes of the DG and the combined hippocampal subfields are given in Table 1 for the tetanised and unstimulated hemispheres of each of the three rats. As can be seen by comparing the tetanised and untreated hemispheres, the induction of LTP in perforant path–granule cell synapses had no statistically significant effect on the volume of the hippocampus or DG.

**Ultrastructural analysis**

Each synapse was identified primarily on the basis of the presence of a post-synaptic density (PSD) with vesicles in close proximity to the pre-synaptic zone. There are two main categories of PSDs: macular (with a continuous PSD) and those with a perforation in the PSD (perforated). Segmented PSDs are a subset of perforated PSDs (Sorra and Harris,
In the present investigation PSDs located on dendrites originating from non-spiny interneurons (Harris, 1994) were ignored. Fig. 4A–D shows four non-consecutive serial EMs from a series of 130 sections. Fig. 4E–G are 3D-reconstructions from this series, taken from the MML of the potentiated DG (approximately 80–100 μm from the border of granule cell bodies) aligned as described above using IGL Trace software; macular and segmented PSDs are present. Fig. 4A, B (EM numbers 21 and 23) shows a dendrite (D) and four thin spines (sp 1, 2, 3 and 4) and their associated macular synaptic zones which consist of both the pre-synaptic and post-synaptic electron densities. These sections together with others from the series (not shown) produced the dendritic reconstruction shown in Fig. 4E (in two rotational positions), with the PSDs indicated in red. Fig. 4C, D shows EM numbers 28 and 31 of the series from the same dendritic segment. These contain a perforated/segmented PSD (pPSD) on a Msp, indicated by the asterisk in both EM numbers 28 and 31. The stalk of the spine is also shown. Note that in EM number 31 there is a spinule protruding from the PSD into the pre-synaptic bouton and a spine apparatus (SA) can be seen in the spine head. The spine and PSD together with the pre-synaptic density seen in EMs 28 and 31 are shown reconstructed in three dimensions in Fig. 4F, which is composed of other sections (not shown) from the same series. The segmented form of the PSD (red) together with the spinule is clearly seen in this reconstruction, as is the concave form of the spine head.

Only axo-spinous and shaft synapses were analysed in this study. Four categories of synapses were subjectively classified according to their post-synaptic contact, three according to whether the dendritic spine was ‘mushroom,’ ‘thin,’ or ‘stubby.’ The fourth category comprised synapses in which the presynaptic bouton contacted the dendrite directly and was termed a ‘shaft’ synapse. Although there is no absolute classification of thin and mushroom (M)sp, a spine was classified as mushroom if its head was appreciably wider than the width of the neck (as in Fig. 4F); thin, if its length was greater than the width of its neck and head, and stubby, if the width of the neck was similar to its length (Peters and Kaiser, 1970). Generally here, the volume of a thin spine was approximately 10 times less than the volume of a Msp. Examples of the four categories are shown in Fig. 4G, which is a complete reconstruction of over 130 serial ultrathin sections of a dendritic segment from the MML (a different series from that shown in Fig. 4E, F); PSDs, both macular (mPSD) and segmented (sPSD) are shown in red. A diagrammatic representation of the four categories of synapse is shown in Fig. 4H.

Synapse density: Stereological analysis

Stereological measurements of synapse density in the MML were made from: (i) potentiated (left) and (ii) unstimulated (right) hemispheres of the tetanised rats, and (iii) from the stimulated but unpotentiated (left) and unstimulated (right) hemispheres of the control rat. No distinction was made between synapse type (macular or perforated/segmented), or the nature of the spine contact when calculating overall synaptic density. Individual synaptic density values for each of the three tetanised hemispheres, and the three contralateral hemispheres showed little variation, and when the mean synaptic densities (Fig. 5A) were estimated, these showed no differences between the three groups (one-way ANOVA, $P>0.05$):
(i) 303 ± 20 per 100 μm$^3$ in the right (unstimulated) hemisphere (hippocampi from three animals, with three serial section series consisting of 75–95 serial thin sections; 500 synapses were counted);

(ii) 315 ± 26 per 100 μm$^3$ in the potentiated hemispheres (hippocampi from three animals; five serial section series consisting of 82–130 serial sections; 750 synapses counted);

(iii) 303 ± 28 per 100 μm$^3$ in the stimulated but not potentiated hemisphere of the control rat (one animal; three serial section series consisting of 73–94 serial sections; 450 synapses were counted).

Percentages of the four categories of synapses (contacting spines or shafts) in the MML of potentiated and control tissue: Serial section analysis and 3D reconstructions

The possibility of synaptic or dendritic spine remodelling cannot easily be ascertained from the stereological

Table 1. LTP is not associated with a change in the volume of dentate gyrus in the tetanized hemisphere$^a$

<table>
<thead>
<tr>
<th></th>
<th>Untetanised</th>
<th>Tetanised</th>
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<tr>
<td></td>
<td>R-DG</td>
<td>R-total Hippocampus</td>
</tr>
<tr>
<td>Mean volume</td>
<td>8.63 ± 0.50</td>
<td>30.53 ± 3.88</td>
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<tr>
<td>Volume ratio (DG/sum hippo %)</td>
<td>28.5 ± 3.82</td>
<td>31.70 ± 3.50</td>
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$^a$ Mean volumes (± S.D.) in mm$^3$ of the left (L) and right (R) hippocampi and left (tetanised) and right (untetanised) DG estimated by use of the Cavalieri method. The boundaries of the DG (cell body layer and molecular layer) and of the total hippocampus (including CA1, CA2, CA3, DG, polymorph cell and hilar regions, but excluding the subiculum) were defined bilaterally in each rostro-caudal section (see Fig. 1A). Total hippocampal and total DG volumes for each hemisphere were derived by multiplying the total surface areas of each structure in each section by the section thickness (t). Statistical comparisons in volume data between regions, hemispheres and animals were performed using multiple t-tests. There are no significant differences between hemispheres, nor between whole hippocampus and dentate gyrus, or for the volume ratios of DG:hippocampus. Hippo, hippocampus.
measurements presented in Fig. 5A. Accordingly, 3D reconstructions were performed on serial sections (up to 130 per reconstruction) taken at random from the MML. Sufficient reconstructions were made to obtain a minimum of 100 of each of the four categories of synapse in each of the potentiated, unstimulated, and stimulated but not potentiated states. The number of synapses per unit area is presented in Fig. 5B as percentages of the total number of synapses. The percentage of thin spines is significantly increased ($P<0.01$) in the MML of the DG of the potentiating hemisphere compared with either the equivalent region in the unstimulated contralateral hemisphere or in the stimulated but not potentiating hemisphere of the control rat. Conversely, the percentage of both stubby and shaft synapses decreased significantly ($P<0.01$) in the potentiating hemisphere compared with
DISCUSSION

This is the first detailed 3D reconstruction study of changes in synaptic and dendritic spine morphology associated with late LTP in vivo. In order to ensure that our morphometric estimations were not influenced by volume changes of hippocampal tissue we have compared the volumes of both hippocampus and DG in each hemisphere and have confirmed that there are no significant differences in tissue volume following the unilateral induction of LTP. This lends strong support to the conclusion that our stereological measurements, showing no change in synapse density per unit volume of tissue the DG 6 h after the induction of LTP, can be interpreted as indicating no change in the number of synapses in the regions measured. However, 3D reconstructions of serial sections demonstrate that LTP is associated with significant changes in the size and shape of the synaptic density specialisations, in particular the PSD volume of which we have measured, and in the proportion of the different types of spines.

The finding that induction of LTP in the DG in vivo does not lead to a change in synapse number 6 h after the tetanus is consistent with the conclusion reached by Sorra and Harris (1998) in their analysis of synapse number in area CA1 2 h after the induction of LTP in vitro. Changes

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The finding that induction of LTP in the DG in vivo does not lead to a change in synapse number 6 h after the tetanus is consistent with the conclusion reached by Sorra and Harris (1998) in their analysis of synapse number in area CA1 2 h after the induction of LTP in vitro. Changes
in synaptic structure and number are likely to depend critically on the post-induction interval since the phase of LTP which requires enhanced protein synthesis (both in vivo and in vitro) begins 2–5 h after induction of potentiation (Frey et al., 1988; Frey and Morris, 1997), arguing that more prominent structural changes, if any, are likely to occur after this period. Hence comparisons of morphometric parameters from different laboratories need to consider the time after tetanisation at which the analyses were performed. Previous data from one of our laboratories (Stewart et al., 2000) indicated an increase in synapse density after induction of hippocampal LTP in vivo. However those data were obtained 24 h after stimulation, and used analyses based upon a two-section disector, rather than 3D reconstructions. We cannot conclude that the restructuring observed here 6 h after induction precludes changes in synapse density in the MML at other times. Nor can we rule out the possibility suggested by Sorra and Harris (1998) that both synaptogenesis and synapse elimination occur, resulting in no net change in synapse number or size.

Significant increases also occur in total volume and area of the 3D reconstructed spines following LTP induction, and in the volume and surface area of the postsynaptic density. Unperforated (macular) synapses were almost all located on thin spines whereas synapses with perforated/segmented junctions occurred on Msps; the volume and area changes occurred in both types of synapse. An increase in the area of the PSD may reflect a role in enhanced synaptic efficacy, perhaps through the accommodation of additional receptors (Malenka and Nicoll, 1999), and/or an increase in the population of recycling vesicles as discussed by Marrone and Petit (2002). Although we have not made quantitative measurements our data appear to show qualitatively that LTP is associated with an alteration in size and shape of the PSD, together with a transformation in the shape of the spine head from relatively flat or convex to a more concave form in potentiated tissue, as also observed by Weeks et al. (1999, 2000, 2001, 2003) in the DG of the freely moving rat. If our qualitative observations are correct they would support earlier findings by Desmond and Levy (1983), determined by single section analysis for DG in vivo, and in hippocampal slices for area CA1 (Chang and Greenough, 1984) and CA3 (Petukhov and Popov, 1986). As controls we have used: (i) the unstimulated hemispheres and (ii) a stimulated but untetanised hemisphere. The changes we observed in the anaesthetized animal are therefore attributable to tetanic stimulation. The LTP-associated changes in the incidence of concave synapses reported by Weeks et al. (2003) in the DG of the unanaesthetised rat were not seen when the induction of LTP was blocked with ketamine, a competitive NMDA antagonist. We conclude that our results reflect the induction of LTP, rather than nonspecific changes related to tetanic stimulation, or to an interaction between anesthesia and tetanic stimulation.

The alterations in the proportions of the four categories of synapse (on thin, stubby and mushroom spines, and on dendritic shafts) 6 h after the induction of LTP result from a significant increase in the proportion of synapses on thin spines and a concomitant decrease in synapses on shaft and stubby spines. In contrast, the proportion of Msp synapses is unchanged after LTP though the area of perforated synaptic densities on Msps increases significantly, as does their volume.

Our interpretation of the changes in synapse and spine structure 6 h after the induction of LTP in vivo is illustrated diagrammatically in Fig. 8. LTP is accompanied by a transition from stubby spines and shaft synapses to thin spines (Fig. 8(1)), with an overall increase in the length of thin spines (Fig. 8(2)). In thin spines the mPSD becomes larger and appears more concave as
the pre-synaptic contact zone becomes more convex. At the same time, Msps expand, with a larger and more concave PSD and a correspondingly more convex pre-synaptic density (Fig. 8(3)). These processes may be
reversible in the absence of stimulation or in physiological conditions such as hibernation (Popov and Bocharova, 1992; Popov et al., 1992, 2003). The idea of a possible transition of shaft to stubby synapses, and stubby to thin synapses, is consistent with earlier in vitro and in vivo studies in area CA1 (Fiala et al., 1998; Kirov and Harris, 1999; Harris et al., 2003). Our findings would suggest that in the DG of the intact animal 6 h post-induction, there is remodelling of pre-existing synapses rather than splitting of dendritic spines and formation of new synapses. We did not observe an increase in the proportion of perforated/segmented synapses as reported for DG by Geinisman et al. (1992) or in the work of Muller et al. (2000). However, it is possible that the discrepancy may be explained by the different post-induction intervals at which the various studies were performed. The studies by Geinisman et al. (1992) were carried out more than 24 h post-initial LTP induction. In ex vivo studies in area CA1 by Muller et al. (2000) and Toni et al. (1999, 2001) an increase in the proportion of perforated PSDs was reported at 30 min post-tetanus, but there was a return to control levels 2 h later.

The EM data presented here only partially support the many recent confocal microscope studies and high-resolution time-lapse imaging of hippocampal tissue, which have revealed the remarkable plasticity and motility of dendritic spines both during development (Dunaevsky and Mason, 2003), and after LTP (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Nikonenko et al., 2002). We have not found evidence for an increase in spine formation reported in the latter studies; our data are in broad agreement with the work of Harris and her colleagues (1999a,b; Kirov and Harris, 1999; Ostroff et al., 2002; Sorra and Harris, 1998) in area CA1, and with the confocal microscope study of Hosokawa et al. (1995), also in area CA1, in which spine size and number were examined in acute hippocampal slices before and after chemically induced LTP. The latter experiments suggested that one of the major forms of structural change involves growth of a subpopulation of small spines, consonant with our finding of an increase in the proportion of long thin spines. Our results are also consistent with the observations of Emptage et al. (2003) who failed to find new spine or filopodia formation following an imaging study of LTP at individual spines in areas CA1 and CA3 of organotypic slices.

Recent studies have provoked a major shift in our understanding of the dendritic spine, from a stable storage site of long-term memory to a dynamic structure which is capable of undergoing rapid morphological variations (Segal and Andersen, 2001). The possibility that spine motility could also contribute to the anchoring or removing of glutamate receptors at spine heads, and may control the efficacy of existing synapses has also been discussed by Segal and Andersen (2001). Whilst the precise mechanism of spine and synapse remodelling remains unclear, it seems likely that it is closely correlated with a change in synaptic strength following stimulation (Nikonenko et al., 2002). A common mechanism involving changes in intracellular Ca\(^{2+}\) concentration may control both formation/elongation and pruning/retraction of spines (Segal 2001; Goldin et al., 2001). LTP may also involve activation of

**Fig. 8.** Schematic interpretation of the changes in synapse and spine structure 6 h after the induction of LTP in the DG in vivo. Synaptic vesicles and mitochondria are shown in the pre-synaptic bouton; the PSD is dark grey at the head of the spines. Stubby spines and shaft synapses are transformed into Tsps (1), whilst existing Tsps grow larger and acquire concave heads (2). As a consequence, Tsps grow more numerous, whilst stubby spines and shaft synapses decline in number. Msp heads also become more concave (3), with no change in density but there is an increase in the PSD volume.
protein synthesis on polyribosomes located in dendrites (Steward and Schuman, 2001; Ostroff et al., 2002; Goldin and Segal, 2003; Bradshaw et al., 2003), and a later phase associated with an enhanced F-actin content within the dendritic spine (Fukazawa et al., 2003). The role of actin appears to be to maintain stability of synaptic structure (Halpain et al., 1998), a stability which is disrupted by the intense glutamate receptor activity following induction of LTP. It is also possible that the remodelling of spines reflects a redistribution of synapses, since a single bouton may innervate more than one spine (Toni et al., 1999; Geinisman et al., 2001). An increase in the proportion of longer thin spines would increase the surface area available for such contacts whilst leaving the overall number of synapses of the MML unchanged. In addition longer thin spines could reach more distant axons. Such structural modifications could underlie the alterations in synaptic efficacy seen in LTP.

In summary, the quantitative 3D data presented here argue that morphological changes 6 h after the induction of LTP in the DG of the anaesthetised rat reflect synapse and dendritic spine remodelling rather than a change in the overall number of synapses.

Acknowledgments—This work was supported in part by grants BBSC 108/S08513 and BBSC 108/NEU15416 (to M.G.S) and The Leverhulme Trust (grant F00269G) and Russian Foundation for Basic Research (grant 48890a to V.I.P.). We thank Dr. John C. Fiala (Boston University, Boston, MA, USA) for consultations on use of the IGL Trace programme, and for helpful comments on the manuscript.

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(Accepted 16 June 2004)
(Available online 20 August 2004)