OPTICAL IMAGING OF CONTEXTUAL INTERACTIONS IN V1 OF THE BEHAVING MONKEY

Masaharu Kinoshita\textsuperscript{1}, Charles D Gilbert\textsuperscript{1} and Aniruddha Das\textsuperscript{2,*}

1. The Rockefeller University, 1230 York Ave, New York, NY 10065
2. Columbia University, Department of Neuroscience, 1051 Riverside Drive, Kolb Research Annex, Unit 87, New York, NY 10032

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* Author for correspondence prior to publication
Phone: 212 543-6931 x 200
Fax: 212 543-5816
e-mail: ad2069@columbia.edu

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Abstract

Interactions in primary visual cortex (V1) between simple visual elements such as short bar segments are believed to underlie our ability to easily integrate contours and segment surfaces. We used intrinsic signal optical imaging in alert fixating macaques to measure the strength and cortical distribution of V1 interactions amongst collinear bars. A single short bar stimulus produced a broad-peaked hill of activation (the optical point spread) covering multiple orientation hypercolumns in V1. Flanking the bar stimulus with a pair of identical collinear bars led to a strong nonlinear suppression in the optical signal. This nonlinearity was strongest over the center bar region, with a spatial distribution that cannot be explained by a simple gain control. It was a function of the relative orientation and separation of the bar stimuli in a manner tuned sharply for collinearity, being strongest for immediately adjacent bars lying on a smooth contour. These results suggest intracortical interactions playing a major role in determining V1 activation by smooth extended contours. Our finding that the interaction is primarily suppressive when imaged optically, which presumably reflects the combined inhibitory and excitatory inputs, suggests a complex interplay between these cortical inputs leading to the collinear facilitation seen in the spiking response of V1 neurons. This disjuncture between the facilitation seen in spiking and the suppression in imaging also suggests that cortical representations of complex stimuli involve interactions that need to be studied over extended networks and may be hard to deduce from the responses of individual neurons.

Keywords

contour integration; intracortical interactions; lateral interactions; intermediate level vision
**Introduction**

The perceptual qualities of local visual features are strongly influenced by the surrounding visual context. This context-dependence is believed to subserve processes of contour integration and surface segmentation, steps along the way to the full parsing of a visual scene. One sees a similar contextual dependency in the responses of single neurons in the primary visual cortex, suggesting an important role of this cortical area in visual spatial integration. Contextual modulation is seen when a simple stimulus feature such as a short oriented bar segment is embedded in a more complex surround (Blakemore and Tobin 1972; Bishop et al. 1973; Maffei and Fiorentini 1976; Nelson and Frost 1978; Gilbert and Wiesel 1990; Knierim and Van Essen 1992; Gulyas et al. 1987). Such contextual influence is now believed to be universal for V1 neurons, especially in the upper cortical layers that relay information to higher visual areas (reviewed e.g. Gilbert 1998). One particular class of modulatory interactions that is very powerful is that amongst arrays of collinear or parallel bars. A neuron’s response to a short bar element can be facilitated up to an order of magnitude by collinear flanks, with the facilitation decreasing as the flanks are moved apart, rotated, or shifted away from collinearity. Parallel flanks placed side-by-side lead to a similarly orientation- and distance-tuned suppression (Kapadia et al. 1995, 2000; Polat and Sagi 1994). This dependence on the geometry of the flanks reflects the orientation- and spatial specificity of long-range horizontal connections within V1 (Gilbert and Wiesel 1989; Stettler et al. 2002; reviewed, e.g. Fitzpatrick 2000) although feedback from higher visual areas has also been proposed to play a role (Angelucci et al. 2002). The geometry of interactions amongst such bar stimuli mirrors, closely, the influence of collinear or parallel
flanks on our perception of simple edges (Polat and Sagi 1994; Kapadia et al. 2000). Such intracortical interactions are believed to contribute importantly to the integration and saliency of contours (Field et al. 1993; Kapadia et al. 1999; Li and Gilbert 2002) as well as the processing of simple textures and texture boundaries (e.g. Wolfson and Landy 1995, 1999).

Earlier experiments showing iso-orientation facilitation in V1 were based on extracellular electrode recordings. This restricted the studies to suprathreshold spiking responses of single neurons or small groups of neurons with no direct measure of the spatial distribution of contextual modulation over cortex (but see Bringuier et al. 1999). Optical imaging, on the other hand, visualizes patterns of activation over extended regions of cortex (e.g. Frostig et al. 1990; Grinvald et al. 1994; Lu and Roe 2007; Bosking et al. 2002; Xu et al. 2005). Further, the intrinsic optical imaging signal is believed to reflect dendritic input including inhibitory and subthreshold excitatory activation covering entire dendritic fields of cortical neurons, thus complementing the information available using extracellular electrodes (Das and Gilbert 1995). In the present study we used optical imaging of intrinsic activity in V1 of awake, behaving monkeys (Grinvald et al. 1991; Vnek et al. 1999; Siegel et al. 2003), coupled with electrode recordings, to study the interactions within simple arrays of oriented bars. In particular, we studied the activation induced by short bars presented either by themselves or flanked by pairs of similar bars in various collinear and oblique arrangements. Comparing these optical images with each other as well as with the corresponding electrode recordings allowed us to measure the suppressive or facilitatory interactions of the particular contextual configurations tested, and to analyze this interaction.
in terms of the relative orientations and separations of the individual bar elements forming
the stimulus arrays.

Materials and Methods:

All optical images and electrode recordings were obtained from three hemispheres of two
alert behaving adult male *macaca mulatta* (weight 5.0 - 6.5 kg).

1: Surgical preparation and routine maintenance of animals.

The animals were first prepared for behavioral training (on a fixation task: see below) by
surgically implanting a head post. After a period of a few weeks, once the animal had
learned to maintain adequate fixation, a shallow recording chamber of surgical stainless
steel with a removable glass window was implanted over the animal’s visual cortex. The
chamber was positioned over opercular V1 close to the V1-V2 border and the lunate sulcus.
A craniotomy was performed to expose a region of cortex about 20 mm in diameter and the
dura resected and replaced with an artificial dura of a clear, soft silicone sheet that allows
both optical imaging and electrode recordings (Shtoyerman et al. 2000). All surgeries were
performed under aseptic conditions, under general anesthesia, following the guidelines
provided by the National Institutes of Health. The animal’s end-tidal CO₂, EKG, EEG and
respiration rate were monitored during each surgical procedure. Appropriate analgesics and
antibiotics were given postoperatively to prevent infection and to minimize discomfort.

To minimize the risk of infection inside the recording chamber we followed a strict regimen
of opening and cleaning the recording chamber every day, under sterile conditions. The
chamber was opened and rinsed with sterile saline. The chamber was filled with warm agar (1.2 - 2% in sterile saline) and then, depending on the planned procedure, either a: sealed with a glass window for optical imaging; or b: closed with a perforated glass window for electrode recording; or c: sealed with a protective stainless steel cap to put the animal back in its cage. On the (few) occasions when we saw signs of infection, either systemic or topical antibiotics were applied after microbiological examination of the fluids inside the chamber.

2: Behavioral training of animals and control of eye fixation.

All training was done with operant conditioning using positive reinforcement with water or juice rewards. No aversive conditioning techniques were employed.

Training: The animals were trained on a simple fixation task as follows. The monkey pulled a lever on the primate chair to display a fixation spot. The animal’s task was to maintain fixation while holding the lever until the spot dimmed after a randomized duration (1-5 sec); at this point the animal had to release the lever within a brief time interval (e.g. 250 ms) to get a reward. The spot was made progressively smaller and the dimming step progressively subtler through the course of the animal’s training. The monkey also learned to ignore distracting stimuli (moving gratings and other visual stimuli that would be used later during experiments). By the final stage of the training, the animal would routinely maintain fixation for periods of 4 – 5 seconds. By this stage, the fixation spot size (less than 0.1º) and the subtlety of the dimming step were such that human observers would find it hard to detect dimming when looking as little as 0.25º away from the spot.
Eye tracking: The animal’s fixation was monitored using an eye tracker based on video imaging of the animal’s eye under IR illumination. The eye-tracking algorithm fits an ellipse to the image of the pupil (once every video field, at 30 frames/sec. i_rec Software and system: Matsuda et al. 2000). Our combination of presenting the animal with a difficult fixation task while monitoring eye position with this IR tracker has proven capable of controlling fixation to an estimated standard deviation of 0.1°; (Fig A1, A3 in Appendix) and of being able to return repeatedly to specific positions in visual space with the same accuracy (Fig A3 in Appendix). The diameter of fixation window was smaller than 0.85° (typical 0.75°) for small bar stimulus or smaller than 1.1° for wide area grating stimulus.

All visual targets were displayed on a cathode ray tube monitor (SONY GDM-520) placed at 150 cm from the animal in the dark room. The display area was 1088 x 816 pixels, 39.2 x 29.4 cm. A refresh rate was 100Hz. The display area was filled with uniform gray (3.2 cd/m², background). The fixation spot was 80 cd/m² and dimming to 35 – 65 cd/m². Stimulus contrast was calculated as

Contrast (%) = 100 x ( “stimulus luminance” – “background luminance” ) / ( “stimulus luminance” + “background luminance” ) .

All behavioral and control software were custom written. Overall experimental control (monitoring eye position, delivering rewards to the animal and synchronizing the optical imaging and electrode recording systems) was governed by the primary behavioral control software (Kaare Christian, Rockefeller University). Visual stimuli were generated using
STIM (Kaare Christian) while the communication between behavioral control software and the optical imaging system was through a custom serial port interface.

3: Optical imaging.

Instrumentation: The intrinsic cortical activity was optically imaged using standard techniques (Das and Gilbert 1995). The cortical surface visible through the glass window and artificial dura of the recording chamber was illuminated with light at 610 or 577 nm wavelength (interference filter, Oriel, 30 nm). Images of the brain surface were digitized and accumulated at video rates (Imager 2001 system from Optical Imaging Inc., with Bischke CCD-6012P camera and Matrox IM-640 imaging board) synchronized with the animal’s behavior through a serial communication line from the behavioral control software.

Acquisition: Once the animal learned to perform the fixation task correctly on more than 80% of trials we started optically imaging intrinsic visually driven activity in the animal’s cortex. With the animal seated in the primate chair, head fixed, the imaging camera was positioned over the cortical surface with the help of identifying surface vascular landmarks. The camera was then focused ~ 500 μm below the cortical surface when imaging cortical activation to defocus surface blood vessels and thus minimize their contributions to vascular artifacts (Ratzlaff and Grinvald, 1991; Bonhoeffer and Grinvald, 1996). The optical imaging data was acquired for two months after making the craniotomy, before dura regrowth obscured the imaging area.
Each trial started with the animal pulling a lever to show a fixation spot on a blank screen (Fig 1). The imaging cycle was triggered by the animal achieving fixation. 2 – 4 image frames of the cortical surface, each 400 ms, were first acquired with the screen still blank to be used as the reference image (“blank screen”) for that trial. The visual stimulus (grating or bar) was then presented at an eccentricity appropriate for the imaged region of V1, i.e. from the vertical midline to ~ 5º horizontally and from 0º to ~4º in inferior hemifield vertically, and the induced intrinsic cortical activity was optically imaged for a further 4 – 8 400-ms image frames. The animal had to maintain fixation until the spot dimmed. The animal’s fixation was monitored through each trial and the trial aborted if the animal broke fixation. The imaging cycle was repeated 8 to 20 trials for each stimulus condition, interleaved randomly. The minimum inter trial interval was 2000 ms.

On-line image analysis provided a reliable monitor of the quality of imaging data. Each image frame was digitized and stored individually on disk (that is, one data file contained data from only one trial/one stimulus condition) for more extensive processing off line.

**Processing:** Each image frame was divided by the base reference image for that trial (i.e. the sum of the initial 2- 4 “blank screen” images) to give the normalized stimulus-induced optical signal compensated, to first order, for inhomogeneities in illumination and slow changes in ongoing unrelated cortical activation.

if the reference image is given by: $f(x,y)$

the stimulus-induced image can be modeled as a fractional change:

$f(x,y) + \varepsilon(x,y) * f(x,y)$

and thus the divided image: $(1 + \varepsilon(x,y))$
where x, y are coordinates along the cortical surface. This normalized optical image gives, in principle, the stimulus-induced change in absorption.

A few outlying trials, caused by vascular artifact or by errors from the imaging machinery, were excluded from the analysis. We calculated a standard deviation of each divided image for each frame of each trial. If the standard deviation of the frame image exceeded 0.2% of the raw image intensity range, the trial containing that frame was excluded from our analysis. Only a few trials were excluded from our analysis. For example, Fig 8, which is the summarized results, consists of more than 2000 trials, but only 4 trials were excluded.

Minimizing any movement of the cortical surface; compensating for residual movement:

Particular care had to be exercised to minimize all relative movement between the camera and the cortical surface. This was achieved as follows. 1: The frame for the optical imaging setup was constructed using heavy-duty optic rail hardware (Newport Research) and stable custom-machined components. 2: During the recording session the animal’s head was attached to the optical imaging frame through a specially designed rigid head post. 3: Even after these structural modifications we found some slight residual lateral movement of the cortical surface with respect to the optic axis of the camera. Such lateral movements (leading to lateral drifts of ~ 50 to 100 μm over the course of a 2-hour recording session) were particularly problematic because of the frame-by-frame image division that went into our image processing. The cortical surface vasculature forms the most prominent feature of the raw image frame, a few orders of magnitude stronger than any stimulus-induced optical activation patterns. With cortical movement the vascular pattern in one image frame gets divided by a slightly shifted version of the same pattern, highlighting the edges of the
pattern and throwing the vasculature into high relief, obscuring any stimulus-induced activation (Fig 2). We corrected for this residual cortical movement as follows. For each recording session we set the first image frame of the entire session as the “template” frame. Every subsequent image frame was then automatically aligned with this template, offline. This was done using a gradient descent algorithm that shifted each frame so as to maximize the cross correlation of a given frame’s vascular pattern with the template (Roe 2007). These “shift-corrected” images were then used for further processing to bring out stimulus-specific optical signals relatively uncontaminated with vascular artifacts (Fig 2). This general procedure was followed for all the stimulus-induced activation patterns below.

Stimuli and corresponding optical maps:

1: Orientation map: gratings of 1 – 3 cycles / degree, of 4 or 8 orientations, drifting at 1 degree / sec were displayed in 1.5º to 5º wide square area. The shift-corrected normalized images for the different orientations were combined pairwise to get orthogonal difference images (“Horizontal” image divided by “Vertical” image, “45º” image divided by “135º” image etc). These images were then band pass filtered by convolving with Gaussian kernels (high pass: 750 μm FWHM: full width at half maximum; low pass: 12 μm FWHM) and combined to give a vector “polar” map of orientation preference and signal strength (Das and Gilbert 1995). A mosaic of such orientation maps was constructed over the exposed V1 surface.

2: Absolute visuotopic axes: The absolute X and Y coordinates were mapped using horizontal and vertical “candy striped” stimuli respectively – i.e. grating stimuli (4 orientations) placed behind a square wave mask of alternating clear and neutral gray
windows (H or V: 1 cycle / degree, duty cycle 50%. Fig 3) (Blasdel and Campbell 2001).
The edges of the mask – positioned at integer X and Y coordinate positions – thus defined
the family of H and V axes. The corresponding optical images defined the absolute
visuotopic coordinates on V1.

3: Bar stimuli: With the absolute visuotopic coordinates mapped on V1, bar stimuli (length:
0.25°, 0.5° or 0.2°, width between 0.04° ~ 0.083°) were positioned with respect to the
animal’s fixation point so as to be roughly centered within the imaged region of cortex. The
bars were swept back and forth perpendicular to their orientation, within a region of visual
space of the same size as the bar length; i.e. the 0.25° bar was swept over 0.25°, the 0.5° bar
over 0.5°. The corresponding optical images (“optical point spreads”) were low pass filtered
by convolving with a Gaussian kernel (48 μm FWHM). All optical images were found to
have a linear variation of the local mean signal value over the image plane, as though each
image were sitting on a tilted baseline of signal intensity. This is likely to be due to the
small residual shifts between images acting upon the non-uniform illumination, as follows:

Let the pattern of illumination over the cortical surface = f(x, y).

In this particular case, with illumination via optic fibers pointed at the cortical surface, the
profile is roughly parabolic, i.e. a second-order polynomial in cortical coordinates (x, y):

\[ f(x,y) = F_0 - (A(x - x_0)^2 + B(y - y_0)^2) \]

where \( F_0 \) is the peak value of the illumination at the cortical coordinate \((x_0, y_0)\).

Any small lateral shift \((\Delta x, \Delta y)\) in the cortex with respect to the camera (and the source of
the illumination that is fixed to the camera) gives an illumination profile:

\[ f(x+\Delta x, y+\Delta y) = f(x,y) + \frac{\partial f(x,y)}{\partial x} * \Delta x + \frac{\partial f(x,y)}{\partial y} * \Delta y \]

which = \[ f(x,y) - 2A(x - x_0) * \Delta x - 2B(y - y_0) * \Delta y \]
i.e. a plane as a function of (x, y) added to f(x,y). Thus with the inevitable small lateral
shifts in the cortex – and our process of “shift-correction” - our processing step of dividing
signal images by the “blank screen” image will in general add a tilted plane of intensity to
the resultant (Fig 4a).

To correct for this tilt in the intensity we selected ‘baseline’ regions of the image far from
the stimulated region – typically around the image perimeter – and fitted a regression plane
through those locations. This regression plane defined the ‘tilted baseline.’ The tilted plane
was unlikely to reflect a real vascular change since the planes were random in their tilt
direction from image to image on any trial. This regression plane was then subtracted from
the full image to give the baseline-corrected response (Fig 4b-e).

4: Electrode recordings:

For electrode recording sessions the regular glass window for optical imaging was replaced
with a perforated glass window and the chamber filled with 2 % agar in sterile 0.9% saline.
A hydraulic electrode advancer (Narishige), on a small light-weight micromanipulator
fabricated in the university machine shop, was mounted on the animal’s head using a set of
screws embedded in the acrylic cap at the time of the initial surgery. Electrodes were of
etched tungsten coated with a fine insulation of borosilicate glass (Merrill and Ainsworth
1972) that penetrate the artificial dura well without leaving large perforations. The signals
from the electrodes were filtered, amplified and passed to a window discriminator (A-M
Systems) for detection of spike events. The computer maintains a record of each spike
occurrence, eye position and reward.
Results:

Our findings are based on optical images and electrode recordings obtained from three hemispheres of two adult macaques, over a two-month period of daily recording sessions on each hemisphere. Each recording session yielded between 400 and 1,000 successful trials. A critical requirement of our experiments was to have the animal maintain accurate fixation during each trial. This was achieved by requiring the animal to perform a demanding fixation task, which ensured fixation to better than 0.25º during each trial.

Analysis of the IR eye tracker data, as well as of the cortical extent of optical signals induced by small visual stimuli confirmed that the animal held fixation with an averaged standard deviation of eye drift of less than 0.11º. Further, the animal could return reliably to the same point, with the same accuracy, day after day (see Methods and Appendix).

After obtaining maps of orientation and retinotopy covering the imaged region of V1, we started imaging responses to short bar stimuli (length and drifting distance = 0.25º or 0.5º for the near periphery at 3.6º - 4.6º eccentricity). For each trial we divided the stimulus-induced image by the image obtained during the blank screen display immediately preceding the stimulus and then corrected for any shift or tilt in the intensity baseline (see Methods). This gave the absolute intensity of the intrinsic optical signal, i.e. the stimulus-induced change in cortical reflectance. At this eccentricity the optical signal from a single 0.5º bar formed a roughly elliptical patch (“optical point spread;” Fig 5) with a width ranging from 1.8 mm to 2.8 mm (Full Width at Half Maximum of a 2-d elliptical Gaussian fitted to the optical signal patch, measured along the minor axis) and a shape that depended
on the orientation of the stimulus bar. Localized orientation maps obtained by vectorially combining the optical signals from bars of different orientations matched the full orientation map over the same region of cortex. A 0.25° bar at the same eccentricity gave an optical spread that was roughly circularly symmetrical with a diameter of 1.5 mm – 2.8 mm and a shape independent of the stimulus bar orientation.

Our primary finding was that the optical point spreads of single bar stimuli were suppressed when the stimulus bar was flanked by collinear bars placed immediately adjacent. **Fig 6** shows a typical example of the optical signals induced by **a**: a single oriented (horizontal, 0.25°) bar (‘single-bar’ image); **b**: a pair of identical bars flanking the (empty) location of the single-bar (‘flanks-alone’) and **c**: center together with flanks forming a collinear array moving in synchrony (‘three-bar’). Notably, the three-bar image appeared distinctly weaker over its center, than even the single-bar image (**Fig 6e**).

To see if the reduced signal strength in the three-bar image could be explained as a form of multiplicative gain control we compared the three-bar image with the ‘linear sum’ of the single-bar plus flanks-alone (**Fig 6c** bottom). The three-bar image was significantly weaker than this ‘linear sum’ but only over the region of the center bar, not the flanks. The suppression, ‘SUPP’, defined as the difference signal (‘linear sum’ MINUS ‘three-bar’) (**Fig 6g**) appeared as a subtractive reduction at the center, rather than a multiplicative gain control extending over the entire image.
Since the suppression was confined to the region of the center bar, we decided to quantify it as the reduction in the effective ‘contribution’ of the center bar to the three-bar image. We defined this ‘contribution’ as the difference between the three-bar image and flanks-alone image (Fig 6i). A 2-d Gaussian fitted to this difference image was both lower in amplitude and narrower in width relative to a similar Gaussian fitted to the single-bar image (0.39 X in amplitude (X = the amplitude for the single bar, Fig 6h), 0.56 X in peak width). These numerical ratios comprised our measure of the suppression in the three-bar array. We then systematically investigated the effect of varying array geometry (bar separation, relative orientation) and contrast.

The observed suppression of the optical point spread was strongest when the flanking bars were immediately adjacent to the central bar and diminished monotonically with increasing separation between flanks and center (Fig 7). We found this relationship over all of our recording sites (Fig 8). The peak height of the difference image was suppressed to 0.12 – 0.37 X at the closest bar separation (Fig 8b1, b3, b5). As the flanking bars were further separated from the central bar, the suppression was greatly reduced, and was negligible at the separation over 0.8 - 1.0º. The most pronounced peak width suppression (0.52 – 0.68 X) was seen at the sites away from the fovea (eccentricity 3.6º ~ 4.6º, Fig 8b2, b4), but was not seen closer to the fovea (eccentricity 1.6º ~ 1.9º, Fig 8b6). This suppressive effect of flanking bars was independent of stimulus contrast (data over a range of 20% ~ 80% contrast, Fig 8a1, a2).
We measured the specificity of the suppression for the relative configuration of the central and flanking bars by changing the orientations of all three bars together while holding their positions fixed. Thus 0° orientation gave a collinear configuration, 90° a parallel side-by-side configuration, and so on (Fig 9). In each case we compared the image induced by the three-bar array (three 0.5° bars of identical orientation) with the images induced by the center alone or the flanks alone. Only the array of 0° bars – i.e. a collinear array of horizontal bars – showed flank-induced suppression. The other three non-collinear arrays of bars – at 45°, 90° or 135° – showed no or weak suppression. For each of these non-collinear arrays the amplitude of the difference image was indistinguishable from that of the corresponding single-bar image (apart from signal artifacts due to large blood vessels). We found the same orientation-specificity of flank suppression to hold across different bar sizes, contrasts and eccentricities (Fig 9f). At the collinear (0°) configuration, the amplitude at the center bar of the difference image was reduced to 0.12 ± 0.08 X (standard error of mean (SEM), number of recording sessions = 5). At non collinear (45°, 90° and 135°) configuration, the corresponding value was 0.69 ± 0.05 X (N = 15).

A comparison of the images obtained while moving the center and flanking bars in-phase vs. counterphase (Fig 10) provided additional evidence that the flank-induced modulation reflects intracortical interactions relevant to contour integration. When the bar elements formed a continuous contour, as happens under the in-phase condition, we observed that the flank-induced nonlinearity (the peak height of the difference image) was suppressed to 0.39 X (Fig 10b). On the other hand, when we broke the linkage between the contour elements by moving them in counterphase, the resultant image was remarkably close to the linear...
sum of the single-bar and flanks-alone, with neither suppression nor facilitation of the signal arising from the center bar (the peak height of the difference image was 0.96 X, Fig 10d). This counterphase signal was thus not only stronger than the in-phase signal, it was also stronger than the single-bar image (Fig 10e). Two important points follow. First, the observed nonlinearity was present when center and flanks formed a smooth contour. If such a geometric coherence was lost (as in the counterphase), the contributions from center and flanks added linearly. Second, the optical image was capable of being stronger than the single-bar image and in-phase three-bar image indicating that saturation is not an issue either in the neuronal or in the optical signal (Fig 10f; the average of 44 normalized in-phase three-bar signal strength at the center bar location).

While collinear flanks suppressed the optical point spread from a single bar, they had the expected facilitatory effect on the spiking responses of upper layer V1 neurons. Using standard extracellular microelectrodes (etched tungsten in glass), and guided by the V1 surface vascular pattern we recorded neuronal spiking responses from neurons in the same region of V1 that we had imaged, using the same visual stimuli employed for the optical imaging data (i.e. the same set of moving collinear bar and flank stimuli with bar lengths, bar separations, movement traverse and stimulus contrasts identical to those used for the optical imaging). As reported previously (Kapadia et al 1995), we found that the presence of collinear bars tended to facilitate rather than suppress the responses of neurons to short bars of their preferred orientation. This facilitation was strongest at the closest approach of the flanks to the center stimulus bar (e.g. Fig 11) and dropped off with increasing separation between flanks and center. The spatial extent of the facilitation measured with...
extracellular electrodes matched quite closely the extent of the inhibition measured optically. Over the 6 neurons (recorded from hemisphere-1) tested for flank-modulation, 2 showed facilitation (peak factors of 2.89X & 1.48X respectively), 1 showed suppression (0.70X) and the others showed no modulation.
Discussion:

Our results indicate that the V1 intrinsic optical imaging signal gives a valuable measure of the intracortical interactions underlying V1 processing of complex visual stimuli, complementing results obtained with extracellular recordings. The nonlinearity in cortical activation in a three-bar array, as visualized optically, showed a dependence on the spatial relationship of the central and flanking bars that reflects the dependence found earlier with extracellular electrode recordings. Thus, consistent with earlier electrode recordings, the interaction is strongest with bars that are collinear and at their closest distance of approach, and drops off as the bars are moved apart or away from collinear. This strengthens earlier suggestions that such interactions form part of the cortical machinery of contour integration underlying our marked ability to perceive smooth edges and object boundaries in visual space (Field et al. 1993; Kapadia et al. 1995; Li and Gilbert 2002). But further, the optical imaging extends these earlier results considerably by revealing the pattern of interaction not just for single neurons but rather over a large area of the cortical surface.

In the imaging signal we found that the interaction between center and flanks in the three-bar array manifested itself as a tuned suppression of the signal strength relative to the linear sum of responses to the array components (i.e. single-bar + flanks-alone images). This suppression was seen over a wide range of stimulus contrasts (Fig 8) (cf. Carandini and Sengpiel 2004) and control experiments with counterphase as vs. in-phase three-bar evoked higher signal strengths showing that signal saturation was not an issue (Fig 10). The observed suppression appeared not as a result of multiplicative gain control extended over
the full extent of the three-bar, but rather, was confined to the region of the center bar (Fig 6g).

The sign of the nonlinearity measured by optical imaging showed a mismatch with that seen in spiking. Whereas collinear bars facilitated spiking (Fig 11; Kapadia et al. 1995), their effect in the intrinsic optical signal image was suppression at the center of the three-bar array.

An intriguing possibility underlying this mismatch is that collinear flanks may enhance spiking responses through a net withdrawal of inhibition rather than an increase of facilitation in the local neuronal network. Such a mechanism of contextual enhancement, by reducing net local metabolic demand, would be manifested as a reduced optical image strength. The imaging signal, reflecting metabolic activity, is dominated by synaptic inputs rather than spiking output (Mathiesen et al., 1998). Further, inhibitory PSPs, with their associated metabolic demands, make positive contributions to the imaging signal comparable to excitatory PSPs (Mathiesen et al., 1998). Thus if the visual context of a smooth contour reduces both excitatory and inhibitory inputs but in a way that shifts the overall balance towards excitation, the strength of the optical signal would decrease along with the increase in spike firing. Such a suppression of inhibition may therefore be as important a contribution towards the facilitation of neuronal responses by salient contours (Kapadia et al. 1999, 2000, Li and Gilbert 2002) as the direct excitation provided by long range horizontal connections (Stettler et al. 2002).
An alternative but less likely explanation for this mismatch between increased spiking and reduced imaging response is that it is only an apparent mismatch; namely, that it is due to the limited spatial resolution of intrinsic signal optical imaging (approximated by a Gaussian of $\sigma \sim 140$ micron; Polimeni et al. 2006) averaging across a flank-induced sharpening of orientation tuning (sharpening due to the longer effective stimulus: Henry et al 1974; Orban et al. 1979; Chen et al. 2005). By this alternative explanation, the collinear flanks facilitate responses (both spiking and local intrinsic signal) in cortical columns of neurons whose orientation matches that of the stimulus bar, while reducing responses in neighboring columns of neurons with more distal orientations further along the flanks of the orientation tuning curve. The limited spatial resolution of the optical imaging technique is then argued to average out this differential response across multiple orientation columns to give a net reduction in the signal strength over the point spread image.

This alternative explanation is unlikely, however, for two reasons. First, it requires profound flank-induced suppression at off-center orientations to counterbalance the facilitation at the peak and give a net reduction in the average signal; this is not seen either in the literature (with spiking) or in our recordings (optical imaging: Fig 12). Second, the spatial resolution of optical imaging is adequate to reveal flank-induced orientation sharpening. Such sharpening would have an orientation scale dependence comparable to the width of orientation tuning in macaque V1, i.e. $\sim 40$ deg (median full width at 1/sqrt(2) max, Schiller et al., 1976). This corresponds to a spatial scale of $\sim 170$ microns on cortex, which would be clearly resolvable in optical imaging. Indeed, the distribution of imaging signal intensity as a function of the underlying orientation columns does reveal weak flank-
induced orientation sharpening (Fig 12d, e). Despite this sharpening, however, the signal evoked by the collinear three-bar stimulus remained profoundly sublinear relative to the sum of responses to the stimulus components at all orientations. This suggests that mechanisms based on flank-induced sharpening of the orientation tuning are unlikely to account for the observed sublinear summation of the three-bar optical imaging signal.

Visualizing contextual interactions with optical imaging provides an additional dimension to the study of visual spatial integration that is complementary to measures based on spiking activity. The results of intrinsic signal optical imaging speak to a dynamic interplay between subthreshold excitatory and inhibitory signals that depends on the precise spatial arrangement and relative orientations of the elements that make up complex shapes. Optical imaging affords a unique tool for exploring these interactions over the extended spatial distribution of the response on cortex. Understanding this interplay is important for the further study of the way in which experience and top-down influences modulate contextual interactions in primary visual cortex (Ito and Gilbert 1999; Crist et al. 2001; Li et al. 2004).
Appendix

A: The accuracy with which the animal maintained fixation.

We took measurements while the animal performed a difficult contrast discrimination task which demanded that it foveate the fixation point over the entire duration of each trial. Our IR eye tracker monitored eye fixation and discarded trials where the animal broke fixation. Two lines of evidence give us confidence that the animal was maintaining fixation to a standard deviation of about 0.1° (standard deviation of eye position in each dimension, X or Y during fixation periods).

1: The eye tracker readings. The standard deviation (SD) in X & Y eye positions (over trials each lasting 4 – 6 sec; sampling rate: 30 Hz):

- SD in eye X position: i.e. orthogonal to stimulus drift: 0.06° (mean SD over 1306 trials. SDs for individual trials ranged from 0.002° to 0.11°; max eye drift: 0.57°)
- SD in eye Y position, i.e. along stim drift: 0.11° (mean SD over 1306 trials. SDs for individual trials ranged from 0.002° to 0.20°; max eye drift: 0.79°).

2: The precision and reliability of measured optical point spreads and spiking RF profiles.

(NOTE: optical point spreads were averaged over more than 10 trials per stimulus location, spiking RFs over more than 5, interleaved randomly over all locations in both cases.)

- 0.25° –long stimulus bars gave reliable, well-separated optical images on V1 with little overlap (Fig A1). Optical point spreads ranged upwards from 1.5 mm in width (Full Width at Half Max: FWHM: Fig 5, main text) which corresponds to 0.5° on
the cortical surface (cortical magnification factor = 3 mm / degree, measured from the visuotopy map. See Fig 3, main text). A point spread of 0.5° is comparable to the median RF size in upper layers of macaque V1 at this eccentricity (e.g. Kagan et al. 2002), as expected if the optical point spread reflected the population response of all neurons responding to the 0.25° stimulus bar.

Next, as seen in Fig A2, we could return reliably to the same locations, day after day, with the same accuracy apparent in the point spreads measured on any one day.

- Electrode recordings of stimulus-driven neuronal responses gave tight 2-d RF profiles with widths as low as 0.32 deg (range: 0.32 – 1.3 deg, mean: 0.62, N=10). See Fig. A3

The sizes of these measured spiking RFs or optical point spreads indicate that fixation errors cannot be larger than 0.1° (SD). If we model the intrinsic RF or point spread as $exp (-x^2 / \sigma^2)$, i.e. a Gaussian of standard deviation $\sigma$, we can model the measured point spread or
spiking RF as a convolution of the intrinsic Gaussian with another Gaussian of SD $\sigma_c$ representing the smearing out due to fixation errors in each trial as well as trial-to-trial variability. This gives a new Gaussian of width $\sigma_1 = \sqrt{\sigma^2 + \sigma_c^2}$. Since the measured RFs and point spreads had widths (standard deviations “$\sigma_1$”) comparable to the known RF widths for macaque V1 layer 2/3, i.e. “$\sigma_1$” ~ “$\sigma$” expected, the effective convolution width $\sigma_c$ must be less than 0.5$\sigma$. ($\sqrt{1^2 + 0.5^2}$ ~ 1.1). This suggests that fixation errors and trial-to-trial variability in fixation had a SD than ~ 0.1°. (Note, that for a Gaussian, \exp(-x^2 / \sigma^2) the FWHM: Full Width at Half Max and the SD, $\sigma$, are related through a constant factor: FWHM = 1.66 $\sigma$)
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Disclosures

None of the authors have any competing financial interests in the results of this study.
References:


Figure Legends:

Fig 1: Sequence for one imaging cycle: The trial starts with the animal pulling a lever, displaying a fixation spot (A). Once the animal has achieved fixation (B), the initial “blank screen” baseline optical image is acquired for 2 – 4 image frames each 400 ms in duration. The visual stimulus (grating or bar) is then flashed on (C); it remains stationary for 1 – 2 400-ms frames before starting to drift (D). The animal has to maintain fixation until the fixation spot dims (E) after 4 - 8 400-ms frames, 1.6 – 3.2 sec.

Fig 2: Reducing vascular artifacts by aligning all image frames to first frame:

a: Single normalized image frame (image frame divided by “blank screen” base reference frame) without shifting individual frames into alignment. b: Same normalized image after aligning each component frame to a common template. c: Ocular dominance image obtained without aligning frames. Small relative movements between “right eye” and “left eye” frames acts as an “edge enhancer” throwing vasculature into high relief. d: Same ocular dominance image after aligning all frames with a common template.

Fig. 3: Horizontal and vertical axes mapped on V1, 1º spacing.

a: Visuotopic V1 map of vertical axes, optically imaged with vertical “candy stripe” stimulus consisting of 0.5º-wide vertical strips of drifting grating alternating with strips of neutral gray. b: Visuotopic V1 map of horizontal axes, obtained with a horizontal “candy stripe” stimulus. Note that the edge of the grating is visible, lower right. c: Mapping absolute visuotopic axes (vertical) on V1. Individual visuotopic images of vertical stripes on V1 were joined into a mosaic using vascular landmarks to blanket the central imageable
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area of the craniotomy. The absolute x-coordinates of individual lines (broken line) were obtained by comparing with the optical images of small single stimulus bars at positions defined with respect to the monkey’s fixation point. The “candy striped” grating pattern is schematically drawn as the inset. **d: Absolute visuotopic horizontal axes.** Same as c but horizontal stripes. The absolute y-coordinates are indicated by broken lines. Each image in this figure consisted of 58 – 190 recording trials.

Fig 4: Fitting tilted baseline to point spread images. a: Schematic showing the effect of adding a tilted baseline to a model Gaussian point spread. b: Raw point spread image showing distinct gradient of darkening in the baseline towards the upper left. c: Regression plane is fitted to the part of the image lying outside the dashed circle. d: The fitted plane. e: Image after subtracting the regression plane. (All images on the same gray scale).

Fig 5: Optical images, i.e. optical point spreads generated by 0.5° and 0.25° stimulus bars. a: Point spreads generated by 0.5° bar: (top left): horizontal bar drifting vertically back and forth over a 0.5° region; (top right): vertical bar drifting horizontally. Contours mark 50% image density. Lower left: local optical map obtained by combining optical point spreads from bars at 4 orientations 0°, 45°, 90° and 135° into a “polar” map of orientation with hue encoding orientation preference – see key below – and brightness proportional to vector signal strength (Das and Gilbert 1995). Lower right: orientation map obtained using full field drifting gratings from the same region of V1. Note the match with the local orientation map around the arrowhead. Grid at 1 mm spacing is superimposed for ease of comparison. b: Point spreads generated by 0.25° bar. (Top): horizontal bar moved back and
forth vertically over 0.25°. (Bottom): The 50% contour (blue) is superimposed over the full orientation map, along with the 50% contour for the point spread from a 0.25° vertical bar drifted horizontally (yellow). The background orientation map is same as Fig 5a bottom right map, but its color saturation is reduced for emphasizing the contour lines. Two contour lines were well overlapped in 0.25° bar width condition.

Fig 6: Flanking collinear bars suppresses the optical point spread from a single bar: a:

Image on top: Optical point spread from a single 0.25° bar (horizontal bar as indicated by key above, 20% contrast, drifting back and forth over 0.25°). Image density encodes optical signal strength i.e. percent change in cortical reflectance, as shown in gray scale key below.

Graph below: Cross section profile at S1 (see inset d). (Scale bars: in image, bar: 1 mm; for profile, X-axis scale bar = 1 mm, Y-axis scale bar = 0.1% cortical reflectance change) b:

Optical image and signal strength profile from flanks alone. c: Optical image and signal strength profile (blue line) from three-bar array; bar separation = 0.2°. The gray line profile represents the ‘linear sum’ of the response profiles of the single-bar (a) and the flanks-alone (b). d: The contours for cross section profiles shown in a-c and g (S1), e (S2: cross section at center bar location) and f (S3: cross section at the flanks). On each profile, signal strength was averaged over about 0.4 mm width across the broken line. e: Cross section profiles at S2. Blue line: three-bar array. Red line: single bar. f: Cross section profiles at S3. Blue line: three-bar array. Green line: flanks alone. g: The profile of collinear suppression (black line: ‘SUPP’ defined = ‘linear sum’ MINUS ‘three-bar’). h: Top: Single-bar optical point spread, same as in a. The box below shows 3-D perspectives of the same image and of the Gaussian fitted to the optical image by least squares. The inset shows the cross-
section profile through the fitted Gaussian (black) superimposed on a cross section of the image (gray) i: Top: Difference image obtained by subtracting “flanks alone” (b) from “three-bar image” (c). Below: 3-D perspectives of this difference image and the fitted Gaussian. Inset: profiles through image and fitted Gaussian. Gaussian height & width as fractions of the single-bar Gaussian fit (shown as thin dashed line). All scale bars as in a: i.e. horizontal: 1mm, vertical: 0.1% cortical reflectance change.

Each image in this figure is average of 13 – 16 recording trials.

**Fig 7:** Flank suppression drops with increasing bar separation: a: Optical signal from three-bar array, with center bar of the same size and location as in Fig 6, but with flank bar separation = 0.8°. b: Flanks alone. c: Top: Difference image for 0.8° bar separation (Fig 7a minus Fig 7b). Bottom: Gaussian (black) fitted to difference image (gray) with height, width measured as fraction of single-bar Gaussian fit (shown as thin dashed line). d: An example response profile of one recording session. Left: Suppression of peak height as a function of flank separation. Heights of Gaussians fitted to difference images, normalized by the height of single-bar Gaussian fit. Right: Suppression of peak width as a function of flank separation. Widths of fitted Gaussians similarly normalized to single-bar Gaussian width. (Normalized value = 1 implies no nonlinear suppression; 0: full suppression. Error bar represents the standard error of the mean (SEM))

Each image in this figure is average of 8 – 15 recording trials.

**Fig 8:** Population results of the signal suppression depending on the bar separation.
Summarized results recorded from 3 hemispheres. a1: normalized peak heights are plotted against the bar separation. Each curve represents a result taken from one recording session. In each recording session, each stimulus condition was repeated at least 8 trials (in most case repeated more than 10 trials). The blue curve represents result of low contrast (≤ 30%) stimulus condition. The red curve is high contrast (> 30%) condition. In any contrast condition, the response profiles were similar. The stronger suppression observed at the closer bar separation. Eccentricities of the recording sites were between 3.6° and 4.6°. These data were recorded from hemisphere-1 (b1) and hemisphere-2 (b3). The error bar was omitted in this plot for the visibility of the graph. a2: normalized peak width for each recording session. Other conditions are same as a1. b1, b3, b5: averaged normalized peak heights. b2, b4, b6: averaged normalized peak width. b1, b2: averaged data recorded from hemisphere-1 (monkey-1). 5 recording sessions (recorded in 3 days) were averaged. Eccentricities of the recording sites were between 3.6° and 4.6°. Stimulus bar length was 0.25°. Stimulus contrasts were between 20% and 80%. b3, b4: data from hemisphere-2 (monkey-2) averaged of 7 recording sessions (recorded in 3 days). Eccentricity was 4.3°. Bar length was 0.25°. Contrasts were between 20% and 60%. b5, b6: data from hemisphere-3 (monkey-2) averaged of 9 recording sessions (recorded in 3 days). Eccentricities were between 1.6° - 1.9°. Bar length was 0.2°. Contrasts were between 65% and 85%. The abscissa of each graph represents the bar separation in degree. Each error bars in b1-b6 represents SEM across the sessions.

Fig 9: Flank suppression is orientation specific: a (“0°”): Left: Optical point spread from a single 0.5° horizontal bar. Center: Difference image, obtained by subtracting optical
image due to collinear flanks alone from image due to a horizontal array of three collinear horizontal bars moving in synchrony (stim contrast: 90%; flanks at separation: 0.1°). Right: Signal strength profiles along the diagonal line (white broken line on the left image). Broken line curve: profile for single bar; Solid line curve: difference image. [Scales: image: 1 mm. Graph: abscissa: 2 mm; ordinate: 0.1% cortical reflectance change. Image is shown at saturated contrast.] b (‘45°’): Same as top row, but for stimulus bars oriented at 45°. The center bar and flank bars had the same center positions as in the horizontal bar stimuli in the top row. The signal profiles (right) were also taken at the same diagonal line shown in Fig 9a. c (‘90°’); d (‘135°’): Same as second row, but with 90° and 135° bars respectively. e: One recording session example of heights of Gaussians fitted to difference images (Fig 9a-d center), normalized in each case to the corresponding single-bar Gaussian fitted to single-bar image (Fig 9a-d left), as a function of stimulus bar orientation. (Fitted Gaussians are not shown but it was calculated as indicated in Fig 6h and 6i) The error bar represents SEM. Number of trials were 11-13 for each orientation condition. f: The averaged Gaussian heights over multiple recording sessions, as a function of bar orientation. The error bar represents SEM. 5 recording sessions (in 4 days) were performed on two monkeys. Stimulus contrast were 80% to 90%. The eccentricities were 1.7° to 4.2°. The bar length were 0.2° to 0.5°.

Fig 10: Suppression by flanks moving in phase and in counterphase with central bar.: a: Three-bar optical image and its cross section profile (black line). Same experiment as in Fig 6. The gray line profile represents the ‘linear sum’ of the response profiles of the single-bar and the flanks-alone. (same as Fig 6c: shown again for ease of comparison) b:
corresponding difference image, signal profile and fitted Gaussian (same as Fig 6i). c: Three-bar image but with flanks moving in counterphase to the center bar and its cross section profile. Black line: counterphase profile. Gray line: ‘linear sum’. d: Difference-image for three-bar counterphase array, signal profile and fitted Gaussians; heights, widths normalized to those for single-bar image (thin dashed line). Scale bars as in Figs 6, 7: horizontal bar = 1mm; vertical = 0.1% change in cortical reflectance. e: Comparison of the cross section profiles. Black line: counterphase three-bar (Fig 10c). Solid gray line: in-phase three-bar (Fig 10a). Broken gray line: single-bar (Fig 6a). The strongest signal was seen in counterphase stimulus. This means that both in-phase three-bar and single-bar optical signal were not saturated. All cross section profile in this figure is measured at S1 location indicated in Fig 6d. f: Population data for the relationship between in-phase signal strength and counterphase strength. The signal strength was measured in the region around the peak position of the single-bar image (width 0.5 ~ 1.0 mm) and averaged for each stimulus condition (each condition was repeated for 11 ~ 20 trials). The in-phase signal strength was normalized by the maximum counterphase signal in the same recording session. The average of 44 in-phase condition data is plotted. The error bar represents a standard deviation.

Each image in this figure is average of 11 - 14 recording trials.

**Fig 11: In electrode recordings, collinear flanks facilitate suprathreshold spiking responses of V1 neurons:** An example of single neuron response. a: Dark gray: peri-stimulus time histogram (PSTH) of spiking response to single-bar at the preferred orientation of the recorded neuron. b: Light gray: The response to the single bar is
facilitated by collinear flanks which do not, by themselves, stimulate the neuron (e). Dashed line shows the response to the single bar, for comparison; bar below PSTH indicates stimulus duration. d: Neuron response to single-bar (dark gray), and to three-bar (light gray) as a function of the separation between flanks and center (PSTH averaged over the time interval 100 – 400 ms after stimulus onset). The facilitation by the collinear flanks is strongest at the closest approach between flanks and center. The t-test was significant (P<0.01) for comparison between “center bar alone” and each of 0.2°, 0.4°, 0.6°, 0.8° separation and not significant between “center bar alone” and 1.0° separation. The error bar represents SEM. Number of trials was 11 - 12 for each condition. e: Normalized differential response, which was calculated as in Fig 7d. Flanks-alone response was subtracted from three-bar response and this difference was divided by the single-bar response.

Fig 12: Suppression of three-bar optical imaging signal relative to the linear sum is not explained by selective orientation-tuned suppression induced by the flanks. a: Profile of signal intensity along test lines lying along (left) or across (middle) the axis of the 3-bar image. Color coded as shown (green: single-bar; gray: flanks alone; red: 3-bar; blue: linear sum). Test lines shown on inset image (of 3-bar response). b: ROIs for calculating orientation tuning, shown on a single-bar and 3-bar image. c: ROI aligned over underlying orientation map. d: Orientation tuning calculated within ROI for the different bar combinations (color code as in panel a). Top: shown with image scale starting from zero; bottom, scaled to magnify single-bar and 3-bar results. e: Same, normalized to single-bar signal.
Figure Legends for the APPENDIX:

Fig A1: Precision of the fine-grained optical map of retinotopy:

a: Individual point spreads from 0.25° bars located on a grid with 0.25° spacing: We optically imaged V1 responses to single 0.25° stimulus bars, (each drifting within a 0.25° region in visual space) centered at specified locations with respect to the fixation point. Here we show a (single day’s) set of 21 individual optical images, each corresponding to a different bar center location (average ~ 10 trials per location, interleaved). The images are arranged by stimulus position: neighboring positions represent a horizontal or vertical shift of 0.25° in visual space. (Images labeled by the bar location; gaps in the image: where the bar was shifted by 0.5°. Colored contours mark the 66% peak height. Arrow marks the point spread due to a stimulus bar centered at (1.5°, -0.5°); the same stimulus location is used again in Fig A2.)

b: Neighboring stimuli 0.25° apart form distinct point spreads on cortex. All the 66% contours of individual point spreads shown in Fig A1a are combined and superimposed on the vascular map of cortex. Note, for example, the well-separated set of contours (red) marking point spreads lying along the vertical axis at x=1.75. The few irregular contours with large areas of overlap with their neighbors come from point-spread images distorted by large underlying blood vessels.

NOTE: In this set of images we show the 66% contours rather than the 50% contours as in the main text, to get a sharp estimate of point spread peaks. This also minimized the distortion of the outline by big blood vessel artifacts for the few images where such artifacts were prominent (see position 2.25, -0.5)
Fig A2: Point spreads from a given spatial location superimpose closely on each other from day to day:

a: Point spread for stimulus at location (1.5, -0.5) with respect to the animal’s fixation point (see arrow, Fig A1a).

b: The contour from Fig A2a, superimposed on the vascular map of cortex taken during the same imaging session.

c: Point spread for a stimulus bar at the same location (1.5, -0.5) with respect to the animal’s fixation, imaged on a different day. The point spread appears shifted because of a slight shift in the camera position.

d: The 66% contour from Fig A2c, superimposed on the vascular map of cortex taken during the same recording session. The contour falls on the same location with respect to the underlying vascular map as the contour in Fig A2b.

Fig A3: 2-D RF histogram of extracellular spiking response, recorded with metal electrode through artificial dura.

Top: individual PSTHs arranged by stimulus position (stimulus: 0.1° x 0.1° square flashed over a 5 x 5 grid with 0.1° spacing; 9 trials each position, randomly interleaved).

Bottom: 2-D RF profile, mapped as average spike rate for initial (40 – 140 ms) or sustained (200 – 600 ms) phase of neuron response. Contour maps were drawn using a smoothed surface spline-fit to 2-D RF profile, with contours at 10 spikes / sec steps. Contour at 50% peak (thick line) gives an RF dimension = 0.28° (Full Width at Half Maximum).
peak height (normalized)

peak width (normalized)

flank separation (degrees)

contrast <=30%  blue
contrast > 30%  red
a. center bar alone

RF

b. bar with collinear flanks

separation: 0.2 deg

c. flanks alone

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d. Spikes / sec

50 spk/sec

0 500 msec

0 500 msec

0 500 msec

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e. normalized difference

0 20 40 60 80 100

0.2 0.4 0.6 0.8 1.0

0.0 1.0 1.6

0.2 0.4 0.6 0.8 1.0

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center bar alone

0.2 0.4 0.6 0.8 1.0

0 80 60 40 20 0

center bar alone

0 500 msec

0 500 msec

0 500 msec
150.0 Spikes / sec.

800 msec.

Initial: 40 - 140 ms

Sustained: 200 - 600 ms

0.1 deg