Optical images of visible and invisible percepts in the primary visual cortex of primates

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We optically imaged a visual masking illusion in primary visual cortex (area V-1) of rhesus monkeys to ask whether activity in the early visual system more closely reflects the physical stimulus or the generated percept. Visual illusions can be a powerful way to address this question because they have the benefit of dissociating the stimulus from perception. We used an illusion in which a flickering target (a bar oriented in visual space) is rendered invisible by two counter-phase flickering bars, called masks, which flank and abut the target. The target and masks, when shown separately, each generated correlated activity on the surface of the cortex. During the illusory condition, however, optical signals generated in the cortex by the target disappeared although the image of the masks persisted. The optical image thus was correlated with perception but not with the physical stimulus.

Before measuring optical images of primary visual cortex (area V-1) during illusory stimulation, it was essential to see first whether a nonillusory stimulus was correlated with the optical image. We began with the technique of sweeping a grating in both eyes separately, and then subtracting the two images from each other to create an ocular dominance difference image (1–3). The results are shown in Fig. 1*a*. Next, we examined the effect of a flickering bar in the appropriate retinotopic position in one eye. We then subtracted an image of the same piece of cortex, unstimulated. The results (Fig. 1*b*) show that the flickering target created a strip of activity on the cortex that correlated with the flickering bar in the visual field. One can furthermore see in Fig. 1 *c* and *d* that the amount of optical signal within the activated region corresponds to the variance predicted by Hubel and Wiesel (4) in the ocular dominance domain (red outlines from ocular dominance boundaries). By moving, widening, and varying the orientation of the bar we examined correlated changes in position and size of the image (data not shown). A nonillusory stimulus therefore generates activity in V-1 that is predicted by the known functional anatomy of the early visual system.

Under certain circumstances, a visual stimulus may be rendered invisible by another stimulus (5, 6), and this effect is known to be present in monkeys as well as in humans (7–11). Illusions of this kind are known as visual masking effects. Here we used a type of visual masking in which a flickering target (a bar) of 50-ms duration is preceded and succeeded by two counter-phase flickering masks (two bars that abut and flank the target, but do not overlap it) of 100-ms duration (10). This cyclic illusion (Fig. 2) proceeds so that either the target or the mask is invariably on the screen, but never both. (This illusion, the "standing wave of invisibility" can be seen dynamically on the worldwide web at http://cortex.med.harvard.edu/~macknik.)

Methods

Stimuli. Stimuli were displayed at 100% contrast on a Mitsubishi Diamond Scan monitor. White stimuli on a black background were used in the images shown, but black on white stimuli generated similar images.

Subjects. Experiments were done in two juvenile male rhesus monkeys, each with similar results. Optical imaging and electrophysiology techniques for recording from anesthetized paralyzed animals were conducted by using standard techniques that have been described (12).

Image Processing. Images were sampled at a rate of 32 Hz by using a custom 8-bit analog video system using a 720-nm light source. Each stimulus was recorded for 20 sec, resulting in an average image of 640 frames per condition. Each image had a resolution of 512 × 480 pixels and was approximately 1.2 cm across on the cortical surface. Images shown here have been further processed with MATLAB using standard techniques (1, 3, 12–14). Specifically, they have been cropped to a size of about 1 cm^2 and smoothed with a Gaussian filter having a standard deviation of 4 pixels, and their look-up tables have been normalized and equalized. Additionally, the ocular dominance image in Fig. 1 *a* and *c* was smoothed a second time with a Gaussian kernel (with a SD of 25 pixels) to highlight large-scale variations in brightness across the image (which are presumably unrelated to ocular dominance), and these large-scale variations then were subtracted to create a more refined final map of ocular dominance (3, 13, 14). The edges of ocular dominance bands in Fig. 1 *c* and *d* were calculated with the "Laplacian of Gaussian" edge-finding function in MATLAB after creating a binary image of Fig. 1*a*.

Results and Discussion

We examined the cortical images generated by the target alone, the masks alone, and the target and masks presented together in the illusory condition. As in Fig. 1*b*, the target alone generated a strip of activity on the surface of the cortex (Fig. 3*a*). The masks, presented alone, also generated activity on the cortex in the form of two strips of activity separated by a space of lesser activity (Fig. 3*b*). Finally, the target and masks presented together in rapid alternation generated an image similar to that seen when the masks were presented alone (Fig. 3*c*), as if the neural representation of the target had been suppressed.

From the unprocessed data we statistically compared the change in reflectance for the central 100 columns of pixels in the target only condition (Fig. 3*a*), where target activation was evidently the strongest, to both the mask only condition (Fig. 3*b*) and target and mask condition (Fig. 3*c*). To do this comparison, we calculated a three-way ANOVA ($P < 0.0001$) between conditions and tested post hoc contrasts with a Tukey's multiple comparison analysis: the target only condition had significantly ($P < 0.0001$) higher change in reflectance than either the mask only or target and mask conditions. To ensure that the results were not a function of variability between measurements of the three conditions, we recalculated the statistical results after normalizing the mean value of the first column in each of the raw images to zero and found the same level of statistical significance.

It seems clear that any cortical inhibitory circuits brought into play by the masks did not give rise to optically detectable activity in

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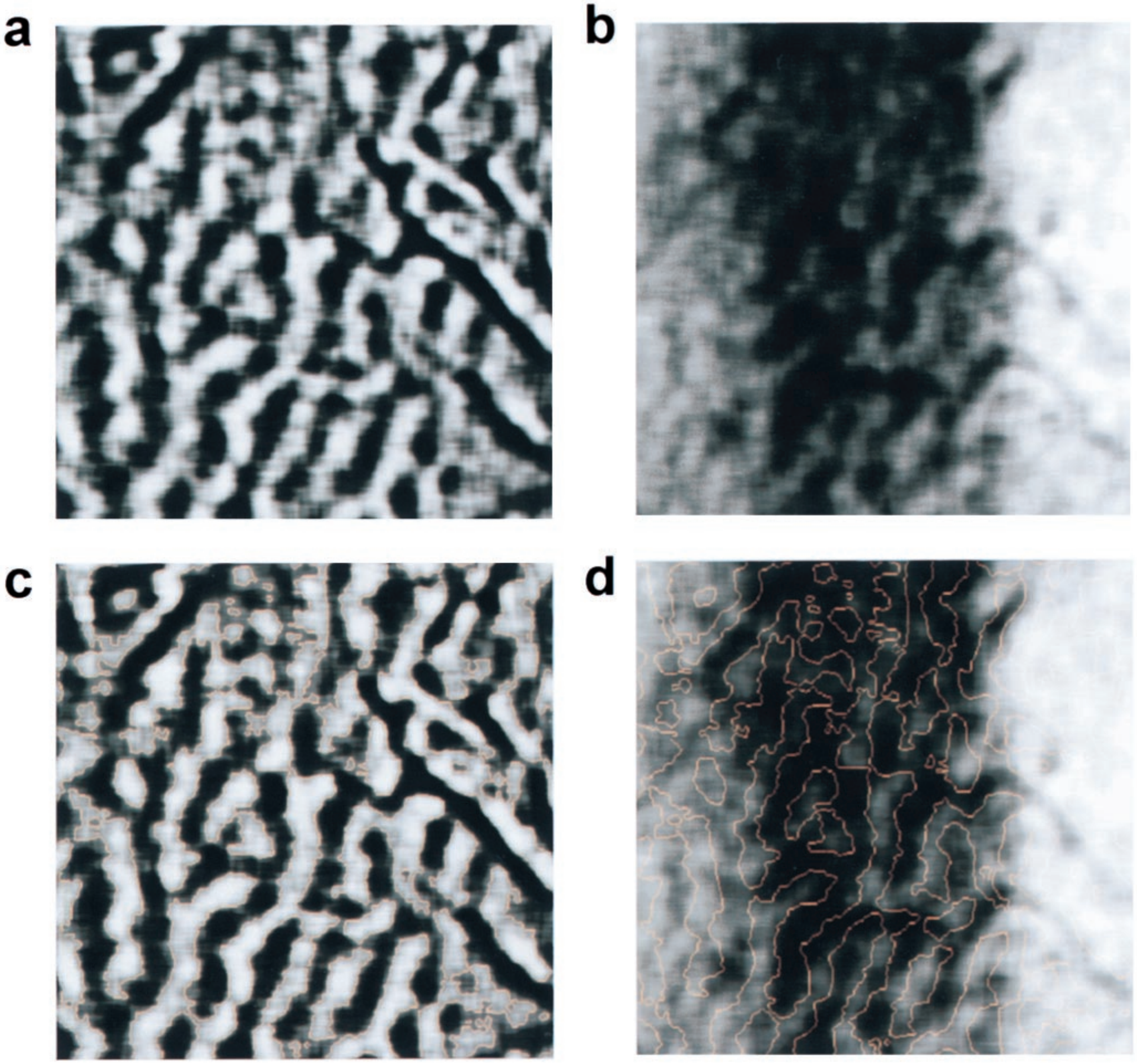

Fig. 1. The functional anatomy of area V-1 with nonillusory stimuli. (*a*) Ocular dominance bands within a 1 cm^2 patch of cortex that was 6° in the visual field periphery and subtended approximately 2° of visual space (as measured with electrode penetrations at each edge of the image). The vertical meridian is oriented along the lower edge of this image. (*b*) The same patch of cortex now stimulated with a flickering bar having a width of 0.48° and an orientation of 102°, with an image of the cortex unstimulated subtracted. (*c*) The edges of the ocular dominance bands are highlighted by an edge-finding algorithm (red lines). (*d*) The ocular dominance edges from *c* now overlaid on the image from *b*. Notice that the patchy signal within the stripe of activity matches the ocular dominance pattern.

either Fig. 3 *b* or *c*. Inhibition previously has been shown to generate negative or undetectable optical signals in the cortex (15, 16). The explanation for this surprising result may be that, if inhibition is occurring cortically, it involves fewer than one-fourth the number of neurons as excitatory processes (17). This numerical difference could be further amplified by the tendency of inhibitory neurons to synapse onto excitatory neurons in morphological positions that evoke the greatest inhibitory impact, such as on the dendritic shafts, on the cell body, or on the initial segment (17, 18).

In summary, neurons in the early visual system do not seem to solely reconstruct the visual scene in terms of the organization of the local functional anatomy, but instead seem to, in addition, reflect a stage in the construction of perception itself. An optical image of area V-1 therefore can be used in some situations to ascertain the current perceptual state of the subject.

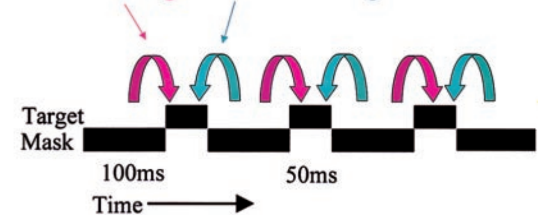

Fig. 2. The time course of events during the standing wave of invisibility illusion. A flickering target (a bar) of 50-ms duration is preceded and succeeded by two counter-phase flickering masks (two bars that abut and flank the target, but do not overlap it) of 100-ms duration that are presented at the time optimal to both forward and backward mask the target (10).

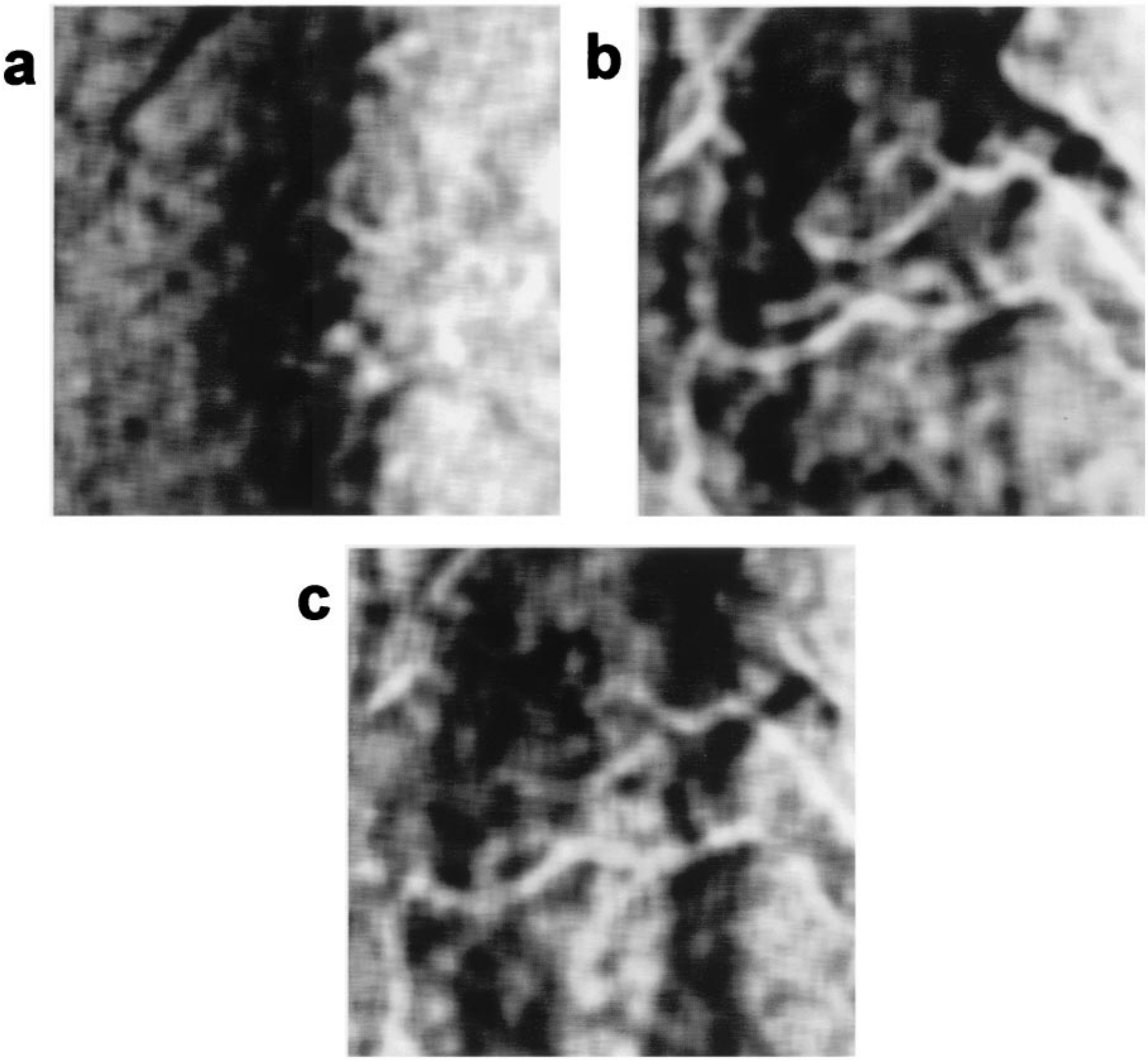

Fig. 3. Demonstration of perception-specific signals using illusory stimuli in the same patch of cortex. (*a*) A visual target with a width of 0.12° and 102° orientation. (*b*) Response to a masking stimulus presented alone. (*c*) Response to the target and mask presented together as the ''standing wave of invisibility.'' Notice that the representation of the target seen in *a* is now missing, just as it is invisible perceptually.

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