ESSAY: AMERSHAM BIOSCIENCES & *SCIENCE* PRIZE

AMPA Receptor Dynamics and Synaptic Plasticity

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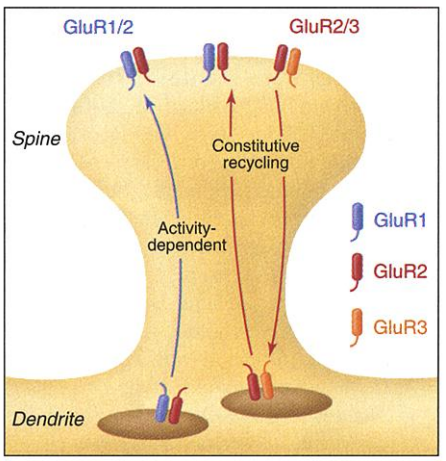
We have about 10^{12} neuronal connections (synapses) in our brain, but how exactly do we remember? Long-lasting, activity-dependent changes in synaptic efficacy are thought to provide the cellular basis for information processing and storage in the brain (*1*). The first experimental support for this idea came from the discovery of long-term potentiation (LTP) (*2*). LTP is a long-lasting enhancement of excitatory synaptic strength in response to brief high-frequency synaptic stimulation. Because LTP may provide the means for us to learn and remember (*3*), its molecular and cellular basis has been intensively investigated (*4*). However, there has been little consensus regarding the persistent changes in synaptic strength responsible for LTP.

In the mammalian central nervous system, excitatory synaptic transmission is mostly mediated by ionotropic glutamate receptors. There are three classes of these receptors: AMPA, NMDA, and kainate-type (*5*, *6*). AMPA receptors (AMPA-Rs) mediate fast ongoing synaptic transmission in response to presynaptic glutamate release. Activation of NMDA receptors (NMDA-Rs) requires both presynaptic neurotransmitter release and postsynaptic depolarization. This results in Ca^{2+} influx, activation of signaling pathways such as the Ca^{2+}-calmodulin–dependent protein kinase II (CaMKII) pathway, and long-lasting changes in synaptic strength, predominantly in AMPA-R–mediated transmission as exemplified by LTP.

How do synapses get stronger after LTP? One way is through the rapid delivery of AMPA-Rs to synapses. To directly test this hypothesis, I monitored the distribution of AMPA-Rs at high resolution in living neurons over time (*7*). First, I fused green fluorescent protein (GFP) to the extracellular amino terminus of GluR1, one of the subunits of the AMPA-R. I then expressed the recombinant GluR1-GFP receptor in organotypic hippocampal slice cultures (*8*) using Sindbis virus, and examined the distribution of fluorescent recombinant receptors by two-photon laser scanning microscopy (*9*). Surprisingly, although GluR1-GFP was located throughout the dendritic trees of CA1 pyramidal neurons, very little (<1%) appeared in dendritic spines where most excitatory synapses are located.

Could LTP induction cause a redistribution of GluR1-GFP to dendritic spines (synapses)? I placed a stimulation electrode close to the fluorescent dendrites expressing GluR1-GFP and delivered a tetanic stimulation, which induces robust LTP in these neurons. Interestingly, GluR1-GFP rapidly redistributed in the dendrites, with some fluorescent receptors traveling into dendritic spines. These results demonstrate that AMPA-Rs can be rapidly recruited to spines in response to LTP induction (*7*).



Two synaptic delivery pathways for AMPA-Rs. Both pathways depend on the subunit composition of AMPA-Rs. To establish plasticity, GluR1/GluR2 heteromers are delivered to synapses to potentiate synaptic transmission. To maintain synaptic transmission, GluR2/GluR3 heteromers replace existing synaptic AMPA-Rs independent of neuronal activity.

Amersham Biosciences and *Science* are pleased to announce the 2001 grand prizewinner of the Amersham Biosciences & *Science* Prize for Young Scientists. The grand prize has been awarded to a regional winner from North America, Song-Hai Shi.

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Next, I asked whether the receptors that had redistributed to spines after LTP induction were actually participating in synaptic transmission. To address this question, I developed an electrophysiological "tag" for recombinant AMPA-Rs (*10*) so that I could monitor the function of synaptic receptors.

There are four different AMPA-R subunits: GluR1 to GluR4 (*5*, *6*). GluR2 has an Arg in the channel pore region instead of the Gln present in GluR1, GluR3, and GluR4. This single amino acid difference determines the current–voltage (*I-V*) relation of the receptor (the direction of ion flow at a given voltage) (*11*). As a consequence, AMPA-Rs containing a GluR2 subunit allow ion flow into or out of the cell, depending on the cell's membrane voltage. In contrast, AMPA-Rs lacking the GluR2 subunit only allow ion flow into, but not out of, the cell. Most endogenous AMPA-Rs in the hippocampus contain GluR2 (*12*). When I overexpressed GluR1-GFP in neurons, homomeric AMPA-Rs exclusively composed of the same receptor subunit, GluR1, were formed. In the absence of GluR2, the fate of this recombinant receptor could be assayed electrophysiologically. Synaptic delivery of the homomeric recombinant AMPA-R resulted in altered synaptic transmission (inwardly rectified) as measured with whole-cell patch-clamp recording.

With this electrophysiological assay, I showed that recombinant AMPA-Rs were delivered to synapses by LTP induction (*10*). Consistent with the established role of CaMKII as a necessary and sufficient mediator of LTP, coexpression of constitutively active CaMKII with GluR1-GFP also resulted in synaptic delivery of the recombinant receptors. Interestingly, this process was not dependent on the phosphorylation of GluR1 at Ser^{831} by CaMKII (*13*), but was dependent on the interaction between the GluR1 carboxyl terminus and PDZ domain proteins. Mutation of the PDZ binding site of GluR1 blocked its synaptic delivery and the expression of LTP. These data provide strong evidence that postsynaptic delivery of AMPA-Rs is the primary mechanism underlying LTP and begin to reveal a detailed molecular flow chart for this process.

I also examined the synaptic trafficking of other AMPA-Rs (*14*). Depending on the subunit composition of the receptor, there

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were two different AMPA-R synaptic delivery mechanisms. Potentiation of synaptic transmission required delivery of AMPA-Rs containing GluR1 and CaMKII to synapses, whereas maintenance of synaptic transmission required that AMPA-Rs containing GluR2 be constitutively exchanged with existing synaptic AMPA-Rs. Such rules were also validated for the heteromeric AMPA-Rs (containing a mixture of subunits) normally found in CA1 neurons (see the figure).

These data lead to a compelling model. Synaptic transmission is maintained at a given level by recycling of a relatively constant number of GluR2-containing AMPA-Rs at the synapse. Plasticity-inducing stimuli initially cause a net addition of GluR1-containing AMPA-Rs, which may eventually be replaced by GluR2-containing AMPA-Rs, resulting in a long-lasting increase in synaptic transmission (*14*). This two-pathway delivery model also addresses an important signal transduction problem in cell biology: How is an appropriate number of cell surface receptors established and maintained? Other signal transduction pathways could also have two mechanisms for receptor delivery: one responding to external cues and regulating the number of receptors, and the other replacing existing surface receptors, thus maintaining a steady-state number of receptors at the cell surface.

Our work on the synaptic trafficking of AMPA-Rs provides critical insights into synaptic plasticity and stability. To gain an even more detailed understanding of synaptic plasticity, we need to determine the molecules that mediate and modulate these two modes of AMPA-R synaptic delivery. Importantly, these studies attempt to define the molecular signature of synaptic plasticity so that we can discover when and where this process occurs in the brain during behavioral modification. Thus, we may eventually be able to answer the question, how do we remember?

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2001 Grand Prize Winner

Song-Hai Shi

Song-Hai Shi was born in a small Chinese village in 1973 and attended Tsinghua University in Beijing from 1991 to 1996. Accepted into the Department of Biological Sciences and Biotechnology, Dr. Shi studied mathematics, physics, and chemistry as well as experimental biology. He then came to the United States to pursue graduate training in the Joint Genetics Program at Cold Spring Harbor Laboratory (CSHL) and the State University of New York, Stony Brook. Working with Roberto Malinow at CSHL, Dr. Shi investigated the mechanisms of long-term potentiation. His research on the synaptic regulation of AMPA receptors in hippocampal pyramidal neurons was published in two first-author papers in *Science* and one in *Cell*. After receiving his Ph.D., Dr. Shi joined Dr. Yuh Nung Jan's laboratory in May 2001 to pursue postdoctoral work at the University of California, San Francisco.

Regional Winners

Europe: Åsa Apelqvist, for her essay, "Analysis of Hh, Notch and Fgf Signaling During Pancreatic Development, Cell Differentiation and Function," based on her research in the laboratory of Helena Edlund at Umeå University, Sweden. Dr. Apelqvist was born in Gällivare, Lapland, Sweden, and studied at Umeå University where she received her bachelor's degree. She joined the Edlund group in 1994 and began her graduate studies on pancreas development. Dr. Apelqvist was awarded her Ph.D. in 2000 and is currently pursuing postdoctoral research in Seung Kim's laboratory at Stanford University.

The second European regional winner is Friedrich Frischknecht, for his essay, "How Smallpox Spreads and What It Tells Us About Cell Motility," reporting research from the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. From 1990 to 1996, Dr. Frischknecht studied biochemistry at the Freie Universität Berlin (FUB) and undertook research at the MRC Laboratory of Molecular Biology in Cambridge, UK. In 1996 he joined Michael Way's group at the EMBL as a predoctoral fellow and obtained his Ph.D from FUB in 2000. Dr. Frischknecht is now a postdoctoral fellow with Robert Menard at the Institut Pasteur in Paris, France.

North America: Matthew L. Albert, for his essay, "Resurrecting the Dead: Dendritic Cells Cross-Present Apoptotic Cells. A Pathway for the Activation of Tumor-Specific Killer T Cells," based on his research at The Rockefeller University. Dr. Albert received his Sc.B. in Chemistry from Brown University in 1992, his Ph.D. in immunology from The Rockefeller University in 1999, and his M.D. from Cornell University Medical College in 2000. Dr. Albert is currently a Clinical Scholar in Robert Darnell's laboratory at The Rockefeller University and is also developing clinical trials using dendritic cells that cross-present apoptotic cells as a tumor vaccine.

Japan: Masaki Hiramoto for his essay, "The Second Function of Receptors in Patterning: Receptors that Present Ligands and the Chemotropic Hypothesis," reporting research carried out in Dr. Hotta's laboratory at the University of Tokyo, Japan. Dr. Hiramoto grew up in Akita and Kanagawa and then moved to Tokyo to pursue his undergraduate and then graduate studies. For his doctoral work with Dr. Hotta's group, he examined mechanisms of axon guidance. Dr. Hiramoto is currently investigating the positional information required for neural network formation, with assistance from the Precursory Research for Embryonic Science and Technology program of the Japan Science and Technology Corporation.

All Other Countries: Itamar Simon for his essay, "Time of Replication: Regulation and Significance," based on his doctoral research with Howard Cedar's group at Hebrew University Medical School in Jerusalem, Israel. Dr. Simon pursued undergraduate studies at Hebrew University between 1989 and 1992, and as a graduate student investigated the precise control of replication timing in animal cells. He is currently a postdoctoral fellow in Richard Young's laboratory at the Whitehead Institute.

The full text of essays by the regional winners and information about applying for next year's awards can be viewed on *Science* Online at www.sciencemag.org/feature/data/pharmacia/prize/winning.shl.