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Functional columns in the primate prefrontal cortex revealed by optical imaging *in vitro*

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Abstract

Although the functional column has been implicated in the dorsolateral prefrontal cortex (DLPFC) of primates, its dynamics and even existence are still uncertain. We performed optical recording with a voltage-sensitive dye (RH482) in brain slices obtained from the principal sulcal region (area 46) of macaque monkeys. Columnar activity was evoked by electrical stimulation of the middle layer (lower layer III or layer IV); this activity consisted of two components: probably action potentials and excitatory postsynaptic potentials. The width of the columnar activity was saturated when the current intensity of stimulation exceeded a certain level, that is, approximately 900 μm at the peak with this intensity. The stimulation of different sites within the same slice activated different columnar activities with only slight overlaps. Furthermore, similar columnar activity appeared when a different site within the area with columnar activity was stimulated. These findings suggest that the primate DLPFC consists of functional columns formed by excitatory synaptic connections.

Keywords: Functional column; Optical recording; Prefrontal cortex; Primate; Voltage-sensitive dye

1. Introduction

In primates, the dorsolateral prefrontal cortex (DLPFC) plays important roles in various cognitive functions such as thinking, planning, and decision making (Fuster, 1997). Moreover, neuroanatomical studies have demonstrated that the DLPFC has a columnar organization (Goldman-Rakic and Schwartz, 1982; Arikuni et al., 1988; Watanabe-Sawaguchi et al., 1991), and the column in the DLPFC has been considered a functional unit, representing various cognitive functions mediated by the DLPFC (Goldman-Rakic, 1995; Mountcastle, 1997). Consistent with this premise, studies applying the 2-deoxyglucose (2DG) method have demonstrated that local cerebral glucose use shows patch- or column-like bands in the principal sulcal region during working memory tasks (Matsunami and Kubota, 1983; Friedman and Goldman-Rakic, 1994). Indirect evidence for functional columns/modules in the DLPFC has also been obtained by a single-neuron study (Constantinidis et al., 2001) and pharmacological inactivation studies (Sawaguchi and Goldman-Rakic, 1994; Sawaguchi and Iba, 2001). However, the existence and dynamics of functional columns in the DLPFC is still uncertain.

A useful methodology for examining functional columns in the cerebral cortex is optical recording (Grinvald, 1985; Momose-Sato et al., 1999; Petersen and Sakmann, 2001). In particular, optical recording with voltage-sensitive dyes in brain slices is suitable for revealing the existence and dynamics of functional columns (Petersen and Sakmann, 2001). Therefore, we performed optical recording with a voltage-sensitive dye in brain slices from the principal sulcal region (area 46) of macaque monkey DLPFC. We obtained evidence that the primate DLPFC consists of functional columns, formed by excitatory synaptic connections.

2. Materials and methods

2.1. Slice preparation and experimental procedures

Animals were treated in accordance with the guide for care and use of laboratory animals (National Institutes of Health, Bethesda, MD, USA) and the guide for our institute (Hokkaido University, Japan).

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Six adult Japanese monkeys (*Macaca fuscata*, both sexes) were used. Each monkey was treated with ketamine hydrochloride (10 mg/kg, i.m.) and then deeply anesthetized with sodium pentobarbital (approximately 25 mg/kg, i.v.). A craniotomy was performed over the prefrontal cortex. Tissue blocks were then obtained from the principal sulcal region (area 46; Walker, 1940) of the DLPFC under aseptic conditions (Fig. 1A). As soon as possible, the brain tissue was cut coronally into 400-μm thick slices using a microslicer (DSK-3000W; Dosaka, Kyoto, Japan). Each coronal slice was incubated in artificial cerebrospinal fluid (ACSF, containing 125 mM NaCl, 26 mM $NaHCO_3$, 5 mM KCl, 1.2 mM KH_2PO_4, 1 mM $MgSO_4$, 2 mM $CaCl_2$, and 10 mM glucose, with a mix of 95% O_2/5% CO_2, pH 7.4) for at least 2 h at room temperature (approximately 25 °C) and then stained with the voltage-sensitive dye RH482 (NK3630, 0.1 mg/ml in ACSF) for 20 min. To wash out unbound dye, stained slices were perfused with ACSF for >30 min before starting the optical recording. The slice was mounted on a microscope (BX51WI; Olympus, Tokyo, Japan), and cortical layers (usually lower layer III or layer IV) of the principal sulcus area were stimulated electrically (single pulse, 0.3 ms in duration) using a bipolar tungsten electrode (100 μm outer diameter, <50 μm sharp tip). We identified each layer visually through the optics and were able to discriminate layer IV. The intensity of stimulation was usually 300 μA because this was sufficient to induce significant changes in the optical signal (see Section 3). The perfused ACSF was warmed between 31 and 33 °C for recording sessions.

A 150-W tungsten-halogen lamp was lit using a stabilized DC power supply. Heat absorption and band-pass (700 ± 30 nm) filters and a mechanical shutter were inserted in the light path between the source and a condenser lens. The neural activities elicited by electrical stimulation were converted to absorbance changes associated with membrane potential changes and were detected with metal-oxide semiconductor (MOS) image sensors (9 mm × 9 mm, 128 × 128 pixels) at a sampling rate of 1700 Hz (0.6 ms/frame) using an optical recording system (SD 1001; Fuji Film Microdevices, Tokyo, Japan). Images were obtained through a 4× objective lens (XLFLUOR 4×/340, NA = 0.28) and 0.6× relay lens. To reduce noise, eight consecutive stimuli were averaged, with each stimulus separated by an interval of 5 s. Each image (I) was subtracted from an initial control image (I_0) in each pixel. This difference image ($\Delta I = I - I_0$) was amplified 800 times by the optical recording system. The optical signal was converted into an 8-bit numerical value (i.e., 0–255) for quantitative analysis.

In some recording sessions ($n = 5$), the excitatory amino acid antagonist, kynurenic acid (KYN, 10 mM) or the sodium channel blocker tetrodotoxin

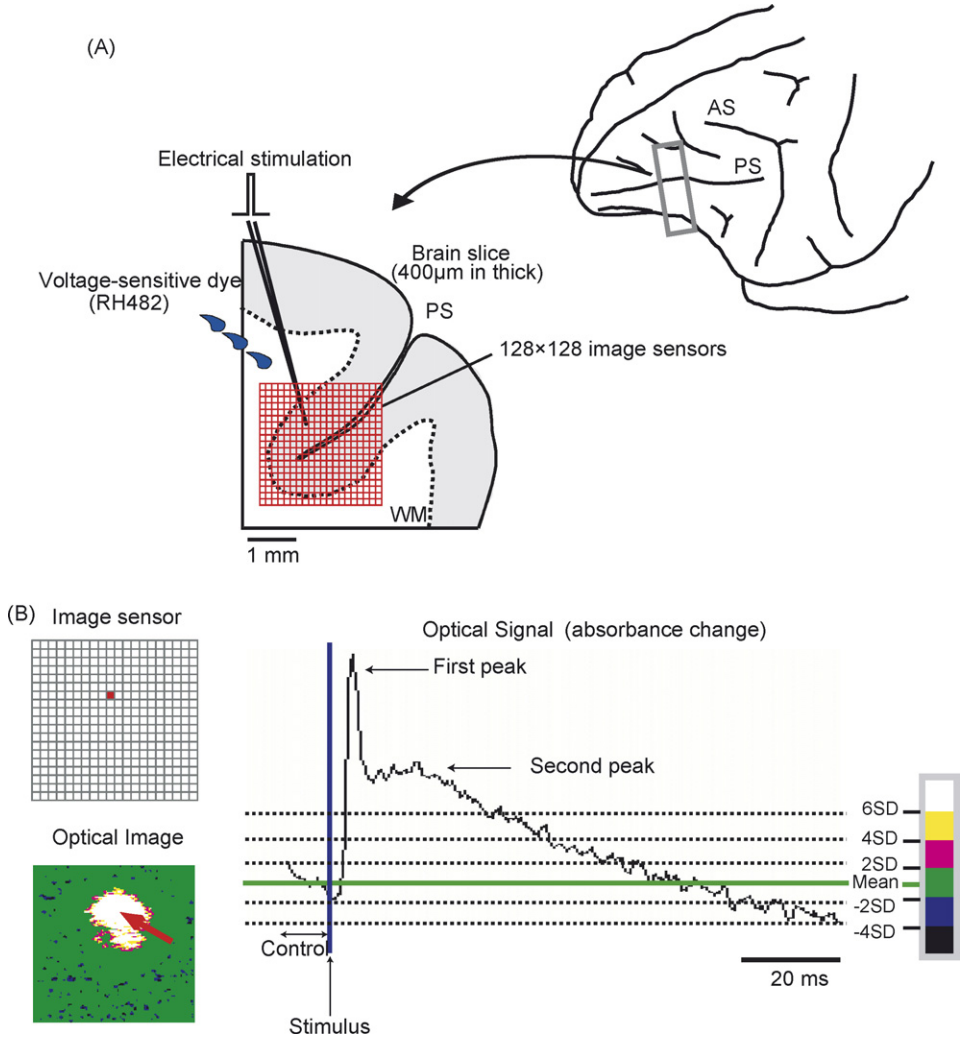

Fig. 1. Experimental procedures—(A) coronal slices (400-μm thick) were obtained from the principal sulcal region (area 46) of the dorsolateral prefrontal cortex (DLPFC) of macaque monkeys. Each slice was stained with a voltage-sensitive dye (RH482). Cortical layers (usually, layer III or IV) were electrically stimulated. The optical signal was detected through an optical filter by an optical recording system with a 128 × 128 pixels MOS image sensor covering an area of 3.75 mm × 3.75 mm. (B) The method used to detect significant changes in the optical signal (absorbance change). An optical signal was detected by the image sensor. The mean and S.D. of the optical signal (8-bit value) for each area/pixel were calculated at a sampling rate of 0.6 ms for about 20 ms before the onset of stimulation as a control. When the optical signal at a given area at a given time (e.g., red closed square in the upper-left figure) after the onset of stimulation exceeded ±2 S.D. of the control level, the optical signal was considered to have changed significantly. Different levels of change in the optical signal are indicated by different colors. PS, principal sulcus and WM, white matter.