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5. M. M. Kater, G. M. Koningstein, H. J. J. Nijkamp, A. Banerjee, G. M. Koningstein, H. J. J. Nijkamp, A. Banerjee, 5.2 Å, a = 60.9 Å, c = 79.0 Å, and b = 92.9° with a tetramer in the asymmetric unit (16). Data were collected to 2.5 Å (Table 1, data set Native-1) on a twin San Diego Multi- 

6. Escherichia coli ENR is a homotetramer (M ~ 28,000 per subunit) that was prepared from an overexpressing E. coli strain (2, 5). Crystals of the ENR-NAD+ (crystal form A) belong to space group P212121, and have unit cell dimensions of a = 74.0 Å, b = 81.2 Å, c = 79.0 Å, and b = 92.9° with a tetramer in the asymmetric unit (16). Data were collected to 2.5 Å (Table 1, data set Native-1) on a twin San Diego Multi-

7. Crystals of the ENR-NAD+ -diazaborine complex (crystal form B) belong to space group P6322 and have unit cell dimensions of a = 74.0 Å, b = 80.9 Å, c = 328.3 Å, α = β = 90°, and γ = 120° for the thienodiazaborine complex, and a = b = 80.6 Å, c = 325.3 Å, α = β = 90°, and γ = 120° for the benzodiazaborine complex in a dimer in the asymmetric unit (16). Data sets were collected on the ENR-NAD+ -thienodiazaborine complex to 2.2 Å and on the ENR- 

8. Crystals of the ENR-NAD+ -diazaborine complex (crystal form B) belong to space group P6322 and have unit cell dimensions of a = 74.0 Å, b = 80.9 Å, c = 328.3 Å, α = β = 90°, and γ = 120° for the thienodiazaborine complex, and a = b = 80.6 Å, c = 325.3 Å, α = β = 90°, and γ = 120° for the benzodiazaborine complex in a dimer in the asymmetric unit (16). Data sets were collected on the ENR-NAD+ -thienodiazaborine complex to 2.2 Å and on the ENR- 

9. Crystals of the ENR-NAD+ -diazaborine complex (crystal form B) belong to space group P6322 and have unit cell dimensions of a = 74.0 Å, b = 80.9 Å, c = 328.3 Å, α = β = 90°, and γ = 120° for the thienodiazaborine complex, and a = b = 80.6 Å, c = 325.3 Å, α = β = 90°, and γ = 120° for the benzodiazaborine complex in a dimer in the asymmetric unit (16). Data sets were collected on the ENR-NAD+ -thienodiazaborine complex to 2.2 Å and on the ENR- 

10. Several cycles of rebuilding and refinement gave a final R factor for the model of 0.157 (52,346 reflections in the range 10 to 2.2 Å, 7836 atoms including 324 water molecules), with an rmsd of 0.01 Å for bonds and 2.9° for angles. The average B factor for the dimer is 24 Å2 (20 Å2 for main-chain atoms, 20 Å2 for diazaborine atoms). For the ENR-NAD+ -thienodiazaborine complex, 24 Å atoms superimpose with an rmsd of 0.3 Å, whereas for the two ENR-NAD+ -diazaborine complexes, 216 Å atoms superimpose with an rmsd of 0.2 Å.


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19. Responses to subjective contours in visual cortical areas V1 and V2 in adult cats were investigated by optical imaging of intrinsic signals and single-unit recording. Both V1 and V2 contain maps of the orientation of subjective gratings that have their basis in specific kinds of neuronal responses to subjective orientations. A greater proportion of neurons in V2 than in V1 show a robust response to subjective edges. Through the use of subjective stimuli in which the orientation of the luminance component is invariant, an unmasked V1 response to subjective edges alone can be demonstrated. The data indicate that the processing of subjective contours begins as early as V1 and continues progressively in higher cortical areas.

Orientation Maps of Subjective Contours in Visual Cortex

Bhavin R. Sheth, Jitendra Sharma, S. Chenchal Rao, Miriganka Sur

Responses to subjective contours in visual cortical areas V1 and V2 in adult cats were investigated by optical imaging of intrinsic signals and single-unit recording. Both V1 and V2 contain maps of the orientation of subjective gratings that have their basis in specific kinds of neuronal responses to subjective orientations. A greater proportion of neurons in V2 than in V1 show a robust response to subjective edges. Through the use of subjective stimuli in which the orientation of the luminance component is invariant, an unmasked V1 response to subjective edges alone can be demonstrated. The data indicate that the processing of subjective contours begins as early as V1 and continues progressively in higher cortical areas.

Contours that are perceived under stimulus configurations in which the stimulus lacks any physical discontinuity (such as a luminance border) are termed subjective or illusory contours. Subjective contours and subjective shapes can be perceived in a manner analogous to the perception of luminance

Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology (MIT), Cambridge, MA 02139, USA.

*To whom correspondence should be addressed at Department of Brain and Cognitive Sciences, MIT, E25-235 Cambridge, MA 02139, USA. E-mail: mrsur@wocmit.edu.


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29. We thank the support staff at the Synchrotron Radiation Source at Daresbury Laboratory for assistance with station alignment. Supported by grants from the UK Biotechnology and Biological Sciences Research Council (BBSRC) and Medical Research Council (D.W.R. and A.R.S.). C.B. is funded by a Zeneca Agrochemicals--supported CASE award. J.B.R. is a BBSRC David Phillips Research Fellow. The Krebs Institute is designated a BBSRC Biomedical Science Centre.

2 August 1996; accepted 15 October 1996
more, whether cells responsive to subjective edges are organized into maps, and how the organization relates to that of cells responsive to luminance edges, remains open. We now demonstrate that cells in both V2 and V1 of cats are responsive to subjective edges, and cells with the same subjective orientation preference are clustered to form maps that bear a systematic relation to maps of orientation preference for luminance edges.

Cats perceive subjective contours (4) and are able to discriminate fine differences in the orientation of subjective edges (5). We investigated the ability of subjective contours to drive cells in V1 (area 17) and V2 (area 18) of adult cat visual cortex (6) using intrinsic signal optical imaging (7) and single-unit recording. To quantitatively compare cortical responses to subjective and luminance stimuli, we used luminance gratings composed of light and dark bars (or thinner lines), and subjective gratings composed of orthogonal inducing lines (Fig. 1A). If neurons in visual cortex signal the subjective orientation and ignore the orthogonal inducing lines (luminance edges), the locations of orientation domains imaged with luminance and subjective gratings should match closely (Fig. 1A, left). Thus, for each pixel comprising the optical map, the orientation preference for subjective and luminance gratings should be nearly identical. A histogram of pixel count showing the difference in orientation preference obtained with the two types of stimuli should therefore yield a histogram with all of the pixels comprising the bin at 0° orientation difference (Fig. 1B, left). Conversely, if neurons respond solely to the inducing lines and ignore the orthogonal subjective edges (Fig. 1A, right), maps for luminance and subjective gratings should be complementary (compare, for example, the hypothetical maps at upper left and lower right of Fig. 1A). A histogram of pixels showing the difference in orientation preference between the two maps would therefore show all pixels in the bins at ±90° orientation difference (Fig. 1B, right). In either instance, pixels at an orientation difference intermediate between 0° and ±90° would represent combined responses to both subjective and luminance components of the subjective grating (Fig. 1B). The single-neuron responses that underlie such optically imaged maps are shown in Fig. 1C. The tuned response of a cell to a luminance grating is shown in vector form in Fig. 1C (left), whereas possible responses to subjective gratings with orthogonal subjective and luminance orientations are shown in Fig. 1C (right). A cell could respond to the subjective component alone (Fig. 1C, right, “pure subjective response”), to the inducing lines alone (Fig. 1C, right, “pure luminance response”), or to a combination of the two (Fig. 1C, right, “combined responses”). These response types would be represented in the orientation difference histograms of Fig. 1B as lying, respectively, in the 0°, ±90°, or intermediate orientation difference bins.

Responses from a patch of V2 (8) to gratings of similar luminance and subjective orientations are shown in Fig. 2, A and B, respectively. Their orientation domains overlap in spatial location and extent. The composite luminance and subjective grating maps (9), combining the responses at all stimulus orientations (10), are shown in Fig. 2, C and D. We determined the relative contributions of the subjective and luminance components of the subjective grating to the cortical response by computing the difference in orientation preference between luminance and subjective gratings for each pixel (a map of “subjective signal strength”) (11). The resultant difference
map is shown in Fig. 2E (regions with an orientation difference less than ±22.5° are shown in black, regions with an orientation difference greater than ±67.5° are shown in white). The orientation difference map shows that a substantial portion of V2 (coded in black) has a preference for similar luminance and subjective orientations. The map also demonstrates that the orientation preference of neurons for the luminance and subjective components of subjective gratings varies smoothly across the cortical surface (darker shades of gray represent progressively stronger preference for the subjective edges relative to the inducing lines). Neurons with a given response preference are clustered, and neurons with adjacent preferences are organized, in specific places, in a radial, pinwheel-like manner (Fig. 2F shows a typical radial organization at a higher magnification). The density of pin-
wheels is 2.3 per square millimeter. The difference histogram (Fig. 2G), quantifying the difference map of Fig. 2E, shows that a large proportion of pixels respond to similarly oriented luminance and subjective gratings. These results, which are representative of maps from V2 in three animals, demonstrate that many neurons in V2 respond in an orientation-specific manner to subjective gratings, and that these neurons are organized into maps of orientation preference that bear a systematic relation to those for luminance gratings.

The spacing between inducing lines is important in the perception of subjective contours (5). We doubled the spacing between the inducing lines (and also doubled the thickness of each individual line in order to maintain the same overall stimulus luminance) and observed a marked change in the V2 response. The same cortex that showed a clear response to subjective edges composed of a high density of inducing lines (Fig. 2G) now gave a strong response to the inducing lines with little or no response to the subjective edges in the stimulus (Fig. 2H). Thus, changing the density of inducing lines changes the physiological response of V2 to subjective gratings.

Single-unit recordings in V2 (12), in which the same stimuli were used as for optical imaging, showed that individual neuron responses were consistent with the imaging results. A V2 cell that responded best to the same orientation of luminance and subjective gratings is shown in Fig. 2I (i and ii). Apart from such neurons that respond to subjective edges alone, V2 also contains neurons that respond to a combination of subjective edges and inducing lines (Fig. 2I (iii)) or to the inducing lines alone (Fig. 2I (iv)) in the subjective grating. Of all cells recorded in V2 that responded to subjective gratings (38 of 57 cells), 21 cells showed a response to subjective gratings with a preferred orientation within ±10° of the preferred orientation of luminance gratings (Fig. 2J), indicating that these cells responded almost exclusively to the subjective edges in the subjective grating. For another 13 cells, responses to subjective gratings were tuned to an orientation intermediate between ±10° and ±70° of their preferred luminance grating orientation, indicating that these cells carried signatures of both luminance and subjective components in their responses (13). Together, the majority of V2 neurons in our sample (60% of cells) conveyed information about subjective orientations (14).

We next examined V1 responses to subjective gratings constructed with stimulus parameters derived from the known stimulus selectivity of V1 cells (15) (four animals). Maps of V1 in response to luminance gratings and to subjective gratings with a high spatial frequency of inducing lines are shown in Fig. 3, A and B, respectively. The orientation difference map showed the presence of regions (Fig. 3C, coded in shades of gray) whose response depended to a varying extent on the orientation of subjective edges. In specific regions of the map, these cells were also organized in a pinwheel-like manner. Although the orientation difference maps in V1 and V2 demonstrated that fewer pixels in V1 than in V2 prefer the same luminance and subjective orientation (compare Figs. 3C and 2E), the orientation preference for luminance and subjective components varied smoothly across the V1 map as well. The density of pinwheels in V1 (2.8 per square millimeter) was comparable to that in V2. The difference histogram quantifying Fig. 3C peaked at ±90° orientation difference but had other local maxima, some fraction of pixels at 0° orientation difference, and a high fraction of pixels with intermediate orientation differences (see legend to Fig. 3D). Thus, a demonstrable proportion of cells in V1 respond to subjective edges alone or in combination with luminance edges in the subjective grating (16).

Single cells in V1, as in V2, showed a range of responses to subjective gratings, although the proportion of cells with the various types of response was different in the two cortical areas. Figure 3E shows cells in V1 that responded almost exclusively to the orientation of subjective edges (i), to a combination of both luminance and subjective edges in the subjective grating (ii), or to the inducing lines alone (iii). Of the cells in V1 that responded to subjective gratings (35 of 43 cells), 3 had a preference for subjective grating orientation that was less than ±10° offset from the cell’s preferred luminance grating orientation, and 15 others showed a preference for intermediate orientations. Thus, the single-cell data confirm that a reasonable proportion of cells in V1 (42% in our sample) respond to either the subjective component alone or in combination with the luminance component of the subjective grating (17, 18).

To further examine the responses of V1 neurons to subjective edges, we devised stimuli to differentiate as unambiguously as possible...
sible between cortical responses attributable to the subjective and luminance parts of the stimulus. We imaged the response of visual cortex to a pair of stimuli that had identical inducing line orientation, identical direction of motion (orthogonal to the inducing lines), and that differed in a single stimulus parameter—the orientation of the subjective edge (Fig. 4A). We found distinct dark and light patches in V2 and V1 (19) corresponding to orientation domains for the two orthogonal subjective orientations. If the cortex did not contain columns segregated for subjective orientation preference, subtracting the response to one subjective stimulus orientation from that to the orthogonal orientation would result in a homogeneous optical map (the orientation signal from the inducing lines is the same for both stimuli, and subtraction of the two optical images will eliminate the common luminance signal). However, the resultant map consists of response clusters that are elicited by the subjective orientations (Fig. 4A). The orientation-specific response of a cell in V1 to the luminance-invariant subjective grating is shown in Fig. 4B. A majority of cells (11 of 13 cells) recorded in V1 showed an unequivocal response to a particular subjective orientation (Fig. 4C); 8 of these cells responded well to the inducing lines in the conventional subjective grating stimulus (with orthogonal inducing lines). Thus, the subjective response component of V1 cells appears to be unmasked when examined with subjective grating stimuli in which the orientation of the luminance component is invariant. When subjective gratings with orthogonal inducing lines are used, the important difference between V1 and V2 might be not whether V1 cells have a subjective component to their responses, but rather the extent to which a luminance component is expressed in, and masks, responses of V1 cells to subjective edges.

Cells in both areas V1 and V2 of cats are orientation-selective and cluster to form columns whose cells share the same orientation preference for luminance edges (20, 21) (Figs. 2C and 3A). We have confirmed that V2 contains neurons that respond to the orientation of subjective edges (2, 22), and we now show that (i) V1 also contains neurons that respond to the same kind of subjective stimuli, and (ii) in both areas, these neurons are clustered in discrete columns and organized into maps of subjective orientation preference. The maps show, in places, a radial, pinwheel-like arrangement of orientation differences (between preferred subjective and luminance orientations). A sizable number of cells in both V1 and V2 respond to at least a combination of subjective and luminance edges in the subjective grating. Specifically, V1 and V2 contain cells that respond almost exclusively to the subjective edges, the inducing lines alone, or a combination of the two. However, the relative proportions of these cells differ between the two areas: a greater proportion of cells in V2 than in V1 show a nearly exclusive response to subjective edges. At the same time, the majority of V1 cells can potentially convey information about subjective orientations, as revealed through the use of a luminance-invariant subjective stimulus. Subjective orientations appear to be signaled in a distributed manner, through the activity of large numbers of neurons in V1 and V2.

Our study shows that even a complex visual attribute, such as subjective contour orientation, can be extracted and systematically represented in the earliest stage of visual cortex, V1 (23). The processing of subjective contours continues to the next cortical stage, V2 (24), with more neurons providing the perceptual signals related to a subjective orientation. The presence of neurons responsive to subjective edges in V1 and V2, their clustering into modules, and their organization into maps argues for an intricate level of network organization that far exceeds the complexity associated with these areas so far (25).

Fig. 4. Responses in V1 to a stimulus in which the subjective orientation was varied without varying the orientation of inducing lines (luminance-invariant subjective grating). (A) Areas V1 and V2 of visual cortex contain clusters of neurons that respond preferentially to subjective orientations. Stimuli (left and middle) with identical oblique inducing line orientation and identical directions of motion orthogonal to the inducing lines (arrows), but with orthogonal subjective edge orientation (labeled 0° and 90°) were presented in a randomly interleaved manner. The optically imaged map (right) was obtained by subtracting the response to the stimulus at 90° from that at 0°. Dark patches show regions that prefer a 0° subjective grating over a 90° grating; vice versa for light patches. The arrows show the V1/V2 border. (B) Responses of a cell in V1 in another animal to the stimuli in (A). The abscissa shows the stimuli used (alternating vertical and horizontal subjective gratings moving in one of four different directions). The cell showed a consistently higher response to a vertical subjective grating (0° subjective orientation) than to a horizontal subjective grating (90° subjective orientation) for all directions tested. The cell’s spontaneous activity was subtracted from the responses. (C) Orientation indices [normalized orthogonal response expressed as percent (27)] for V1 cells (n = 13) recorded with the stimulus in (A). Lower percentage values indicate sharper orientation tuning.

REFERENCES AND NOTES

6. Adult cats (n = 18) were used in these experiments. All experiments were performed under protocols approved by MIT’s Animal Care and Use Committee. Anesthesia was induced with ketamine [15 mg per kilogram of body weight, intramuscular (im)] and xylazine (1.5 mg/kg, im) and maintained with isoflurane (typically 0.5 to 1.5% in 70/30 N2O/O2) delivered through a tracheal cannula. Cats were paralyzed with an intravenous combination of gallamine triethiodide (3.6 mg/hour) and tubocurarine chloride (0.15 mg/hour) and artificially respired to maintain end-tidal CO2 volume at ~4% at a partial pressure of 30 ± 3 mm Hg. The animal’s electroencephalogram and heart rate were monitored continuously to ensure adequate anesthesia. Crucotomy followed by duralysis was performed to expose visual cortex. For imaging area V2, we centered our chamber at A4 and recorded from an area that extended approximately from A5 to A7 in the anteroposterior direction and between L0.5 to L3.5 in the mediolateral direction. For imaging V1, the chamber was centered at P5 and a similar expanse of cortex was exposed. For imaging V1 and V2 simultaneously, the chamber was centered at A0 (R. J. Tusa, L. A. Palmer, A. C. Rosenquist, J. Comp. Neurol. 177, 213 (1979); R. J. Tusa, A. C. Rosenquist, L. A. Palmer, ibid. 185, 657 (1979)).
7. Techniques for intrinsic signal imaging were similar to those described by Girveld et al. [A. Girwald, E. Lieke R. D. Frostig, C. D. Gilbert, T. N. Wiesel, Nature 324, 361 (1986) and used by us previously (26)]. A stainless-steel recording chamber was positioned over the skull surrounding the craniotomy, filled with silastic oil, and then sealed with a quartz plate. A video camera (CCD-5024N Bishke, Japan, RS-170, >80 dB signal to noise ratio) consisting of a BS6 by 480 array of pixels equipped with a tandem-lens macroscope [E. H. Ratzlaff and A. Grinvald, J. Neurosci. Methods 36, 127 (1991)] was positioned over the craniotomy. This arrangement provided a resolution of 75 pixels per millimeter. Data were collected by means of an imaging system (Optical Imaging). The camera signal was amplified by a video enhancement amplifier; a baseline image was subtracted from each stimulus response image in analog form and then
We compared orientation strengths (magnitude of maximum signal as determined by our previous experiments) for these pixels. Thus, the response to subjective gratings in V1 was stronger for subjective gratings than for luminance gratings for this subset of pixels, although they occupied a small portion of V1.

Receptive field properties of a subset of V1 cells whose luminance and subjective orientation preference differed by ±45° or less were studied in detail. Three cells were complex; one was a simple cell. Two cells were end-stopped, four cells were non-end-stopped. Six cells whose locations were identified were encountered at depths ranging from 300 to 1200 μm from the surface. Among these responses is an extensive cytoskeletal reorganization and subsequent nuclear responses in host cells. Among these responses is an extensive reorganization of the actin cytoskeleton, requiring a high spatial frequency (0.5 cycle per degree) and low drift velocity (4° per second), which V1 neurons prefer, and a grating of low spatial frequency (0.15 cycle per degree) and high drift velocity (12° per second), which V2 neurons prefer [21]. By distinguishing the portion of the optically imaged region that responded preferentially to the slower drift rate, higher spatial frequency stimulus (V1) compared to the higher drift rate, lower spatial frequency stimulus (V2), we could locate the V1/V2 boundary accurately. The physiological border coincided with the anatomical border between areas 17 and 18 as demonstrated by marker lesions and histology. J. A. Movshon, I. D. Thompson, D. J. Tolhurst, Arch. Psychiatr. Nervenkr. 165, 559 (1963); ibid., 160, 165 (1962); A. Leopold and N. K. Logothetis, Nature 379, 549 (1995).

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(December 20, 1996)
Science 274 (5295), 2110-2115. [doi: 10.1126/science.274.5295.2110]

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