electric potential varies with distance, since this is affected by the local geometry and charge distribution of the protein-solution dielectric interface. Second, CTX is not a point charge, but rather a protein with a spatial distribution of charges. Finally, the total charge alteration resulting from a single point mutation depends on an unknown number of polypeptide chains making up the oligomeric channel complex. In spite of these uncertainties, it is instructive to make some plausible assumptions and calculate the distance from Glu^{422} to the CTX binding site. We use Debye-Huckel theory to approximate the electric potential variation with distance from a point charge, and we assume an effective valence of +5 for CTX. Then, considering that the local electric fields are mainly confined to aqueous regions of dielectric constant 80, we calculate a distance of 17 Å between position 422 and the CTX binding site, for a homotetrameric channel. Although this is a very crude estimate, it is a plausible one, falling squarely within the range of distances over which electrostatic focusing mechanisms operate in cases of known macromolecular structures (*18–21*).

These results have identified the physical mechanism by which Glu^{422} influences CTX binding energy in the outer mouth of the *Shaker* K^+ channel: via the electrostatic potential arising from surface charges some 10 to 20 Å distant from the toxin binding site. The effect of Glu^{422} is therefore local, and so we conclude that position 422 of the *Shaker* K^+ channel must reside on the extracellular side of the membrane. This information is in harmony with the standard folding models of this channel (*10, 11*), and it places a strong constraint upon more refined models of this transmembrane folding topology. Moreover, it localizes a region of the channel sequence near the external ion entryway. This region can be used as a starting point for future efforts to identify amino acid residues directly involved in the binding of the toxin, and, eventually, in the ionic conduction process itself.

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Long-Term Potentiation in the Motor Cortex

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Long-term potentiation (LTP) is a model for learning and memory processes. Tetanic stimulation of the sensory cortex produces LTP in motor cortical neurons, whereas tetanization of the ventrolateral nucleus of the thalamus, which also projects to the motor cortex, does not. However, after simultaneous high-frequency stimulation of both the sensory cortex and the ventrolateral nucleus of the thalamus, LTP of thalamic input to motor cortical neurons is induced. This associative LTP occurs only in neurons in the superficial layers of the motor cortex that receive monosynaptic input from both the sensory cortex and the ventrolateral nucleus of the thalamus. Associative LTP in the motor cortex may constitute a basis for the retention of motor skills.

IT IS GENERALLY BELIEVED THAT learning and memory processes are subserved by some form of synaptic plasticity within the central nervous system. Long-term potentiation (LTP), that is, the long-lasting enhancement of synaptic transmission, occurs in the hippocampus (*1*), and this LTP is thought to participate in mnemonic processes for cognitive events (*2*). However, in humans learning and retention of motor skills are subserved by other areas of the brain (*3*), perhaps in the motor cortex (MCx) (*4*) and/or in the cerebellum (*5*).

We have recently demonstrated that tetanic stimulation of the sensory cortex (SCx) can induce LTP in motor cortical neurons (*6*), which suggests that the corticocortical (CC) input from the SCx to the MCx is involved in the acquisition of motor skills. In that study, tetanic stimulation of the ventrolateral nucleus of the thalamus (VL) never produced homosynaptic LTP in motor cortical neurons (7), and this has been confirmed in many cells in the present experiment. Furthermore, when a motor cortical neuron received short-latency excitatory postsynaptic potentials (EPSPs) from both the SCx and the VL, tetanic stimulation of the SCx produced homosynaptic LTP in SCx input but did not produce heterosynaptic LTP in VL input (five cells) (*6*), suggesting that VL input is not related to motor learning. However, elimination of CC input to the MCx in the cat produces retardation in learning motor skills, but elimination of CC input after the learning does not eliminate previously acquired motor skills (*8*). This observation suggests that, in addition to the synaptic plasticity in the CC pathway, there are plastic processes in some additional pathways projecting to the motor cortex. Simultaneous tetanic stimulation of two inputs in the hippocampus, one of which does and the other of which does not produce LTP, can produce associative LTP in the latter input (*9*). We now report that associative LTP can also be induced in the thalamic input to the MCx by combined tetanic stimulation of the SCx and the VL.

Results were obtained from 14 young adult cats anesthetized with Nembutal. Five platinum-in-glass microelectrodes were implanted in the SCx (area 2), and four metal electrodes were placed in the VL for microstimulation. Intracellular recordings were made from neurons in the MCx (area 4) with a glass microelectrode filled with 3*M* KCl and a double-barreled, closed chamber technique (*10*). Stable intracellular recordings were obtained from more than 500 cortical neurons. Of these, 18 neurons that

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satisfied the following criteria were accepted for analysis: (i) they responded with EPSPs to stimulations through one of the electrodes in both the SCx and the VL (*11*), (ii) the latency of at least one of the EPSPs was shorter than 3.0 ms, (iii) the membrane potential was stable and at least −50 mV, and (iv) recordings lasted for more than 10 min.

Intracellular recordings from these accepted cells lasted 32.6 ± 26.2 min (mean ± SD, n = 18); five of these cells were recorded for more than 60 min (maximum, 93 min). The input resistance of these cells ranged from 12 to 20 megohms (*12*). EPSPs produced by stimulation of the SCx and the VL were recorded for at least 3 min as controls (*13*). Tetanic stimulation (200 Hz, 20 s) was then delivered simultaneously through the electrodes in the SCx and the VL (*14*).

Figure 1 illustrates a typical example of the associative LTP induced by combined tetanic stimulation of the SCx and the VL. Amplitudes of the EPSPs produced by stimulation of the SCx and the VL were stable during 7 min of control recordings. The amplitudes of SCx-induced EPSPs decreased immediately after the tetanic stimulation, possibly because of short-lasting inhibitory effects produced by the CC input (*6*). Then the amplitudes gradually increased and stabilized at 203.0 ± 5.6% (mean ± SEM) of the control (n = 6, $P << 0.01$). The amplitudes of EPSPs produced by VL stimulation were not markedly decreased immediately after the tetanic stimulation, and they stabilized at the potentiated level within 3 min (184.1 ± 1.6%, n = 6, $P << 0.01$). In this example, a second tetanic stimulation was delivered 10 min after the first tetanus, and the amplitudes of the VL-induced EPSPs were further increased, stabilizing after 6 min at 205.8 ± 2.2% relative to the control (n = 16, $P << 0.01$). In contrast, the second tetanus did not produce a further potentiation of SCx-induced EPSPs. These LTPs lasted for more than 30 min, until the recording electrode came out of the cell.

Of the 18 cells accepted for analysis, eight cells exhibited associative LTP that was similar to the example shown above, and in one cell only the CC input was potentiated. We believe that we are studying true LTP because all of these LTPs lasted until the electrode escaped the cell (range, 9 to 73 min), suggesting that even longer periods of potentiation could be observed if the intracellular recordings were continued. In the remaining nine cells neither VL nor SCx inputs were potentiated.

We examined the depth distribution of the neurons in which associative LTP was induced histologically, utilizing lesions made during the experiments, by inserting a metal electrode into the recording site (Fig. 2A). Associative LTP was induced only in cells in layer III, although not all the cells in the superficial layers produced associative LTP; in four superficial cells, both VL and CC inputs were not potentiated, and in one cell only the CC input was potentiated. None of the cells in the deeper layers were potentiated, even though cells in deeper layers (V and VI) of the MCx form synapses with the SCx afferents (*15*).

To further characterize those cells in which the tetanic stimulations were ineffective in producing LTP, we plotted the latencies of the CC and the thalamic EPSPs of the layer III neurons against the magnitudes of the EPSP potentiation (Fig. 2B). LTP was induced exclusively in those neurons in which the latencies of CC- and VL-induced EPSPs were 2.1 and 2.2 ms or less, respectively. Previous results suggest that EPSPs with these latencies are elicited monosynaptically (*16*). The latency of the VL EPSP of the superficial layer neuron, in which only the CC input but not the VL input was potentiated, was 3.45 ms (Fig. 2B, arrow). Two other neurons that did not produce associative LTP received VL input with latencies shorter than 2.2 ms; however, the latencies of the CC EPSPs were longer than 2.1 ms. Of five deep-layer cells (not shown in Fig. 2B), three received short-latency EPSPs from both the SCx and the VL, but these EPSPs were not potentiated. Thus we conclude that associative LTP can be induced only in a selected group of superficial layer neurons that receive monosynaptic inputs both from the SCx and from the VL.

It has been proposed that the rise time of an EPSP (that is, from the start to the peak of EPSPs) reflects the distance between the site of the activated synapse and the recording electrode (*17*), although these synapses can be located on the different dendritic branches. Of eight neurons that produced associative LTP, seven cells showed clear single-peak EPSPs in response to both CC and VL stimulation. In all of these seven cells, the difference in the rise time between VL- and CC-induced EPSPs was very small, 0.13 ± 0.07 ms (mean ± SD), the maximum difference being 0.3 ms. Our results can be interpreted to suggest that associative LTP was produced when terminals of VL and SCx projection fibers were located in close proximity to each other and that CC

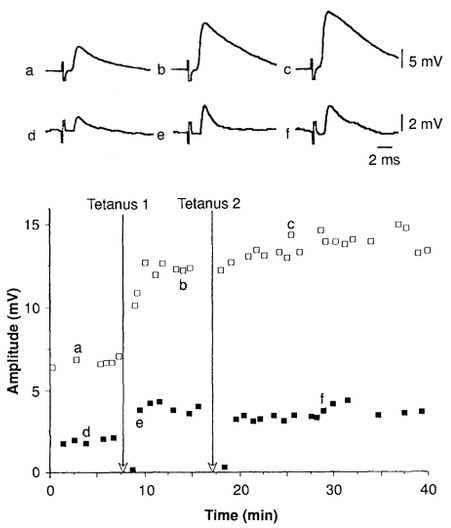

Fig. 1. Examples of EPSPs (**top**) and the time course of associative LTP in a motor cortical neuron (**bottom**); a, b, and c designate monosynaptic EPSPs elicited by thalamic stimulation; d, e, and f, monosynaptic EPSPs elicited by SCx stimulation before and after tetanus. Both EPSPs were augmented significantly (see text) after combined tetanic stimulation (200 Hz, 20 s) of the SCx and the thalamus. Letters in the time course graph (a through f) indicate the points at which the examples were taken. No hyperpolarizing current was passed throughout the recording period. The membrane potential (−65 mV) and the input resistance (15 megohms) of this cell were stable throughout the recording period. (□) Thalamocortical EPSP; (■) corticocortical EPSP.

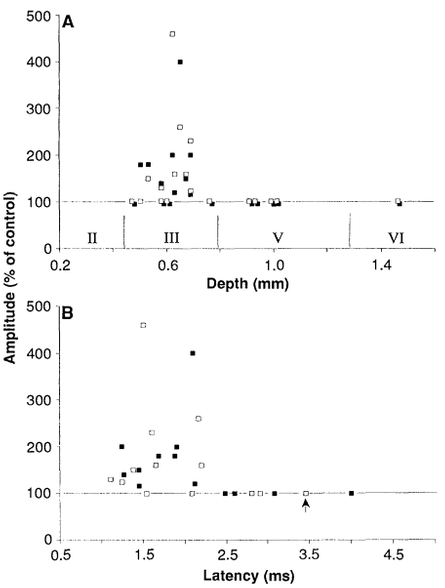

Fig. 2. (**A**) Relation between the depths of the recorded cells from the cortical surface and the magnitudes of the LTPs induced. Roman numerals indicate cortical layers. The amplitude of EPSPs is expressed as a percentage of the control EPSP amplitude. (**B**) Relation between the latency of EPSPs and the magnitude of LTP in the layer III cells is shown in (A). Layer V and layer VI cells are not included in this graph (see text). The arrow indicates the latency of the thalamic EPSP in the neuron in which only the cortical input, but not the thalamic input, was potentiated. (□) Thalamocortical EPSP; (■) corticocortical EPSP.

input produced local postsynaptic changes in the target neuron that increased efficiency of VL input heterosynaptically (*18*).

Our results indicate that associative LTP is induced only in neurons in the superficial layers, which contain only noncorticofugal neurons, many of which are local interneurons. These neurons receive nonspecific input from the VL (*19*) but specific input from the SCx (*20*). This result suggests that associative LTP is produced only in intracortical neurons, which then modulate the activity of a small group of cortical efferent neurons.

One possible role of associative LTP in motor learning may be the following: In the early stages of learning a motor skill, a set of movements is practiced slowly, with heavy reliance on sensory feedback. After repeated practice, the movement becomes faster and smoother, and eventually the sensory feedback becomes unnecessary. At this stage, removal of the SCx does not impair the learned motor skills (*8*). It is possible that the function of CC LTP is to facilitate learning and retention of motor skills and the latter function is transferred to VL LTP as the learning proceeds.

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11. Glass microelectrodes were advanced through the MCx while the SCx was being stimulated (30 μA, 0.2 ms, 1 Hz) through all the SCx electrodes. Whenever a neuron responded to SCx stimulation with a short-latency EPSP, the electrode responsible for the EPSP was determined. Then the VL was stimulated (100 μA, 0.2 ms, 1Hz) to determine whether the same cortical neuron received short-latency EPSPs from a single electrode in the VL.
12. We estimated the membrane resistances by measuring the difference in the amplifier's bridge balance inside and outside the cells. Brief pulses were occasionally passed during the recordings to determine if there was a change in membrane resistance induced by the tetanic stimulation; in no instance was a change in resistance observed.
13. When the recording was stable, 3-min control was sufficient because further observation (up to 20 min) showed that the amplitude of the EPSPs did not change from the initial amplitude.
14. The intensity of each tetanic pulse was identical to that used to evoke control EPSPs. The interval between each pair of SCx and VL stimuli, during the tetanic stimulation, was set so that the peak of the VL-evoked EPSP was preceded by the peak of the SCx-evoked EPSP by 1 to 2 ms. Because the potentiating effects of tetanic stimulation of the SCx are known to be long-lasting (*6*), each stimulating site in the SCx and VL was used for tetanic stimulation only once in each experiment.
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The Mechanism of DNA Transfer in the Mating System of an Archaebacterium

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The genetic transfer system in the extremely halophilic archaebacterium *Halobacterium volcanii* is the only archaebacterial mating system known. The mechanism of genetic transfer of this archaebacterium was studied by using the immobile plasmids pHV2 and pHV11 as cytoplasmic markers. It was found that the cytoplasms of the parental types do not mix during the mating process, that each parental type can serve both as a donor and as a recipient, and that cytoplasmic bridges, with dimensions of up to 2 micrometers long and 0.1 micrometer in diameter, were formed between the parental types. These bridges appear to be used for the transfer of DNA from one cell to another. If so, this archaebacterial mating system is different from both eubacterial conjugation and eukaryotic sexual cell fusion.

FINDINGS IN COMPARATIVE TAXONOMY led to the division of prokaryotes into distinct urkingdoms, that of the eubacteria and that of the archaebacteria (*1*). This division was based on a comparison of T1 catalogs of 16*S* and 23*S* ribosomal RNAs, characteristic features of the cell envelope, membrane lipids, and the DNA-dependent RNA polymerases. A similar comparison between archaebacteria and eukaryotes appears to indicate several common characteristics. For example, as in yeast, some archaebacteria contain introns in transfer RNA genes (*2*), and the RNA polymerase of archaebacteria shows a considerable immunological cross-reactivity to the RNA polymerase of yeast (*3*). Most of the archaebacteria grow in unusual ecological niches: methanogens are strictly anaerobic, the sulfur-metabolizing archaebacteria are thermoacidophilic, and the halobacteria grow only in concentrated salt solutions.

The archaebacterial genome resembles the eubacterial genome in its size and complexity (1×10^3 to 3×10^3 kb) (*4*). However, the lack of a well-developed genetic analysis system has restricted functional studies of the archaebacterial DNA. The extremely halophilic archaebacteria of the genus *Halobacterium* are probably the best model system for the study of the genome organization in archaebacteria. These bacteria are easy to grow and maintain, and auxotrophic mutants are readily obtainable.

The existence of a natural genetic transfer system in *Halobacterium volcanii* has been demonstrated (*5*). This transfer system has the following characteristics: it requires physical contact between the two parental cells, the two parental cells have to be alive, and no distinction can be made between donor cells and recipient cells. This system is the only genetic transfer system known so far for archaebacteria.

Eukaryotic organisms and eubacteria use different basic strategies for cell contact–dependent DNA transfer. In all eukaryotes, two haploid cells fuse to form one diploid cell. In eubacteria, cell contact–dependent DNA transfer (conjugation) involves pore formation between juxtaposed donor and recipient cells (*6*). We examined the basic strategy of cell contact–dependent DNA transfer in archaebacteria.

Cytoplasmic markers are used to distinguish between the two DNA transfer mech-

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