Research report

Visual responses to contrast-defined contours with equally spatial-scaled carrier in cat area 18

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Article history:
Received 18 February 2011
Received in revised form 14 June 2011
Accepted 15 June 2011
Available online 1 July 2011

Keywords:
Visual cortex
Illusory contours
Second-order cue
Form cue invariance
Spatiotemporal energy model

Abstract

Contrast-defined contours are one type of second-order contours, across which there are no differences in luminance. Although they can be always perceived, their responses have been only investigated when the spatial frequency of carrier, the background texture whose contrast is modulated to form contours, is much higher than that of contrast-defined contours, due to the interference of responses to luminance contours in other cases.

In the present study, we examined visual responses in cat area 18 to the contrast-defined contours with carrier at same spatial frequency equal to neuron’s preferred value for luminance contours, by establishing a control stimulus including all the luminance components but lack of the contrast contour information. Using single unit recording and intrinsic optical imaging, we demonstrated that contrast gratings with equally spatial-scaled carrier induced responses in a proportion of cat area 18 neurons with the preferred orientation similar to that for luminance contours, and the responses generated orientation maps similar to those for luminance contours.

Our finding suggests that early visual cortex can process second-order contours regardless of the spatial frequency of carriers, in a way similar to the processing of luminance contours. This uniform manner of early visual processing might underlie the visual detection of both luminance contours and non-luminance second-order contours.

1. Introduction

Detecting the boundaries of different objects is a primary visual task. To extract the boundaries delineating objects with texture (such as zebra, kraits snake), visual system relies on the processing of high-order cues. Generally, contours defined by differences in luminance are classified as first-order contours; contours defined by non-linear differences in the pattern of luminance are classified as second-order contours [7,12,15]. Second-order contours can be recognized by many species from invertebrates to mammals [19]. Many studies have focused on the visual responses to second-order contours [3,4,26,27]. In those studies, second-order stimuli were conventionally created by modulating non-luminance properties of a background texture, such as contrast defined grating created by modulating the contrast of a luminance grating (Fig. 1A and B) [25,40], and abutting lines created by shifting the phase of parallel lines [11,22,28,33,34]. The background texture whose contrast, phase or other non-luminance properties are modulated to form contours is referred to as carrier.

Like luminance contours, it has been known that second-order contours can evoke responses representing their orientations in a proportion of neurons in early visual cortex, when the spatial frequency of carrier is far beyond neuron’s spatial selective range for luminance contours, in other words, higher than that of second-order contours within that range [37,42]. Whereas when the spatial frequency of the carrier is equal or comparable to that of second-order contours, and resultantly falls in neuron’s spatial selective range, the responses to second-order contours are mixed with those to luminance contours and have not been demonstrated. Objects with coarse texture abound in environment. The environmental background of objects usually contains textures at both high and low spatial frequencies, So it might be important for visual system to process second-order contours with carrier at low spatial frequency. We are curious how these contours are represented in the brain, and whether they can be processed in early visual cortex as well. Previous studies showed controversial results. Some previous studies suggested that early visual cortex neurons might be unresponsive to this kind of second-order contours, because these neurons had a selective range of carrier spatial frequency for second-order contours totally beyond that for luminance contours [41,42]. Other studies in cat LGN [25], area 17 (A17) and area 18 (A18) [39] suggested that neurons exhibited an overlap rather

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doi:10.1016/j.brainresbull.2011.06.004
than a clear segregation between those two ranges. However, these results are not sufficient to conclude that second-order responses contribute to the overlap. Up to date, although second-order contours can always be perceived regardless of the spatial frequency of carrier, it remains unknown whether they can be processed in a unified detection system. We address this question by examining responses to contrast-defined contours with equally spatial-scaled carrier in cat area 18 and the orientation tuning properties of the responses.

One main obstacle for investigating second-order responses is the difficulty in separating the responses from those to luminance components. Previous studies have shown that simply removing the responses to carrier will not get rid of all the responses to luminance contours in second-order stimuli [38]. According to the well-accepted spatiotemporal energy model [1,9,14,18], early visual responses to luminance contours depend on the linear differences in luminance in neurons on-off receptive fields. Mathematically, these differences are the linear summation of the differences contributed by all the Fourier component gratings of the stimulus. In brief, the responses to luminance contours in early visual cortex depend on the Fourier component gratings of stimulus [5,8,14,37]. For second-order stimulus, the Fourier component gratings include not only those arising from carrier, but also different ones (Fig. 1D–F) [25,38]. Even when the responses to carrier are removed or avoided, there might be responses from other Fourier components at different orientations, higher or lower spatial frequencies. However, subtracting the sum of responses to individually displayed Fourier component gratings from total responses to second-order stimulus will not give the responses to second-order contours either, because the cross-inhibition might be significant when the Fourier component gratings are displayed together [13,16]. Therefore, in previous studies, as mentioned above, the spatial frequency of carrier was always set much higher than neuron’s spatial selective range to ensure that the Fourier component grating with lowest spatial frequency fell out of that range and did not elicit responses [15,25,29]. But this strategy is not adequate for the examination of the responses to second-order contours with carrier at relatively lower spatial frequencies.

In present study, we established a method to separate responses to second-order contours from those to luminance contours, and examined cat A18 responses to second-order contours when the spatial frequency of the carrier was equal to that of the second-order contours at neurons preferred value (see Section 2). Our test stimulus (abbreviated as T) is conventional “contrast grating” stimulus consisting of a stationary, sinusoidal luminance grating whose contrast is modulated by a drifting oblique (45°) grating (Fig. 1B), which can be considered as the sum of three Fourier component gratings, G_{H}, G_{L}, and G_{S} (Fig. 1D–F, see Section 2). By shifting the phase of G_{H}, we got the control stimulus for T (correspondingly abbreviated as T′) with Fourier component gratings at same orientations, spatiotemporal frequencies but lack of contrast grating information, as shown in Fig. 1C. As a plus, since all the Fourier components are contained in one control stimulus, their cross inhibition should be included in responses to T′.

Using electrophysiological single unit recording technique, we demonstrated that in some cat A18 neurons responses to T and T′ are significantly different, and these differences in responses to T and T′ reflected the orientation of the contrast grating in T. Then using intrinsic optical imaging method, we showed the responses to contrast gratings generated orientation maps similar to those for luminance gratings.

2. Materials and methods

2.1. Animal preparation and maintenance

Experiments were carried out on 7 normal adult cats, approved by the Animal Ethical Committee, Institute of Biophysics, Chinese Academy of Sciences. Care was taken to minimize any pain and discomfort of the cats, in compliance with the National Institutes of Health guidelines on the care and use of laboratory animals. The procedures for animal preparation and maintenance were conventional and have been described previously [36]. Briefly, anesthesia was induced with ketamine
hydrochloride (20–30 mg/kg, i.m.). Surgery was performed to enable continuous infusion of sufentanil (0.13 μg/kg/h), propofol (2–6 mg/kg/h), gallamine triethiodide (10 mg/kg/h) and glucose (200 mg/kg/h) in Ringer’s solution through a foreleg venous cannula (~4.0 ml/h) and artificial ventilation through a tracheal cannula. Expired CO₂, body temperature, electrocardiogram and electroencephalogram were continuously monitored and maintained at appropriate levels. A craniotomy and durotomy were performed to expose the surface of cortical areas 18 [21,32]. The eyes were focused at a distance of 57 cm with appropriate spectacle lenses.

2.2. Visual stimuli

The contrast-defined contours (see Fig. 1A for an illustration frame), which consist of a stationary carrier (e.g. the vertical grating in Fig 1A) whose contrast is modulated by a drifting contrast grating (e.g. the tilted grating in Fig 1A), are widely used as the second-order stimuli in the relevant studies [25,31,37]. In convention, the spatial frequency of the carrier is much higher than that of the contrast grating, so that the resultant spectral energy is outside the neuron’s luminance defined pass-band. Distinctively, in the present study, the spatial frequencies of both the carrier and the contrast grating are set to the optimum measured with luminance gratings (see Fig. 1B). In any case, the luminance profile of the composite stimuli can be described as:

\[ I(x, y, t) = I_0 \left( 1 + C_1 \sin(2\pi f_1/x \cos \theta_1 + y \sin \theta_1) + 1 + C_2 \sin(2\pi f_2/x \cos \theta_2 + y \sin \theta_2) - f_1 \right) \]

where \( I_0 \) is the mean luminance; \( C_1, f_1, \theta_1 \) are the contrast, the spatial frequency and the orientation of the carrier, respectively; \( C_2, f_2, \theta_2 \) and \( f_2 \) are the contrast, the spatial frequency, the drifting direction and the temporal frequency of the contrast grating, respectively.

Mathematically, this expression can be always transformed into a linear summation of three first-order component gratings. In this study, the spatial frequency of carrier and contrast grating was set equal, and the relative angle between the carrier and contrast grating was set as 45°. Hereof the parameters are specified as \( f_0 = f_1 = f_2, \theta_a = \theta, \theta_d = \theta - \pi/4, \) \( C_{1,2} = C \) and \( \phi_1 = 1 \), and the transformation turns to be:

\[ I(x, y, t) = I_0 \left( 1 - 0.25 \times \cos 2\pi \left( 1.85y (x \cos \left( \frac{\theta - \pi}{4} \right) + y \sin \left( \frac{\theta - \pi}{4} \right) \right) - f_1 \right) \]

\[ -0.25 \times \cos 2\pi \left( 0.77y (x \cos \left( \frac{\theta - \pi}{4} \right) + y \sin \left( \frac{\theta - 3\pi}{4} \right) \right) - f_1 \right) \]

\[ + 0.5 \times \sin 2\pi \left( 0.5y (x \cos \left( \frac{\theta - \pi}{4} \right) + y \sin \left( \frac{\theta - 3\pi}{4} \right) \right) \]

where \( \theta \) is the drifting direction of the contrast-defined contours, \( f_1 \) is the spatial frequency of the both static carrier and the contrast grating. The three terms in the formula represent a high spatial frequency drifting grating (GS, 1.85y, \( \theta - \pi/4 \)), a low spatial frequency drifting grating (GS, 0.77y, \( \theta - 3\pi/4 \)) and a stationary grating (GS, \( \theta - \pi/4 \)), respectively (for each component grating, the abbreviation, the spatial frequency, and the drifting direction or orientation are given in the accompanying bracket).

Besides the test stimuli described above (abbreviated as T; see Fig. 1B), another set of composite stimuli were taken in the present study as the control (abbreviated as C; see Fig. 1C). In respect of a test stimulus (T = GS + GS + GS + GS), the corresponding control stimulus is also composed of three first-order component gratings, of which GS and GS are exactly as same as those in the test stimulus, while the spatial phase of GS is shifted by 180° (i.e. T = GS + GS + GS + GS). Hence the control stimulus possesses the same Fourier magnitude spectrum as the test stimulus does, but does not display the salient contrast-defined contours which are clearly visible in the test stimulus. Therefore, the neuronal responses to stimuli T and C could be compared with each other to indicate the activity evoked by the second-order cue. In addition, luminance gratings are used as the first-order stimuli for reference.

In the experiments all these stimuli were generated online by C++ programming on a personal computer with a plug-in graphics accelerator card (WinFast Quadrax FX3400, Leadtek Research, Taiwan) and displayed on a CRT monitor with a mean luminance of 44.2 cd/m² (Hiyama HM204D. 22”. 800 × 600 pixels, 150Hz, Japan). For single-unit recording, the stimuli were presented within a circular window subtending 30° in diameter, covering the receptive field optimally; for optical imaging, full screen stimuli were used. In both the electrophysiological and optical imaging experiments, the data were collected monocularly.

2.3. Single-unit recording and data analysis

49 neurons from 5 cats were recorded in the experiments. Epoxyite-insulated tungsten electrodes (THC, Bowdoin, ME) were used to record neuronal activity in the cortex. The signals were acquired and processed with a 16-channel neurophysiology workstation (Tucker-Davis Technologies, Alachua, FL). When a single unit was isolated, the receptive field was approximately plotted with hand-held stimuli. Cells were selected for further study only if they exhibited clear orientation selectivity. Before experiments, preliminarily tests were performed to measure neuronal responses to drifting grating with spatial frequencies from 0.05 cpd to 0.8 cpd, and temporal frequencies from 0.5 Hz to 8 Hz at octave steps. The optimal spatial and temporal frequencies estimated by the preliminary test were taken as the parameters \( f_1 \) and \( f_2 \) for all the stimulus patterns in subsequent main tests. Most of neurons recorded in the study have an optimal spatial frequency of 0.1 cpd or 0.2 cpd (42 out of 49 neurons), and an optimal temporal frequency greater than 2 Hz (45 out of 49 neurons). A main test consisted of 60 trials. Each trial was composed of three component luminance gratings (GS, GS and GS), T and T drifting in 16 directions every second were interleaved and presented with the optimal temporal frequency. The contrast of T and T was set as 100%. Accordingly, the contrast of GS, GS, and GS were 25%, 25% and 50%, respectively. All the different conditions were pseudorandomly interleaved trial by trial, and the spatial phase of the stimuli was shifted by 90° after each trial to exclude the possible influence of any phase preference. For each sweep, the stimulus remained stationary for 250 ms and then drifted for 500 ms. The firing rates during the drifting period were taken for plotting tuning curves. The differences between the responses to T and T were determined by Students paired t test at 16 directions. We classified neurons as contrast grating responsive if the responses to T were significant stronger than those to T, and calculated the responses to second-order contours as the differences between responses to T and T. Spontaneous activities were subtracted from responses to luminance gratings for comparison with responses to second-order contours.

When plotting the direction tuning curves for neuron A and neuron B (Fig. 2E–H), the orientations of the peaks were estimated from a fit to a modulated von Mises function [30], using the Fit program from Matlab Toolbox (matlab. Mathworks, Natick, Massachusetts). The model function was the sum of two classic of Mises functions representing two peaks located at same orientation, which is described as following:

\[ R(x) = A + B_1 \exp \left( k_1 \cos(x - \theta_1) \right) + B_2 \exp \left( k_2 \cos(x - \theta_2 + 180° - \theta_1) \right) \]

where R is the response, x is the direction of stimulus motion, A is a constant, B1 and B2 scale the height of the individual peaks, \( \theta_1 \) and (\( \theta_1 - 180° \)) is the location of each peak and k1 and k2 are inversely related to the bandwidths of each peak.

The measurements of orientation tuning properties were based on vector averaging, due to the relatively weak strength of responses to second-order contours. Preferred orientations were determined by the vector sum of responses. Estimates of bandwidth were measured as circular variance, given by the following equation [15,23]:

\[ CV = 1 - \frac{\sum_{i=1}^{N} R_i \exp(2\pi i \theta)}{\sum_{i=1}^{N} R_i} \]

where \( R_i \) is the strength of response for the given orientation, the numerator of the second formula is the magnitude of the vector sum. To adjust \( R_i \) to positive value, for each neuron, the original responses at all orientations were subtracted by the maximum. The value of circular variance ranges between 0 and 1, where 0 represents equal responsiveness across all orientations (180° bandwidth), and 0 indicates responsiveness to only one orientation.

2.4. Optical imaging

A stainless steel chamber was cemented onto the skull around the exposed cortex, filled with sterile silicone oil and sealed with a glass cover. Images were acquired from the region using an Imaging 3001/F system (Optical Imaging, New York, NY) with 546 nm (green) illumination. The reason for using 546 nm illumination was to improve the signal/noise ratio, and allow for the recording of relatively weaker second-order contour responses [6]. But on the other hand, the green illumination caused the higher contrast artifact of vascular patterns than traditional red illumination. The image data acquisition and processing procedures were largely conventional [6]. Raw data were collected in dimension of 504 × 504 pixels (~1 mm/100 pixel in distance on cortical surface) and temporally binned to 2 frames online. 100–200 trials of stimulus were in a test to enhance the signal-to-noise ratio by image averaging. Each trial was composed of luminance gratings with saturated contrast (drifting in eight orientations), T and T (and T drifting in horizontal and vertical orientation, see the small panels in Fig. 5C and D) and the different stimulus conditions were pseudorandomly interleaved trial by trial. The spatial frequency of \( f_1 = 0.2 \) cycles/degree and the temporal frequency of \( f_1 = 4 \) Hz were adopted for all the stimulus patterns. Each sweep lasted about 30 s, including 3 pre-stimulus period, 8 s stimulus duration, 3 s post-stimulus period and 16 s inter-stimulus interval.

2.5. Image analysis

Besides trial averaging, the so-called ‘first frame analysis’ was applied to reduce slow noise of biological origin [6]. For each stimulus condition, the frames
acquired within the pre-stimulus period were time averaged and the mean image was subtracted from all subsequent frames. Thereafter, visually evoked activities were time averaged over a window of about 2–11 s following the stimulus onset and results for pairs of orthogonal stimuli were subtracted to yield difference maps. Without any spatial filters used, maps for luminance grating, T and T’ are shown in Fig. 5A, C and D, displaying the relative preference of each location in the image for one (darker pixels) or other (lighter pixels) of a pair of stimulus conditions (horizontal or vertical movement). Moreover, an additional image (Fig. 2B) was produced by subtracting the difference map for control stimuli (Fig. 2D) from that for test stimuli (Fig. 2C). Since a test stimulus and its corresponding control stimulus are identical in their Fourier magnitude spectra but distinct from each other in the appearance of contrast-defined contours, this image is utilized to visualize the activity evoked by the second-order cue in the test stimuli.
Correlation analyses were employed to quantify the similarity in spatial pattern of maps. The blood vessels were binarized by setting a threshold for blood map. The mask was manually improved to mask out most of blood vessels in Map C and Map G (Fig. 5A). The correlation coefficient ranges from −1.0 (between two spatially complementary areas) to 1.0 (between two identical areas). The distribution of the coefficients between all corresponding sub-areas (squares of 100 × 100 pixels, shifted along either x- or y-axis in steps of one pixels) were further plotted to examine whether the correlation is uniformly distributed in whole images. Smaller (50 × 50, 25 × 25, 10 × 10 pixels) and/or non-overlap sampling windows were also used to measure the correlation in sub-areas between maps.

3. Results

3.1. Contrast grating responses demonstrated by electrophysiological single unit recording

A total of 49 neurons in cat area 18 were recorded in the experiments. Twenty of them exhibiting significant differences between the responses to T and T′ were considered responsive to contrast gratings in T (t-test, p < 0.05).

Fig. 2 displays the responses to contrast gratings in two typical neurons (left and right, neuron A and B, respectively). The PSTHs (per-stimulus temporal histogram) in Fig. 2A and B shows the strong responses to T and T′, with contrast gratings in T nearest to neurons preferred orientation (67.5° and 22.5°, for neuron A and B, respectively). Averaged firing rate over the stimulus duration to T and T′ was plotted against the direction of contrast grating (Fig. 2C and D). The difference in responses to T and T′ was significant during the whole stimulus period (Fig. 2A and B) and at various orientations (Fig. 2E and F).

The direction tuning curves to contrast grating (Fig. 2E and F) were obtained as the differences between those curves to T and T′, which were similar to the tuning curves to luminance gratings (Gx, Gy, Gz, Fig. 2G and H) both in shape and in orientation preference. The orientation of fitting peaks (see Section 2) for contrast grating was near those for luminance gratings (30°, 44°, 38° and 40° for contrast grating, Gx, Gy, and Gz in neuron A, and 69°, 74°, 54° and 70° in neuron B). The maximal firing rates were also compared. In neuron A, the responses to contrast gratings were significantly smaller than those to Gx and Gz, but comparable to those to Gy (12.9 ± 4.4 spikes/s, 56.8 ± 3.4 spikes/s, 23.9 ± 3.0 spikes/s, 19.2 ± 2.9 spikes/s, respectively); In neuron B, the responses to contrast gratings were comparable to those to Gx, Gy and Gz (15.8 ± 3.5 spikes/s, 18.3 ± 2.7 spikes/s, 10.4 ± 2.0 spikes/s, 11.8 ± 2.2 spikes/s, respectively). It needs to note that for estimating the contribution of each attribute to neuron’s responses to T, the comparison might bias towards the luminance gratings (Gx, Gy, Gz), due to the absence of the cross inhibition, when those gratings were individually displayed.

Fig. 3 shows the summary results from all 20 responsive neurons. For the responses to contrast grating and luminance gratings, the overall direction tuning curves were obtained by averaging individual tuning curves which were normalized by the maximum of responses to Gx and aligned to the corresponding preferred orientation (see Section 2). The curve to contrast grating (Fig. 3A) was similar to those to luminance gratings (Gx, Gy, Gz, Fig. 3B), with the correlation coefficients of 0.82, 0.77, and 0.72 respectively (Fig. 3C). On average, the responses to contrast gratings were significant but weaker than those to luminance gratings. The normalized responses to contrast grating, Gx, Gy at 0° direction (Fig. 3A and B) were about 13%, 31%, 62% of maximal responses to Gx.

In each of 20 responsive neurons, we compared the preferred orientation for contrast gratings with that for luminance grating (Gx), represented by the orientation of the vector sum of responses at varying orientations. The average difference was not significant different from zero (5.7 ± 6.5°, n = 20, p < 0.05). Fig. 3C shows most of the points representing each individual neuron distribute around the diagonal indicating equality in orientation preference (linear correlation, r = 0.78) and within the lines indicating 45° difference.

The tuning bandwidths of each individual neurons, estimated with circular variance (CV), are shown in Fig. 3D (see Section 2). Generally, responses to contrast grating exhibited broader orientation tuning, manifested as greater values of circular variances, when compared to luminance grating (Gx) responses. By visual inspecting the direction tuning curves of each individual neurons, it was found that 14 neurons (with CV < 0.7) exhibited clear orientation tuning like neuron A and neuron B, while the other 6 neurons exhibited flat or noisy curves.

3.2. Neuronal population responses to contrast grating demonstrated by optical imaging

We used conventional intrinsic optical imaging method to map the cat A18 population responses in two cats. Maps to contrast gratings and those to drifting luminance gratings were then compared in their spatial patterns, to confirm the single unit similarity in orientation preference at the neuronal population level.

Fig. 4 shows the maps from one cat. Differential maps obtained by subtracting maps to perpendicular stimuli were used in the comparison. Fig. 4A shows the differential map to luminance gratings at cardinal orientations (Map G, vertical minus horizontal). The black/white crosses were added manually to the light/dark areas indicating preference in vertical/horizontal stimuli. The differential map to cardinal contrast gratings (Map C, vertical minus horizontal) calculated as (Map T − Map T′) is shown in Fig. 4B. The dark/light areas indicated the contrast grating activation. Although the signal evoked by contrast grating was much weaker, as shown with black/white crosses duplicated from Map G, similarity in the spatial pattern of Map G and Map C can still be found, indicating similar domains preferred vertical/horizontal contrast grating and luminance grating. The differential maps to cardinal T (Map T) and T′ (Map T′) are shown in Figs. 4C and D.

Correlation analyses were employed to quantify the similarity in spatial pattern between maps, after masking out the blood vessels. Fig. 5A shows that the mask sheltered most of blood vessels both in Map C and Map G. Positive correlation can be found between Map C and Map G (0.39 and 0.51 for two cats respectively), whereas negative correlation can be found between Map T and Map G (−0.25 and −0.25). When Map C was compared to rotated Map G (by 180°), the correlation was about 0 (0.05 and −0.04). The positive correlation between Map C and Map G was relatively weak but meaningful, when considering that Map C was susceptible to interference for its relatively weak signal. To examine whether the correlation between Map C and Map G is uniformly distributed in whole images, from the same example used in Fig. 4, the coefficients between all 100 × 100 (pixels) corresponding sub-areas were plotted to histograms (Fig. 5B–D). For Map C and Map G, almost all sub-areas had positive coefficients clustered around the mean of 0.49 (Fig. 5B), indicating most of sub-areas in two maps have similarity in their spatial patterns. For Map T and Map G, the coefficients distributed in a broader range from −0.9 to 0.9 (mean = −0.20, Fig. 5C). In Fig. 5D, the distribution of coefficients between Map C and rotated map G shows little correlation between the different patterns formed by dark/light regions in similar size (mean = 0.09). Similar results can be found in the other cat.

To confirm the positive correlation between Map C and Map G was resulted from the consistent orientation preference of neurons in area 18, we further examined whether the similarity was orientation specific. Correlation analyses were employed between differential map to cardinal contrast grating and differential maps to luminance gratings at varying orientations (every 22.5° from...
**Fig. 3.** Statistics on area 18 neurons \( n=20 \) which were significantly responsive to contrast gratings. (A) Normalized tuning curve to contrast gratings. (B) Normalized tuning curves to luminance gratings \( G_s \) (black), \( G_h \) (dark gray) and \( G_s \) (light gray). For each cell, the four tuning curves were individually normalized in respect to the maximal response to \( G_s \) and aligned to the corresponding preferred direction. The normalized curves were averaged over different cells to show the overall situation that the orientation tuning of cortical neurons was similar for contrast and luminance gratings. The spontaneous activities were subtracted prior to the normalization. Error bars indicate standard errors. (C) Comparison between the preferred orientation to luminance gratings \( G_s \) (ordinate) and that to contrast gratings (abscissa), determined by the calculation of vector sum of responses. Almost all the data points fall between the two dashed lines indicating an orientation difference of 45°, while none is close to the dotted lines indicating an orientation difference of 90°. (D) Comparison between the circular variance (see methods) to luminance gratings \( G_s \) and that to contrast gratings. For almost all the cells, the orientation tuning was broader (with a higher CV value) for contrast gratings. The gray square dots in (C, D) represent the two neurons shown in Fig. 2.

**Fig. 4.** Optical images of cortical area 18 in responses to different visual stimuli. Differential maps were obtained from subtraction of activities evoked by stimuli drifting in horizontal and vertical directions. The stimulus patterns are schematically depicted with small panels left to the images, with dark arrows marked the moving direction of the luminance gratings in (A) or the contrast gratings in (B) and (C). (A) Map G, differential maps obtained with conventional luminance gratings with saturated contrast and optimal spatial frequency. The darker areas (marked with white crosses) indicate stronger responses to horizontal movement, whereas the lighter areas (marked with black crosses) indicate stronger responses to vertical movement. (B) Map C, differential maps to contrast grating, produced by subtracting differential map to \( T \) and \( T' \). The crosses in (A) are duplicated at identical locations in (B), to show the similarity between Map G and Map C in the spatial pattern of cortical activities evoked by luminance and contrast gratings. (C) Map T, the differential map to \( T \). (D) Map \( T' \), the differential map to \( T' \). Scale bar: 1 mm. Gray scale (right): reflectance change. P, posterior; L, lateral; A, anterior; M, medial.
−90° to 90°, Fig. 6). In both cats, the correlations were well “tuned” by the difference in orientations of the contrast grating and the luminance grating. The highest correlation can be found near 0° difference (−11° and −9° for two cats, respectively, estimated by fitting to von Mises function).

The distribution of the coefficients between maps were also examined with various sampling windows (100 × 100, 50 × 50, 25 × 25, 10 × 10; overlap or non-overlap; see Section 2), which shows that the positive correlation between map to contrast grating and map to luminance grating was uniformly distributed in sub-areas and orientation-specific independent of the measurements. For both cats, the means of coefficients in all sub-areas, obtained with all kinds of sampling windows, are shown in Supplementary Table 1.

4. Discussion

Our electrophysiological and imaging results have shown that contrast gratings with equally spatial-scaled carrier contributed responses to a proportion of cat A18 neurons, the responses had the preferred orientation similar to that for luminance contours and generated orientation maps similar to those for luminance contours.

Our results showed in some cat A18 neurons the contribution of second-order contours to neurons total responses was convincingly significant, sometimes even comparable to the contribution of the three Fourier components and generating obvious peaks (such as in neuron B, Fig. 1D–F), suggesting that this contribution was non-ignorable. As some previous study suggested, this contribution might be more significant, when the spatiotemporal frequencies of second-order contours are set lower [25,35,39,42]. On average, the responses to the contrast grating were much smaller than those to luminance gratings. This is in agreement with previous studies. The maximal firing rates (from 5 to 22 spikes/second varied in different neurons) were comparable to those to second-order with carrier at higher spatial frequencies in cat A17 and A18 [20,41,42]. The differences in preferred orientation of neurons for second-order contours and luminance contours distributing within ±45° were also consistent with those results and acceptable due to the relatively smaller responses [15]. Using optical imaging method, we further confirmed that neurons responsive to contrast grating in cat A18 had similarity in their orientation preference for contrast grating and luminance grating. The strength of signal was even weaker when compared with results obtained from electrophysiological recording due to the involvement of the non-responsive neurons, which is also comparable to previous results [24,37]. In addition, the activation of contrast gratings can be found in whole cat A18, implying the responsive neurons uniformly distributed to be organized to a functional map.

These results suggest that some early visual cortex neurons can detect the non-luminance differences across the second-order contours, generate orientation-selective responses to second-order contours, even when the spatial frequency of carrier is lower enough for its luminance contours activating neurons. The processing of second-order contours with equally and comparably spatial-scaled carrier in early visual cortex is similar to that of the second-order contours with carrier at higher spatial frequencies, and that of luminance contours.

![Figure 5](image1.png)

**Fig. 5.** Distribution of cross-correlation coefficients between differential maps to different visual stimuli: (A) Masked differential maps to luminance (left) and contrast (right) gratings. The mask was determined upon the distribution of blood vessels on a surface view of the imaged region, and manually improved to mask out most blood vessels. (B) Significant positive correlation between Map G and Map C. (C) Weak negative correlation between Map G and Map T. (D) Little correlation between Map C and a control map which was obtained by rotating Map G by 180°. Arrows point to the means of cross-correlation coefficients.

![Figure 6](image2.png)

**Fig. 6.** The orientation specificity of the correlation between map to contrast grating and that to luminance grating. The correlation between differential map to contrast grating at cardinal orientation and differential maps to luminance gratings at varying orientations (every 22.5° from −90° to 90°) were plotted against the difference in orientations of two gratings. A von Mises function was fit to the correlations. The left plotting represent the same example used in Figs. 4 and 5.
4.1. The differences in T and T′ responses were accounted for by contrast-defined contours

In present study, we measured contrast grating responses by comparing responses to T and T′, which were different in the phase of G11 and resultantly in contrast grating. Although T and T′ look very different, few known differences in T and T′ other than the contrast grating seem to induce the differences in responses to T and T′. Firstly, the differences in phase should not bring absolute differences in average luminance, because we have averaged responses to T and T′ covering whole temporal periods. Secondly, T and T′ have Fourier component gratings with same contrasts, orientations and spatiotemporal frequencies, so they should be the same in the responses accounted for by the linear filter; in the cross inhibition among responses to different Fourier components, and in the vector sum of Fourier components representing the velocity and direction of pattern motion. More than others, the differences in responses to T and T′ had the peak values when the contrast grating at the neurons preferred orientation, strongly suggesting that they were accounted for by the contrast grating. Similar assumption is well accepted and applied in the researches on pattern motion processing and illusory contour processing [2,17,33].

Our success in separating responses to second-order contours on the other hand indicated that the responses to T′ represented most of responses arising from contributers other than contrast grating. In our experimental conditions, either the responses to all three Fourier components or the cross inhibition (can be deduced from data in Fig. 2) among them were significant, when the carrier spatial frequency was set as the neurons optimal value for luminance contours. These findings confirm that the responses to second-order stimulus in early visual neurons are not the simple sum of responses to carrier and those to second-order contours intuitively, but consist of the responses to all luminance Fourier gratings, responses to second-order gratings, and the cross-inhibition between responses to all gratings at different orientations. Neither carrier nor the three individual Fourier component gratings should be the proper control for the purpose of separating second-order contour responses from luminance contour responses. This principle should be followed when recording other second-order contour responses, as presented previously [38].

Mathematically, this method is standard and applicable to all types of “contrast grating” stimuli with varying carrier spatiotemporal frequencies, angular separations between the contrast grating and the carrier luminance grating, but it still needs to be mention that responses to contrast-defined contours when examining with our method, might be underestimated because of the neglect of the inhibition of Fourier components to the second-order contours, and the neglect of the responses to slight second-order contours at same orientation in control stimulus.

4.2. Neuron's spatial selective range for carrier eliciting second-order contour responses

An important feature of second-order responses is their selectivity for carrier spatial frequency [20,29,38,42]. Neurons spatial preference and selective range for carrier eliciting second-order contour responses, which are determined by the mechanisms underlying detection of non-linearity [25], are different from those for luminance contours based on on-off receptive fields [10,14].

It has been reported that for neurons in cat LGN [25], A17 and A18 [42], the optimal spatial frequencies for carrier eliciting second-order responses were much higher than those for luminance contours and varied in a wide range. This preference for higher spatial frequency of carrier is consistent with visual experiences, and our impression that less responsive neurons and a little smaller responses have been observed in our conditions, when the spatial frequency of carrier was much lower than that in previous studies. However, those studies also reported that in cat A17, A18, neurons spatial selective range for carrier eliciting contrast contour responses was totally beyond that for carrier eliciting luminance contour responses [41], implying the second-order contours with equally spatial-scaled carrier will not have the chance to be processed in early visual cortex. This is inconsistent with our visual experience and the visual responses recorded in our experiments when the spatial frequency of carrier was optimal for it eliciting luminance contour responses. Many differences in recording methods might account for this discrepancy. One possibility is that neurons have varied spatial selective ranges, but to avoid responses to luminance contours, previous studies only tested the neurons with second-order stimulus with carrier at much higher spatial frequencies and resultantly only neurons with higher spatial preference for carrier have been selected. To make it sure needs direct comparison. More similar to our results, other studies employed in cat LGN [25] and cat A17, A18 [39], primate MT [20] showed an overlap rather than a total segregation between the two selective ranges.

So far, compared with previous implication that early visual cortex neurons can only alternatively respond to luminance contours or second-order contours depending on carrier spatial frequency [29,38], our results suggest that early visual cortex neurons can simultaneously respond to luminance contours and second-order contours when the spatial frequency of carrier is relative lower. At population level, the non-linear mechanism's selective range for carrier spatial frequency should be much wider than previously thought. The mechanism underlying this selective range and preference for carrier remains unknown.

The selectivity for carrier is one of the important characteristics of second-order contour responses. This result provides new information for the understanding of mechanism underlying second-order processing, which is more consistent with the subcortical origin of the responses [25] than the cortical filter model [4]. According to previous results [20], the overlap between the selective range for carrier and that for luminance grating might be larger in higher cortex. Interesting questions can be asked including the mechanism underlying this difference and the role of higher cortex in second-order contour processing, by measuring second-order contour responses which are not mixed with first-order contour responses.

4.3. The role of cat A18 in signaling contrast grating's orientation

Cat A18 neurons can respond to second-order contours with orientation preference similar to that for luminance contours. The response to these two kinds of contours formed similar orientation maps. This “form-cue invariance” [2] suggested that the orientation information of second-order contours have been included in cat A18 activities just as they were luminance contours with different orientations and contrasts or they were second-order contours with carrier at higher spatial frequencies. Although under our condition, the responses to second-order contours were smaller than and mixed with those to luminance contours, it is still reasonable to suppose that these information have the potential to be extracted in a separate pathway or decoded latterly in higher brain in a way similar to that for luminance contours. Thus, our results suggest that based on calculating the non-linear differences in neurons' on-off receptive fields, cat A18 might be capable of signaling the orientation of second-order contours and supporting their perception. Whereas whether the perception of second-order contours depends on cat A18 signaling or there are other higher processing independent of this cat A18 signaling needs further investigation on this kind of second-order responses in higher brain areas.
In summary, with the application of the well-known spatiotemporal energy model, we successfully removed the responses to luminance contours and demonstrated orientation-selective responses in some early visual cortex neurons to second-order contours with carrier at same spatial frequency. This manner of processing in early visual cortex is similar to that for luminance contours and that for second-order contours with carrier spatial frequency much higher, suggesting uniform processing might underlie the visual detection of luminance contours and second-order contours including some illusory contours with either finer or relatively coarse background textures.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

We thank the advices from Yi Wang, Curtis L. Baker Jr, Anna Wang Roe, Jianyong Wu, Xiaoying Huang and Ying Liu. We also thank Xiaoian Li for his cooperation in setting up optical imaging system. This work was supported by the National Basic Research Program of China (2005CB724301), the National High Technology Research and Development Program of China (2007AA02Z313), the National Natural Science Foundation of China (509408020) and the Chinese Academy of Sciences (KSCX1-YW-R-32).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.brainresbull.2011.06.004.

References