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Weakened feedback abolishes neural oblique effect evoked by pseudo-natural visual stimuli in area 17 of the cat

Wei Shen[a], Zhiyin Liang[a], Tiande Shou[a,b,c,*]

[a] Vision Research Lab, Center for Brain Science Research, School of Life Sciences, Fudan University, Shanghai 200433, PR China
[b] Department of Ophthalmology and Visual Science, Eye & ENT Hospital, Fudan University, Shanghai 200433, PR China
[c] Laboratory of Visual Information Processing, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, PR China

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ABSTRACT

The psychological oblique effect, a well-known phenomenon that humans and some mammals are more visually sensitive to cardinal (vertical and horizontal) contours than to oblique ones, has commonly been associated with the overrepresentation of cardinal orientations in the visual cortex. In contrast to the oblique effect, however, Essock et al. [E.A. Essock, J.K. DeFord, B.C. Hansen, M.J. Sinai, Oblique stimuli are seen best (not worst!) broad-band stimuli: a horizontal effect, Vision Res. 43 (2003) 1329–1335] reported a psychological 'horizontal effect', in which visual stimuli dominated by oblique orientations were best perceived by human subjects when tested with unique natural broad-band stimuli. In this study, using optical imaging and the similar visual stimuli, we found an overrepresentation of cardinal orientations, i.e. the neural oblique effect, but not 'horizontal effect', in area 17 of the cat. In addition, the oblique effect was abolished by GABA administration in area 21a due to the preferred orientation shifting (6.0%) and decrease of orientation selectivity strength of neurons (26.9%) in area 17. These results indicate a neuronal basis of the oblique effect when animals watch a more natural scene, whereas no evidence was found for the 'horizontal effect'.

The oblique effect, a well-known phenomenon that humans and some mammals are more visually sensitive to cardinal (vertical and horizontal) contours than to oblique ones, has repeatedly been observed by psychological, physiological and behavioral studies. This effect has always referred to the overrepresentation of cardinal (vertical and horizontal) orientations in the visual cortex [1,14,6,24,3,26,11,22,8,12]. Recently, using fMRI and optical imaging methods, it was revealed that more neurons preferentially respond to cardinal contours than to oblique ones in human and animal primary visual cortex [5,26,11,22,8,12]. Furthermore, Liang et al. reported that the neural oblique effect in cortical area 17 was enhanced by activation of area 21a in the cat through neurons' preferred orientation shifting [12]. However, Essock and colleagues [4] reported, in contrast to the oblique effect, a 'horizontal effect' in which that visual perception was dominantly at oblique orientations were best seen by human subjects when tested with natural broad-band stimuli consisting of multiple spatial frequencies, whose power distributed as same as most natural scenes [4]. It is of interest to address the following two questions: (1) whether the 'horizontal effect' exists in the primary visual cortex of the cat, when the similar natural broad-band stimuli are applied; (2) if the 'horizontal effect' exists, whether the feedback projection from area 21a to area 17 also influences that effect. In the present study, the stationary pseudo-nature stimuli were recruited and the preferred orientation maps of cats' area 17 were optically imaged in normal condition, as well as when the excitability of area 21a neurons were manipulated by local application of γ-amino-butyric acid (GABA), a basic inhibitory neurotransmitter of the brain.

Eleven normal adult cats of either sex were used in the current study. All animal use procedures were performed in strict accordance with the Guide for the Care and Use of Laboratory Animals described by the U.S. National Institutes of Health, and all experiments were designed to minimize the number of animals used and their suffering. Animals were initially anesthetized with ketamine (20 mg/kg). Then, anesthesia was maintained with i.v. pentobarbital sodium given at a loading dose of 4 mg/kg followed by an infusion of 3 mg/(kg h) throughout the experiment. After intravenous and tracheal cannulations were performed, the cat was placed in a stereotaxic apparatus. Gallamine triethiodide (8–10 mg/(kg h), Flaxedil; Shanghai Dongfeng Chemicals Factory, Shanghai, China) was then used for immobilization and animals were artificially respired using a pulmonary pump. The animals'

* Corresponding author at: Vision Research Lab, Center for Brain Science Research, School of Life Sciences, Fudan University, Shanghai 200433, PR China.
Tel.: +86 21 65642355; fax: +86 21 65643528.
E-mail address: tdshou@fudan.edu.cn (T. Shou).

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physiological conditions were kept in normal ranges throughout the experiment. Thus, the end-tidal CO_2 was kept at 4% and body temperature of animals was at 38 °C. Electroencephalogram (EEG) and electrocardiogram (ECG) were continuously monitored.

Area 21a was exposed at Horsley-Clarke coordinates P1-7, L7-13 corresponding to retinotopical projection of 0–10° above the horizontal meridian [20]. A stainless steel chamber was fixed on the skull covering area 17 at P0-10, L0-L5, [21] where the central contralateral visual field (0–10°) was also represented. After removal of the dura, the chamber was filled with warm (>25 °C) silicone oil and sealed with a transparent glass for the following intrinsic signal optical imaging.

Area 21a was reversibly inactivated by microinjection of 1.0 μl of 100–400 mM GABA (Sigma, USA) as the description in previous study [7,12]. As a control 1.0 μl of phosphate-buffered saline (PBS, pH 7.4) was also injected at the same site. Solutions were injected slowly within 4 min and the needle of the micro-syringe was held for 10 min before withdrawal. The injection sites were centered 0.5–1.0 mm beneath the pial surface mainly at cortical layer II–III. Previous studies have proven that 1.0 μl of 100 mM GABA tends to diffuse over a region of 1.5 mm in diameter in the mammalian cortex [9]. Finally, the location of the centers of the injection sites was assessed histologically counterstained with Nissl staining. Only data of animals with correct injection locations within area 21a were included in the study.

For optimal spatial frequency of most neurons in area 21a is lower than the one of most neurons in area 17 of cats [16,17,19], a new pseudo-nature visual stimulus with a single orientation combined with multiply spatial frequencies (SF = 0.0016–8.65 cycle/degree, slope = 1), which is similar to the one used by Essock et al. [4], was adopted in the current study to elicit most neurons both in area 21a and area 17. The patterns of visual stimuli were broadband isotropic images that were constructed with a combination of broad amplitude spectrum (amplitude spectrum slope = −1.0 [25]) and different random phase spectra. The visual isotropic images were filtered to create an increment of amplitude within a narrow orientation band (1° extent) centered at one of 4–6 orientations. The inverse two-dimensional Fourier transform was then applied to convert these frequency-domain representations to the space domain for display (Fig. 1). The visual stimulus was repeatedly presented on the screen of a high resolution monitor (FlexScan F931, EIZO NANAO, Japan) positioned 57 cm in front of the cat's eyes for 2 s with 10 s blank interval in between. The mean luminance of the screen was 15.1 cd/m^2 and the root mean square contrast of the stimulus was 0.7.

An intrinsic signal optical imaging system was used to record functional orientation maps of area 17. The vessel map of the cortical surface was obtained with green light (540 nm) shining on the cortex. Orientation maps evoked by the visual stimuli were detected under illumination with red light (640 nm) when the camera was focused 500 μm underneath the pia. Data acquisition started 1 s before the onset of the stimulus and total 5 frames (1 s/frame) were recorded during a 5 s stimulus period followed by a 10 s interval. The order of stimulus orientation presentation in each trial block was randomized. Cortical intrinsic signals were averaged for 32–64 times.

To obtain a single-condition map, the cortical image obtained with a specific stimulus was divided by the 'cocktail blank' cortical image. Cocktail blank refers to the mean of the responses elicited by all stimuli of different orientations. To remove the high and low spatial frequency noise, images were filtered with a high pass of 960 μm and a low pass of 216 μm. For quantifying response strength of a map, the averaged difference of the 10% maximum and the 10% minimum in brightness was computed and divided by the first frame. The resultant value was defined as the response strength (RS) of a given map. Essentially, RS represents the averaged contrast of a given orientation map [18].

Orientation preference and orientation bias (selectivity strength) in a composition map were calculated by pixel-wise vector summation of the responses from several single orientation maps as follows:

$$\mathrm{Mo} = (a^2 + b^2)^{1/2},$$
$$\theta = 0.5 \times \tan^{-1}(b/a),$$
$$a = \Sigma_i[R_i \times \cos(2\varphi_i)], \quad b = \Sigma_i[R_i \times \sin(2\varphi_i)],$$

where θ and Mo are preferred orientation (ranged from 0° to 180°) and orientation bias, which ranged from 0 to 1, bias > 0.1 meaning significant orientation selectivity according to the circular statistics [2] of each pixel in a given orientation map, respectively. R_i is the signal strength (pixel luminance) and φ_i is the stimulus orientation corresponding to the ith map of several maps evoked by different stimulus orientations. Thus, the color-coded composition map was constructed for quantifying both preferred orientation and orientation bias.

In order to reduce noise/artifacts, all images were corrected to minimize interference from blood vessels. Specifically, some areas occupied by larger blood vessels (>250 μm in diameter) and their immediate surround (∼100 μm) were excluded for analysis. Regions within 0.2 mm from the edge of the craniotomy containing bone or the dura and those out of focus of the camera were not included for analysis. Accordingly, an area of about 7 mm^2 of cortex per hemisphere in average was quantitatively analyzed.

In this study, using new pseudo-nature visual stimuli to mimic a natural scene, we found that inactivation of area 21a by GABA application caused a decrease not only in response and orientation selectivity, but also in the neural oblique effect of neurons shown in functional optical imaging in the cat's area 17. The results indicate

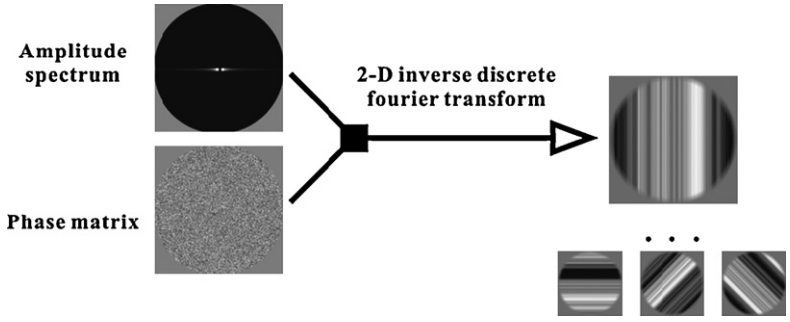

Fig. 1. The stimulus patterns constructed by taking a broad amplitude spectrum (amplitude spectrum slope = −1.0 [23]) combined with different random phase spectra to make broadband isotropic visual images (0.7 root mean square (rms) contrast and 15.1 cd/m^2 mean luminance). The isotropic visual images were filtered to create an increment of amplitude within a narrow orientation band (1 extent) centered at one of four orientations (0°, 45°, 90°, 135°). An inverse Fourier transformation was then applied to convert these frequency-domain representations to the space domain for display.

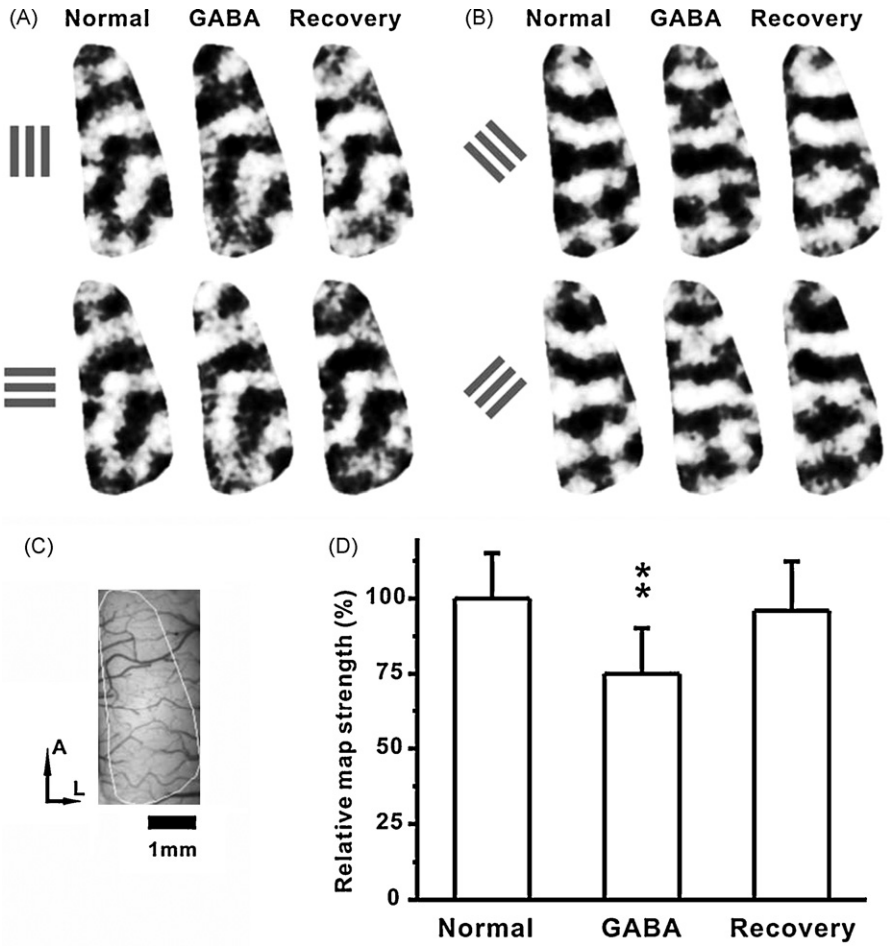

Fig. 2. Impact of inactivation of area 21a on response strength in area 17. (A and B) Four single-orientation maps obtained in area 17 before (left column), during (middle column), and after (right column) inactivation of area 21a by the injection of 1 μl 400 mM GABA in a cat (#0106), respectively. Orientations of the pseudo-nature stimuli are represented as simple gratings on the left of each row of images. (C) Surface view of the imaged region of area 17. The region of interest (ROI) is denoted by white lines. A, anterior; L, lateral. Scale bar, 1 mm. (D) Comparison of mean relative response amplitudes of maps obtained before, during, and after area 21a inactivation from 11 cats. Error bars indicate standard deviation (hereafter, referred as S.D.). ** represents $P < 0.01$. Note that, visually evoked responses of neurons in area 17 significantly decreased by 25.0% (t-test, $P \ll 0.01$) when area 21a was inactivated.

that area 21a plays an important role in modulating form processing in area 17 via feedback projections.

Local injection of GABA into the ipsi-lateral area 21a resulted in reversible decreases in response to pseudo-nature visual stimuli of area 17 cells in 11 cats studied. Fig. 2A and B shows typical single orientation maps elicited by pseudo-nature visual stimuli in area 17 before, during and after area 21a activation by injecting 1 μl 400 mM GABA in a cat. Dark areas in the maps indicate the regions where concentrated with highly activated cells. In all animals the GABA injection caused a significant 25.0% reduction in mean response amplitude ($N = 11$, t-test, $P < 0.01$, Fig. 2D). Thus, the result reveals that area 21a has an excitatory or positive feedback influence on neurons in area 17 of the cat, in agreement with the previous results with traditional grating stimuli in area 17 obtained by single cell recording and local cooling of area 21a [23].

Fig. 3A shows the composition orientation maps of area 17 obtained before, during and after inactivation of area 21a by injecting 1 μl 400 mM GABA in the same cat in Fig. 2. Qualitative comparison revealed that there were significant differences of preferred orientation in some positions between the two preferred orientation maps obtained before and during GABA application (Fig. 3B). Data analysis based on 11 animals showed significant changes in the composition of orientation maps indicating that neurons in 6.0% pixels (or area) within ROI in area 17 changed their preferred orientation by larger than 45° during 21a inactivation, compared with the control (Fig. 3C, Pearson's correlation test, $P = 0.042 < 0.05$, $N = 11$). Furthermore, the proportion of neurons with high neuronal orientation bias or selectivity strength decreases significantly (Fig. 3E). As an overall result of 11 cats, the average orientation biases of neurons within ROI decreased by 26.9% significantly (t-test, $P < 0.01$, $N = 11$) due to inactivation of area 21a (Fig. 3D and F). Thus, the results indicate that area 21a plays an excitatory role in form information processing via enhancement of orientation selectivity in area 17.

The psychological oblique effect has commonly been associated with more visual cortical neurons preferentially respond to cardinal contours than to oblique ones. In this study we confirmed the neural oblique effect by using the pseudo-natural visual stimuli, but failed to observe a 'horizontal effect', proposed earlier by Essock et al. [4]. In the color-coded composition maps (Fig. 4A and B), cardinal overrepresentation of neurons' preferred orientation in area 17 of a cat in the normal condition (Fig. 4C) was significantly abolished by inactivation of area 21a due to GABA application (Fig. 4D). For the 11 cats studied, the mean cardinal representation area (52.1 ± 4.4%) was +4.4% higher than that of the oblique (47.9%) in area 17 under normal condition (Fig. 4F), while this value turned to be 48.8 ± 13.3%, which was 2.4% lower than the oblique (51.2%) under the 21a inactivated condition. The change in cardinal repre-

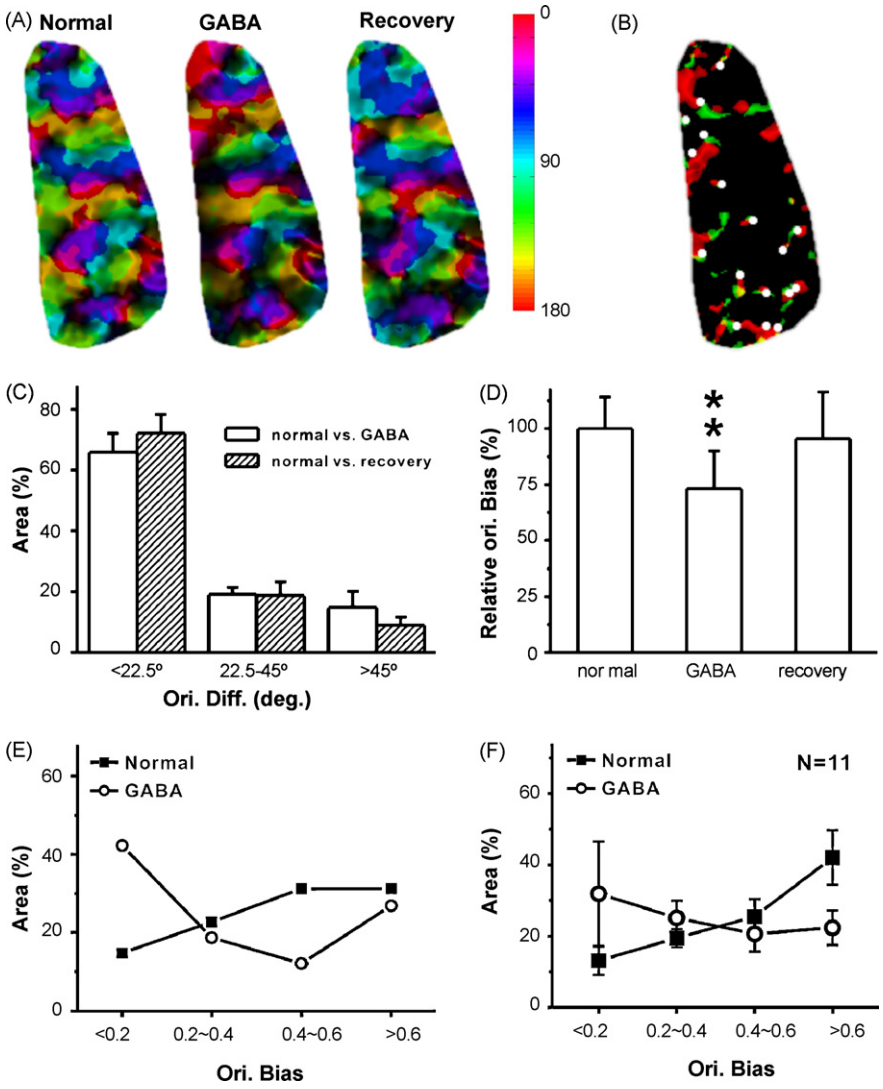

Fig. 3. Impact of inactivating area 21a on orientation selectivity within ROI of area 17 in a cat. (A) Preferred orientation maps obtained before (left column), during (middle column), and after (right column) inactivation of area 21a by injections of 1 μl 400 mM GABA, based on the data shown in Fig. 2A and B. The colors indicate the values of preferred orientation according to the color scale on the right, and the brightness indicates orientation biases. (B) Differential map of preferred orientation angles. Red regions indicate the sites where the difference between preferred orientation angles obtained before and during inactivation of area 21a was more than 45° (the maximal difference was 90°), and green regions indicate the sites where the difference between preferred orientation angles obtained in normal and recovery conditions was more than 45°. Superimposed red and green regions are shown in yellow. Pinwheel centers are shown as white dots (radius = 72 μm). The dark regions indicate the areas with preferred orientation angle differences of less than 45°. (C) Averaged distribution histograms of angular differences between orientation maps obtained before and during inactivation of area 21a (open columns), and angular differences between orientation maps for normal and recovery conditions (hatch columns), based on 11 cats. Note that preferred orientation of about 6.0% ROI did change significantly when inactivating area 21a (Pearson's correlation test, $N = 11$, $P < 0.05$). (D) Comparison of average orientation biases within ROI of area 17 before, during, and after area 21a inactivation. Note that the average orientation bias decreased significantly by 26.9% (t-test, $N = 11$, $P < 0.01$) when inactivating area 21a. Asterisk indicates significant difference at a level of $P < 0.01$. (E and F) Comparison of orientation bias distribution within ROI in area 17 before and during 21a inactivation for the cat in A (E) and for 11 cats studied (F). Note that pixels having higher biases decreased significantly due to application of GABA in area 21a.

sentation was statistically significant (t-test, $N = 11$, $P = 0.01 < 0.05$). This means that inactivation of 21a abolished the overall neural oblique effect. The result indicates that the feedback projection from area 21a to area 17 can enhance the neural oblique effect, but not the 'horizontal effect' in area 17 under normal condition such as when the animal is watching a more natural scene.

The advantage of utilizing optical imaging for studying the oblique effect is its large recording area, where the activities of millions of neurons are simultaneously studied with a fine spatial resolution (24 μm in the current study), compared to the single neuron electrophysiological recording method with a lower sampling number and largely biased penetrations, which may result in discrepancies in the strength of the neural oblique effect [15,10,11]. Recently, an increasing number of studies have been reported, in which optical imaging was used to reveal the neural oblique effect in different cortical areas, including areas 17, 18 and 21a [26,22,13,8,12]. In this study we confirmed the neural basis of the neural oblique effect rather than the psychological 'horizontal effect' reported by Essock et al. [4]. It is possible that the 'horizontal effect' was based on somewhat different neuronal mechanism due to different criterions and subjects they used in the psychological experiments.

The fact that the inactivation of area 21a caused disappearance of the oblique effect in area 17 is interesting and in agreement with the report that activation of area 21a enhanced the effect in area 17 [12] although the visual stimuli used in their study were different from ours. Given that area 21a demonstrates a neural oblique effect 4.6 times higher than that of area 17 [8], it is likely that the co-excitation of areas 21a and 17 via reciprocal corticocortical connections may contribute to the psychological oblique effect.

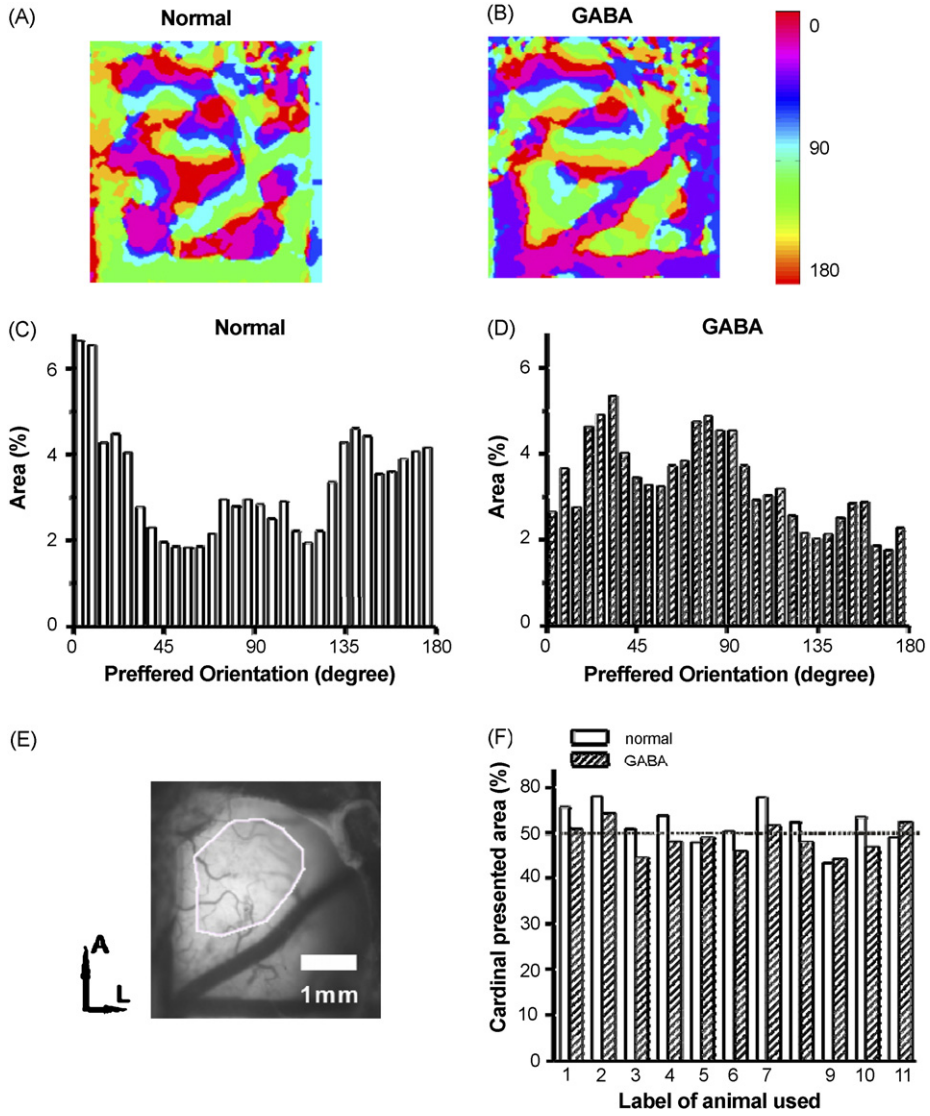

Fig. 4. Neural oblique effect, found in area 17 (but not 'horizontal effect') and its abolition due to application of GABA in area 21a. (A and B) Color coded composition orientation maps recorded under normal (A) and GABA (1 μl, 400 mM) injection (B) conditions. (C and D) Preferred orientation distribution histograms of pixels in the same ROI in area 17 under normal (C) and GABA injection (D) conditions. Note that a W-shaped distribution indicates the neural oblique effect; an overrepresentation of cardinal orientation appears in (C), while the cardinal dominant distribution disappears in (D). (E) Surface view of the area 17 of the cat studied. ROI denoted by solid line. A, anterior; L, lateral. Scale bar, 1 mm. (F) Summary results of the change in oblique effect caused by inactivation of area 21a by GABA for 11 cats studied. Note that the cortical area representing ROI in area 17 preferring 0° and 90° was larger than the area preferring 45° and 135° on average under normal conditions and that the cardinal overrepresentation was totally abolished due to GABA application in area 21a.

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