the big payoffs will come in an understanding of basic mechanisms of channel operation. For instance, close examination of ionic interactions with the K^+ channel inactivation ball shows that this type of gating represents the physical plugging of the cytoplasmic face of the pore and that nonspecific electrostatic forces focus this positively charged domain into its pore-blocking site (8). The slowing of inactivation by adenosine 3′,5′-monophosphate–dependent protein kinase phosphorylation of an equivalent domain on the Na^+ channel (9) leads to testable speculations about electrostatic steering as the basis for functional modulation by phosphorylation. Recently, point mutations were described that lead to a profound change in Na^+ channel selectivity, a phenomenon that requires the creation of sites for simultaneous binding of two Ca^{2+} ions (10).

Most of the questions addressed up to the present have been "minimalistic," in that they have focused on fundamental issues of mechanism and structure studied on simplified channels in heterologous expression systems, largely uncontaminated by biological reality. One of the most difficult challenges for more neurobiologically minded scientists will be to understand the structure and function of channels in their native cellular environments. The recent cloning of huge numbers of ligand-gated channel subunit isoforms, for instance, points to what an awesome task it will be merely to identify the subunit composition of a given channel in a given cell. A combination of antisense techniques, pharmacological manipulation, and single-channel analysis has recently begun to sort out this combinatorial nightmare in neuronal acetylcholine receptors (11).

The approach of genetic manipulation of ion channels as an analytical tool is still very young. It holds great promise in two directions, downward to the level of macromolecular structure and upward to the level of cellular function. The field's youth ensures that even primitive answers to the questions posed will remain exciting for some time yet, and that the inevitable frustration arising from the dearth of hard structural data will not flummox its practitioners in the near future.

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Bench to Bedside: The Glutamate Connection

Dennis W. Choi

Like so many other areas of neuroscience, the field of excitatory amino acid transmitter research has seen a rush of new information during the past year. Glutamate or related amino acids mediate fast synaptic transmission at the majority of excitatory synapses throughout the brain and spinal cord. An exhilarating appreciation of the role of this signaling process in health and disease is now unfolding.

Building on the pioneering efforts of Heinemann and colleagues, who cloned the first glutamate receptor subunit 3 years ago, ionotropic receptor subunits have been cloned faster than they can be definitively named (1). GluR1, GluR2, GluR3, and GluR4 subunits (alternatively referred to as GluRA through GluRD or α1 through α4 by other laboratories) exhibit functional and binding properties akin to those of the native α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA)–preferring glutamate receptors defined by earlier pharmacological studies. GluR5, GluR6, and GluR7 as well as KA-1 and KA-2 may be components of high-affinity kainate receptors. NMDAR1 together with its growing family of siblings (the ζ series) and cousins NMDAR2A, NMDAR2B, NMDAR2C, and NMDAR2D (the ε series) are all probably components of heteromeric NMDA receptors.

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Correlations between molecular structure and function of these receptors are emerging. The AMPA receptor subunits GluR1 through GluR4 can each be expressed by alternative mRNA splicing in two different versions, flip and flop. Although these two versions have only minimally different sequences in a 38–amino acid transmembrane region, flip forms show less desensitization and hence carry larger currents. Consistent with current work on voltage-gated channels, critical amino acid residues determine the Ca^{2+} permeability of AMPA receptor channels and the sensitivity of the NMDA receptor channel to Mg^{2+} block (2).

Much has also been learned about the intracellular events set in motion by glutamate receptor stimulation. These are proving to be complex: a remarkably intricate and interacting network of enzyme cascades, messenger compounds, and changes in gene expression capable of exerting lasting influence over excitatory synaptic behavior. In addition to ionotropic receptors, glutamate activates multiple metabotropic receptors coupled through G proteins to inositol phosphate or adenosine 3′,5′-monophosphate (cAMP) formation (3). Furthermore, glutamate stimulates an increase in intracellular free Ca^{2+}, which triggers the production of potential intercellular messengers such as fatty acid metabolites or nitric oxide (4).

Consistent with the spirit of the "Decade of the Brain," this basic information may also lead with surprising directness to advances in the clinical arena. Neurology, neurosurgery, and psychiatry are all concerned with numerous diseases with known phenotypes but unknown pathogeneses. The current knowledge explosion in the glutamate receptor field points to new molecular sites where a specific disturbance might cause disease or where a therapeutic intervention might be targeted.

One mechanism capable of connecting abnormalities in the glutamate system to disease is excitotoxicity—the ability of glutamate or related compounds to induce neuronal death, often after receptor overstimulation. Degeneration of human motor neurons in neurolathyrism is caused by ingestion of the chick pea excitotoxin, β-oxalylaminoalanine (5). A newly recognized clinical syndrome characterized by seizures and brain damage has been linked to the digestion of a kainate receptor agonist, domoate, found in contaminated mussels (6). Excitotoxicity is also strongly implicated in the brain or spinal cord damage induced by insults such as hypoxia-ischemia or trauma. NMDA antagonists can protect against damage in numerous experimental models of stroke (focal brain ischemia) consistent with in vitro studies indicating that NMDA receptor overactivation is a critical step in neocortical or hippocampal

neuronal death induced by brief exposure to high concentrations of glutamate (*7*). NMDA receptor overactivation is rapidly lethal, probably because the high Ca^{2+} permeability of its associated membrane channel triggers cellular Ca^{2+} overload.

However, more recently antagonists of AMPA-kainate receptors have also shown considerable promise as neuroprotective agents, especially in models of cardiac arrest (global ischemia) in which NMDA antagonists are ineffective (*8*). The basis for this reversal of receptor roles remains to be defined but may be partly related to downmodulation of NMDA receptors by factors such as extracellular acidity (*9*). Another interesting possibility is raised by a report that global ischemia selectively repressed GluR2 mRNA levels in the hippocampal CA1 region (*10*). Since the GluR2 subunit limits the Ca^{2+} permeability of the channels gated by heteromeric AMPA receptors, this depression might enhance the pathogenicity of AMPA receptor currents.

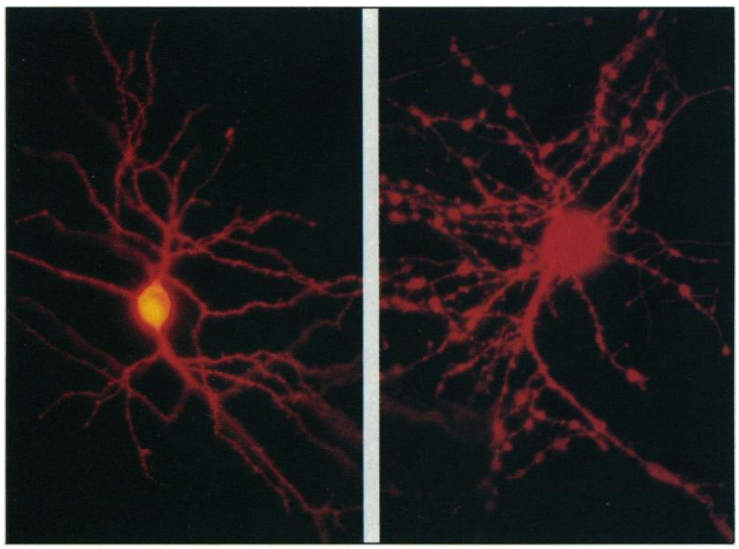
Excitotoxicity may also be triggered in other diseases associated with impairment of cellular energy metabolism failure. One example is the childhood MELAS syndrome—mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (*11*). MELAS is characterized by disturbances in mitochondrial energy metabolism, often due to a mutation in a highly conserved portion of the gene for mitochondrial transfer RNA (*12*). Children with MELAS show multiple episodes of localized brain dysfunction reminiscent of strokes but with little relation to vascular territories; brains examined after death exhibit spongy degeneration. Might the brain damage occurring in MELAS patients occur by a process intermediate between that occurring in stroke and that occurring in more gradual neurodegenerative diseases?

Indeed, a broader search for excitotoxicity in human diseases would do well to sift systematically through the many conditions associated with premature neuronal degeneration. The triggering factor need not be agonist degeneration or energy failure; other precipitations might include increased glutamate release, decreased glutamate uptake, or alterations in events occurring downstream from receptor activation, such as reduced Ca^{2+} buffering or free radical scavenging. Furthermore, certain growth factors, such as nerve growth factor or basic fibroblast growth factor (*13*), or certain gene products, such as heat shock proteins (*14*), may impart cellular resistance to excitotoxic damage. Disturbances in such protective systems might allow normal amounts of glutamate to become lethal.

Several intriguing clues to the possible participation of excitotoxicity in common neurodegenerative diseases have emerged in the past year or two: (i) Synaptosomes prepared from neural tissue of patients with amyotrophic lateral sclerosis show a selective deficit in glutamate uptake (*15*); (ii) 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–induced destruction of nigrostriatal dopaminergic neurons, the best experimental model of Parkinson's disease to date, can be attenuated by NMDA antagonists (*16*); and (iii) the β amyloid protein that accumulates in Alzheimer's disease can potentiate excitotoxic degeneration (*17*). Are these and other findings pieces of a larger puzzle or are they distractions rooted in coincidence? Speculations of excitotoxic involvement in neurodegenerative diseases have been with us for a long time. Excitotoxicity would be a convenient mechanism to explain the shared pathogenesis implied by the concurrent presentation of elements of amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease in natives of Guam (*18*).

Another important question, which will be explored during a symposium at the upcoming annual meeting of the Society for Neuroscience in Anaheim, is whether excitotoxicity participates in the pathogenesis of AIDS dementia. Lipton and colleagues have reported that the HIV-1 envelope protein gp120 can induce NMDA receptor–dependent degeneration of neurons in retinal cultures (*19*), and the spinal fluid of patients with AIDS dementia has markedly elevated concentrations of the endogenous NMDA agonist, quinolinate (*20*).

Mechanisms besides excitotoxicity might also tie abnormalities in the glutamate system to disease. Molecular evidence hints at an exquisite orchestration of glutamate receptor expression during brain maturation: For example, a transient preponderance of flip-type AMPA receptors that might be essential in enhancing a neurotropic or synapse-pruning action of glutamate (*21*). Disturbances in this orchestration during critical periods of nervous system development could produce altered synaptic connectivity, selective neuronal loss, or even a failure of normal programmed neuronal death (*22*) in an inborn neurological or psychiatric disease. In addition, changes in glutamatergic transmission independent of cell death might cause disease symptomatology. The anticonvulsant properties of glutamate antagonists have been recognized for a decade, and more recent data suggest that these drugs may also be able to ameliorate certain movement disorders. A major area for future exploration is the possibility that subtle abnormalities in the glutamate system might underlie difficulties with memory, perception, cognition, or personality. These problems are difficult to approach in animal models, so clinical observations may provide unique glimpses of the relation of the glutamate system to integrated brain function.



Excitotoxic neuronal injury induced by oxygen-glucose deprivation. (**Left**) Cultured cortical neuron labeled with the lipophilic fluorescent tracer dil. (**Right**) Dendritic swellings seen after 50 minutes of oxygen-glucose deprivation. [Photograph by M. Bateman and M. Goldberg]

Research in the glutamate field thus promises to form a bidirectional connection—bench to bedside and back—to the benefit of us all. An active medicinal chemistry pipeline presently exists, bringing advances in receptor biology to bear on the development of practical drugs. Besides NMDA and AMPA antagonists, interest in AMPA receptor–enhancing drugs has been sparked by the finding that aniracetam, a drug reported to improve cognition in some experimental paradigms, can slow the desensitization kinetics of AMPA receptor–gated channels (*23*), as can the clinically useful antihypertensive drug diazoxide (*24*). Effort is

also focused on identifying methods for reducing excitotoxicity without interfering with receptor activation. Areas of current emphasis include free radical scavengers, gangliosides, growth factors, protease inhibitors, inhibitors of nitric oxide production, and inhibitors of glutamate release; this list will undoubtedly expand as the mechanisms underlying excitotoxicity are elucidated. A key bench-to-bedside connection to watch over the next months is the chromosomal mapping of relevant normal and disease genes. The locations of GluR1 through GluR4 have just been reported (*25*). None of these correspond directly to a known disease gene, but several possibilities exist, and much more mapping remains to be done.

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Are Adult Learning Mechanisms Also Used for Development?

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During the next decade significant progress in the study of learning and memory storage is likely to come from the recognition that certain of its mechanisms are shared with those used for neural development.

Perhaps the most interesting clues to shared mechanisms are evidenced in the current revisions in our thinking about how connections in the vertebrate brain are formed during development. Until 10 or 15 years ago, most neurobiologists believed, as Roger Sperry proposed for cold-blooded vertebrates, that connections in the brain are formed independent of activity or experience and are programmed by a set of recognition molecules on each pre- and postsynaptic neuron of the synapse (*1*). It is now clear that Sperry's conception only applies to pathway selection and target region selection, the first two steps of a three-step developmental program for synapse formation. Much as Sperry predicted, axons such as those of the retinal ganglion cells grow out from the retina and select the correct pathway—the optic nerve—by means of a set of molecular guidance cues. The axons next leave the optic pathway to reach their target region (the tectum in cold-blooded vertebrates such as fish, the lateral geniculate nucleus and superior colliculus in mammals) by a similar series of molecular interactions. These two steps appear to depend only on molecular recognition events and seem not to require activity (*2*). However, research from M. Stryker, C. Shatz, J. T. Schmidt, and M. Constantine-Paton shows that on reaching their targets, there is a third stage, cellular selection, whereby each presynaptic axon is matched to a specific postsynaptic target neuron through activity-dependent mechanisms to produce the point-to-point order required for the mature function of sensory relay regions (*3*). Indeed, many of these connections can be modified by activity throughout the adult life of the organism (*4*), illustrating a temporal continuity between development and learning.

How does activity, in the form of action potentials, contribute to the functional and anatomical correction of the cellular selection process? The excitation of a target cell by the synchronous firing of a group of presynaptic fibers seems to strengthen those synapses with presynaptic fibers that are active together and to weaken those with presynaptic fibers that are inactive or are asynchronous with the excitation of the target cell (*3*, *5*). These changes in function connectivity are accompanied by anatomical changes. As the axons mature they are remodeled by selectively withdrawing some branches and growing new ones (*5*).

How does the postsynaptic cell detect the synchrony or asynchrony in the incoming presynaptic activity? And how does it send one type of signal back to all concurrently active presynaptic inputs so as to strengthen them, but at the same time send back a signal to the inputs that are not active so that they are weakened and ultimately eliminated? The specific mechanisms for these activity-dependent changes are not known, but the best candidates for mediating these processes are those that are utilized for certain forms of learning and memory storage in the adult animal.

In the adult brain some synaptic connections undergo an associative increase in synaptic strength called long-term potentiation (LTP) as a result of a high-frequency train of action potentials produced synchronously in a small population of neurons. LTP lasts for hours and, under some circumstances, for days and weeks and is thought to be important for some types of learning.

At synapses capable of associative LTP, the presynaptic terminals release the neurotransmitter glutamate, which then binds to two classes of postsynaptic receptor, the *N*-methyl-D-aspartate (NMDA) and the non-NMDA receptors. It is the unique character of the NMDA receptor that gives this form of LTP its associative properties. For the NMDA receptor channel to open, two conditions must be met simultaneously: (i) the receptor must bind glutamate, and (ii) the postsynaptic cell must be depolarized. At the resting membrane potential, the NMDA receptor channel is normally blocked by Mg^{2+}, and this block is only removed when the postsynaptic cell is depolarized. Adequate depolarization is achieved only by the synchronous firing of many presynaptic neurons, activating many non-NMDA receptors on the target cell. Thus, synchronous activity among several presynaptic axons can produce sufficient depolarization on a common target cell. This depolarization unblocks the NMDA receptor channel and

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