REFERENCES

Al-Anzi, B., Sapin, V., Waters, C., Zinn, K., Wyman, R.J., and Benzer, S. (2009). Neuron *63*, this issue, 329–341.

Aronne, L.J., Wadden, T., Isoldi, K.K., and Woodworth, K.A. (2009). Am. J. Medicine *122*, S24–S32.

Baker, K.D., and Thummel, C.S. (2007). Cell Metab. *6*, 257–266.

Bharucha, K.N. (2009). Pediatr. Res. *65*, 132–137.

Gao, Q., and Horvath, T.L. (2007). Annu. Rev. Neurosci. *30*, 367–398.

Gronke, S., Mildner, A., Fellert, S., Tennagels, N., Petry, S., Muller, G., Jackle, H., and Kuhnlein, R.P. (2005). Cell Metab. *1*, 323–330.

Gutierrez, E., Wiggins, D., Fielding, B., and Gould, A. (2007). Nature *445*, 275–280.

Schlegel, A., and Stainier, D.Y.R. (2007). PLoS Genet. *3*, e199. 10.1371/journal.pgen.0030199.

Taguchi, A., and White, M.F. (2008). Annu. Rev. Physiol. *70*, 191–212.

Tecott, L.H. (2007). Cell Metab. *6*, 352–361.

Wu, Q., Zhao, Z., and Shen, P. (2005). Nat. Neurosci. *8*, 1350–1355.

The Pre/Post LTP Debate

John E. Lisman[1,*]
[1]Department of Biology and Volen Center for Complex Systems, Brandeis University, Waltham, MA 02454-9110, USA
*Correspondence: lisman@brandeis.edu
DOI 10.1016/j.neuron.2009.07.020

The pre/post debate involves the question of whether long-term potentiation (LTP) is mediated by enhancement of release, enhancement of postsynaptic receptors, or both. Recent papers have presented evidence for purely postsynaptic or purely presynaptic changes, and a paper by Ahmed and Siegelbam (in this issue of *Neuron*) suggests a mechanism by which release is enhanced. This debate is increasingly constrained by technical advances that allow central synapses to be studied with increasing precision. A possible of way of reconciling conflicting evidence is suggested.

Could there be more disagreement than this? Roger Nicoll, a leading figure in the field of long-term potentiation (LTP), recently wrote a review (Kerchner and Nicoll, 2008) declaring victory for the postsynaptic hypothesis of LTP at hippocampal CA1 synapses. According to this hypothesis, the addition of AMPAR to the postsynaptic membrane makes the synapse more powerful, there being no significant role for presynaptic changes. But in a recent issue of this journal (Enoki et al., 2009), Alan Fine's group declared that LTP is due to increased release of vesicles from the presynaptic terminal, there being no significant postsynaptic changes. And in this issue of *Neuron* (Ahmed and Siegelbaum, 2009), Steven Siegelbaum's group provides evidence for a molecular mechanism by which LTP could enhance the release of vesicles.

The question of whether LTP is expressed presynaptically or postsynaptically has been pursued for over 20 years. How can such a seemingly simple question still be unanswered? One reason is that the field still lacks a clear picture of how central synapses work. Quantal analysis, a method that provided a straightforward way for dissecting presynaptic and postsynaptic processes at the neuromuscular junction (NMJ), has proven to be ambiguous at central synapses. At the NMJ, an increase in the probability of a quantal response implies a change in the presynaptic release machinery. Thus, when early studies on LTP showed a dramatic increase in the probability of response, the presynapticists declared victory. However, in a dramatic turnaround nicely described in Nicoll's review, it was then shown that the increase in probability could be due to postsynaptic changes, at least in the case of "silent synapses." At such synapses, there is initially no response at negative voltages (an NMDAR-mediated response is evident at positive voltages). After LTP, AMPARs are added to synapse, making the synapse responsive at negative voltages. Thus, the probability of response goes from zero to a finite value through a postsynaptic mechanism. As will be discussed later, another standard rule of quantal analysis at the NMJ, that addition of postsynaptic receptors increases quantal size, may not always be correct at central synapses.

Although quantal analysis has proven problematic, the pre/post debate has been exciting to watch because of the introduction of stunning new methods. Technical advances over the last few years now make it possible to study postsynaptic and presynaptic events with unprecedented precision. Thus, the debate between the presynapticists and postsynapticists is not just a rehash of the same old issues, but a debate in which each side is increasingly constrained by new findings.

Has Two-Photon Uncaging "Proven" Postsynaptic Involvement?

Kerchner and Nicoll start their review by summarizing classic data that pointed to a postsynaptic mechanism for LTP

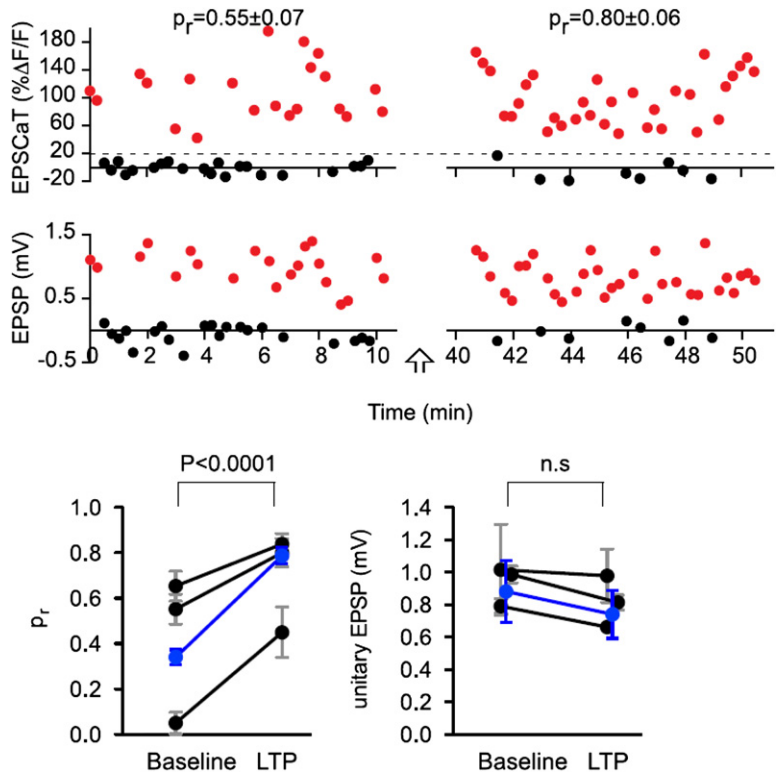

Figure 1. Effect of LTP on the Optical and Electrical Responses at a Single Synapse of the CA1 Hippocampal Region (Schaffer Collateral)

(Top) Optical (EPSCaT) and electrical EPSP responses from a single spine show successes (red) and failures (black). The successes are more likely after LTP (right) than before (left). (Bottom) Summary of four experiments showing change in probability of response produced by LTP (left) and no change in the amplitude of successful responses (right). From Enoki et al. (2009).

expression, but rapidly come to the "proof" provided by recent work using two-photon uncaging of glutamate (Matsuzaki et al., 2004). Indeed, this work seems definitive because the presynaptic cell is left out of the picture altogether. Rather, a two-photon laser is used to supply glutamate to a submicron region near an identified dendritic spine. With this method, uncaging pulses can be used both to test the strength of the identified synapse and (when given in conjunction with postsynaptic depolarization or after Mg^{2+} removal) to induce LTP at that synapse. After induction, the response generated by test pulses becomes larger, making a strong case that all the machinery needed for LTP is on the postsynaptic side.

The Case for a Purely Presynaptic Mechanism

So why doesn't this settle the issue? The production of glutamate by two-photon uncaging seems definitive, but the temporal and spatial glutamate profiles could differ from those produced by actual vesicle release. Wouldn't it be more convincing to study the LTP induced by normal synaptic function? This has now been done for the first time at single synapses by Enoki et al. and the results, at least superficially, don't agree with those obtained by the uncaging method. Recall that the response at individual synapses is probabilistic. What Enoki et al. find is that the average amplitude of successful EPSPs (this is termed "potency") does not increase after LTP induction (Figure 1). The only observed change is the probability of response (p_r). Because the synapse is functional before LTP induction, the change in p_r cannot be attributed to activation of silent synapses. According to the argument used by Enoki et al., the addition of AMPAR to the postsynaptic membrane would enhance potency (I will return later to this argument). They therefore conclude that the addition of AMPAR to the postsynaptic membrane is at most a minor aspect of the LTP story.

The ability of Enoki et al. to study LTP at single synapses is a major accomplishment. A problem that has bedeviled the study of central synapses is that almost all studies have been done on populations of synapses. Even if one stimulates only a single input axon, this axon is likely to make multiple synapses with the postsynaptic cell being recorded from. In the whole literature on slice physiology in the hippocampus, there have been few instances where it has been experimentally possible to examine the electrical responses generated at a single identified spine. Conti and Lisman (2003) described one such case and four such cases are examined in the recent Enoki et al. (2009) study. These recordings were achieved using optical detection of synaptically induced Ca^{2+} elevation in an identified spine. In these cases, there was a perfect correlation of the successes and failures of the electrical and optical responses, providing strong argument that the synapse on the identified spine was the only synapse generating electrical responses. It is under this type of recording condition that Enoki et al. induced LTP and found no change in the average voltage amplitude of successful responses (Figure 1).

Direct Evidence for a Presynaptic Component

Just as technical advances have made it possible to study postsynaptic changes in isolation, it is now possible to directly monitor the release of synaptic vesicles and to examine whether this process is affected by LTP. The first experiments of this kind were conducted in Steven Siegelbaum's laboratory (Zakharenko et al., 2001) and utilized the dye FM1-43 (for details see Ahmed and Siegelbaum, 2009). This dye can be loaded into synaptic vesicles and loses its fluorescence after the vesicle fuses with the presynaptic membrane. After long periods of stimulation, all fluorescence is lost from a presynaptic bouton and the rate at which this occurs allows estimation of the probability of release. Measurements of this kind showed that after LTP induction, the probability of release is persistently enhanced.

After this initial report, there has been substantial further progress in understanding plasticity of the release process. Experiments have verified the key findings using a somewhat different method that utilized mice expressing SynaptopHluorin in presynaptic boutons (Bayazitov et al., 2007). These experiments showed that the enhancement of release develops slowly, making the relative role of presynaptic and postsynaptic changes vary with time after LTP induction. Furthermore, progress has been made in understanding some of the underlying mechanisms. In this issue, Ahmed and Siegelbaum (2009) demonstrate that LTP also enhances release at the perforant path synapses onto CA1 (previous work had been done on Schaffer collateral synapses). The main conclusion is that potentiation at these synapses is due to enhancement of N-type Ca^{2+} channels. The authors utilized ω-Ctx GVIA, a toxin that selectively blocks these channels. When applied before LTP induction, there was no change in the rate of FM1-43 release. However, when applied after LTP induction, the rate of dye release was reduced to a level similar to that in nontetanized slices. Interestingly, at CA1 Schaffer collateral synapses, N-type channels already play an important role under basal conditions; the mechanism by which LTP enhances release at these synapses has yet to be identified.

So what are the arguments against presynaptic involvement in LTP? Kerchner and Nicoll argue that if release is enhanced, the current generated by glial glutamate transporters should be enhanced. The available evidence (Diamond et al., 1998) indicates that this is not the case (but measurements were made less than 20 min after LTP induction, a time at which the presynaptic component might not yet have developed). Furthermore, Kerchner and Nicoll argue that the reported presynaptic changes only occur when induction conditions are "extreme." Indeed, when a standard 50–100 Hz tetanus is used to induce LTP, fluorescent methods show no change in release probability (Bayazitov et al., 2007; Zakharenko et al., 2001). The extreme protocol referred to is a 200 Hz tetanus, and one can argue about its physiological relevance. However, all the papers report that release is also enhanced if a theta burst protocol is used (brief periods of 100 Hz stimuli repeated at 5 Hz). This firing pattern resembles that seen in the hippocampus and should not be characterized as extreme.

In summary, there appears to be strong evidence for both presynaptic and postsynaptic changes after some LTP induction protocols (but not 100 Hz for 1 s). The strongest evidence against postsynaptic changes is the lack of change in the amplitude of successful responses (Figure 1).

Advances in Understanding Quantal Transmission

Before discussing this evidence further, it is useful to review recent progress in understanding quantal transmission at central synapses.

Advance 1: The Ability to Make Stereotyped Vesicles That Generate Stereotyped Quantal Responses Is Present in the CNS, Just as in the NMJ

At the NMJ, evoked responses are the summation of an integer number of quantal responses. Moreover, mEPSCs have a size equivalent to one of these quanta. Although quanta are not exactly stereotyped in size, the SD is a relatively small fraction of the mean (coefficient of variation ~0.2–0.3). But recordings in the CNS generally show highly variable mEPSC amplitude, raising the possibility that vesicle size in the CNS might be more nonuniform than at the NMJ. Recent work (Li et al., 2009), however, shows that if hair cells are hyperpolarized, mEPSCs in the postsynaptic targets are highly stereotyped (CV = 0.29).

Advance 2: mEPSPs and EPSP Can Be Multiquantal

In CNS recordings, mEPSCs have a very skewed amplitude distribution because there are some very large events (Magee and Cook, 2000). Recent work (He et al., 2009) used measurements of capacitance to measure the fusion of vesicles with the presynaptic membrane. This work directly demonstrates that the large mEPSCs involve multiple presynapatic vesicles. These vesicles may either fuse with each other before fusing with the plasma membrane of fuse simultaneously with the plasma membrane. This work on multiquantal mEPSPs complements previous work indicating that evoked release at single hippocampal synapses can also be multiquantal (Tong and Jahr, 1994).

Advance 3: Spine Voltage Can Be Different from That in the Dendrite Because the Spine Neck Resistance Chokes Current Flow

At the NMJ, the amplitude distribution of the evoked responses shows evenly spaced peaks (the distance between peaks equals quantal size). This quantization is usually not evident in CNS recordings and is not obvious in Enoki et al.'s recordings (Figure 1). An underlying assumption of quantal analysis is the linear summation of quantal response. Recent work raises the possibility that this is not the case in dendritic spines (Palmer and Stuart, 2009). A major advance is the use of voltage-sensitive dyes to monitor membrane potential with high spatial resolution. It was found that there is a voltage gradient between spine and dendrite and that it is due to current flow across the spine neck resistance, which can be as high as 500 meg. During the current resulting from a single quantum (20–30 pA), the voltage change in the spine is not large (<15 mV), but for multiple quanta, the voltage change in the spine could be large enough to reduce the driving force for the EPSC, thereby choking off the generation of large synaptic currents. Thus synaptic current could depend sublinearly on the number of vesicles released.

Advance 4: AMPARs Can Be Added to a Synapse without Significantly Affecting mEPSC Amplitude

Computational studies (Raghavachari and Lisman, 2004) have shown that the fast rise time of mEPSCs, their amplitude, and their amplitude variance can be accounted for by the properties of a simple model of glutamate release from a vesicle and the stochastic binding of glutamate to AMPAR in the synaptic cleft. This model further showed that the 20–30 AMPARs that generate the mEPSC are highly localized near the site of vesicle fusion, creating a hotspot of channel opening in a much larger array of AMPARs (many hundreds at large synapses). According to standard quantal analysis, addition of postsynaptic receptors can be detected by an increase in quantal size. According to the new view, this is not necessarily true: quantal size is determined by the density of AMPAR near the release sites; if AMPARs were added to the synapse

at a distant location (e.g., with growth of synapse diameter), quantal size would not be increased.

Reconciling Evidence for Presynaptic and Postsynaptic Involvement in LTP

We now return to the question of whether the lack of change in potency observed by Enoki et al. (2009) (Figure 1) indeed rules out a significant postsynaptic contribution to LTP. Let us start by supposing that the EPSPs generated by single CA1 synapses are uniquantal. The answer is then simple: LTP could enlarge the synapse by addition of new release sites presynaptically and the addition of AMPA channels to the periphery of an enlarged postsynaptic specialization (resulting in no change in AMPAR density). By the logic of Advance 4—that quantal size is determined by local density of AMPA channels—this would not change potency. If, on the other hand, release can be multiquantal, enhancing p_r would lead to more multiquantal responses; if summation is linear, this should lead to an increase in potency, contrary to what is observed. However, if summation is sublinear, as suggested by Advance 3, large responses would be choked off, leading to little change in potency. In summary, the fact that LTP does not affect potency is not a definitive argument against strong postsynaptic involvement in LTP.

The newly available data from the papers reviewed here provide strong constraints on models of LTP expression. Readers interested in a model that deals with many of these complexities and accounts for a broad range of findings regarding both presynaptic and postsynaptic changes should consult Lisman and Raghavachari (2006). More constraints will be available soon as even better methods are applied to the problem. Specifically, methods that break the diffraction limit of light microscopy may make it possible to directly visualize where AMPARs are added to synapses and how synapses grow after LTP induction. The use of quantum dots will make it possible to monitor single-release events before and after LTP (Zhang et al., 2009). Buy your tickets now for the pre/post debate, 2010.

REFERENCES

Ahmed, M.S., and Siegelbaum, S.A. (2009). Neuron *63*, this issue, 372–385.

Bayazitov, I.T., Richardson, R.J., Fricke, R.G., and Zakharenko, S.S. (2007). J. Neurosci. *27*, 11510–11521.

Conti, R., and Lisman, J. (2003). Proc. Natl. Acad. Sci. USA *100*, 4885–4890.

Diamond, J.S., Bergles, D.E., and Jahr, C.E. (1998). Neuron *21*, 425–433.

Enoki, R., Hu, Y.L., Hamilton, D., and Fine, A. (2009). Neuron *62*, 242–253.

He, L., Xue, L., Xu, J., McNeil, B.D., Bai, L., Melicoff, E., Adachi, R., and Wu, L.G. (2009). Nature *459*, 93–97.

Kerchner, G.A., and Nicoll, R.A. (2008). Nat. Rev. Neurosci. *9*, 813–825.

Li, G.L., Keen, E., Andor-Ardo, D., Hudspeth, A.J., and von Gersdorff, H. (2009). J. Neurosci. *29*, 7558–7568.

Lisman, J., and Raghavachari, S. (2006). Sci. STKE *2006*, re11.

Magee, J.C., and Cook, E.P. (2000). Nat. Rev. Neurosci. *3*, 895–903.

Matsuzaki, M., Honkura, N., Ellis-Davies, G.C., and Kasai, H. (2004). Nature *429*, 761–766.

Palmer, L.M., and Stuart, G.J. (2009). J. Neurosci. *29*, 6897–6903.

Raghavachari, S., and Lisman, J.E. (2004). J. Neurophysiol. *92*, 2456–2467.

Tong, G., and Jahr, C.E. (1994). Neuron *12*, 51–59.

Zakharenko, S.S., Zablow, L., and Siegelbaum, S.A. (2001). Nat. Neurosci. *4*, 711–717.

Zhang, Q., Li, Y., and Tsien, R.W. (2009). Science *323*, 1448–1453.

A Nonvisual Look at the Functional Organization of Visual Cortex

Marius V. Peelen[1,*] and Sabine Kastner[1]
[1]Department of Psychology and Princeton Neuroscience Institute, Princeton University, Princeton, NJ 08540, USA
*Correspondence: mpeelen@princeton.edu
DOI 10.1016/j.neuron.2009.07.022

In this issue of *Neuron*, Mahon et al. show that the ventral visual cortex of congenitally blind individuals, who have never experienced the visual world, has an object-category organization similar to that found in sighted individuals. Here, we discuss the implications of this finding for our understanding of the "visual" cortex.

> "… if you take men born blind, who have made use of such [tactile] sensations all their life, you will find they feel things with perfect exactness that one might almost say that they see with their hands.…"
>
> —Descartes (1637; in Gregory and Wallace, 1963)

Visual neuroscience research has predominantly considered the visual cortex as a stimulus-driven, unimodal system. This line of research has revealed