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ENHANCEMENT OF OBLIQUE EFFECT IN THE CAT'S PRIMARY VISUAL CORTEX VIA ORIENTATION PREFERENCE SHIFTING INDUCED BY EXCITATORY FEEDBACK FROM HIGHER-ORDER CORTICAL AREA 21A

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Abstract—It is often suggested that the oblique effect, the well-known phenomenon whereby both humans and animals are visually more sensitive to vertical and horizontal contours than to oblique ones, is due to the overrepresentation of cardinal orientations in the visual cortex. The functional role of feedback projections from higher-order cortical areas to lower-order areas is not fully understood. Combining the two issues in a study using optical imaging here, we report that the neural oblique effect was significantly enhanced (3.7 times higher than the normal) in the cat's primary visual cortex through orientation shifting induced by excitatory feedback from the higher-order cortical area 21a. This suggests that a reciprocal co-excitatory mechanism may underlie the perceptual oblique effect.

Key words: oblique effect, visual cortex, area 21a, area 17, feedback connection, orientation.

The phenomenon that the function of visual system of human and some mammalian animals is more sensitive to vertical and horizontal stimuli than to those obliquely oriented is well known as the "oblique effect," which was confirmed psychologically, behaviorally and physiologically (Campbell et al., 1966; Maffei and Campbell, 1970; Appelle, 1972; Annis and Frost, 1973; Howard, 1982; Shou et al., 1985; Blake and Holopigian, 1985; Heeley et al., 1997; Orban et al., 1984; Mathews and Welch, 1997; Essock, 1980; Weitheimer and Beard, 1998; Li et al., 2003) Recent studies using optical imaging and functional magnetic resonance image (fMRI) have revealed that more neurons responded preferentially to cardinal (vertical and horizontal) contours than to oblique ones in areas 17 and 18 of cats, area 17 of ferrets, primates and even in human primary visual cortex (Yu and Shou, 2000; Wang et al., 2003; Huang et al., 2006; Coppola et al., 1998; Mansfield, 1974; Furminski and Engel, 2000). The anisotropy in the functional distribution of cortical neurons is thought to provide the neural basis of the psychological or behavioral oblique effect. However, the anisotropy shown in these lower visual cortices is rather small (about 5%–7% difference). Recently, a much larger anisotropic difference (about 23% in difference) was found in higher visual area 21a in cats, using optical imaging (Huang et al., 2006).

Area 21a receives its principal inputs from area 17 and sends extensive excitatory feedback projections to area 17 in the cat (Dreher, 1986; Dreher et al., 1996). This area is often considered to be a counterpart to primate V4 or V3v in the ventral (or temporal) visual stream (Burke et al., 1998). The functional role of such feedback projections from higher-order to lower-order cortical areas is at best poorly understood. The main aim of this study was to investigate the relationship of oblique effects between areas 21a and 17. Using optical imaging combined with pharmacological methods we investigated this issue by recording an optical measure of the oblique effect in area 17 of the cat when the excitation of area 21a was reversely altered.

EXPERIMENTAL PROCEDURES

Animal preparation

Nine normal adult cats of either sex, weighing between 2.5 and 3.0 kg, were used in the current study. All experiments involving animals conformed to the policy of the Society for Neuroscience on the Use of Animals in Neuroscience Research. All procedures were performed in strict accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all experiments were designed to minimize the number of animals used and their suffering. The detailed methods used here for surgical preparation and intrinsic optical recording have been described elsewhere (Chen et al., 2003; Huang et al., 2004; Shen et al., 2006) Animals were initially anesthetized with ketamine (25 mg/kg). All pressure points and surgical incisions were infiltrated with lidocaine. During the experiment, anesthesia was maintained with i.v. pentobarbital sodium (loading dose of 4 mg/kg, followed by maintenance with 3 mg/kg/h). After i.v. and tracheal cannulation, the cat was placed in a stereotaxic apparatus (Jiangwan II type, The Second Military Medical University, Shanghai, China). Gallamine triethiodide (Flaxedil; Shanghai Dongfeng Chemicals Factory, Shanghai, China; 10 mg/kg/h) was then used for immobilization and animals were artificially respired using a pulmonary pump. The animals' physiological conditions were kept within normal ranges throughout the experiment. Thus, the end-

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Abbreviations: EEG, electroencephalogram; NMDA, *N*-methyl-D-aspartate; ROI, region of interest.

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tidal CO_2 was kept at a range from 3.5% to 4% by adjusting the rate and/or stroke volume of the pulmonary pump, body temperature was maintained near 38 °C by an automatic temperature control system and electroencephalogram (EEG) and electrocardiogram (ECG) were continuously monitored. EEG records showed a slow wave pattern and the heart rate was maintained between 200 and 260 pulses/min throughout the procedures.

The pupils of the cat were dilated with atropine (0.5%) and nictitating membranes were retracted with neosynephrine (2%). The eyes were carefully refracted and corrected with contact lenses of appropriate refractive power. To reduce the amount of spherical aberration, artificial pupils (3 mm in diameter) were placed in the front of each eye.

Area 21a was localized by its relationship to the suprasylvian and lateral sulci (Tusa and Palmer, 1980; Tusa et al., 1981). Visual cortical area 21a and area 17 were exposed at Horsley-Clarke coordinates P1-7, L7-12 and P0-10, L0-7 respectively (Huang et al., 2004). Special care was taken to ensure that the two exposed areas had similar and overlapped retinotopic fields using the method to measure cortical retinotopic topography described elsewhere (Chen and Shou, 2003). Generally the field-of-view for areas 17 and 21a was within 10° of the fovea. Then, a stainless steel chamber of 16 mm in diameter with a glass cover, whose large field is useful for identifying the precise location of area 17, was cemented onto the skull surrounding the exposed area. After careful removal of the dura, the chamber was filled with warm silicone oil and sealed with a transparent glass window. Usually optical signals were acquired from area 17 and averaged in order to get a functional map of higher quality in the same experiment. Then the drug was administrated into area 21a and optical signals were reacquired from area 17. In some case the administration of the drug could be repeated a number of times.

Area 21a was reversibly activated and inactivated by microinjections of 1.0–1.5 μl 0.2 mM glutamate (Sigma, St. Louis, MO, USA) and 1.0 μl 0.2 mM *N*-methyl-D-aspartate (NMDA, Sigma), and 1.0–1.5 μl 100–400 mM GABA (Sigma) though a microsyringe which was fixed in the stereotaxic apparatus, respectively. As a control we also injected 1.0 μl of phosphate-buffered saline (PBS, pH 7.4) at the same site. Solutions were injected slowly (over a period of 4 min) and the needle of the micro-syringe was withdrawn 10 min after the termination of injection in order to prevent leakage (Huang et al., 2004; Shen et al., 2006). The injection sites were centered in area 21a at a depth of 0.5~1.0 mm beneath the pial surface. Previous studies have indicated that 1.0 μl of 100 mM GABA tends to diffuse over a region of 1.5 mm in diameter in the mammalian cortex (Hupe et al., 1999). To reduce the extent of the mechanical damage to the injected part of area 21a, drug injections were limited to no more than three times per site. Usually the interval between two trials was more than 3 h to guarantee a sufficient recovery. After 3 days of experiments, the animal was killed for histological study. The animal was deeply anesthetized with pentobarbital sodium (25 mg i.v.) and transcardially perfused with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer. The location of the centers of the injection sites was assessed histologically in 50 μm sections counterstained for Nissl substance with Cresyl Violet. Only data from animals with the correct injection locations within area 21a were included for further analysis.

Visual stimulation

The cats were stimulated binocularly with a whole field (30°×40°) of drifting sinusoidal wave gratings (contrast 90%, temporal frequency of 2 Hz) with a spatial frequency of 0.5 cycles/degree. These stimulus parameters elicit responses from most neurons in both areas 21a and 17 as described previously (Chen et al., 2003; Huang et al., 2004). Stimuli were randomly presented at four orientations (0°, 45°, 90°, and 135° with respect to horizontal), each moving at two opposite directions of motion (for total eight directions). The two moving directions of the gratings were always orthogonal to the orientation. The visual gratings stimuli were repeatedly presented on the screen of a high-resolution monitor (FlexScan F931, Eizo Nanao Corporation, Japan) positioned 57 cm from the cat's eyes for 2 s with 10-second blank intervals in between. The mean luminance of the blank screen was 15.1 cd/m^2.

Optical data acquisition

As in our recent studies (Chen et al., 2003; Huang et al., 2004) a slow-scan CCD camera (512×512 pixels, 24×24 μm/pixel; Dalsa, Waterloo, Ontario, Canada) was used to record the optical images of intrinsic signals from the exposed portion of area 17. A macroscope tandem-lens arrangement of two coupled 50 mm lenses (f=1:1.2) was used to achieve a very shallow depth of field (less than 100 μm) in order to minimize blood vessel artifacts and influence of surface layers in the functional maps. However, a vessel map on the cortical surface was obtained with green light (540 nm) shining on the surface of the cortex. Intrinsic signals evoked by the grating stimuli were detected under illumination with red light (640 nm) when the camera was focused on the plane of 500 μm below the pial surface, where was located within layers 2 and 3 of the cortex. Data acquisition started one second before the appearance of the 2 s stimulus and a total of five frames, each of which lasted for one second, were recorded. Since the largest intrinsic signals appear at 3~4 s after the onset of visual stimulus, we only used the fourth frame in a trial as effective data to analyze (Chen et al., 2003). To remove the activity-dependent microvascular changes, the period of stimulus presentation was followed by a 10 s interstimulus interval. The order of stimulus presentation in each trial was randomized to prevent any systematic effects of stimulus presentation order. To reduce the noise in the acquired images, signal averaging was used, with each stimulus being presented 16–64 times.

Defining the region of interest (ROI) of the functional maps in area 17

Determination of the area 17/18 border was based upon differences between these areas in spatio-temporal frequency response (Movshon et al., 1978; Bonhoeffer et al., 1995). Specifically, the location of the boundary between areas 17 and 18 was identified objectively by subtracting the two orientation maps elicited by drifting gratings of spatial frequency 0.58 and 0.14 cycles/deg respectively as described previously (Issa et al., 2000; Hung et al., 2001; Huang et al., 2004).

Before comparing the difference of two maps, a ROI in area 17 was selected and all further comparisons were restricted within it. The following criteria were taken into account. First, in order to reduce noise or artifacts, all images were corrected to minimize interference from blood vessels using the corresponding surface vascular images. Specifically, areas occupied by major blood vessels (>250 μm in diameter) and their immediate surrounding (within 100 μm) were excluded from analysis. Second, regions within 0.2 mm from the edge of the craniotomy containing bone or dura mater were not taken into account. Finally, regions not in the focal plane of the camera due to cortical curvature were not included in the analysis. Altogether, an average area of larger than 4 mm^2 of cortex per hemisphere in nine cats underwent quantitative analysis.

Data analysis

For constructing orientation maps, the functional maps elicited by two gratings of the same orientation and opposite motion directions were added. To remove the high and low spatial frequency noise, optical images were filtered with a high pass of 960 μm and a low pass of 216 μm.

Based on the circular statistics, the orientation selectivity (preferred orientation angles and orientation bias) of each pixel