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Prefrontal cortical cells projecting to the supplementary eye field and presupplementary motor area in the monkey

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Abstract

We examined the location and spatial distribution of prefrontal cortical (PF) cells projecting to the supplementary eye field (SEF) and presupplementary motor area (pre-SMA) using a double retrograde-labeling technique in monkeys (*Macaca fuscata*). The SEF and pre-SMA were physiologically identified based on the findings of intracortical microstimulation and single cell recordings. Two fluorescent tracers, diamidino yellow and fast blue, were injected into the SEF and pre-SMA of each monkey. Retrogradely labeled cells in the PF were plotted with an automated plotting system. The cells projecting to the SEF and pre-SMA were mainly distributed in the upper and lower banks of the principal sulcus (area 46), with little overlap. Cells projecting to the SEF, but not to the pre-SMA, were observed in areas 8a, 8b, 9, 12, and 45. These findings suggest that the SEF and pre-SMA receive different sets of information from the PF cells.

Keywords: Prefrontal cortex; Supplementary eye field; Presupplementary motor area; Primate; Retrograde labeling

1. Introduction

The supplementary eye field (SEF) and presupplementary motor area (pre-SMA) receive projections from the prefrontal cortex (PF) (Huerta and Kaas, 1990; Luppino et al., 1993, 2003; Morecraft and Van Hoesen, 1993; Takada et al., 2004). However, whether the banks of the principal sulcus (PS) are the direct input source to the SEF (Huerta and Kaas, 1990; Luppino et al., 2003) and pre-SMA (Luppino et al., 1993; Morecraft and Van Hoesen, 1993; Takada et al., 2004) remains controversial. Huerta and Kaas (1990) reported that cells in area 46, including the dorsal and ventral banks of the PS, richly projected to the SEF. In contrast, Luppino et al. (2003) reported few projections to the SEF from area 46, especially from the ventral portion; they found labeled cells projecting to the SEF only in a small area of dorsal convexity and in the caudal-most portion of the PS. As for the projection from the PF to the pre-SMA, Luppino et al. (1993) and Morecraft and Van Hoesen (1993) showed direct projections. Recently, however, Takada et al. (2004) reported that such connections were scarce. To resolve these discrepancies, we re-investigated the connectivity between the PF and the SEF and between the PF and the pre-SMA using qualitative and quantitative measurements of projection cells in the PF. Furthermore, if direct projections from the PF to the SEF and pre-SMA exist, it will be interesting to know whether the PF populations projecting to the SEF and pre-SMA overlap. Recent studies in human subjects and in monkeys indicate that the pre-SMA as well as the SEF is involved in higher-order aspects of oculomotor performance (Kawashima et al., 1998; Olson and Gettnner, 1999; Stuphorn et al., 2000; Merriam et al., 2001; Fujii et al., 2002; Isoda and Tanji, 2002, 2003, 2004; Lu et al., 2002; Schall et al., 2002).

In this study, we first identified the precise locations of the SEF and pre-SMA electrophysiologically. We then applied a

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double retrograde-labeling technique. Two different tracers were injected into the SEF and pre-SMA of each monkey, and we quantitatively analyzed the labeled cells in the PF. A preliminary account of this study has appeared elsewhere (Wang et al., 2004a).

2. Materials and methods

Four Japanese monkeys (*Macaca fuscata*) weighing 5.0–8.0 kg each were used in this study. The animals were cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Guidelines for Institutional Animal Care and Use published by our institute. After the monkeys became fully accustomed to sitting quietly in a primate chair during an electrophysiological examination, they were anesthetized with ketamine hydrochloride (5 mg/kg, i.m.) and sodium pentobarbital (30 mg/kg, i.m.) for surgery. Under aseptic conditions, the skull was sufficiently removed to expose the SEF and pre-SMA. The dura mater was left intact. An acrylic recording chamber and head fixation bolts were implanted in the skull. After the surgery, each monkey was given antibiotics and analgesics, and was returned to its cage.

After a 1-week recovery period, electrophysiological examinations began. Stimulus-induced and spontaneous eye movements were monitored with an infrared reflecting monitor system with a resolution of 4 ms (Fujii et al., 2002; Isoda and Tanji, 2002). For intracortical microstimulation (ICMS) and for recording extracellular unit activity, we used glass-insulated Elgiloy microelectrodes with a measured impedance of 0.8–1.0 M. For stimulation, trains of 12–22 cathodal 200 μs pulses were applied at 333 Hz through a constant-current stimulator. Current intensities routinely ranged from 20 to 40 μA for mapping of the pre-SMA and SMA. For the SEF mapping, 22–42 pulses and currents of up to 50 μA were used (Isoda and Tanji, 2002, 2003).

Physiological examinations were performed when the monkeys were fully awake. ICMS was begun when the microelectrode reached a depth of 1.0 mm from the pial surface and was performed thereafter at every 500–1000 μm of electrode advancement. In each position, we mapped the topographical organization based on ICMS effects and on cell receptive fields of somatosensory responses. We also examined visual and somatosensory responses at each position (Matsuzaka et al., 1992; Matsuzaka and Tanji, 1996; Isoda and Tanji, 2002, 2003). The localization of the pre-SMA was based on physiological criteria established previously (Matsuzaka et al., 1992; Matsuzaka and Tanji, 1996). In short, we first mapped the SMA by observing limb movements evoked by ICMS, and by observing somatosensory responses of neurons. Then the pre-SMA was identified just rostral to the face representation of the SMA. In the pre-SMA, complex forelimb-movements by ICMS (20–40 μA; a train of 12–22 pulses,) and visual responses were observed as reported in previous reports (Matsuzaka et al., 1992; Matsuzaka and Tanji, 1996). The SEF was defined as a cortical area in the dorsomedial convexity immediately antero-lateral to the pre-SMA, in which saccadic eye movements were elicited at a low threshold of ICMS (less than 50 μA; a train of 22–42 pulses) (Isoda and Tanji, 2002, 2003). An area that was determined to be the pre-SMA did not overlap with the SEF (cf. Fig. 2). After identifying the SEF and pre-SMA, electrolytic microlesions (anodal currents of 350 μC) were placed to use as reference points for later reconstruction of the electrode tracks. The cortical depths at which ICMS effects were determined were calculated based on the depth readings of the stepping microdrive.

Two different tracers were used, 5% diamidino yellow (DY, Sigma; dissolved in saline), and 5% fast blue (FB, Sigma; dissolved in saline). On the basis of the map constructed by the electrophysiological studies, we selected the sites for tracer injections in the SEF and pre-SMA. Either DY or FB was injected into the SEF and pre-SMA (Table 1). The tracers were pressure-injected with a 1.0-μl Hamilton syringe attached to the same manipulator as was used for microelectrode insertion, thus using the same stereotaxic coordinates used for physiological studies. For the SEF, injections were placed at the depth of 1–1.5 and 2–2.5 mm, and at 1.0–1.5, 2.0–2.5, and 3.0–3.5 mm for the pre-SMA. The amount of tracers at each depth of the penetrating tracks was 0.15–0.3 μl for DY and FB. The survival period of 2.5–3 weeks after each injection was found to be adequate for detecting corticocortical projection cells in *M. fuscata*. Fig. 1A (SEF) and B (pre-SMA) shows photomicrographs of injection sites of fluorescent dyes taken from coronal sections demonstrating maximal spread of dyes. As shown in this figure, both tracers were localized within 1.5 mm in diameter across the longitudinal axis of injection. This indicates that the tracers were injected within the expanse of the SEF and pre-SMA (Isoda and Tanji, 2002, 2003; Matsuzaka et al., 1992). Examples of photomicrographs of DY- and FB-labeling of projection cells are shown in Fig. 1C and D, respectively.

Under deep anesthesia with ketamine hydrochloride (10 mg/kg, i.m.) and sodium pentobarbital (50 mg/kg, i.m.), each monkey was perfused transcardially with 0.1 M phosphate buffer (pH 7.4) containing heparin. The monkey was then perfused with the following solutions: 3% paraformaldehyde in 0.1 M phosphate buffer, 3% paraformaldehyde in 0.1 M phosphate buffer with 10% glycerin, and 3% paraformaldehyde in 0.1 M phosphate buffer with 20% glycerin. The brains were removed, blocked, and stored at 4 °C in a solution containing 20% glycerin, and 3% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for a few days before sectioning. Serial sections were made at 50 μm in the frontal plane using a freezing microtome. Every alternate section was left unstained for examination with a fluorescence microscope and was later Nissl-stained with cresyl violet for cytoarchitectonic examination.

Plotting was performed in sections using a computerized digital plotting system (MD2 Microscope Digitizer, Min-