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Long-term modifications of synaptic efficacy in the human inferior and middle temporal cortex

(Long-term potentiation/long-term depression/*N*-methyl-D-aspartate receptor/visual memory)

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ABSTRACT **The primate temporal cortex has been demonstrated to play an important role in visual memory and pattern recognition. It is of particular interest to investigate whether activity-dependent modification of synaptic efficacy, a presumptive mechanism for learning and memory, is present in this cortical region. Here we address this issue by examining the induction of synaptic plasticity in surgically resected human inferior and middle temporal cortex. The results show that synaptic strength in the human temporal cortex could undergo bidirectional modifications, depending on the pattern of conditioning stimulation. High frequency stimulation (100 or 40 Hz) in layer IV induced long-term potentiation (LTP) of both intracellular excitatory postsynaptic potentials and evoked field potentials in layers II/III. The LTP induced by 100 Hz tetanus was blocked by 50–100 μM DL-2-amino-5-phosphonovaleric acid, suggesting that *N*-methyl-D-aspartate receptors were responsible for its induction. Long-term depression (LTD) was elicited by prolonged low frequency stimulation (1 Hz, 15 min). It was reduced, but not completely blocked, by DL-2-amino-5-phosphonovaleric acid, implying that some other mechanisms in addition to *N*-methyl-D-aspartate receptors were involved in LTD induction. LTD was input-specific, i.e., low frequency stimulation of one pathway produced LTD of synaptic transmission in that pathway only. Finally, the LTP and LTD could reverse each other, suggesting that they can act cooperatively to modify the functional state of cortical network. These results suggest that LTP and LTD are possible mechanisms for the visual memory and pattern recognition functions performed in the human temporal cortex.**

Synaptic plasticity has long been considered as a cellular basis for learning and memory. It was first shown in the hippocampus that brief high-frequency activation of input fibers yields a long-term potentiation (LTP) of the stimulated synapses (1). Since then, LTP has been analyzed in great detail in rodent hippocampus (2), and a similar phenomenon has also been observed in the rat and cat neocortex (3–5). An opposite process, long-term depression (LTD) of synaptic transmission, has recently been described and studied in both hippocampus and neocortex (6–9). It has been widely assumed that LTP and LTD revealed by experiments in subprimate animals underlie the mechanisms of learning and memory in the human. However, there have thus far been no reports of either LTP or LTD in the human neocortex.

Among the many cortical areas that have been implicated in roles in learning and memory, the primate inferior and middle temporal cortex is of special interest with regard to visual memory and pattern recognition (10–13). This cortical region receives input from the primary visual cortex and synthesizes the dissociated features into a coherent mental image that is then stored as a template, presumably through mechanisms involving long-term synaptic plasticity, for subsequent identification of visual objects. Electric stimulation of this area during brain surgery causes recall of visual experience (14). Lesions in this cortex produce a clinical syndrome called prosopagnosia, the impaired recognition of familiar faces (15). A recent study employing positron emission tomography also shows that the inferotemporal cortex is specifically active during visual object identification (16). It is therefore of particular interest to investigate whether this cortical area shows activity-dependent synaptic modifications, which have been postulated to be the underlying mechanisms for memory formation on the basis of the experiments in rodent hippocampus and neocortex. In the present study, we address this issue by using surgically resected human inferior and middle temporal cortex.

MATERIALS AND METHODS

The experiments described in this paper were performed on temporal cortex slices from 14 patients, whose ages ranged from 2 to 55 years old (with an average of 31.6 ± 15.4). All tissues were resected for therapeutic reasons. The anterior part of the inferior and middle temporal cortex, from which the tissue was taken, is shown in Fig. 1*A*. In most cases, the cortices were removed to gain access to more mesial structures (i.e., hippocampus), where focal epileptiform activity was localized. The obtained neocortical tissues were not grossly abnormal when examined electrographically and pathologically, and they did not give rise to abnormal synchronous discharges when maintained *in vitro*. Following the resection, a 5–10-mm tissue block was immediately immersed in ice-cold oxygenated artificial cerebrospinal fluid and was then transferred to the electrophysiological laboratory, where it was cut into 500-μm-thick slices with a vibratome. The slices were kept submerged in a conventional holding chamber for at least 2 h. Recordings were made in an interface chamber maintained at 29–34°C. The artificial cerebrospinal fluid used in both slice preparation and recording contained 124 mM NaCl, 3 mM KCl, 1.3 mM $MgSO_4$, 2.0 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 10 mM glucose and was kept at pH 7.4 by bubbling with 95% O_2 and 5% CO_2.

A bipolar stimulating electrode was placed in layer IV, which appeared as a white band in the middle of the gray matter (Fig. 1*B*). Evoked field potentials and intracellular excitatory postsynaptic potentials (EPSPs) were recorded more superficially above the stimulation site in layers II/III. Stimulation in layer IV activates direct projections from layer IV neurons to layers II/III, recurrent collaterals of layers II/III pyramidal neurons, as well as ascending fibers that pass through layer IV

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Abbreviations: LTP, long-term potentiation; LTD, long-term depression; APV, DL-2-amino-5-phosphonovaleric acid; EPSP, excitatory postsynaptic potential; NMDA, *N*-methyl-D-aspartate.

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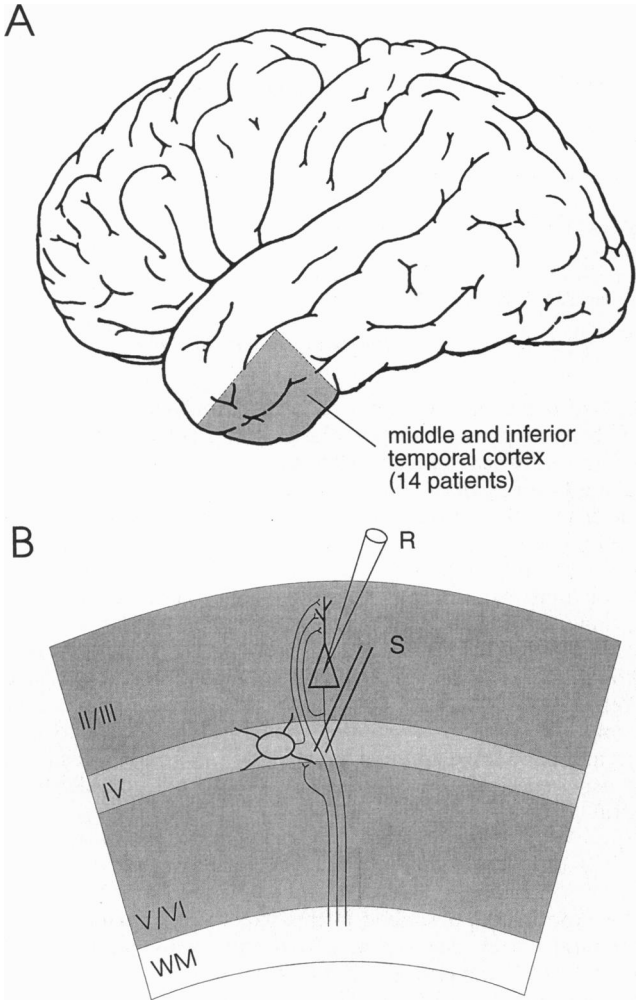

FIG. 1. Location of human neocortical tissue and the stimulation-recording arrangement. (*A*) Human neocortical tissue was obtained from the anterior part of the inferior and middle temporal cortex. (*B*) Recordings were made from layers II/III, and stimulation was delivered at layer IV, which appeared as a white band in the middle of the gray matter. The recording and stimulating electrodes were aligned perpendicular to the pia mater. The major pathways activated by layer IV stimulation are illustrated in the figure and explained in *Materials and Methods*.

en route to layers II/III (Fig. 1*B*). The recording electrodes were filled with 1 M NaCl (1–6 MΩ) for extracellular field recordings or with 2 M potassium acetate (70–150 MΩ) for intracellular recordings.

In each experiment, the input-output relation was first examined. The stimulation intensity was then adjusted to yield a synaptic response of approximately half-maximum. Test stimuli of 200 μs duration were delivered every 30 s. To induce synaptic plasticity, the following three conditioning protocols were used: (*i*) five trains of 100 Hz stimuli, each lasting for 1 s, delivered every 30 s; (*ii*) 10 trains of 40 Hz stimuli, each lasting for 1 s, delivered every 10 s; (*iii*) 1 Hz stimulation delivered for 15 min. The intensity and duration of conditioning stimuli were the same as those of test stimuli. The amplitudes of EPSPs and evoked field potentials were measured as an index of synaptic strength. Only those experiments in which there was no drift in preconditioning base-line responses were included in this study. All response data in each experiment were normalized by expressing them as percentages of the preconditioning base-line average. The time-matched, normalized data were averaged across experiments and were presented as mean ± SEM. A *t* test was used for statistical comparisons.

RESULTS

The synaptic responses in layers II/III to stimulation of layer IV were reliably potentiated following the 100-Hz tetanus. As shown in Fig. 2*A*, 100 Hz tetanus induced long-term potentiation of both intracellularly-recorded EPSPs (upper traces, $n = 2$) and extracellularly-recorded field potentials (lower traces, $n = 10$) in the human temporal cortex. This potentiation could persist for at least 2 h. The averaged time course of 10 field potential experiments showed an initial large enhancement of the synaptic response, which then decayed to a steady potentiated level (Fig. 2*B* upper half). The average amplitude of synaptic responses measured 30 min after the tetanus was 113.8 ± 4.0% of base-line average, which was significantly different from the pretetanic controls ($P < 0.01$). To assess if the 100 Hz-induced LTP was *N*-methyl-D-aspartate (NMDA) receptor-dependent, eight experiments were performed, in which all conditions were exactly the same as those of the above-mentioned control group except that the NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (APV, 50–100 μM; Sigma) was bath applied. As illustrated in the lower half of Fig. 2*B*, the average amplitude of responses measured 30 min after tetanus was reduced by APV application to 101.7 ± 2.1% of base-line. This value showed no significant difference from the pretetanic controls ($P > 0.5$), but it did significantly differ from that measured in the control group not treated with APV ($P < 0.05$).

The functioning of cortical circuits appears also to involve mechanisms that weaken synaptic strength. Without such a mechanism, the whole network would eventually saturate to a single final state, which is inconsistent with the functional operation of the cortex. Kirkwood and Bear (8) have recently reported that prolonged low frequency stimulation (1 Hz for 15 min) leads to long-term depression of synaptic transmission in the rat visual cortex. Using the same conditioning protocol, we were able to induce prominent LTD of both the EPSPs ($n = 2$) and evoked field potentials ($n = 12$) in layers II/III of the human temporal cortex (Fig. 3*A*). The average amplitude of LTD at 30 min after conditioning was 81.9 ± 4.8% of base-line, which was significantly different from the preconditioning controls ($P < 0.01$; Fig. 3*B* upper half). Like LTP seen in this tissue, synaptic depression persisted over 1 h. The LTD was input-specific. When stimulation was delivered at two sites in layer IV that were separated by about 1 mm, LTD was restricted to the conditioned pathway, leaving the response in the unconditioned pathway unaffected ($n = 3$, data not shown). Bath application of 50–100 μM APV reduced, but did not completely block, the 1 Hz-induced LTD in the human temporal cortex (Fig. 3*B* lower half). In the presence of APV, the LTD amplitude at 30 min after low frequency stimulation was 92.1 ± 5.3% of base-line.

Since both the LTP and LTD could be induced in human temporal cortex, it was of interest to analyze the interaction between these two different forms of synaptic plasticity. Low frequency stimulation was found to be able to depotentiate the LTP previously induced by 100 Hz tetanus (Fig. 4 upper half; $n = 2$). Conversely, 100 Hz tetanus could reverse the LTD elicited by 1 Hz stimulation (Fig. 4 lower half; $n = 2$).

The above results showed that human temporal cortex could undergo bidirectional changes in synaptic effectiveness, depending on the conditioning frequency. To examine in more detail the frequency dependence of the synaptic plasticity, we also tested the effect of a 40 Hz tetanus. This conditioning slightly potentiated synaptic transmission, with an average response of 106.1 ± 5.3% at 30 min after tetanus ($n = 14$). Fig. 5 summarizes the dependence of synaptic modification on conditioning frequency. The left data point in the graph is based on the observation that synaptic responses were stable when the stimulus interval was greater than 20 s. The extrapolated line indicates that low frequency stimulation induces

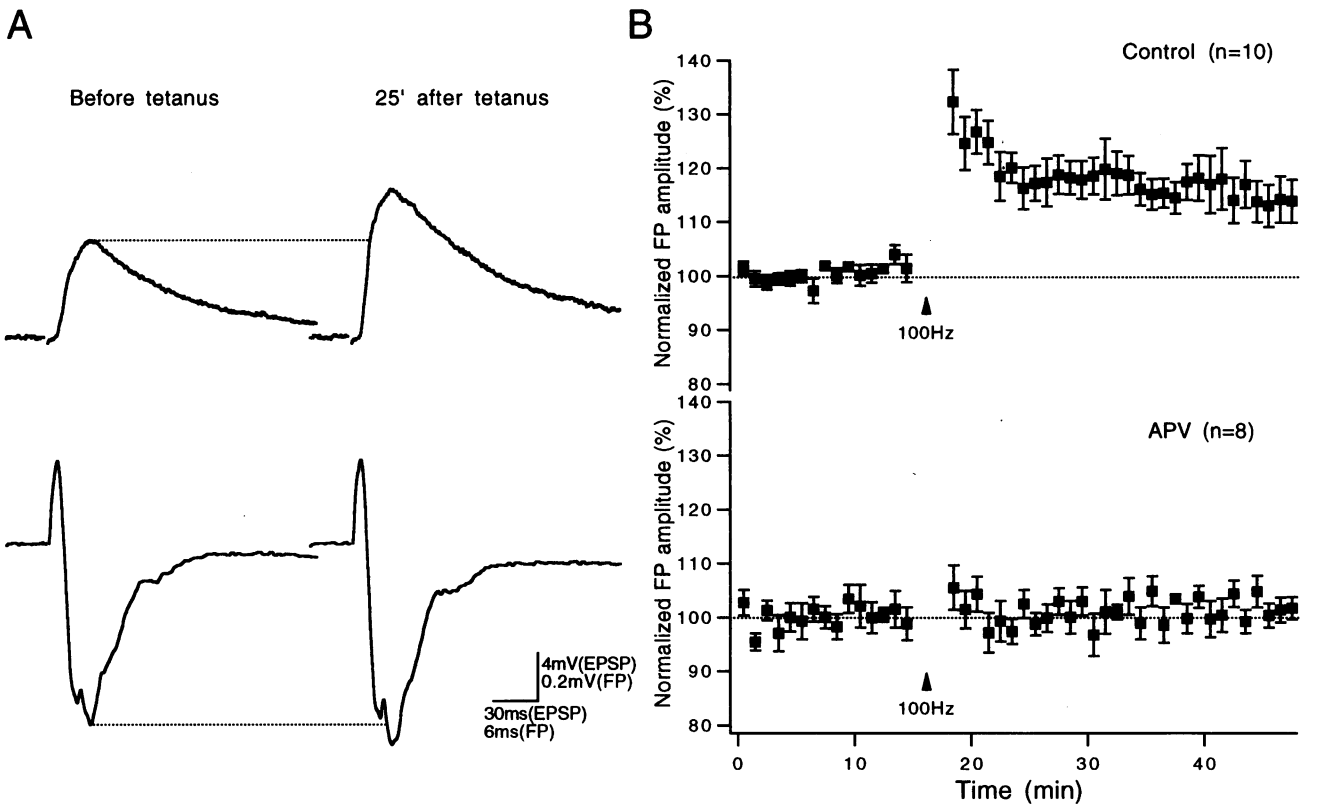

FIG. 2. (*A*) 100 Hz tetanus induced long-term potentiation of both intracellular EPSPs (upper traces) and evoked field potentials (lower traces) in the human temporal cortex. The EPSPs and evoked field potentials were averaged over a 5-min recording period immediately before and 25 min after the tetanus. (*B*) Time courses of the average field-potential responses evoked in the absence (upper half) and presence (lower half) of 50–100 μM APV.

LTD, while high frequency tetanus leads to LTP, with the tendency for the higher frequency of stimulation to induce greater potentiation.

DISCUSSION

The present results demonstrate that the human inferior and middle temporal cortex can undergo bidirectional modifications of synaptic efficacy in response to different temporal patterns of stimulation. As this is the first attempt to study synaptic plasticity in the surgically resected human neocortex, it is necessary to evaluate the validity of the preparation. Most of the tissue used in this study was removed to gain access to the underlying hippocampus, where focal epileptiform activity was localized. The tissue appeared to be relatively normal because no synchronous discharges were recorded either intraoperatively or in the *in vitro* slices. In addition, the firing behaviors of neurons in human neocortex slices have been reported to be similar to those observed in normal cat neocortex (17, 18). GABAergic inhibition has been shown in rodent hippocampus and neocortex to have a strong influence on the induction of both LTP and LTD (6, 19, 20), and a low dose of $GABA_A$ receptor antagonist has often been used to facilitate LTP induction in the rodent neocortex. It is thus possible that LTP and LTD demonstrated here could be a consequence of the impairment of inhibition in the surgically resected human cortex. However, in a previous study of human neocortical slices obtained with the same surgical methods by the same neurosurgical group, McCormick (21) reported that GABAergic inhibition, including both $GABA_A$ and $GABA_B$ receptor components, is well preserved. Taken together, the synaptic modifications documented here appear to be normal functions of the human inferior and middle temporal cortex.

It has been well documented in the hippocampus that NMDA receptor-mediated Ca^{2+} influx plays a central role in LTP induction. Kirkwood and Bear (8, 22) also reported that both LTP and LTD in rodent visual cortex require NMDA receptor activation. Our results from the human temporal cortex show that the 100 Hz-induced LTP is exclusively mediated by NMDA receptors. However, in case of the 1 Hz-induced LTD, other mechanisms in addition to the NMDA receptors appear to be involved. Voltage-gated Ca^{2+} channels and Ca^{2+} release from internal stores have been proposed as other possible routes for Ca^{2+} influx that triggers LTP (23–27). In the hippocampus, supporting evidence for the involvement of R-, T-, and L-type Ca^{2+} channels in the induction of homosynaptic LTD has been reported (28, 29). Whether the same mechanisms contribute to the NMDA receptor-independent LTD shown in Fig. 3*B* remains to be determined.

The frequency dependence of synaptic modification indicates that the excitatory synapses in the human temporal cortex are similar to a theoretically hypothesized synapse by Bienenstock, Cooper, and Munro (BCM), which assumes the following properties: (*i*) the synapse can undergo bidirectional changes in its connection weight; (*ii*) synaptic transmission is potentiated if the input consistently yields a postsynaptic response greater than a critical value (modification threshold) and is depressed if its response is below the modification threshold; (*iii*) the modification threshold is sliding, i.e., it changes as a function of the previous activity of postsynaptic neuron (30). Of all these properties, only the third one is not implicated in our results, and a further examination of it is needed. It has been shown that a cortical network connected with BCM synapses can reproduce all types of plasticity that have been observed in the visual cortex (31). Although originally presented to account for the experience-dependent plasticity in the primary visual cortex, our results suggest that

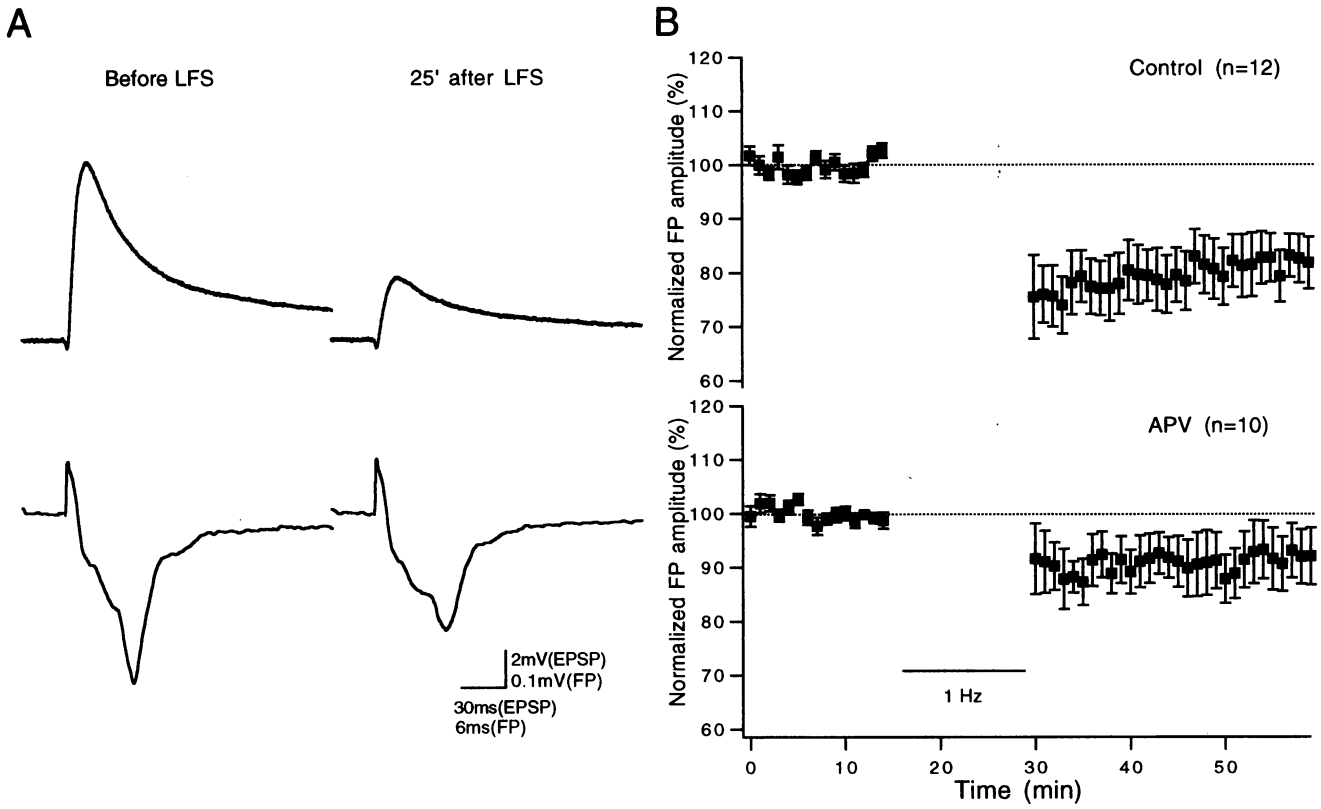

FIG. 3. (*A*) Low frequency stimulation (1 Hz for 15 min) induced long-term depression of both intracellular EPSP (upper traces) and evoked field potential (lower traces). The EPSPs and evoked field potentials were averaged over a 5-min period immediately before and 25 min after conditioning stimulation. (*B*) Time courses of the average field-potential responses evoked in the absence (upper half) and presence (lower half) of 50–100 μM APV.

the BCM theory may also provide a model for visual learning and memory in the human temporal cortex. Unlike neurons in primary visual cortex, single cells in the primate inferotemporal cortex are sensitive to specific complex patterns such as

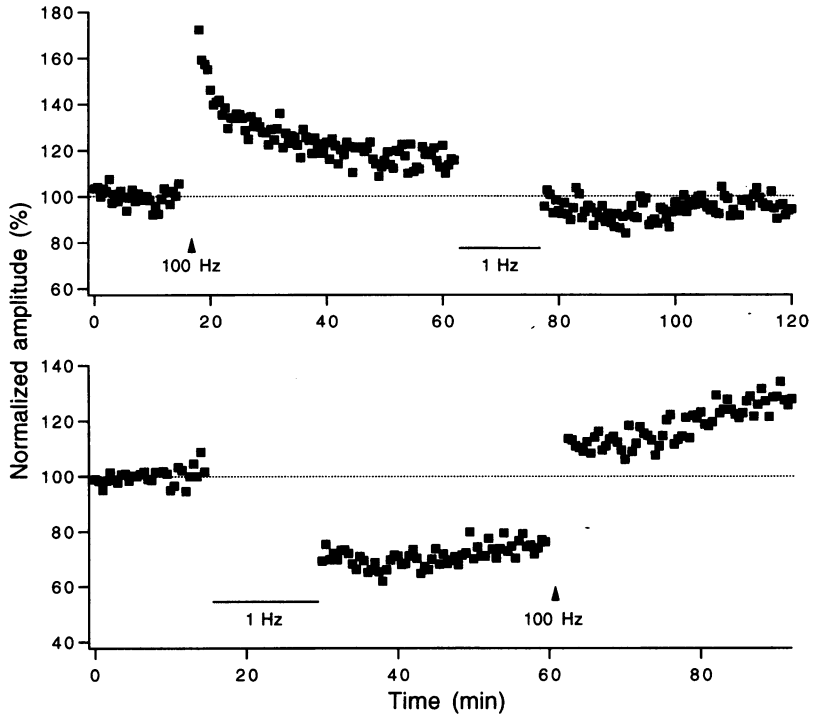

FIG. 4. Interaction between long-term potentiation and long-term depression. The upper graph shows that low frequency stimulation depotentiated the LTP previously induced by 100 Hz tetanus. The lower graph illustrates that 100 Hz tetanus was able to reverse the LTD caused by low frequency stimulation.

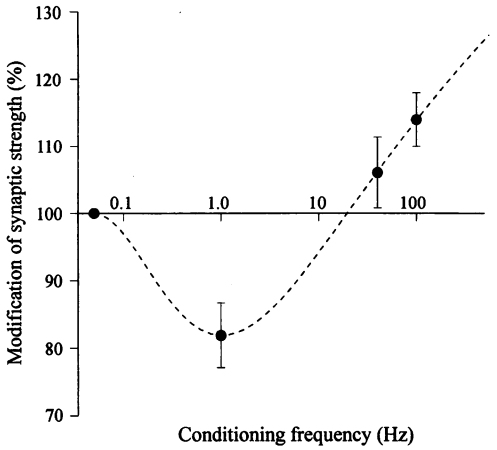

FIG. 5. Relationship between conditioning frequency and synaptic plasticity. The three data points in the graph are the average changes of synaptic responses measured at 30 min after the 100-, 40-, or 1-Hz conditioning stimulation. The leftmost point is given without a standard error bar, as the synaptic response was stable when the stimulus frequency was lower than 0.05 Hz.

human face and hand (32), and therefore their activities are more closely related to higher functions like visual memory and pattern recognition (10, 12, 16). It appears that simple features, which have been dissociated in early stages of visual processing, are "bound" together in the temporal cortex to produce a coherent mental representation of external objects. Since both 40- and 3–4-Hz oscillatory activities have been found in the visually responsive neurons in the inferiotemporal cortex of behaving monkeys (33, 34), the LTP and LTD described in this study may represent two complementary mechanisms whose interaction at the network level leads to the emergence of visual memory and pattern recognition.

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