LTP: Desperately Seeking Resolution

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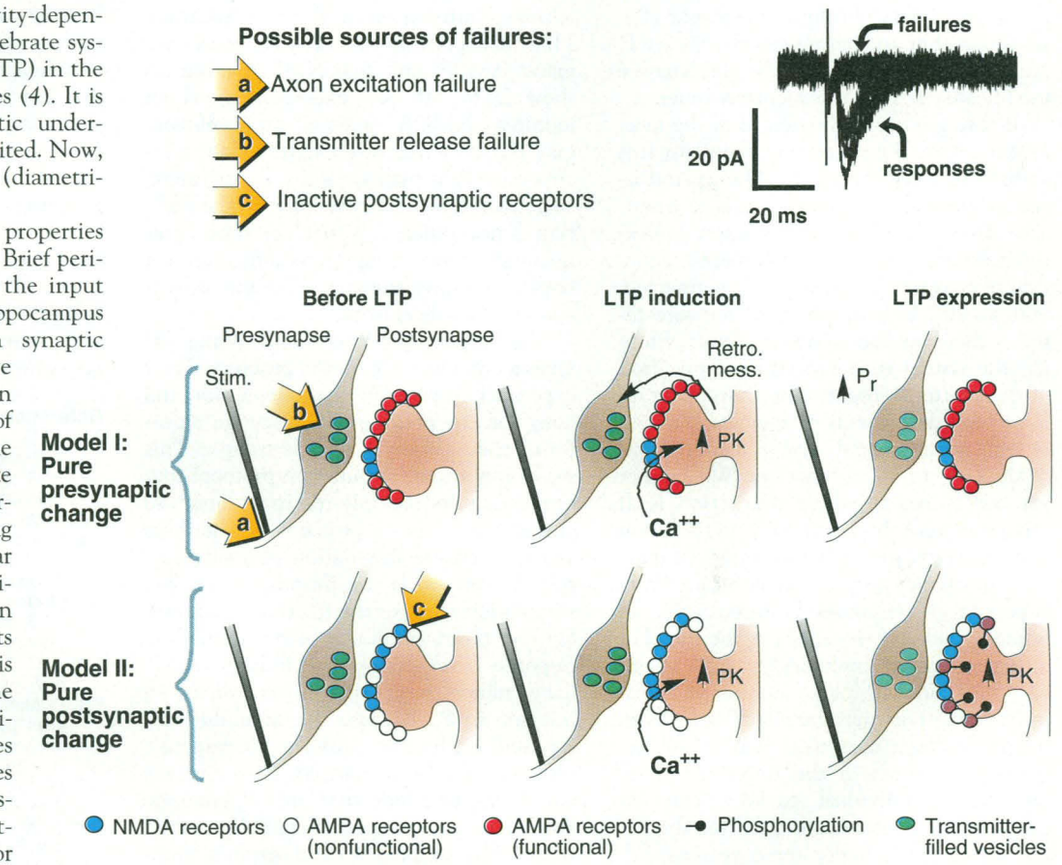
Certain patterns of neuronal synaptic activity produce lasting changes in synaptic function. This concept is central to the working models of many neuroscientists. Such a mechanism may be key in establishing the appropriate connections between developing neurons (*1*) and in the modification of circuits underlying various forms of learning and memory (*2*). Furthermore, this phenomenon may act in a number of neuropathological conditions (*3*). The best characterized example of activity-dependent synaptic plasticity in a vertebrate system is long-term potentiation (LTP) in the CA1 region of hippocampal slices (*4*). It is little wonder that a mechanistic understanding of LTP is anxiously awaited. Now, two new studies (*5*, *6*) propose (diametrically opposed) solutions.

Over the past 20 years many properties of LTP have been identified (*4*). Brief periods of synchronous activity in the input fibers to the CA1 area of the hippocampus can trigger a potentiation in synaptic strength that is selective for active synapses and lasts for hours in vitro or days in vivo. This burst of synaptic activity facilitates the opening of postsynaptic glutamate receptors of the N-methyl D-aspartate (NMDA) subtype, producing a transient rise in intracellular calcium concentrations and activation of postsynaptic protein kinases (*4*). Although such events are considered critical, there is little common preaching on the identity of the persistent modification that makes these synapses stronger. After LTP, are synapses stronger because more neurotransmitter is released, because postsynaptic responsivity is greater, or both? The simplicity and concreteness of this "presynaptic versus postsynaptic" question, along with the anticipated impact of the answer on the field, has led to a seeking frenzy.

As tools improved over the years, the "solution" to this puzzle has vacillated across the synapse like the whims of a prima donna. Early microdialysis data suggested that there was increased release of neurotransmitter after LTP (*7*). However, by using selective glutamate receptor antagonists, two groups found that the increased synaptic efficacy could only be detected at the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) subtype of the postsynaptic glutamate receptor but not at the NMDA subtype (*8*, *9*). Because these receptors are found together on individual synapses, this result argued that, after LTP, there was a purely postsynaptic modification of AMPA receptors; if LTP caused an increase in neurotransmitter release, it should have been detected by both types of postsynaptic receptors. But just when it seemed that the case was cracked, two groups using patch-clamp recording made an unexpected observation while studying the fluctuations in neurotransmission at a small number of synapses (*10*, *11*). This fluctuation from trial to trial is due to the stochastic release of transmitter. On some trials, all stimulated synapses will fail to release transmitter and the response amplitude will be zero (see figure, upper panel). The fraction of trials producing such "failures" has been a classical measure of pre-



Synaptic responses and models of underlying function. (**Upper**) Response or failure? Fifty consecutive responses of a postsynaptic cell to the same stimulus (whole-cell recording). These measurements are made at the resting membrane potential. An unknown number of synapses contribute to these responses. Note the clear distinction between responses and failures. (**Lower**) Two different scenarios, consistent with most data, can explain the decrease in failure rate after LTP. (i) Presynaptic modifications: If failures are purely release failures, then P_r must increase, likely through a retrograde message generated postsynaptically. In this model, synapses undergoing LTP should have few NMDA receptors, to fit with the Manabe and Nicoll data. (ii) Postsynaptic modifications: In this model a significant number of synapses have functional NMDA receptors but nonfunctional AMPA receptors. Transmitter released at such synapses would be recorded as failures, because NMDA receptors do not open at resting membrane potentials. During LTP induction, AMPA receptors could be converted from a nonfunctional to functional state (AMPAfication of AMPA-virgin synapses). Subsequently, neurotransmitter released at these synapses would produce a response at resting potentials, thereby decreasing the failure rate. The responses at such a converted synapse should be smaller than at a synapse transmitting before LTP, to fit with the Stevens and Wang data that the average amplitude of nonfailure responses (which would now include occasional sums from both converted and nonconverted synapses) does not change after LTP. Stim., stimulating electrode; PK, protein kinases; Retro. mess., retrograde messengers.

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synaptic function (*12*). The two groups found a dramatic reduction in failures after LTP, a result that argues for a presynaptic modification.

A compromise solution surfaced with the publication of three studies (*13–15*) that measured failures and responses to individual packets of transmitter. These three studies concluded that, after LTP, changes occurred both presynaptically (failures were confirmed to decrease in frequency) and postsynaptically (the response to a neurotransmitter packet increased). This made some sense for those who know tango, as there are two sides to a synapse that, anatomically at least, are known to change in stride during development.

Now enter two new papers into the fray, one arguing for purely presynaptic changes in the probability of transmitter release (P_r), while another indicating no change in P_r and thus supporting entirely postsynaptic modifications. Why this reentrenchment?

Before getting to the details of the most recent entries, I will try to explain why it is so hard to solve the puzzle. The central issue in question is what happens at a synapse after it undergoes potentiation. Unfortunately, the pre- and postsynaptic function of synapses are inextricably connected, and we still lack the tools to measure directly these functions independently. Probing the system with exogenous application of transmitter or monitoring transmitter release with detectors is problematic because of the strict spatial and temporal constraints of synaptic function: We cannot get our probes to where the action is. If synapses were homogeneous in function and plasticity, we could use statistical measures to extract microscopic constants from a population response. Unfortunately, an unpredictable heterogeneity is the rule: For different synapses on a CA1 neuron, P_r can vary by almost 10-fold; postsynaptic transmitter sensitivity may vary by 50-fold. Even relatively direct measures, such as the frequency of failures or the amplitude of responses to individual packets, can get murky in a heterogeneous pool (see the figure for examples). For these reasons, addressing the pre versus post question has been no holiday.

The new studies by Manabe and Nicoll (*5*) and Stevens and Wang (*6*) attack the problem in clever ways. Manabe and Nicoll use a method for analyzing P_r that is independent of the number of synapses activated and that can detect heterogeneity in transmitter release (*16, 17*). They used the NMDA-channel antagonist MK-801, which is an irreversible open-channel blocker and thus will inhibit responses at a synapse only if transmitter is released. Therefore, in a population of synapses exposed to this drug, the rate at which NMDA receptor–mediated transmission decreases with repeated trials is proportional to P_r. With this method they find no change in P_r during LTP, whereas they do measure a change in P_r during paired-pulse facilitation, a different form of plasticity lasting hundreds of milliseconds that is of presynaptic origin. They conclude that the changes in LTP are occurring in the postsynaptic cell.

How compelling are the conclusions from this rigorous study? Two caveats appear, to me, to be relevant. First, although AMPA-type and NMDA-type receptors are colocalized, there is heterogeneity in their relative contributions at different synapses. Thus, it is possible that only synapses with many AMPA and few NMDA receptors show LTP. MK-801 experiments, which monitor NMDA-mediated transmission, may not be sensitive enough to detect increases in P_r at such synapses. Furthermore, this method will be insensitive to the addition of new synapses [which could be a presynaptic or postsynaptic modification (or both)] that have the same P_r profile as synapses previously existing.

The paper by Stevens and Wang (*6*) takes a different tack on the problem. Their experimental paradigm both argues for, and hangs on the premise that, they are monitoring transmission at a single synapse. This study uses a weak stimulation protocol that produces response failures in at least 50 percent of trials—a protocol, the authors argue, to ensure stimulation of a single fiber. Under such conditions, a tetanic stimulation changes the fraction of failures, but the mean amplitude of the nonfailure response is not changed. If failures are release failures, these experiments indicate an increase in P_r. Because the nonfailure response is unchanged, postsynaptic responsivity must also be unchanged. Thus, Stevens and Wang conclude that only P_r changes. Are there caveats to this carefully crafted study? The authors do not explain completely previous studies showing experiments with similarly high failure rates before LTP and for which the nonfailure response increased in amplitude after LTP (*13, 18*). Furthermore, they fail to consider a postsynaptic scenario for LTP (*15, 19*) in which AMPA receptors are added to synapses with only functional NMDA receptors (see figure) and to synapses already having some functional AMPA receptors. If the newly AMPAfied synapses have a response smaller than the mean of synapses responding before LTP, then the mean amplitude of responses after LTP (which includes occasional simultaneous release at new and old synapses) could remain constant.

Where does this dash through the underbelly of synaptic physiology leave us? In my mind, considering the body of evidence, the presynaptic versus postsynaptic question is and will remain unanswered until more basic synaptic physiology is understood. For instance, assuming slightly different synaptic properties (see figure, before LTP), the decrease in failures during LTP (a fairly consistent finding in this tempestuous field) can be explained by models in which the modifications are purely presynaptic or purely postsynaptic (see figure). Testing such models may require the use of more powerful techniques, for example, optical imaging to monitor the function of individual synapses (*20, 21*). We would also gain insight if candidate molecular targets (see figure) could be perturbed with precise spatial and temporal control, thereby avoiding secondary effects inherent in long-term treatments. Thus, the challenge to understand how synapses get stronger remains. We can only hope that the audience will appreciate the difficulty of the problem and share in our enthusiasm for seeking resolution.

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