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Contrasting forms of synaptic plasticity in monkey inferotemporal and primary visual cortices

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ACTIVITY-DEPENDENT synaptic plasticity was examined *in vivo* in two cortical areas of the adult monkey visual system, the primary visual and inferotemporal cortex, the first and late cortical stages essential for object recognition. Discontinuous high-frequency electrical stimulation of intrinsic horizontal connections in layer 2/3 caused contrasting forms of synaptic plasticity in the two areas. In the inferotemporal cortex, long-term potentiation of extracellular field potentials in layer 2/3 was induced, whereas in the same pathway of V1, identical stimulation protocol elicited long-term depression. The results indicate that susceptibility to synaptic plasticity varies among cortical areas in the monkey brain.

Key words: Inferotemporal cortex; Long-term depression; Long-term memory; Long-term potentiation; Memory formation; Primary visual cortex; Synaptic plasticity

Introduction

The visual cortex in the monkey is divided into more than 30 areas according to structural and functional criteria.[1] Different areas make different contributions to storage and processing of visual information.[2–4] Little is known, however, about whether or how these cortical areas differ in terms of modifiability of their neuronal circuits. In this study we chose the dorsal part of area TE of the inferotemporal cortex (TEd) and the primary visual cortex (V1) for comparison, as the former represents a higher association cortex and the latter represents the primary sensory cortex along the same functional cortical pathway, i.e. the object vision pathway.[4]

The TEd and V1 of the monkey share two organizational features. First, neurones with similar functional properties are clustered in the columnar fashion. In V1, neurones are grouped into columns according to their orientation selectivity and ocular dominance.[5] In the TEd, neurones are arrayed into columns according to their selectivity for visual features of objects such as shapes, colours and textures.[6] Second, these functional columns are linked by an extensive network of intrinsic horizontal axons, mostly running in layer 2/3.[7,8] These horizontal axons can mediate interactions between columns, and their plasticity may be crucial for regulation of the coded representation of visual information within a cortical area.[9] We examined whether activity-dependent modification of synaptic transmission, long-term potentiation (LTP) or depression (LTD),[10–12] occurs in the horizontal axon pathway of the TEd and V1, and if so, how they differ in their tendency to generate LTP and LTD.

Materials and Methods

Recordings of extracellular field potentials (EFPs) were made in the TEd and V1 of three male monkeys (*Macaca fuscata*; 4–6 years old). These monkeys were prepared for repeated experiments.[6] All procedures were conducted in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* (1996) and approved by the animal experiment committee of Osaka University Medical School. The monkey was premedicated with atropine sulfate, and initial anaesthesia was induced by i.v. application of sodium thiopental. After a tracheal tube was inserted, the monkey was artificially respirated, and immobilized with infusion of a saline-based solution containing glucose and pancuronium bromide through a transcutaneous catheter inserted into the calf vein. A small hole for electrode penetrations was made on the skull under subsequent anaesthesia with isofluorane. During recording, anaesthesia was maintained with a mixture of N_2O and O_2. We monitored an electrocardiogram, body temperature and expired CO_2 level throughout the experiments to judge the condition of the monkey. Electrical stimuli (50–200 μA, 50 μs, 0.25 Hz) were applied through bipolar

concentric electrodes with a diameter of 200 μm. The stimulus intensity was adjusted so that a response that was smaller than half of the maximal response was elicited. When two stimulating electrodes were used, the intensity of each stimulus was further adjusted so that the amplitude of EFPs evoked by simultaneous stimulation from the two electrodes was equal to the sum of those evoked by separate stimulation, ensuring that stimulus current from an electrode did not spread to the side opposite to the electrode side. EFPs were recorded with glass-coated elgiloy alloy electrodes (0.5–1.5 MΩ at 1 kHz). Recording signals were filtered (10Hz–5 kHz band-pass), digitized at 20 kHz, and stored on a computer. An electrolytic lesion was made through the electrode at the end of each experiment for later verification of the recording site in Nissl-stained sections, and we confirmed that monophasic and negative EFPs (see Results) were recorded in layer 2/3. The peak amplitude and the maximum initial slope of the EFPs averaged over 10–20 responses were measured off-line.

As a conditioning stimulus, a train consisting of 20–40 pulses at 40–100 Hz was applied every 4 s for 3–5 min with intensity of 100–400 μA. A long-term change in EFPs was defined as a significant difference of mean values of the averaged responses 30–45 min after the tetanic stimulation from those of pretetanic responses ($p < 0.01$, two-tailed t-test or t-test with Welch's correction when necessary).

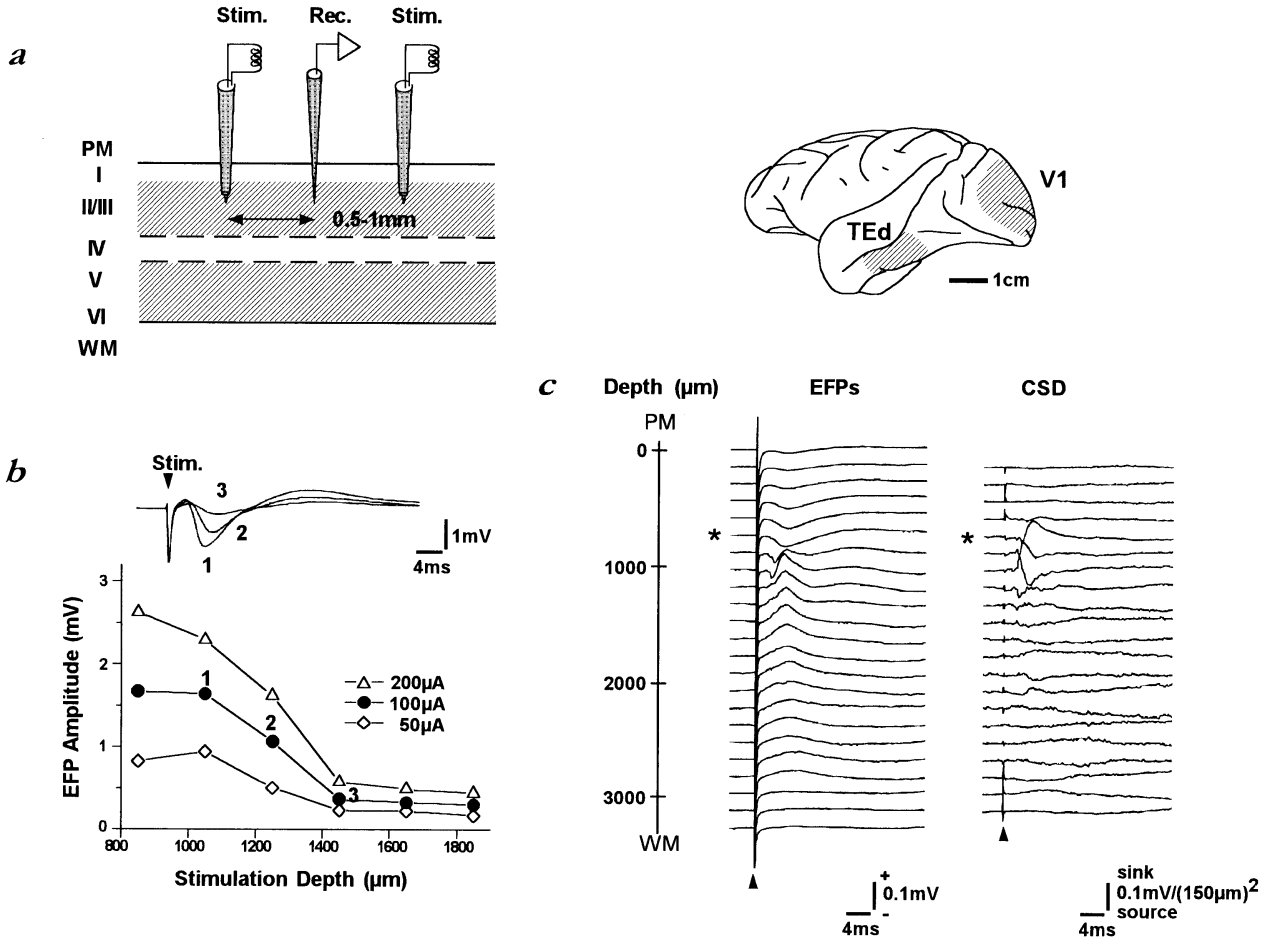

FIG. 1. Characterization of extracellular field potentials (EFPs) evoked by electrical stimulation of the intrinsic horizontal pathway in monkey visual cortices. (**a**) Schematic of experimental procedure. Experiments were performed in either the primary visual cortex (V1) or the inferior temporal cortex (area TEd) (right). A recording electrode was placed in layer 2/3 of the TEd or V1. One or two stimulating electrodes were positioned lateral to the recording site to stimulate intrinsic horizontal axons (left). (**b**), EFPs evoked by stimulation at different depths in the TEd. A recording electrode was fixed in layer 2/3, and a stimulating electrode was moved from superficial to deeper layers. The negative wave almost disappeared when the stimulating electrode was inserted 600 μm further than the depth at which the maximal response was elicited. This was observed over a range of stimulus intensities (50–200 μA) which was used for test stimuli in the following long-term potentiation or depression experiments. This indicates that the evoked EFPs in layer 2/3 are mediated by intracortical connections within layer 2/3. Stimulation depth shown on the abscissa is measured from the initial contact of the stimulating electrode with the cerebrospinal fluid. EFP traces were taken at the indicated depth with a stimulus of 100 μA. Each point represents the average of 6–10 responses in one experiment. (**c**) Depth profiles of EFPs (left) and their current source-density (CSD, right) in the TEd. When the recording electrode was advanced toward the white matter while the stimulating electrode was kept in layer 2/3, EFPs became biphasic in a deeper part of layer 3, and were positive in even deeper layers. A current sink was observed at the depth where a monophasic negative EFP was observed (asterisk). Arrowheads indicate time of electrical stimulation.

Results

We recorded extracellular field potentials (EFPs) in layer 2/3 of the TEd and V1 of anesthetized and immobilized monkeys (see Fig. 1a). Electrical stimuli were applied with one or two electrodes placed 0.5–1 mm lateral to the recording site in each area.

An early part (with ~10 ms latency) of EFPs recorded in layer 2/3 of either the TEd or V1 was monophasic and negative in polarity (Fig. 1b, trace 1). We used this early negative wave as an index of synaptic responses mediated by horizontal connections on the following grounds. First, the negative waves evoked with two stimulating electrodes placed on opposite sides of the recording electrode showed a linear summation by simultaneous stimulation with an appropriate stimulus intensity (<200 μA), indicating that the negative wave was a result of synaptic, but not direct electrical, activation of neurones at the recording site ($n = 9$). Second, the negative wave was maximal after stimulation of layer 2/3, and it was much smaller when deeper layers were stimulated ($n = 6$; Fig. 1b). This indicates that the negative wave is caused by activation of fibres in layer 2/3, rather than fibers originating in or running from deeper layers. Finally, current source–density analyses[13] of EFP depth profiles revealed a massive current sink at the depth where EFPs were monophasic and negative ($n = 3$; Fig. 1c). The above-mentioned properties of the negative wave were observed in both TEd and V1. These results suggest that the negative wave in layer 2/3 largely reflects an ensemble of excitatory synaptic currents mediated by horizontal axons.

Discontinuous brief high-frequency stimulation (see Materials and Methods) of the horizontal pathway in the TEd produced LTP of the negative wave in five of seven experiments (Fig. 2a,b). Both the amplitude and the initial slope of the negative wave increased gradually without a change of the monophasic shape and polarity of the wave, and this potentiation lasted throughout the recording period of the experiments (105–245 min; Fig. 2a). It took 50–70 min for the negative wave to reach the maximum level of potentiation. Normalized amplitudes of the negative wave 10 and 90 min after tetanic stimulation were 97.5 ± 6.1% and 127.6 ± 4.0% (mean ± s.e.m., $n = 5$) of the pretetanic level (Fig. 2b). In the other two experiments, the negative wave remained unchanged. LTD was not observed in any case.

In two experiments the input-specificity of LTP was tested using two stimulating electrodes placed on opposite sides of the recording electrode (Fig. 1a). Stimulus intensity was adjusted appropriately for activation of different groups of horizontal axons converging from the two stimulation sites. Then, a tetanic stimulus was applied to one side. LTP was

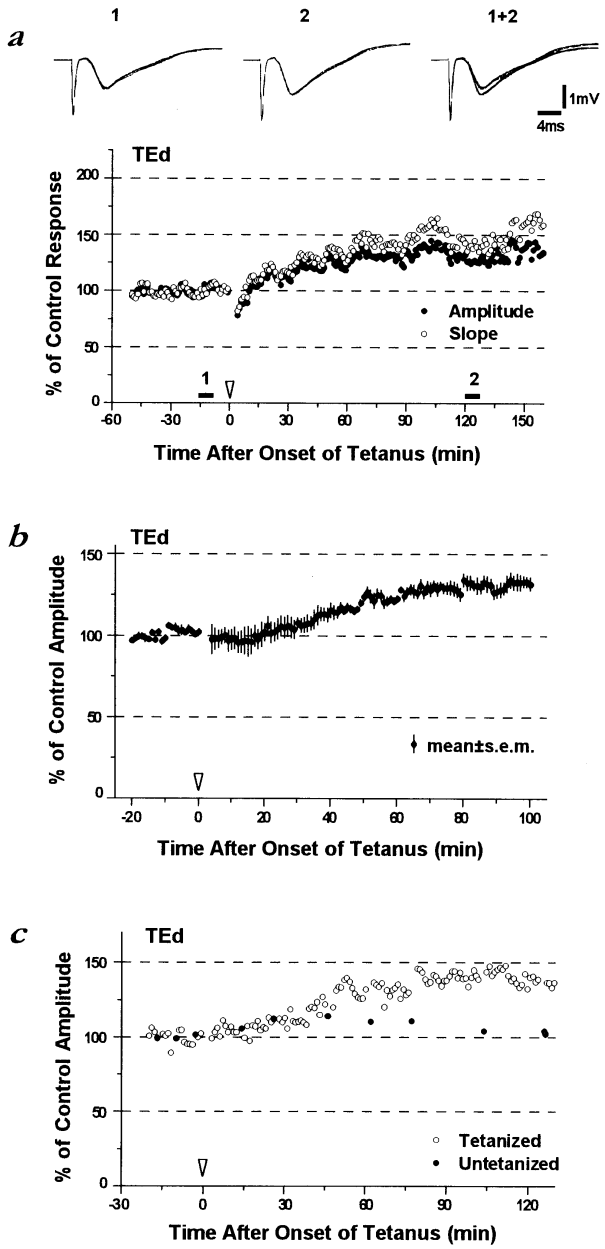

FIG. 2. Long-term potentiation (LTP) of extracellular field potentials (EFPs) elicited by high-frequency stimulation of the horizontal pathway in the TEd. (**a**) Example of time course of changes in the amplitude and initial slope of EFPs. After tetanic stimulation (arrowhead) of the horizontal pathway, the amplitude and initial slope of the negative wave of EFPs evoked from the same pathway increased slowly, and this potentiation lasted for 3 h. The amplitude and initial slope of the negative wave were normalized to those during the period before the tetanic stimulation. Upper traces show field potentials at the indicated time. Current intensities of test and tetanic stimuli were 100 μA and 400 μA, respectively. (**b**) Average of five experiments in which LTP was observed. Each point represents mean ± s.e.m. (**c**) Example of input specificity of LTP in the TEd. Two stimulating electrodes were placed on opposite sides of the recording site to stimulate independent groups of horizontal axons. Before application of tetanic stimulation, the independence of the input sources was confirmed by successful linear summation of the negative waves evoked from the two electrodes. The amplitude of the negative wave evoked from the pathway to which tetanic stimulation was applied (tetanized pathway) increased in a similar manner to that shown in Fig. 2a, but the negative wave mediated by the untetanized pathway did not show a comparable potentiation. In this example, current intensity of test and tetanic stimuli was 100 μA.

observed only in the tetanized pathway, while the negative wave evoked from the untetanized pathway did not undergo LTP (Fig. 2c). This result indicates that LTP in the TEd can be produced in an input-specific manner and rules out the possibility that the observed change might be a result of general changes in responsiveness of postsynaptic cells (e.g. changes in input resistance).

In contrast, identical tetanic stimulation did not potentiate the negative wave in V1, but instead induced LTD (Fig. 3a,b). The amplitude and initial slope of the negative wave decreased during the initial 5–10 min after the tetanic stimulation, although immediately after the tetanus they were the same as the pretetanic level. Once the depression had developed, the negative wave was stable for more than 3 h. We interpreted these observations as showing that this depression was a physiological phenomenon, not an artifact arising from damage to the tissue around the stimulation or recording loci. The time course of the development of the depression was shorter than that of the potentiation in the TEd. The negative wave was already depressed to 60.1 ± 7.0% (mean ± s.e.m., $n = 6$) of the pretetanic level 10 min after the end of tetanus, and was 59.3 ± 7.4% at 90 min. The LTD of the negative wave in V1 was also input-specific. Only the negative wave from the tetanized pathway exhibited LTD, with no changes in the negative wave evoked from the untetanized pathway ($n = 2$; Fig. 3c). The probability of induction of LTD in V1 varied with the intensity of tetanic stimulation. Long-term reduction of the amplitude of the negative wave was more reliably induced when stronger tetanic stimulation was applied (Fig. 3d). In

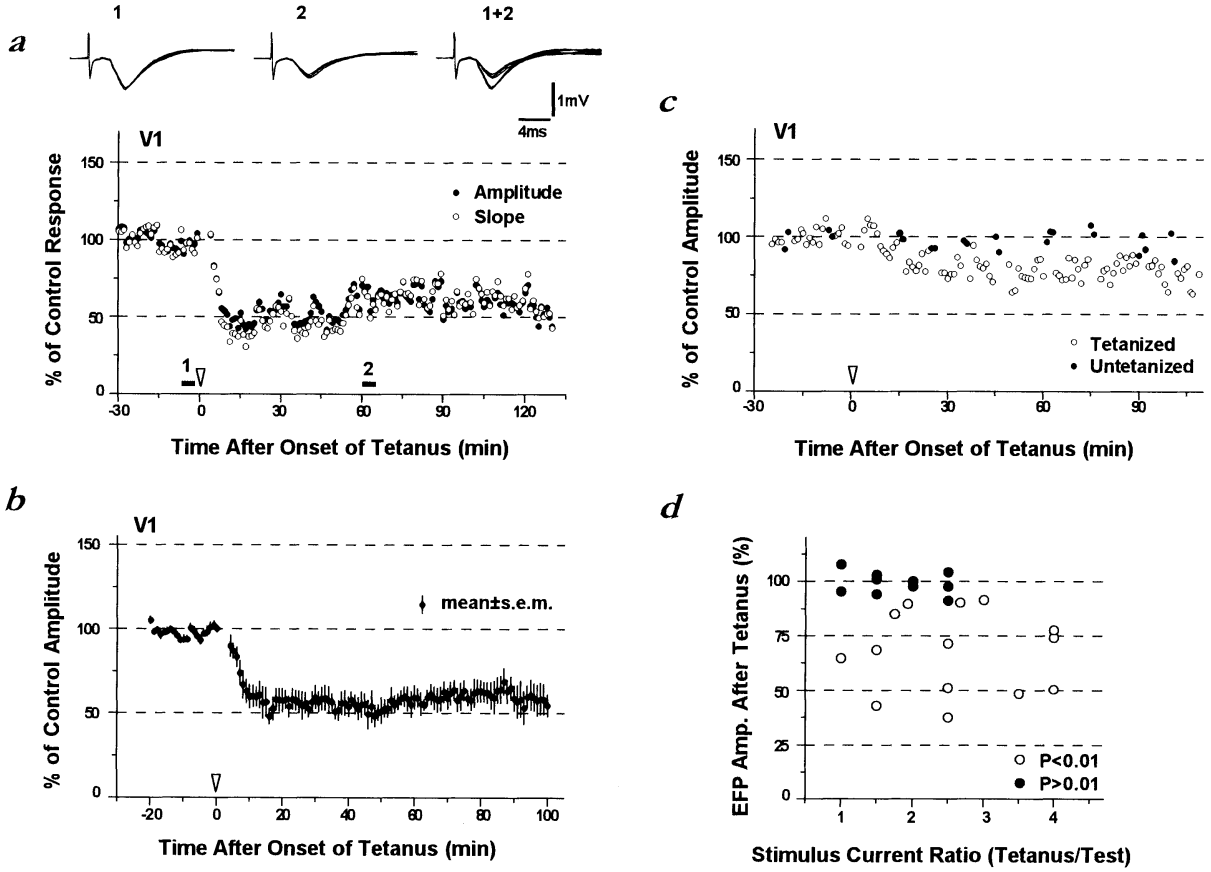

FIG. 3. Long-term depression (LTD) of extracellular field potentials (EFPs) elicited by high-frequency stimulation of the horizontal pathway in V1. (**a**) Example of time course of changes in the amplitude and initial slope of the negative wave. The same tetanizing protocol was used as for the TEd. Both the amplitude and the initial slope were depressed after the tetanic stimulation, and the depression lasted until the end of the recording. Note that responses immediately after the tetanic stimulation remained almost the same as those in the control period. Current intensities of test and tetanic stimulation were 100 μA and 400 μA, respectively. (**b**) Average of six experiments in which the negative wave was depressed to smaller than 85% of the pretetanic level and the recording was made more than 90 min after the tetanic stimulation. (**c**) Example of input specificity of LTD in V1. The negative wave evoked from the tetanized horizontal pathway exhibited LTD, whereas that evoked from the untetanized pathway remained unchanged. Experimental setups were the same as those outlined in legend to Fig. 2b. Current intensities of test and tetanic stimulation were 100 μA and 400 μA, respectively. (**d**) Relationship between the intensity of the tetanizing stimulus and LTD. Ordinate indicates the normalized amplitude of the negative wave measured 30–45 min after tetanic stimulation (% of control), and abscissa indicates the ratio of current intensities of the tetanic and test stimulation. Stronger tetanic stimulation tends to cause LTD more reproducibly than weaker stimulation. Filled circles, the cases in which LTD of EFP amplitude was observed ($p < 0.01$). Open circles, the cases in which no significant change was observed ($p > 0.01$).

none of the experiments, was LTP observed in V1 with the stimulation protocol we used.

Tetanic stimulation and low-frequency stimulation (LFS, 1–3 Hz, 900 pulses), respectively, have been demonstrated to, induce LTP and LTD in the rat and kitten visual cortex *in vitro*.[11,12] However, LFS failed to induce LTD in either the TEd or V1 ($n = 4$ for the TEd, $n = 2$ for V1).

Discussion

We have shown here that synaptic transmission mediated by horizontal axons in the adult primate neocortices can undergo activity-dependent modification. The same tetanizing stimulus protocol produced contrasting effects in the TEd and V1: the negative field potentials evoked from intrinsic horizontal axons in layer 2/3 exhibited LTP in the TEd and LTD in V1. Although the field potentials analysed were considered to reflect mostly excitatory synaptic responses in layer 2/3, they are a sum of postsynaptic responses (i.e. excitatory and inhibitory) from a number of neurones around the recording site. It is uncertain to what extent the changes in the amplitude of the EFPs due to changes in transmission efficacy at excitatory synapses and to what extent changes in inhibitory synapses contribute. The results nevertheless suggest that monkey visual cortical areas are diverse in terms of synaptic plasticity in addition to other characteristics, such as cyto- or chemo-architecture, input–output connections, and functional properties of neurones.

Our contradictory results of tetanus-induced LTD in V1 and failure to induce LTD by LFS might be due to differences in brain areas and synaptic pathways studied, species and ages of animals used, and experimental conditions. Experiments reported previously[11,12] were performed on vertical pathways, i.e. interlaminar pathways within a column. Failure of LFS to produce LTD in the monkey TEd and V1 in the present study is unlikely to be explained solely by difference in species or in experimental conditions (*in vivo vs in vitro*), because LFS is shown to produce LTD in the human temporal lobe *in vitro*[14] and in the rat hippocampus *in vivo*.[15] There is increasing evidence to indicate that susceptibility to synaptic plasticity changes during maturation and aging (see references listed in Ref. 16). Tetanus-induced LTP at inhibitory synapses[17] might also provide a possible account for the observed tetanus-induced LTD of EFPs in V1.

The neuronal mechanism of the difference in synaptic plasticity between the TEd and V1 remains to be clarified. However, differences in the distribution of neurochemicals regulating LTP/LTD induction and expression may partially account for the molecular basis of this difference. For example, the concentration of protein kinase C (γ subtype), which is implicated in expression of LTP,[18,19] and the phosphorylation level of two of its substrates, GAP-43 and an 81 kDa protein, are higher in the TE than in V1.[20,21]

Several lines of evidence suggest that the neocortex, rather than the hippocampus and its associated structures, is the site of storage of long-term recognition memory.[2,3,22–24] Visual long-term memory is considered to be stored in the inferotemporal cortex and functional properties of neurones in this area can be modified even in adulthood[25] (see also Refs 3, 23, 24 for reviews). LTP in the TEd may relate to the modifiable functional properties of neurones involved in long-term visual memory.

Conclusion

In the inferotemporal cortex (area TEd), discontinuous brief high-frequency stimulation of the horizontal pathway in layer 2/3 potentiated EFPs in layer 2/3. In contrast, the same stimulus protocol depressed EFPs in the primary visual cortex (V1). These plastic changes were long-lasting (until the end of experiments, up to 4 h) and induced in an input-specific manner. Thus, cortical areas even belonging to the same hierarchical organization have different susceptibility to synaptic plasticity.

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General Summary
Long-term potentiation (LTP) and long-term depression (LTD) are an activity-dependent, persistent change in efficacy of synaptic transmission. A currently popular hypothesis holds that the natural equivalents of LTP and LTD modify neuronal circuits in the brain and underlie learning and memory process. These phenomena, however, were not previously detected in the neocortex of monkeys, the animal group with the most developed learning abilities. In the present study, we have shown that LTP and LTD can be induced in the monkey neocortex *in vivo,* and that the first and late cortical areas within the same functional pathway (i.e. the object vision pathway) drastically differ from each other in terms of synaptic plasticity. The result provides a new dimension to characterize diverse cortical areas, and opens a way to link systems-level analysis of the primate visual system with studies of LTP and LTD.