Quantal analysis and synaptic anatomy – integrating two views of hippocampal plasticity

John E. Lisman and Kristen M. Harris

The excitatory synapses onto CA1 pyramidal cells have become a model system for understanding the activity-dependent changes in synapses that underlie learning and memory. Here we examine physiological and anatomical results that are relevant to understanding the mechanisms of synaptic transmission and plasticity at these synapses. Three main points are discussed. First, quantal analysis indicates a large heterogeneity of postsynaptic efficacies for different synapses on the same cell. Reconstructions from electron microscopy show that synapse size is also highly heterogeneous. Reasons for suspecting a relationship between synaptic size and efficacy are discussed. Second, physiological evidence indicates that the changes during long-term potentiation are both pre- and postsynaptic. Similarly, several lines of anatomical evidence suggest that plasticity affects the structure of both the pre- and postsynaptic elements. The detailed registration of structures across the synapse and the physical linkage between pre- and postsynaptic elements suggest a 'structural unit hypothesis' for coordinating pre- and postsynaptic modifications. Third, quantal analysis indicates that stimulation of a single axon can release multiple quanta. Anatomical evidence shows that cell pairs can be connected by multiple synapses, suggesting that multiple quanta may be released at independent sites. These results raise the possibility that one component of synaptic plasticity is mediated by changes in the number of functional synaptic sites.

John E. Lisman is at the Dept of Biology and the Center for Complex Systems, Brandeis University, Waltham, MA 02254, USA; and Kristen M. Harris is at the Dept of Neurology and Program in Neuroscience, Children's Hospital and Harvard Medical School, Boston, MA 02115, USA.

It is widely thought that memory is stored by an ensemble of synapses that individually change their efficacies during learning. For this reason, elucidation of the mechanisms underlying long-term potentiation (LTP), a process that involves synapse-specific changes in efficacy[1], is a major goal of current efforts to understand the cellular basis of learning and memory. Most of the work on LTP has been done in the CA1 region of the hippocampus, and substantial progress has been made in understanding some of the processes involved. Induction of LTP is governed by the Hebb rule and involves Ca^{2+} influx mediated by the NMDA receptor/channel complex (reviewed in Brown *et al.*[2]). More recently, the focus of research has turned to the expression of LTP – the mechanisms responsible for enhanced transmission. There are several related questions here. Do the mechanisms involve structural growth or modulation of existing structures? Is the modification presynaptic, postsynaptic or both? Does it take place at a single site of synaptic contact or at multiple sites? Much of the recent work on LTP expression has used the physiological tool of quantal analysis[3–10], but there has also been progress in understanding the anatomy of the synapses involved. Our goal here is to review the physiological and anatomical data, and to make connections between the two where possible.

Heterogeneity of synaptic efficacy and synaptic size

If memories are stored in nerve networks through synapse-specific changes, learning should produce heterogeneity of synapses and experimenters should find evidence of such heterogeneity in regions of the brain that are involved in memory. Both physiological and anatomical studies have observed such heterogeneity in the hippocampus, a region of the brain critical for memory processes[11,12]. The physiological evidence for heterogeneity of synapses comes from analysis of spontaneous miniature excitatory postsynaptic currents (mepcs) in hippocampal CA1 cells. These events are thought to arise from the spontaneous release of a single synaptic vesicle at any of the thousands of synapses on the cell. Figure 1 shows that mepcs recorded in a CA1 cell are highly variable[6,13,14]. One explanation is that the variability results from nonuniformity in the transmitter content of synaptic vesicles. This explanation is, however, inconsistent with histograms of elicited responses resulting from the summation of a variable number of quanta. These histograms sometimes have evenly spaced peaks (see below) that would not occur if different vesicles contained significantly different amounts of neurotransmitter[15,16]. Another explanation could be that mepcs are generated at different electrotonic distances from the soma; theory indicates

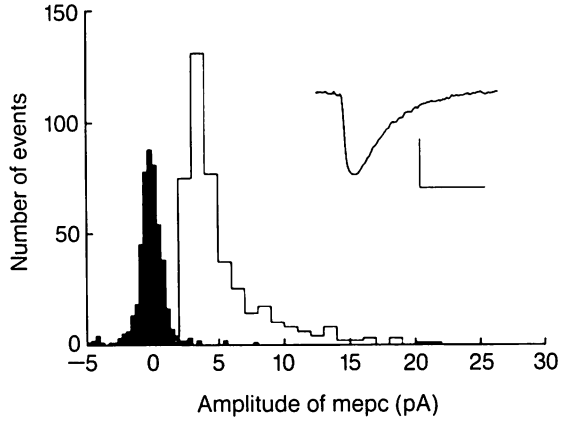

Fig. 1. *Amplitude histograms of spontaneous miniature synaptic currents (mepcs) are very broad, with events ranging from 2 to 20 pA. The width of the distribution (open bars) is much larger than that of the noise (filled bars). Events were recorded using a whole-cell clamp from a CA1 pyramidal cell. The large sized events cannot be attributed to presynaptic action potentials since the size distribution is not altered by tetrodotoxin, as documented in the paper from which the figure was taken. The inset shows the average mepc waveform; calibration 5 pA and 20 ms.* (Reproduced, with permission, from Ref. 6.)

0166 - 2236/93/$06.00

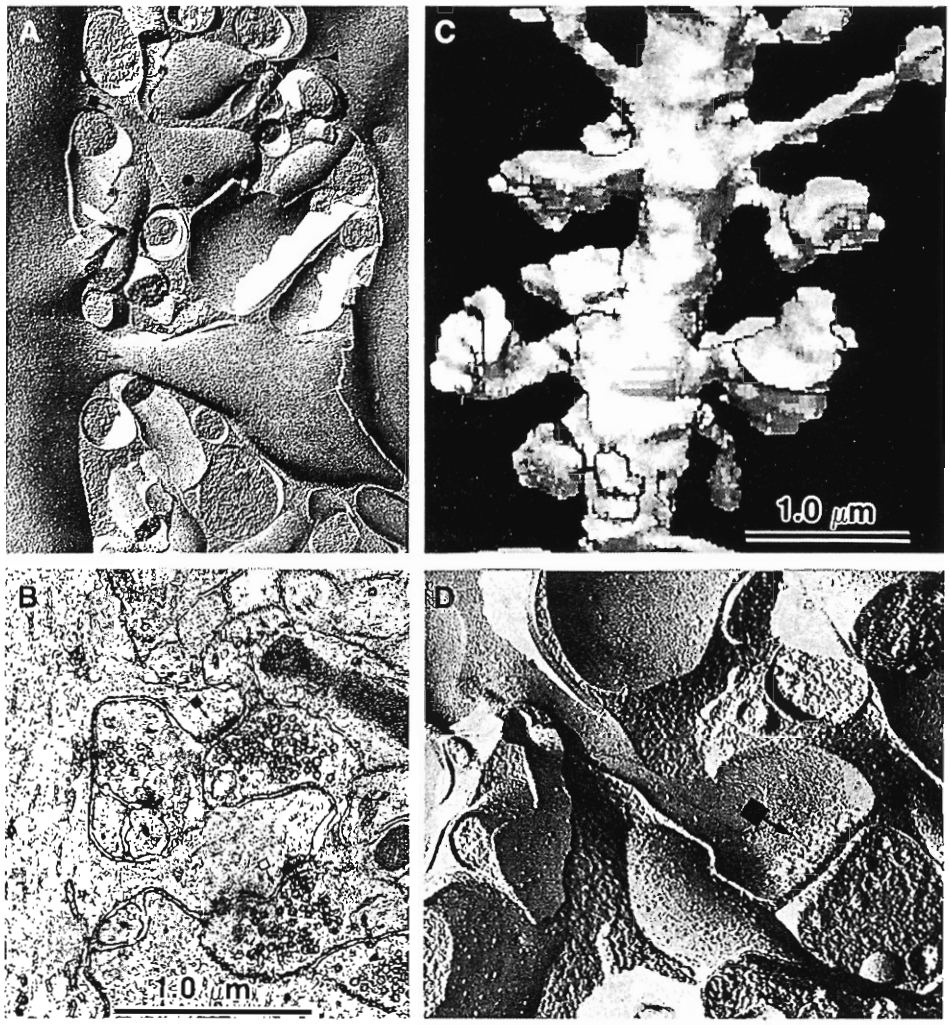

Fig. 2. *Heterogeneity in the size and shapes of dendritic spines and their synapses in hippocampal area CA1.* **(A)** *Cytoplasmic profile (P-face) of a small, thin, or 'pedunculated' dendritic spine (filled square) revealed by freeze-fracture electron microscopy to be near to a large, mushroom-shaped dendritic spine (open square) of the same dendritic segment.* **(B)** *Thin-section view of two spines with similarly diverse shapes that also have different types of postsynaptic densities (PSDs). The smaller spine has a continuous, macular-shaped PSD (filled square), while the larger spine has an electron-lucent perforation in the PSD (open square).* **(C)** *Three-dimensional reconstruction of a segment of CA1 pyramidal cell dendrite revealing multiple spine shapes along its length.* **(D)** *Particle aggregate on the extracellular half of the membrane (E-face) at the site of a synapse on the head of a thin dendritic spine (filled square).* [(A), (B) and (D) are modified from Ref. 18; (C) is modified from Ref. 19.]

that the larger the distance between a synapse and the soma, the smaller the synaptic current at the soma[17]. This is not likely to be the exclusive cause of mepc heterogeneity because similar heterogeneity is seen when mepcs are stimulated by application of hypertonic solution at a fixed dendritic location[5]. Furthermore, little or no correlation is found between the size and the kinetics of mepcs[5,14]. If electrotonic distance were the major factor determining size, then the small mepcs generated distally should have been the slowest because electronic spread results in kinetic slowing[17]. There must therefore be another major cause of size variability. The most likely remaining possibility is that synapses onto the same CA1 cell differ in their postsynaptic responsiveness to transmitter.

Anatomical studies of the synapses that generate the mepcs also provide evidence for postsynaptic heterogeneity. Dendritic spines are the major postsynaptic targets of excitatory synapses that have been studied using quantal analysis. Freeze-fracture profiles (Fig. 2A) reveal that adjacent dendritic spines can have very different shapes. The synapse usually occurs on the spine-head, which is connected to the parent dendrite by a thinner spine-neck (Fig. 2B). Spine volume can be definitively established by three-dimensional reconstruction from serial electron microscope (EM) sections (Fig. 2C). Such reconstructions show that spine volume varies by over an order of magnitude (Fig. 3). Since even neighboring spines can have very different sizes, this heterogeneity must arise through a synapse-specific mechanism.

Does this variability of postsynaptic structure account for the variability in postsynaptic efficacy? This question cannot yet be answered definitely, but the following observations make it likely that efficacy and size are related. Like other excitatory synapses, the synapses onto CA1 cells have specialized regions that are the site of synaptic transmission. In these regions the synaptic cleft widens [about 10–20 nm (Ref. 20)] and there are specialized pre- and postsynaptic structures. Just below the postsynaptic membrane is a structure called the postsynaptic density (PSD) that is characteristic of asymmetric [Gray's Type I (Ref. 21)] synapses (Fig. 2B). The PSD is physically linked to the glutamate receptors/ion channels that mediate synaptic transmission[22,23]. Freeze-fracture studies of the synaptic region show a discrete aggregate of particles [~2800 particles/μm^2 (Ref. 18)] (Fig. 2D), at least some of which are likely to be the glutamate-gated channels that mediate synaptic transmission. The size of the particle aggregates varies from spine to spine by more than an order of magnitude. Since larger spines have larger synaptic regions (Fig. 3), they probably have more synaptic channels and are therefore likely to have greater efficacy.

We conclude that there is both anatomical and physiological evidence for postsynaptic heterogeneity, and that spine size is likely to be one of the factors that determines postsynaptic efficacy. An intriguing possibility is that the heterogeneities in size and efficacy are the anatomists' and physiologists' views of the animal's stored memories.

Coordinated pre- and postsynaptic changes

Work using quantal analysis has sought to determine whether the enhanced synaptic efficacy during

LTP is due to presynaptic changes, postsynaptic changes or both. Recent evidence (Fig. 4; Ref. 10) is based on analysis of responses elicited by minimal stimulation[7,9,10]. In favorable cases, the amplitude histogram shows evenly spaced peaks, as would be expected if the response is composed of a variable number of nearly identical quanta. As shown in Fig. 4, the separation between peaks can almost double during LTP (note the doubling of the abscissa scale in the lower histogram). This increase in separation indicates that the response to each quantum increases, and implies that LTP enhances the postsynaptic response to a quantum of transmitter. Further support for an increase in the quantal response is the increase in the amplitude of spontaneous mepcs during LTP (Ref. 6). However, the data cannot be wholly explained by a change in responsiveness to each quantum of neurotransmitter. In particular, the decrease in the number of failures[4] and changes in the relative size of the different peaks[7,9,10] suggest that there is also an increase in the number of quanta that contribute to the response during LTP, probably due to enhanced release of vesicles (but see Edwards[24]). The physiological results therefore suggest that the enhanced efficacy during LTP is due to both pre- and postsynaptic changes.

Several lines of anatomical evidence also suggest that plasticity involves both pre- and postsynaptic changes. Moreover, the evidence indicates that there must be a mechanism for coordinating pre- and postsynaptic changes. The general line of argument is that while there is enormous variability from synapse to synapse, the size of the presynaptic structure in any one synapse is closely related to the size of its respective postsynaptic structure. The first line of evidence comes from analysis of the spines and presynaptic boutons. As mentioned above, the area of PSD is correlated with the volume of the dendritic spine and this is also the case for other postsynaptic structures such as the smooth endoplasmic reticulum[19,25,26]. Anatomical analysis also shows that the size of presynaptic elements varies. Importantly, a correlation is found between the size of the pre- and postsynaptic structures: the volume of the presynaptic bouton[19] and the total number of vesicles it contains (Fig. 3) correlate closely with the size of postsynaptic structures.

The second line of evidence involves the size of specializations at the synapse. A specialization called the synaptic grid is seen at the presynaptic active zone [especially in ethanolic phosphotungstic acid (E-PTA)-stained material[27]]. From the grid emerge 'dense projections' that may be involved in vesicle docking and release. As shown in Figs 5A–C, the lateral dimensions of the presynaptic grid are very close to that of the postsynaptic density and the ends of the two structures are in register.

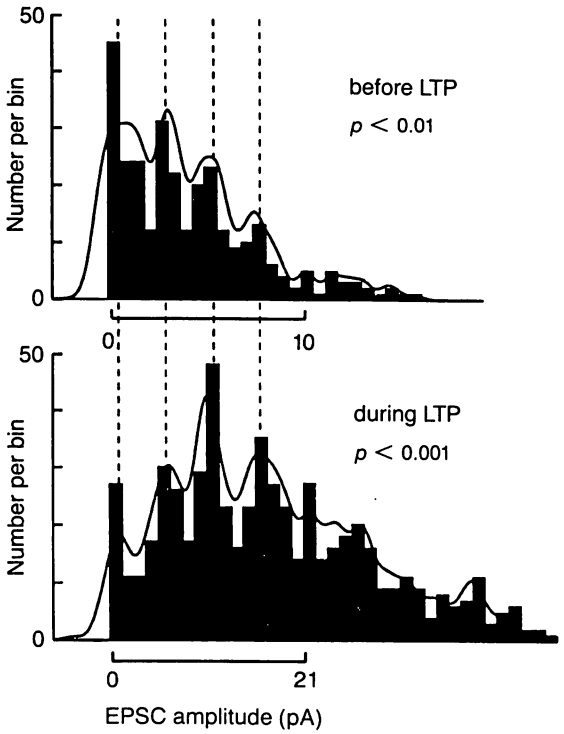

Fig. 4. *Amplitude histograms of responses (excitatory postsynaptic currents; EPSCs) elicited by minimal stimulation before and during LTP. Note the presence of evenly spaced peaks indicating summation of quanta. During LTP, the amplitude between peaks is increased (note doubling of the scale on the x-axis), indicating enhanced postsynaptic responsiveness. During LTP, there is also a reduction of the number of failures (at zero current), indicating enhanced presynaptic release of neurotransmitter. Data were obtained by whole-cell recording of a CA1 pyramidal cell, as described in Liao* et al.[10]

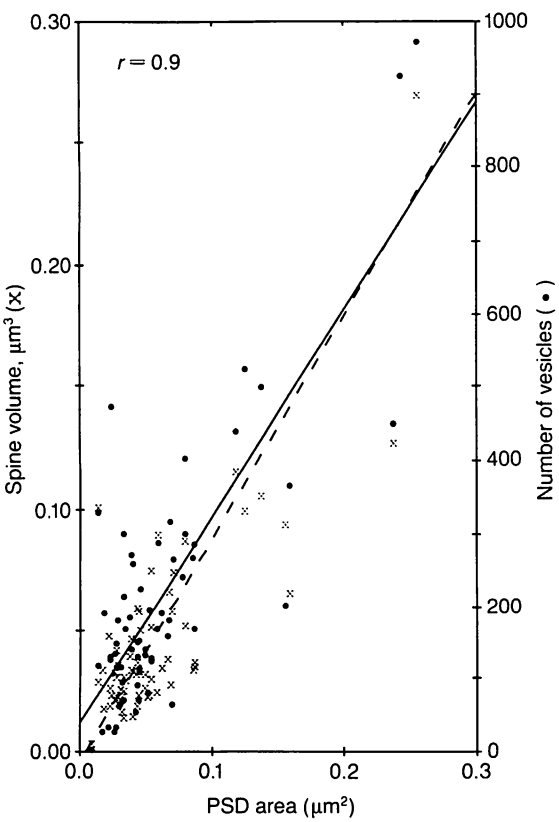

Fig. 3. *Correlation (r) between area of postsynaptic density (PSD; in μm²), spine volume (in μm³) and the number of vesicles in the presynaptic axonal bouton.* (Modified from Ref. 19.)

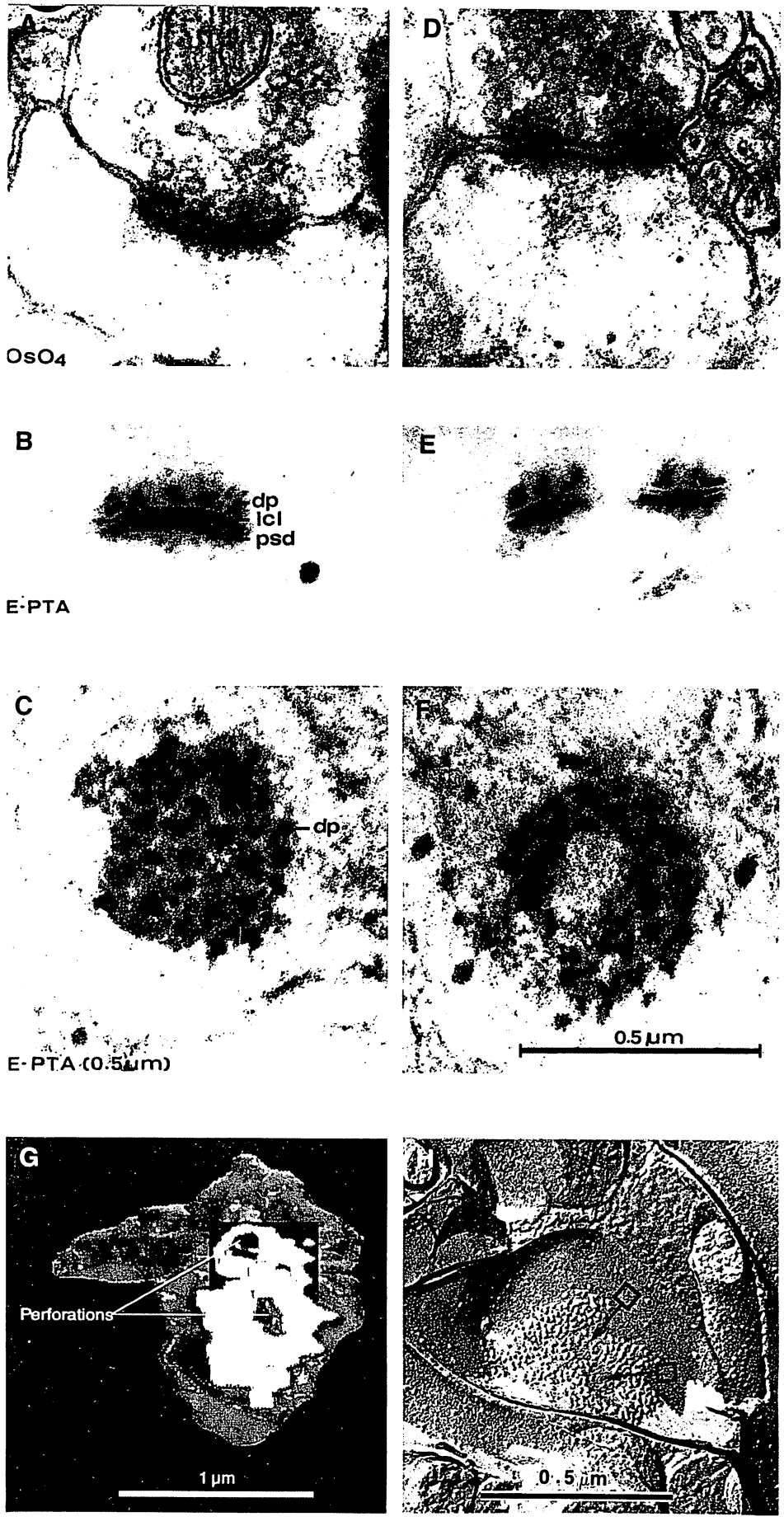

Fig. 5. *Pre- and postsynaptic specializations appear to be matched in size and shape.* **(A)** *Thin section of the head of a dendritic spine (from rat cortex) at a point where the postsynaptic density is contiguous with the dense cleft material, and the presynaptic extent of vesicles.* **(B)** *Presynaptic dense projections* (dp), *intercleft layer* (icl) *and postsynaptic density* (psd) *are coextensive when revealed by the ethanolic phosphotungstic acid protocol (E-PTA).* **(C)** En face *view of the presynaptic grid can be continuous.* **(D)** *Perforation in the postsynaptic density is paralleled by* **(E)**, *a perforation in the presynaptic dense projections, and* **(F)**, *the array of dense projections viewed* en face. **(G)** *Three-dimensional reconstruction of a hippocampal dendritic spine (gray) that contains two perforations in the postsynaptic density (white). A window of translucency was used to reveal the upper perforation through the spine.* **(H)** *Freeze-fracture* en face *view of two particle-free zones in the middle of a particle aggregate; these particle-free zones are located in the E-face, and are thought to be the membrane equivalent of the perforation in the postsynaptic density.* [(A)–(F) are taken from Ref. 27; (G) is modified from Ref. 26; and (H) is from Ref. 18.] *Scale bar shown in* (F) *applies to all figures* (A)–(F).

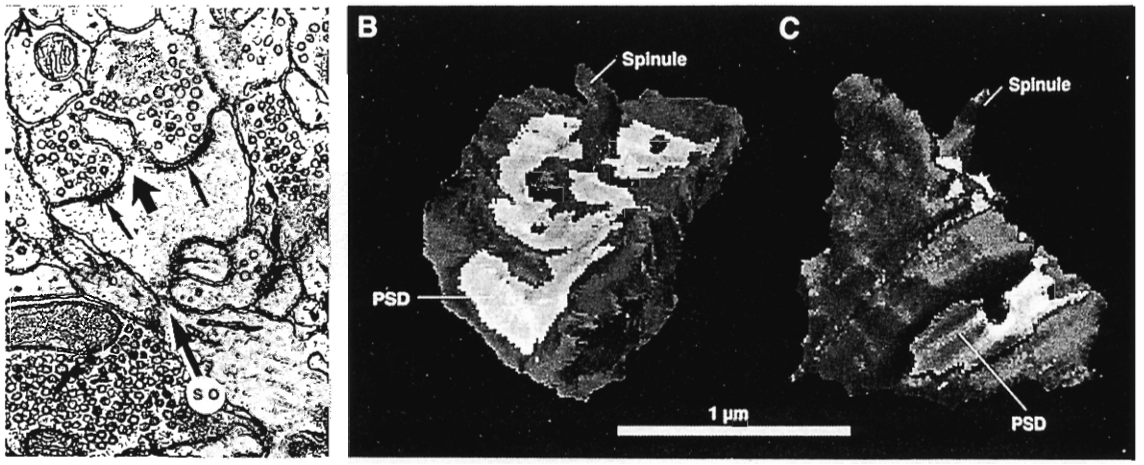

Fig. 6. *Perforated postsynaptic density (PSD) with a complex morphology and a spinule.* **(A)** *Thin section through the middle of a perforation with the spinule projecting out of the perforation.* **(B)** *Three-dimensional reconstruction of the spine (gray) that is viewed from the top of the highly irregular PSD surface.* **(C)** *Side view of the profile of the spinule projecting from the PSD.* (Modified from Ref. 19.)

A third line of evidence involves the perforations of these specializations. Larger PSDs are often seen to have a perforation, a region of low density in the center (Figs 5D,E, see also Figs 2B and 6). Serial EM reconstruction[28] shows definitely that the perforation in the PSD is not an artifact of the sectioning angle (Fig. 5G). Perforations are also seen in the presynaptic grid (Fig. 5F), and these perforations are exactly in register with those in the PSD (Fig. 5E). A perforation can also be seen in the array of freeze-fracture particles in the postsynaptic membrane (Fig. 5H).

Taken together, these anatomical results indicate that a coordinated process governs the growth of pre- and postsynaptic structures. Moreover, the exquisitely detailed registration of structural specializations on the two sides of the synapse further suggests that the synapse is a structural unit. Indeed, there is direct evidence for a strong structural linkage between the presynaptic and postsynaptic cells: when the brain is homogenized, the PSD and the associated glutamate channels remain attached to the presynaptic terminal (reviewed in Heuser and Reese[29]). The molecules involved in trans-synaptic structures are not known, but may involve the adhesion molecules implicated in synapse formation[30–32]. It has been widely speculated that, because induction of LTP is postsynaptic and because there are consequent presynaptic changes, there must be a diffusible retrograde messenger. However, the existence of structural links between presynaptic and postsynaptic cells raises the possibility that coordination of growth might be achieved structurally, without a diffusible messenger[30,33].

It would clearly be of great interest to visualize individual synapses as they undergo LTP and ask directly whether both pre- and postsynaptic growth occurs, but this has not yet been possible. What has been done is to compare the average density and dimensions of synapses in preparations that have undergone LTP with those in preparations that have not (reviewed in Wallace *et al.*[34]). Some of these studies have reported that during LTP there is an increase in the average spine area and PSD length, as measured on single thin sections. There have also been reports of presynaptic changes, specifically a clustering of vesicles closer to the site of release[35,36]. These studies have relied on measurements of single sections, which can lead to misinterpretations as discussed in Harris *et al.*[26]. In another study, serial EM constructions of sample dendrites were used to reveal changes both in spine geometry and a doubling of spine number during LTP (Refs 37–39). Taken as a whole, these results suggest that LTP is associated with both pre- and postsynaptic structural changes.

We have emphasized the possibility that structural changes are involved in plasticity, but processes involving modulation of existing structures are probably also involved. For instance, modulation of existing receptors by phosphorylation, could change synaptic efficacy without changing the synaptic structure. All that can be said at this point is that models of LTP based solely on biochemical changes would seem unlikely given the evidence for structural heterogeneity. In a model proposed by one of us (J.L.), the efficacy of synaptic transmission is stored through activity-dependent changes in the fraction of PSD calmodulin-kinase molecules in the phosphorylated ('on') state[40]. To be consistent with the anatomical evidence, this model would have to be extended to include a process by which the number of calmodulin-kinase switches increases as the synapse grows.

Multiple synaptic sites between pairs of cells

Knowing the number of synaptic sites that connect a pair of cells is critical to understanding synaptic transmission and the way it is affected by plasticity. If multiple sites are involved, LTP induction might convert a non-functional site into a functional one or stimulate the growth of new sites. The multi-peaked histograms of elicited responses (Fig. 4) indicate that the postsynaptic response to a single presynaptic action potential involves the linear summation of multiple quanta. However, these data by themselves leave it unclear whether these quanta are released at the same synapse or at multiple synapses.

Work at other central nervous system synapses, the inhibitory synapse of the goldfish (*Carassius*

auratus) Mauthner cell[41] and the excitatory synapses onto cat motoneurons[16], demonstrates that the response to stimulating a single axon is due to multiple synapses, each of which releases a vesicle with a probability less than one. This implies that elicited responses are due to the summation of single quanta generated at multiple boutons rather than multiple quanta at a single bouton. Indeed, there are both theoretical calculations and experimental evidence suggesting that the thousands of transmitter molecules released by a single vesicle nearly saturate the postsynaptic receptors at central synapses[15,16,42,43]. In this case, it necessarily follows that linearly summating quanta must be due to vesicles released at different sites.

In area CA1, there is now evidence that a single axonal branch can make multiple synapses with its target pyramidal cells[44,45]. In addition, it is clear that these axons themselves are highly branched[44,46], raising the possibility that more than one of these branches synapse onto the same pyramidal cell. Based on this evidence, it is likely that at least some of the quanta in multi-quanta responses are released at different spines. Because of transmitter degradation, reuptake and dilution, the transmitter released at one spine is unlikely to interact with the transmitter released at other spines.

It is more difficult to assess what happens if multiple quanta are released at the same spine. As mentioned above, there are reasons to believe that a single vesicle can release enough transmitter to saturate the postsynaptic receptors locally, but this may not be true for large synapses or when the PSD becomes segmented into different regions. Simulations of diffusion and binding of transmitter within the synaptic cleft are just beginning to be done[47,48], but the number of variables is large, and little can be said with certainty. Serial section reconstruction indicates that the shape of the PSD can be very complex (Fig. 6). Complicating the picture still further, there is often a spinule[49] near the middle of the synapse that extends the lateral dimensions of the extracellular space and must therefore affect the diffusion of transmitter.

There are hints that the complexity of synaptic structure is related to plasticity. Anatomical studies suggest that PSDs are segmented through an activity-dependent process. The best evidence for this comes from work on the dentate gyrus, where across-animal comparisons show that LTP produces an increase in segmented PSDs[50]. If transmitter released at each segment acts only on that segment, the formation of segmented PSDs would effectively increase the number of synapses, a possibility that has important implications for quantal analysis. There is also evidence that the frequency of spines having a spinule increases following LTP (Ref. 36), raising the possibility that spinules may be involved in synaptic growth[49]. Clearly, we are now at the threshold of understanding many fascinating aspects of synaptic plasticity in the central nervous system.

Concluding remarks

There has been substantial physiological and anatomical investigation of CA1 synapses but there has been little or no crosstalk between these approaches. As we have argued here, there are sufficient data to begin to formulate ideas about the relationships between structure and function at these synapses. Experiments that combine physiological and anatomical approaches are becoming technically feasible, and are likely to be done in the near future. The study of central nervous system synapses is at a particularly exciting stage because relatively little is known about fundamental structure–function issues, because new methods are becoming available for attacking these issues and because it is clear that the insights achieved will be important for the understanding of learning and memory.

Selected references

1 Bliss, T. V. P. and Lomo, T. (1973) *J. Physiol.* 232, 331–356
2 Brown, T. H., Zador, A. M., Mainen, Z. F. and Claiborne, B. J. (1991) in *Long-term Potentiation, a Debate of Current Issues* (Baudry, M. and Davis, J. L., eds), pp. 357–389, MIT Press
3 Foster, T. C. and McNaughton, B. L. (1991) *Hippocampus* 1, 1–48
4 Malinow, R. and Tsien, R. W. (1990) *Nature* 346, 175–180
5 Bekkers, J. M. and Stevens, C. F. (1990) *Nature* 346, 724–729
6 Manabe, T., Renner, P. and Nicoll, R. A. (1992) *Nature* 355, 50–55
7 Kullman, D. M. and Nicoll, R. A. (1992) *Nature* 357, 240–244
8 Voronin, L. L., Kuhnt, U. and Gusev, A. G. (1992) *Exp. J. Brain Res.* 89, 288–299
9 Larkman, A., Hannay, T., Stratford, K. and Jack, J. (1992) *Nature* 360, 70–73
10 Liao, D., Jones, A. and Malinow, R. (1992) *Neuron* 9, 1089–1097
11 Scoville, W. B. and Milner, B. (1957) *J. Neurol. Neurosurg. Psychiatr.* 20, 11–21
12 Zola-Morgan, S., Squire, L. R. and Amaral, D. G. (1986) *J. Neurosci.* 6, 2950–2967
13 Bekkers, J. M., Richerson, G. B. and Stevens, C. F. (1990) *Proc. Natl Acad. Sci. USA* 87, 5359–5362
14 Finch, D. M. and Jackson, M. B. (1990) *Brain Res.* 518, 269–273
15 Larkman, A., Stratford, K. and Jack, J. (1991) *Nature* 350, 344–347
16 Redman, S. (1990) *Physiol. Rev.* 70, 165–198
17 Rall, W. and Segev, I. (1985) in *Voltage and Patch Clamping with Microelectrodes* (Smith, T. G., Lecar, H., Redman, S. J. and Gage, P. W., eds), pp. 191–215, Williams and Wilkins
18 Harris, K. M. and Landis, D. M. (1986) *Neuroscience* 19, 857–872
19 Harris, K. M. and Stevens, J. K. (1989) *J. Neurosci.* 9, 2982–2997
20 Peters, A., Palay, S. L. and Webster, H. deF. (1991) *The Fine Structure of the Nervous System: The Neurons and Supporting Cells*, W. B. Saunders
21 Gray, E. G. (1959) *J. Anat.* 83, 420–433
22 Wu, K., Carlin, R. and Siekevitz, P. (1986) *J. Neurochem.* 46, 831–841
23 Fagg, G. E. and Matus, A. (1984) *Proc. Natl Acad. Sci. USA* 81, 6876–6880
24 Edwards, F. (1991) *Nature* 350, 271–272
25 Harris, K. M. and Stevens, J. K. (1988) *J. Neurosci.* 8, 4455–4469
26 Harris, K. M., Jensen, F. E. and Tsao, B. (1992) *J. Neurosci.* 12, 2685–2705
27 Vrensen, G. and Cardozo, J. N. (1981) *Brain Res.* 218, 79–97
28 Cohen, R. S. and Siekevitz, P. (1978) *J. Cell Biol.* 78, 36–46
29 Heuser, J. E. and Reese, T. S. (1977) in *The Handbook of Physiology, The Nervous System I* (Kandel, E., ed.), pp. 261–294, American Physiological Society
30 Bahr, B. A. and Lynch, G. (1992) *Biochem. J.* 281, 137–142
31 Ferns, M. J. and Hall, Z. W. (1992) *Cell* 70, 1–3
32 McMahan, U. J. *et al.* (1992) *Cell Biol.* 4, 869–874
33 Staubli, U. and Lynch, G. (1990) *Brain Res.* 513, 113–118
34 Wallace, C., Hawrylak, N. and Greenough, W. T. (1991) in *Long-term Potentiation: A Debate of Current Issues* (Baudry, M. and Davis, J. L., eds), pp. 189–232, The MIT Press
35 Applegate, M. D., Kerr, D. S. and Landfield, P. W. (1987) *Brain Res.* 401, 401–406
36 Schuster, T., Krug, M. and Wenzel, J. (1990) *Brain Res.* 523,

171–174
37 Andersen, P., Blackstad, T., Hulleberg, G., Vaaland, J. L. and Trommald, M. (1987) *Proc. Physiol. Soc.* PC50, 288P
38 Andersen, P., Blackstad, T., Hulleberg, G., Trommald, M. and Vaaland, J. L. (1987) *J. Physiol.* 390, 264
39 Trommald, M., Vaaland, J. L., Blackstad, T. W. and Andersen, P. (1990) in *Neurotoxicity of Excitatory Amino Acids* (Guidotti, A. and Costa, E., eds), pp. 163–174, Raven Press
40 Lisman, J. and Goldring, M. A. (1988) *Proc. Natl Acad. Sci. USA* 85, 5320–5324
41 Korn, H. and Faber, D. S. (1987) in *Synaptic Function* (Edelman, G. M., Gall, W. E. and Cowan, W. M., eds), pp. 57–108, John Wiley
42 Clements, J. D., Lester, R. A., Tong, G., Jahr, C. E. and Westbrook, G. L. (1992) *Science* 258, 1498–1501
43 Faber, D. S., Young, W. S., Legendre, P. and Korn, H. (1992) *Science* 258, 1497–1498
44 Andersen, P. (1990) in *Progress in Brain Research* (Storm-Mathisen, J., Zimmer, J. and Otterson, O. P., eds), pp. 215–222, Elsevier
45 Sorra, K. E. and Harris, K. M. (1991) *Soc. Neurosci. Abstr.* 17, 1156
46 Tamamaki, N. and Nojyo, Y. (1991) *J. Comp. Neur.* 303, 435–442
47 Busch, C. and Sakmann, B. (1992) *Cold Spring Harbor Symp. Quant. Biol.* 55, 69–80
48 White, G., Levy, W. B. and Steward, O. (1990) *J. Neurophysiol.* 64, 1186–1198
49 Tarrant, S. B. and Routtenberg, A. (1977) *Tissue Cell* 9, 461–473
50 Geinisman, Y., Morrell, F. and deToledo-Morrell, L. (1990) *Brain Res.* 507, 325–331