Functional architecture and synaptic plasticity in the monkey inferior temporal cortex

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Abstract. Area TE of the inferior temporal cortex in the monkey has a columnar organization. Most neurons in the TE respond selectively to particular visual features of objects, and those with similar stimulus preferences are clustered in vertical arrays roughly 0.5 mm in width. Evidence for the columnar organization has been obtained in anesthetized monkeys, but is now extended by experiments in awake monkeys. Columns interact with each other via intra-areal horizontal axons which connect laterally displaced sites in the TE. High-frequency electrical stimulation of horizontal axons evokes long-term potentiation (LTP) of synaptic transmission efficacy mediated by this axon system. In the primary visual cortex, however, an identical stimulus protocol produced long-term depression in the same fiber system. The success of evoking LTP in the TE in vivo will allow us to investigate in future studies whether and how LTP affects stimulus selectivity of TE neurons and the functional columnar organization of this area. The results also suggest that visual cortical areas are diverse not only in their anatomical structures and receptive field properties, but also in their synaptic plasticity.

Key words: columns, horizontal axon, long-term depression, long-term potentiation, primate, visual memory, visual recognition.

Introduction

Cellular/subcellular-level analysis of synaptic plasticity and systems-level analysis of cognitive functions represent two major research fields in neuroscience today, yet there is scant interaction between them. In studies on experimental models of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), research is mostly directed toward elucidating the subcellular and molecular bases of these phenomena, and rodents are commonly used as subjects (reviewed in [1–4]). The number of studies on the biological significance of LTP and LTD is increasing but still small [4–9]. In contrast, monkeys are a favorite subject for systems-level studies of cognitive functions because of their highly developed perception, recognition and motor control. The nature of synaptic plasticity in the monkey brain has not been explored despite the fact that many aspects of cognitive functions involve learning and memory components.

Several lines of evidence suggest that the neocortex, rather than the hippocampus and its associated structures, is the site for storage of long-term recognition memory in the primate [10–13]. The natural equivalents of LTP and LTD are thought to

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modify neuronal circuits in the brain and underlie certain types of memory (e.g., spatial memory in rodents). If this hypothesis holds for visual recognition memory, LTP or LTD should occur in the monkey visual cortices, particularly in the inferior temporal cortex where long-term memory of visual stimuli is stored and can be reorganized even in adulthood (Fig. 1) [14,15].

A goal of our current project is to bridge the gap between the two research fields discussed above. We started our analysis at an intermediate level of brain organization, i.e., neuronal circuit level, to outline the functional and anatomical architecture of area TE of the monkey inferior temporal cortex. Neurons in this area are arranged into columns according to their selective responsiveness to visual features of objects [16]. In this article, we present new evidence, obtained in awake monkeys, of the columnar organization of the TE [17]. Perpendicular to the columnar array of neurons, intrinsic (i.e., intra-areal) horizontal axons traverse the TE cortex [18]. Horizontal axons can mediate interaction between columns and are suggested to be important for regulating representation in some cortical maps [19,20]. We investigated whether LTP and/or LTD can occur at synapses activated by signals from horizontal axons in the TE.

**Columnar organization in the inferior temporal cortex**

Most neurons in the TE selectively respond to a particular range of stimuli which constitute visual features of objects [21–23]. Preferred object features vary among neurons, but are not as diverse as the number of the neurons. Two adjacent neurons recorded with a single electrode respond maximally or almost maximally to the same or similar stimuli [16]. The most effective stimuli or suboptimal stimuli, however

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*Fig. 1. Five major cortical areas along the visual pathway critically involved in object recognition. V1 the primary visual cortex; V2 and V4: two prestriate cortices; TEO and TE: cytoarchitectonic areas TEO and TE of the inferior temporal cortex. The brain is from a Japanese macaque. Numbers indicate approximate distance (mm) from the external meatus. A: anterior; P: posterior; D: dorsal; V: ventral.*
lightly differ between adjacent neurons. The effective stimulus features vary among cortical penetrations 1 mm apart across the cortical surface. Neurons with similar, but not identical, stimulus selectivities are thus clustered locally within the TE [16,24,25].

Neurons with similar selectivities are arranged in columns in the TE: neurons selective for the same or similar stimuli are distributed vertically across most of the cortical layers, but are localized within a tangential extent of 0.4–0.5 mm (Fig. 2) [16]. Preferred stimuli are shared by neurons within a column, although the optimal stimulus or response tuning properties differ among neurons as is already evident from the results of simultaneous recordings of adjacent neurons. Neighboring columns respond to distinct object features. Several columns with similar stimulus selectivities appear to exist, based on the finding that in tangential penetrations, the same stimuli activated two or three clusters of neurons which were separated by a cluster of neurons unresponsive to the stimuli tested or ones selective for other stimuli. The number of columns in one TE is estimated to be 1,300–2,000 [15,16]. The clustering of TE neurons with similar stimulus selectivities has recently been confirmed by optical imaging techniques [26].

Evidence in awake monkeys of the columnar organization of the TE

The evidence summarized in the preceding section was obtained in anesthetized monkeys. The procedure used to determine the stimulus preference of TE neurons in anesthetized monkeys (referred to as the simplification procedure or reduction process in [15] and [27]) is time-consuming and cannot readily be applied to awake monkeys.

In the present experiments we implanted five guide cannulae perpendicular to the cortical surface of the TE. The cannula tips were placed below the dura mater, but above the brain surface (Fig. 3A). Recording electrodes were aimed repeatedly at the same site through these cannulae. We first searched for effective stimuli for neurons

![Fig. 2. Columnar organization of the TE. Neurons with shared stimulus selectivities are grouped together to form a column 0.4–0.5 mm wide.](image-url)
Fig. 3. Evidence for awake monkeys of the columnar organization of the TE. A: Experimental setup. Fibers guide cannulae (two of them are shown in this Figure) were implanted perpendicular to the cortex surface of the TE. Through these cannulae, neuronal recordings were made under both anesthetized and awake conditions. B: Correlation coefficients for normalized responses were calculated among pairs of neurons in the same (r_{same}) and different (r_{diff}) penetrations. C: Distribution of r_{same} and r_{diff} under awake conditions. Coefficients are significantly larger within the same penetration than between different penetrations, suggesting that neurons with similar response profiles are clustered along vertical penetrations.

along a penetration under anesthetized conditions. For some neurons, critical stimuli features were determined using the simplification procedure. To repeat the same experiments for the five cannulae, penetration-specific effective stimuli were determined. We then devised a stimulus set by combining 40 effective stimuli for neurons along the five penetrations with another 40 stimuli which were arbitrary chosen. Two to 14 days after the experiments under anesthetized condition responses of TE neurons were recorded through the five cannulae under awake conditions. While the monkey fixated a small spot on a color LCD monitor, the predetermined set of 80 stimuli was presented in a pseudorandom order.

We recorded 62 neurons under anesthetized conditions and the same number under awake conditions. For each neuron, responses to the 80 stimuli were averaged over seven to 10 stimulus presentations, and were normalized to its own maxim response. Correlation coefficients were then calculated for normalized response magnitude among pairs of neurons in the same and different penetrations (r_{same} or r_{diff}, respectively; Fig. 3B). The average of r_{same} was 0.30 ± 0.09 whereas r_{diff} w:
averaged to 0.00 ± 0.05 (Fig. 3C). Twenty-seven percent of \( r_{\text{same}} \) were larger than 0.5, whereas only 2% of \( r_{\text{diff}} \) exceeded 0.5. Under anesthetized conditions, the average of \( r_{\text{same}} \) was smaller (0.14 ± 0.07) than that under awake conditions, but was significantly larger than the average of \( r_{\text{diff}} \) (0.00 ± 0.03) (p < 0.0001, t test). These results show a stronger correlation among neurons within a vertical penetration than among neurons at different penetrations under both awake and anesthetized conditions, indicating that neurons in the TE are organized into columns according to their stimulus selectivity.

**Horizontal axon system**

As in other cortices, vertically running axons across cortical layers and horizontal axons running roughly parallel to the cortical surface are the two major components of intrinsic neuronal connections in the TE [18]. Horizontal axons are present in all layers, and are most numerous in layers 2 and 3. Some of them extend over several millimeters within the TE. These long-range horizontal axons give rise to a few to more than 15 patches of terminal arborization. These patches are columnar in shape in the coronal plane, spanning from layer 1 to layer 3 or sometimes to layer 5, and are 0.5 mm wide on average. Neurons at a given site in the TE are thus preferentially connected to a particular set of neurons which tend to be distributed in columnar clusters, and are not diffusely and randomly connected with other TE neurons.

Most horizontally projecting neurons are pyramidal neurons [28]. The contribution of GABAergic neurons to the horizontal axon system is small and limited to short-distance projections (<1 mm). Horizontal axons are therefore predominantly excitatory in their direct synaptic interactions.

The size of patches of horizontal axon terminal arborization roughly corresponds to the width of columns measured in unit recording experiments [16] and by optical imaging [26]. The spacing of nearby patches is also consistent with the spacing of multiple clusters of neurons with similar stimulus preferences [16]. Relations between patches of horizontal axon terminal arborization and functional columns, however, have not been analyzed in the TE.

**Long-term potentiation and depression in the primate neocortex**

The evidence that modification in horizontal axons changes a coded representation in the primary visual and motor cortices [19,20] prompted us to investigate whether synaptic transmission mediated by horizontal axons in the TE is modifiable [29].

Extracellular field potentials were recorded in layer 2/3 of the TE, and stimulating electrodes were placed 0.5–1 mm lateral to the recording site to activate horizontal axons. A single electrical stimulus (50 μs duration, 100 μA amplitude, negative monopolar) evoked monophasic negative field potentials with a peak latency of around 5 ms in layer 2/3. The results of analyses of depth profiles of field potentials and current source-density, effects of stimulus position, together with the results of anatomical analyses of horizontal axons discussed above, suggest that this potential largely reflects ensemble excitatory synaptic currents in layer 2/3 (Y. Murayama, I.
Fujita, M. Kato, unpublished observation).

After a high-frequency conditioning stimulus (20–40 pulses at 40–100 Hz, applied every 4 s for 3–5 min, 100–400 µA), the amplitude (Fig. 4) and initial negative slope of field potentials evoked by a single stimulus gradually increased over 50–70 min to a maximum, and this potentiation lasted for more than 3 h [29]. The slow time course of LTP in the TE contrasts with that found in rodent hippocampus where maximal level of potentiation is induced immediately after a conditioning stimulus.

When a new memory is stored into a cortical area, patterned neural inputs are thought to cause changes in synaptic strength at distributed synapses. It has been proposed that the neuronal network accumulates small changes caused by input activity to individual synapses, and that it gradually adjusts the distribution of synaptic strength to find a state where both new and already existing memories can coexist [30,31]. The slow time course of LTP in the TE may reflect the properties required for the gradual adjustment.

When the same experiments were performed in the primary visual cortex (V1), an identical stimulus protocol did not potentiate field potentials in the V1, but instead caused a depression. This depression developed over 5 to 10 min and remained stable until the end of recording. Steady-state depression with a smooth initial development indicates that the depression is a physiological phenomenon and not an experimental artifact due to, for example, damage to the stimulating site. In both areas, field potentials evoked from an unconditioned pathway were not changed in their amplitude and waveform. Thus, both LTP in the TE and LTD in V1 were homosynaptically induced and maintained [29].

**Fig. 4.** LTP and LTD in the horizontal axon pathways of the TE and V1. Changes in the amplitude of extracellular field potentials evoked by electrical stimulation of horizontal axons are plotted against time. Arrowhead indicates time of application of a conditioning tetanic stimulus. After the tetanic stimulation field potentials are potentiated in the TE, whereas those in V1 are depressed.
The present demonstration of LTP and LTD in the TE and V1 represents the first in any part of the monkey brain. It is surprising that, despite their widely proposed role in memory function, LTP and LTD were not previously experimentally shown in monkeys, the animal group with the most developed learning abilities.

Stimulus selectivity of neurons in the columnar organization of the TE can be changed by learning of novel visual stimuli [15,32]. Intrinsic horizontal axons are crucial for maintaining a cortical map in the primary visual cortex [19,33] (but see 34 for another interpretation). The ability of the TE and V1 to undergo changes in functional connections in the horizontal axon system may provide a clue to the mechanism for these changes induced by learning or altered sensory inputs.

The same conditioning stimulus protocol produced contrasting effects in the TE and V1. This suggests that monkey visual cortical areas are diverse in the nature of their synaptic plasticity in addition to other characteristics such as cyto- or chemoarchitecture, input and output connections, and functional properties of neurons. The cellular and molecular basis of the difference between the two areas remains to be clarified, although differences in the distributions of some neurochemicals related to synaptic plasticity may partially account for this: the concentrations of protein kinase C (γ subtype) which is implicated in expression of LTP [35,36], and the phosphorylation level of its substrates (protein F1, also known as GAP-43, and a 81 kDa protein) are higher in the TE than in the V1 [37,38].

Conclusions

The confirmation of the columnar organization of the TE of awake monkeys now takes us a step further to accepting that this organization plays a role in object recognition. The success of evoking LTP and LTD in vivo provides us with a unique opportunity to investigate whether and if so how LTP and LTD alter functional properties of cortical neurons through examination of visual responses of neurons before and after expression of LTP or LTD, and opens a way to link systems-level analysis of the primate visual system with studies on LTP and LTD. The primate visual system represents a case where the organization of the system, functional and anatomical architecture of each cortical area, characterization of neuronal properties, and behavioral capacity have been extensively studied. Bringing the paradigm of LTP and LTD into studies on the primate visual system is expected to yield new developments in both research fields.

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References

27. Fujita I. Columns in the inferotemporal cortex: machinery for visual representation of objec


4. McClelland JL, McNaughton BL, O'Reilly RC. Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. Psychol Rev 102:419–457.


