COMMENTARY

Mary B. Kennedy On Beyond LTP

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In a challenging and insightful commentary, Sanes and Lichtman (1999) courageously point out in print what has often been discussed at recent meetings: LTP seems to have lost its usefulness as a paradigm for studying synaptic plasticity, much less learning and memory. The authors then offer two broad explanations for the current profusion (and confusion) of potential molecular mechanisms for LTP. The first is the simple suggestion that “Many molecules are required to mediate the process.” The second is that a variety of historical, experimental, and biological “factors have thwarted attempts to pinpoint a minimal cadre of essential molecules among a larger group of candidates.” Sanes and Lichtman favor this second, less optimistic explanation. I will argue that the truth lies somewhere in between.

Sanes and Lichtman suggest that one reason we have a clear molecular explanation for, say, the action potential but not for LTP is that “the molecular biologists and biochemists studying action potentials knew what they were trying to explain.” In contrast, they correctly point out that it has “remained a challenge to supply a clear definition, description or function” for LTP. I agree that the inability to measure with a microelectrode alone the subtle mechanistic differences between one form of long-lasting synaptic potentiation and another has prevented a clear definition of LTP. The problem is magnified because the *Nature* of modern *Science* of the *Cell* biology of the *Neuron* has been to equate function with what is measured by a microelectrode. Thus, the trend has been to accept almost any manipulation that alters the tetanus-induced increase in EPSP slope as a legitimate functional study of LTP. It seems evident that a new paradigm is needed. In fact, many investigators have been quietly forging new ways of investigating synaptic regulation in the CNS, despite the frustration of having their work often relegated to non-functional status.

In theory, there are a finite number of ways to change the strength of synaptic transmission. For example, the probability that a presynaptic action potential causes release of a vesicle of transmitter can be altered. An able group of molecular biologists and biochemists have made great strides in understanding this facet of synaptic regulation by investigating the molecular mechanisms of vesicular transmitter release. These studies, starting with descriptions of the proteins in synaptic vesicles (Reichardt and Kelly 1983) and interacting proteins in the plasma membrane (Bennett and Scheller 1994), have lead inevitably to exciting insights into the mechanisms of controlled synaptic vesicle fusion (Sutton et al. 1998; Chen et al. 1999). The next step will be to clarify the sites of regulation by calcium and by protein phosphorylation (Bennett 1997; Bajjalieh 1999).

Another way that synaptic strength can be altered is to change the magnitude of the current-through-activated postsynaptic glutamate receptors. Here again, molecular biologists and biochemists have defined

the sequences and structures of glutamate receptors (Boulter et al. 1990, 1992; Nakanishi 1992; Seeburg 1993), the locations of their ion channels (Wo and Oswald 1995), and mechanisms by which their current can be regulated (Roche et al. 1996; Barria et al. 1997). A third way is to add or subtract glutamate receptors from the postsynaptic membrane so that the total current produced by release of transmitter is changed. Recent evidence indicates that AMPA receptor insertion and removal can occur in central synapses and may be regulated by NMDA receptors (Shi et al. 1999). Make no mistake! Each of these kinds of studies will eventually lead to insights into some form of LTP. They also point to a more subtle and complex view of the synapse than appears in the usual microelectrode paradigm.

The mechanisms that regulate vesicle fusion, magnitude of the current through glutamate receptors, and addition or removal of receptors from the postsynaptic membrane can be thought of as likely core programs or first-order causes (to use the Sanes and Lichtman terms) of LTP and LTD. But these are mechanisms of expression of synaptic plasticity. What about the more elusive core programs for induction or maintenance of LTP [see commentary by Sweatt (1999) for definitions of these terms]? Here, I think Sanes and Lichtman have missed a profound insight arising from the many studies implicating multitudes of molecules in LTP induction; thinking is more complicated than firing an action potential. The kinds of synaptic LTP or LTD that would be important for processing information or storing memories in the brain must result from integrating many different, often subtle, signals impinging on a neuron. Furthermore, the changes in synaptic strength that store information must occur in a context that preserves the stability or homeostasis of the brain as an interacting system of neurons. Control engineers would say that the brain, as a dynamic system of neurons, and each neuron, as a dynamic system of molecules, must be robust to a wide variety of changing signals from the environment (Doyle et al. 1992). In processing these signals, they must maintain function within a limited range of parameters so the system doesn't swing out of control. An epileptic seizure is an example of the brain slipping out of the permissible range of parameters so that the system of neurons oscillates and loses function. Similarly, excitotoxic neuronal death is an example of synapses slipping out of a permissible range of parameters so that the neuron is irreversibly damaged.

Robust control systems usually comprise a large number of highly interacting parts (e.g., Lenski et al. 1999). The parts are arranged to provide both redundancy and feedback at every level of control. Therefore, it is not surprising that the signal-transduction pathways that can induce LTP or LTD are complex, highly interacting, and apparently redundant. Very likely, many molecules *can* mediate the process, which ones depending on exactly which process you are talking about. But how do we study such complicated pathways? I would argue that much progress has already been made in understanding them; but, again, the progress hasn't been made with microelectrodes. As in the studies of vesicular release and postsynaptic receptors, a group of able molecular biologists and biochemists (myself included) have investigated the organization of signal-transduction machinery at the synapse, starting with a description of the most prominent signaling proteins in neurons (Greengard 1979; Kennedy 1983) and then in the postsynaptic density (Kennedy 1997; Ziff 1997). These studies have revealed a highly organized

meshwork of interacting signaling proteins within postsynaptic spines (Kornau et al. 1995, 1997; Sheng 1996; Chen et al. 1998; Kennedy 1998; Naisbitt et al. 1999; Tu et al. 1999). Fortunately, our understanding of the signaling potential of this system of molecules is greatly aided by studies in many other cell types. All cells seem to share a basic repertoire of signaling molecules that interact in a large, but finite, variety of signaling motifs (e.g., Bray 1995, 1998; Cobb and Goldsmith 1995; Cohen et al. 1995). Control of cell division in an embryo or in the mature immune system and decisions about different cell fates during development are also mediated by large interacting sets of signaling molecules. Most of us feel that we are approaching the end of the necessary descriptive phase in the study of signal-transduction mechanisms. Soon, in fact, the availability of sequences of whole genomes will permit us to catalog all of the potential signaling molecules in neurons. How will an understanding of synaptic plasticity emerge from this body of data?

By regarding the molecular complexity of LTP as more of a headache than a reality, Sanes and Lichtman have missed the importance of a wonderful new tool; the computer. Over the past 5 years, the computing power available on a desktop has skyrocketed. Consequently, it is now possible to create simulations of complex biological pathways of a size that would have overwhelmed desktop computers a few years ago. Biochemists are notoriously skeptical of computer models of large metabolic pathways and with good reason. In the past, many such models have incorporated so many arbitrary variables that the resulting simulations often bore little resemblance to experimental reality. The task now is to develop efficient programming methods, gather appropriate quantitative data to plug into the simulations, and finally, to devise appropriate and efficient experimental tests of different simulations. A number of groups have already begun assembling programming methods (Weng et al. 1999). One effort that seems especially promising for simulating synaptic transmission and signal transduction in a spine is based on a program originally conceived and written in the Salpeter laboratory at Cornell University (Bartol et al. 1991; Stiles et al. 1996); the recently expanded and newly termed MCell (T. Bartol, J. Stiles, T. Sejnowski, M. Salpeter, E. Salpeter, unpubl.; www.mcell.cnl.salk.edu). However, devising appropriate experimental tests of complex simulations will be particularly important because it will provide the means to trim away inappropriate models and home in on those that simulate real biological systems. The experimental tests will need to examine both the predicted biochemical network and the predicted synaptic output. Thus, they will likely incorporate temporal and spatial measurements of reaction points in the biochemical network, such as fluctuations in calcium concentration and phosphorylation of particular proteins, as well as electrophysiological measurements of synaptic output.

The tone of the Sanes and Lichtman article seemed ultimately pessimistic to me. However, I don't feel pessimistic at all about the future of molecular studies of synaptic plasticity. To quote Bray (1997), one of the pioneers in analysis of complex signal transduction, "The data are accumulating and the computers are humming. What we lack are the words, the grammar, and the syntax of the new language." We have nearly assembled all of the words, now it's on to the grammar and the syntax.

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