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?

Eric R. Kandel and Thomas J. O'Dell

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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allows the influx of Ca^{2+}, which in turn initiates the enhancement of synaptic transmission by leading to the activation of at least three different protein kinases.

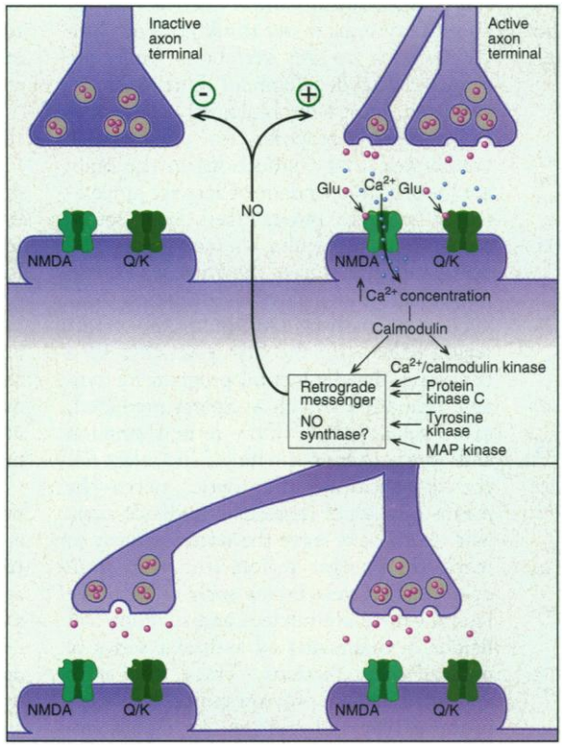
Whereas the induction of associative LTP depends on postsynaptic depolarization, the subsequent maintenance of LTP seems to also involve an increase in presynaptic transmitter release (6). The transfer of information from the postsynaptic cells, where LTP is induced, to the presynaptic terminal, where it is maintained, is thought to involve a retrograde messenger.

What is the nature of this retrograde signal? Because presynaptic spines lack the conventional machinery for release, the retrograde messenger is thought to be a substance that is synthesized on demand and rapidly diffuses out of the postsynaptic cell across the synaptic cleft and into the presynaptic terminal (7). Two such molecules now have been examined: arachidonic acid (8, 9) and nitric oxide (NO) (9–11). The evidence for neither of these messengers is fully compelling, but at the moment the evidence is stronger for NO. We therefore will here discuss NO as an example of how a retrograde messenger could serve similar functions during learning and development (10). However, there may well be several such messengers. For example, growth factors might serve as messengers during development.

Nitric oxide, a soluble gas, is generated by the Ca^{2+}-calmodulin–sensitive enzyme NO synthase. Specific inhibitors of NO synthase block the induction of LTP when injected into the postsynaptic cell in hippocampus (9–11), and NO increases spontaneous release of transmitter in cultured cells, indicative of a presynaptic effect (9).

Exogenous NO produces potentiation in slices only when it is given in conjunction with weak presynaptic activity (12). Thus, not only must the postsynaptic cells normally be active to allow Ca^{2+} influx through the NMDA receptor, but presynaptic cells must also be active to respond to it. Thus, synapses that exhibit associative LTP appear to have two independent associative mechanisms (see figure): a postsynaptic one that is derived from the properties of the NMDA receptor and a presynaptic one (similar to that in associative learning in *Aplysia*) that derives from the activity-dependent enhancement of second messenger pathways in the presynaptic terminals by the retrograde messenger (13).

Understanding of how out-of-phase activity leads to weakening of synaptic connections is much less satisfactory than our understanding of LTP, but a few clues are beginning to emerge. Stimuli that produce LTP in active fibers in hippocampus can also produce long-term depression (LTD) in neighboring fibers that are inactive or are active out-of-phase. How this occurs is not known. Perhaps NO could lead to depression of transmitter release from inactive or asynchronously active axons, while causing potentiation of release from fibers that are synchronously active (see figure). In fact, NO can cause depression of LTP and cause LTD in the cerebellum, albeit by a different mechanism (14). However, different retrograde messengers may be involved in depression and facilitation.



Learning mechanisms for development. One model by which a single retrograde messenger could lead to strengthening of active connections and weakening of inactive connections. (**Top**) Active presynaptic terminals engaged by the retrograde messenger are enhanced, while inactive terminals are inhibited. (**Bottom**) In the long term, this leads to regression of inactive terminals and stabilization or growth of new synapses from active presynaptic fibers.

In both vertebrates and invertebrates, learning proceeds through stages, much as does synapse formation. Unlike short-term changes, which result from covalent modifications of preexisting proteins, the long-term changes result from transcriptional activation and new protein synthesis, and are accompanied by growth of new or retraction of preexisting connections.

Are these adult learning mechanisms utilized during development? One clue that associative LTP might be operative during development has come from the work of M. Constantine-Paton and her colleagues. When activity is blocked by tetrodotoxin in the frog, the optic nerve fibers grow into the tectum but do not segregate to form eye-specific stripes (15), indicating that segregation requires that neighboring fibers are active together. Temporal summation of synaptic excitation from the activity of neighboring fibers in the retina would assure that depolarization produced in a common target cell is sufficient to remove the Mg^{2+} block from the NMDA receptor, allowing Ca^{2+} to flow into the postsynaptic cell and activate Ca^{2+}-dependent second messengers. In fact, segregation of stripes is blocked by antagonists of the NMDA receptor (16). Constantine-Paton and her colleagues have now turned to a mammal (the rat) and found a similar NMDA-sensitive process in another target of the optic nerve, the superior colliculus. There is a sharp rise in the amount of mRNA for both the NMDA receptor and NO synthase in the second postnatal week, and this parallels the refinement of the topographical map in the superior colliculus (17). It will now be interesting to see whether inhibitors of NO synthase produce alteration in cellular selection, like those seen after NMDA receptor blockade.

Thus, there is some evidence, albeit incomplete, that LTP (or a closely related phenomenon) may be important for developmental fine tuning of synaptic connections. There is also evidence that NO can act on synchronously active presynaptic terminals to enhance transmitter release in the adult organism, although there is as yet little evidence for the involvement of NO in the strengthening or regression of connections during development. Nevertheless, NO and other diffusible messengers are interesting candidates for both the strengthening and weakening of connections, because the problems posed by development and learning are so similar in their requirements that the idea of a common set of retrograde messengers seems economical and attractive.

Indeed, the requirements for activity-dependence and retrograde messengers represent only two of a number of points of similarity between learning-related synaptic modulation and synapse formulation. Other similarities include the ability to regulate the growth of new connections and the retraction of inappropriate synaptic terminals through the control of gene expression. This, in turn, derives from the

broadest similarity between learning and neural development: the recruitment of cellular programs of growth by signaling molecules.

These cellular programs can include alterations in the display of cell surface adhesion molecules. For example, long-term sensitization training in *Aplysia* causes a transcription-dependent down-regulation of NCAM-related cell adhesion molecules on the surface of sensory neurons (*18*). If this were generally the case, then in a sense, Sperry's circle is squared. Activity initiated by experience could lead to the release of signaling molecules that engage transcriptional control mechanisms, which modulate cell surface receptors so as to regulate cell-cell interactions.

The development of stem cell techniques for homologous recombination in mice (*19*) has provided a useful tool for testing genetically whether a particular mechanism is important for LTP in the hippocampus of the adult organism and whether LTP in the hippocampus is causally required for learning (*20*). These gene ablation methods can now be extended to determine whether synaptic modulation during development resembles memory storage only phenotypically or whether they actually share common molecular mechanisms (*20*). We should therefore soon be in a position to see whether solutions to the problems of learning and memory will yield, as an extra bonus, insights into synapse development and vice versa. If the study of learning and synapse development prove to be mutually reinforcing on the molecular level, then the Decade of the Brain, which we hope will relate molecules to mind, will be off to a particularly good start.

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The Physiology of Memory: Recordings of Things Past

Robert Desimone

Our conception of the process of memory—how we remember and recognize—is now undergoing a revolution similar to the dramatic changes seen over the past two decades in our understanding of how sensory information is processed. Sensory systems are now known to comprise a large number of separate cortical areas with complex interconnections; this complexity replaces the old notion of a primary sensory area with one or two cortical subsidiary areas. Likewise, memory is being fractionated as a result of recent studies—psychological, physiological, and anatomical (*1*).

This fractionation of memory systems was inevitable, as it now appears that most or all of the adult brain undergoes learning-dependent changes. Each biological change contributes to one or more of the numerous memory systems, which are defined behaviorally or psychologically. One class of memory system underlies declarative, or explicit, memories, which are the memories of specific facts and events. Within this class, it is useful to distinguish short-term (working memory) processes from long-term ones, and recognition processes from recall, as well as numerous material-specific systems, such as memory for faces, words, objects, and so on. The other class underlies nondeclarative, or implicit, memories, which include stimulus-response "habits," perceptual learning, conditioning, cognitive and motor skill learning, various types of priming, and habituation.

The mnemonic contributions of a given brain structure are usually closely related to its non-mnemonic functions. For example, the responses of neurons in premotor cortical areas change during visuomotor conditional learning (*2*); neurons in prestriate visual areas concerned with stimulus orientation respond in relation to working memory for the orientation of visual stimuli (*3*); the responses of neurons in the inferior temporal cortex, which is important for visual discrimination, change as visual objects become more familiar (*4*); and prefrontal and posterior parietal areas concerned with spatial relations contain neurons that respond in relation to working memory for spatial location (*5*). Likewise in humans and animals, lesions of the cerebellum, a motor control structure, impair the acquisition of classically conditioned motor responses (*6*); lesions or disease of portions of the striatum, which normally functions in sensorimotor integration, impair stimulus-response learning of habits (*7, 8*); lesions of inferior temporal cortex, an area important for visual discrimination, impair visual recognition and associative memory (*8, 9*); and lesions of superior temporal cortex, an area important for auditory discrimination, impair auditory recognition memory (*10*).

The medial temporal lobe is a major site of multimodal convergence, and it contains neurons that are sensitive to the configuration of many environmental stimuli as well as to the behavioral context in which events occur (*11*); thus, it is not surprising that this region is critical for forming long-term explicit memories (*8, 12*), which depend on just this sort of configurational information. Although the ultimate storage sites for explicit memories appear to be in the cortex, the medial temporal lobe plays a critical enabling, or buffering, role necessary for storage to take place. The hippocampus, ventromedial temporal cortex, and amygdala may each make selective contributions to explicit memory in the medial temporal lobe (*13*).

With such an abundance of memory mechanisms, are there any common physiological underpinnings? Neuronal record-

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