Contrast invariance of functional maps in cat primary visual cortex

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Neurons in cat primary visual cortex (V1) are clustered according to their preference for stimulus position, orientation, spatial frequency, and eye of presentation, thereby giving rise to functional maps. It is not known, however, whether a similar arrangement is present for stimulus contrast. Neurons in cat V1 vary considerably in their contrast responses, and might be clustered in a systematic fashion in this respect. Additionally, stimulus contrast might affect other functional maps. For example, there has been debate over whether the contrast threshold of neurons in cytochrome oxidase blobs is lower than elsewhere. Here we have imaged intrinsic signals to measure orientation maps in cat V1 at a range of contrast levels. We determined, on a pixel-by-pixel basis, contrast-response functions and orientation tuning curves. The fit parameters describing contrast responses were more or less uniform: We found no regions where neurons have lower contrast threshold than elsewhere. If such regions do exist, their functional maps must be substantially weaker than maps of orientation preference. Moreover, we found that contrast has no impact on maps of orientation preference: The orientation selectivity of each pixel is invariant with stimulus contrast. The contrast invariance that we demonstrate at the level of maps is well known at the level of single neurons. It suggests that neurons contributing to a pixel response generally have similar orientation preferences or similar contrast responses. The latter explanation is likely to hold in pinwheel centers, where preferred orientation of nearby neurons can differ markedly. In summary, our data suggest that contrast is represented uniformly over the surface of cat V1, and changes in contrast do not affect maps of orientation preference.

Keywords: cortex, orientation, contrast, selectivity, imaging

Introduction

In cats and in other carnivore and primate species, the preferences for visual attributes by neurons in primary visual cortex (V1) vary in a more or less orderly manner across the surface (Hubel & Wiesel, 1974), giving rise to functional maps. Visual attributes determining maps are stimulus position, orientation, spatial frequency, and eye of presentation (Hübener & Bonhoeffer, 2002). Is a similar arrangement present for contrast? Neurons in V1 vary considerably in their responsiveness to contrast (Albrecht & Hamilton, 1982; Sclar, Maunsell, & Lennie, 1990). If neurons with higher and lower responsiveness were spatially segregated, contrast might be represented in form of a map.

Responsiveness to contrast is assessed by measuring a contrast response, a function relating stimulus contrast and neuronal response; in V1 neurons this function is commonly sigmoidal in shape, and saturates to an asymptotic value (Albrecht & Hamilton, 1982; Sclar et al., 1990). Contrast threshold is the contrast required to obtain a given threshold response; given that contrast responses saturate, this threshold response is commonly taken to be half of the maximal, asymptotic response, and the corresponding contrast threshold is called semisaturation contrast.

The physiological evidence for segregation of neurons with different contrast threshold is mixed. In macaque monkey, a study of 2-deoxy glucose uptake has suggested that contrast threshold might be lowest in cytochrome oxidase (CO) blobs (Tootell, Hamilton, & Switkes, 1988). Studies of single neuron responses, however, do not support this distinction (Hubel & Livingstone, 1990). Neurons inside blobs differ from those outside in contrast sensitivity—a measure used in signal detection theory to take into account response variability—but not in contrast threshold (Edwards, Purpura, & Kaplan, 1995). In a nocturnal primate, the distribution of contrast threshold across the surface has been suggested to be uniform (O'Keefe, Levitt, Kiper, Shapley, & Movshon, 1998). In the cat, instead, a preliminary report supports the notion that contrast threshold might be lower in blobs than elsewhere (Schulze, Bonhoeffer, & Hübener, 1999).

Here we address this issue with optical imaging of intrinsic signals in cat V1. We image responses to a range of stimulus orientations and contrasts, and ask whether any...
portions of V1 have contrast responses that are different from those of other regions.

We also ask whether contrast affects the map of orientation tuning. While the orientation selectivity of single V1 neurons is invariant with contrast (Sclar & Freeman, 1982; Anderson, Lampl, Gillespie, & Ferster, 2000), the same need not necessarily hold true for a pixel in a map, whose response reflects the summed activity of multiple neurons. The orientation tuning of the pixel response will be invariant with contrast only if neurons contributing to the pixel share the same orientation tuning or the same contrast response. The first condition, in particular, is unlikely to hold near pinwheels, singularities in the orientation map around which the full circle of preferred orientations can be found (Blasdel & Salama, 1986; Bonhoeffer & Grinvald, 1991). In these regions, nearby neurons can have widely different preferred orientations (Maldonado, Gödecke, Gray, & Bonhoeffer, 1997).

We find that in experiments with high signal-to-noise levels, it is possible to discern maps of orientation selectivity with contrasts as low as 6%-12%. As far as we can tell, these maps are invariant with contrast. Indeed, as is customarily done for single neurons (Sclar & Freeman, 1982), optical responses of each pixel can be described as the product of two functions, one determining orientation selectivity and one describing responses to different contrasts. We find that parameters of the latter do not vary systematically and one describing responses to different contrasts. The orientation tuning of the pixel response will be invariant with contrast only if neurons contributing to the pixel share the same orientation tuning or the same contrast response. The first condition, in particular, is unlikely to hold near pinwheels, singularities in the orientation map around which the full circle of preferred orientations can be found (Blasdel & Salama, 1986; Bonhoeffer & Grinvald, 1991). In these regions, nearby neurons can have widely different preferred orientations (Maldonado, Gödecke, Gray, & Bonhoeffer, 1997).

Portions of this study have appeared as conference abstracts (Carandini & Sengpiel, 2000; Sengpiel & Carandini, 2000).

**Methods**

Methods for physiological preparation and optical imaging of intrinsic signals are standard and have been described in detail elsewhere (Bonhoeffer & Grinvald, 1996; Sengpiel & Bonhoeffer, 2002).

Briefly, adult cats were anesthetized with an initial i.m. injection of ketamine and xylazine. Animals were intubated and artificially ventilated [60-65% \(N_2O\), 35-40% \(O_2\), 0.8-1.1% (1.5-2.0% during surgery) halothane]. Electrocardiogram, electro-encephalogram, end-tidal \(\text{CO}_2\), and rectal temperature were monitored continuously. A trepanation was made above area 17 of one or both cortical hemispheres. A stainless-steel chamber was cemented onto the skull, and the dura was removed. The chamber was filled with silicone oil and sealed with a cover-glass. Animals were paralyzed with a continuous i.v. infusion of gallamine triethiodide (10 mg/kg/h) in glucose-saline. The pupils were dilated with atropine hydrochloride, and the lids and nictitating membranes retracted with phenylephrine. Eyes were refracted using a refractometer and protected with gas-permeable contact lenses with 3.5-mm artificial pupils, which corrected focus for a viewing distance of 50 cm.

Optical imaging of intrinsic signals was performed in area 17 using either a cooled slow-scan CCD camera (ORA2001; Optical Imaging Inc., Mountainside, NJ) or an enhanced differential imaging system (Imager 2001; Optical Imaging Inc.), with the focal plane parallel to and ca. 500 \(\mu\)m below the surface of the cortex. The illuminating light was band-pass filtered at 700 ± 10 nm. The imaged areas subtended about 4 by 3 mm, with a pixel size of (22 \(\mu\)m)^2.

Visual stimuli were generated by a VSG Series Three (CRS, Rochester, UK) and displayed on a 20 in. monitor positioned 50 cm from the animal.

Stimuli were drifting gratings of various orientations (four or eight, between 0° and 180°) and contrasts (Figure 2A). Spatial frequency was constant (0.5-0.75 cycles/deg), and stimuli were presented binocularly. These were inter-leaved with blank-screen presentations. Mean luminance was kept constant at all times (36 cd/m²). Stimuli were flashed on and remained stationary for 9 s, and then drifted at 2 Hz back and forth for 3.6 s; data were collected during this period and the preceding 1.8 s. Each stimulus was presented 24-32 times.

Data were arranged in blocks consisting of a number of trials, with each trial representing one complete set of stimulus and blank-screen responses. Averaging of trials within each block (1 up to 16) was performed online. From responses to the blank screen we could observe clear variation in mean response from block to block. This variation was corrected by subtracting from each stimulus response the response to the blank screen in the same block. Mean responses were computed by averaging across all blocks of each stimulus.

In our analysis, we applied less filtering to the data than is currently common in the optical imaging field. First, we did not employ a “cocktail blank” and we did not compute any difference image; we simply normalized all responses to the average response obtained with blank stimuli (Bonhoeffer & Grinvald, 1996). Second, we applied only minimal high-pass filtering: we only equalized (by sum and subtraction) the overall spatial mean of responses to the same contrast. Indeed, we were interested in how responses change with contrast, and at high contrast the responses were stronger than at low contrast in large regions of the cortical surface. High-pass filtering the data (e.g., by removing the spatial mean) would have removed this signal. Third, we applied only minimal low-pass filtering, with a Gaussian filter having a SD of one pixel. This filter removed the most evident high-frequency noise but still allowed neighboring pixels to show different behaviors. Similar methods have been recently applied in a study of selectivity for orientation and direction (Swindale, Grinvald, & Shmuel, 2003).

From responses to 50% contrast stimuli, we computed the map of the orientation vector (Swindale, 1982; Blasdel & Salama, 1986). The value of this vector at pixel \((x, y)\) is a complex number.
\[ O(x,y) = \frac{\sum_{\theta} R_{\theta}(x,y)\exp(2i\theta)}{\sum_{\theta} R_{\theta}(x,y)} , \]

where \( R_{\theta} \) is the response to orientation \( \theta \) (which is between 0 and \( \pi \)), and the sum runs over the orientations tested. The angle of the orientation vector indicates preferred orientation, whereas the amplitude indicates strength of orientation tuning. The map of this orientation vector is illustrated in Figure 1B, where color indicates preferred orientation, and brightness indicates strength of orientation tuning (Bonhoeffer, Kim, Malonk, Shoham, & Grinvald, 1995).

In the rectangle that is imaged (Figure 1A), there is commonly a central region of interest that yields relevant signals, surrounded by areas that are out of focus because of the curvature of the brain, or covered by dura or by bone. To identify this region of interest, we employed an automated method. First, (as in Ringach, Hawken, & Shapley, 1997) we selected regions where strength of orientation tuning (the amplitude of the orientation vector) was between 0.05 and 1. The result was a “lake” of ones in a “land” of zeros. The lake had many small “islands” and jagged “coasts.” We then applied a median filter with 30 pixels on each side, and considered as the region of interest all pixels that had a nonzero value. The result was a lake with few or no islands, and smooth coasts (contour in Figure 1B).

To obtain a trace of the vasculature, we considered a conventional image of the cortical surface (Figure 1A) and applied the Canny method to it (The MathWorks, 1999). The Canny method finds edges by looking for local maxima of the gradient of the image. It uses two thresholds, to detect strong and weak edges, and includes the maxima of the gradient of the image. We applied the Canny method to it (The MathWorks, 1999). The result is a trace of the most obvious blood vessels (Figure 1D, red traces).

We fitted each pixel response to various orientations and contrasts with functions previously developed to describe single-cell responses (see Results). Parameters of the functions were found by minimizing the mean square distance between model and data.

We measured fit quality by the proportion of variance explained,

\[ \nu = 1 - \frac{\sum_{i}(m_{i} - r_{i})^{2}}{\sum_{i}r_{i}^{2}} , \]

where \( m \) is the model prediction, \( r \) is the mean optical response, and the index \( i \) runs over stimuli. When using this expression to judge the overall quality of a fit, we extended the sum to all pixels.

This definition of fit quality does not take into consideration the noisiness of the data: Even if a model predicted the responses exactly, neural variability and measurement noise would make it impossible to explain 100% of the variance.

We chose this conservative approach because our measurements of response variance are not as reliable as our measurements of response mean. The SD of the responses (across blocks) was computed offline. As the raw data had already been partially averaged online (within blocks), this method underestimates true response variability. To estimate SD more reliably, we averaged over stimuli, arriving at a single estimate of SD for each pixel (Figure 1C).

 Regions of high SD appeared to be strongly correlated with the trace of the vasculature (Figure 1D). Such “vascular interference” has long been recognized (Blasdel, 1992), and is likely to be artefactual (i.e., not related to differences in neuronal responses).

One-dimensional power spectra for the maps of parameters were obtained from two-dimensional power spectra by summing over concentric circles (Muller et al., 2000). To not unduly emphasize high frequencies, we further divided the result by the area of each circle. The power spectrum of the map of preferred orientation \( O_{p} \) was computed.
from the complex map \( \exp(2iO_o) \), with \( O_o \) in \([0, \pi]\). To reduce high frequency edge artifacts, the region of interest was windowed with a soft edge. Windowing reduced but did not eliminate edge artifacts (dashed curve in Figure 9).

**Results**

We recorded optical responses from V1 of four adult cats. Because the nature of our experiments involves low stimulus contrast, we present as examples data from the animal that gave the highest signal-to-noise ratio. Results for the remaining three are similar, and are summarized at the end of the Results section.

Examples of responses to stimuli differing in contrast and orientation are illustrated in Figure 2B. It is possible to discern by eye patterns of orientation selectivity already at contrasts of 12% or lower. As contrast increases, the overall optical response becomes stronger, but the pattern of response to each orientation appears to be constant. This contrast invariance is reminiscent of the properties of single V1 neurons, whose responses grow with contrast, while remaining constant in their orientation selectivity (Sclar & Freeman, 1982).

![Figure 2](https://jov.arvojournals.org/pdfaccess.ashx?url=/data/journals/jov/932828/)

**Figure 2.** Examples of stimuli and optical responses for two orientations and five contrasts. A. A subset of our stimuli, which included four or more orientations and in some cases additional contrasts. B. Optical responses, scaled by subtracting response to blank screen, and dividing by maximal recorded response. Dark shading represents activation.

We therefore ask whether the optical responses can indeed be described as contrast-invariant, and further, whether contrast responses are uniform across the cortical surface or whether they are mapped in some orderly fashion.

**Contrast invariance of orientation selectivity maps**

To test if optical responses are contrast-invariant, we fitted a simple model to the responses of each pixel, and judged whether the predictions of the model are satisfactory.

According to this model, the response of each pixel to a stimulus with orientation \( o \) and contrast \( c \) is the product of two functions:

\[
\text{Response}(o, c) = f(o) \cdot g(c)
\]

where \( f(o) \) depends only on orientation, and \( g(c) \) depends only on contrast.

The function \( f(o) \) determines orientation selectivity. As is commonly done for membrane potential and firing rate responses of single neurons (Carandini & Ferster, 2000), we take this function to be Gaussian:

\[
f(o) = R_{\text{max}} \exp\left(-\frac{(o - o_p)^2}{2\sigma_w^2}\right),
\]

where the triangular brackets express angles between \(-90^\circ\) and \(90^\circ\). This function has three parameters: maximal response \( R_{\text{max}} \), preferred orientation \( o_p \), and tuning width \( \sigma_w \). Their role in determining the shape of the function is illustrated in Figure 3A. As with a similar function recently used by others (Swindale et al., 2003), fitting a Gaussian function has an advantage over the usual method of computing an orientation vector (Figure 1B). This method corresponds to fitting a cosine function; such a function has two parameters (amplitude and phase) rather than three, and thus confounds responsiveness with sharpness of tuning.

The function \( g(c) \) determines the dependence of response on contrast. For this function, we take the expression commonly used for the responses of single neurons (Albrecht & Hamilton, 1982), the hyperbolic ratio:

\[
g(c) = \frac{c^n}{c_{50}^n + c^n}.
\]

Here, \( c_{50} \) is the semisaturation contrast, the contrast at which responses reach 50% of their asymptotic value, and \( n \) is the exponent, which determines the slope of the function. Their role in shaping the responses to contrast is depicted in Figure 3B.

We fitted the model to the responses of each pixel. These fits were obtained by considering the responses to all contrasts and all orientations. Because all parameters were allowed to vary freely from pixel to pixel, we call this the full model. This name will distinguish it from a reduced model that we will employ shortly afterwards.

An example of fit of the full model for one pixel (selected randomly) is illustrated in Figure 4. The model captures the main effects of contrast and orientation on the images, accounting for 92.8% of the variance of the pixel's responses. It correctly predicts that changing contrast scales the curve relating orientation to response (Figure 4A) and...
Figure 3. Model fitted to the responses of each pixel. The model is the product of two functions. A. The function \( f(o) \) that specifies selectivity for orientation is described by three parameters: maximal response \( R_{\text{max}} \), preferred orientation \( o_p \), and tuning width \( o_w \). B. The function \( g(c) \) determining how responses grow with contrast is described by two parameters, semisaturation contrast \( c_{50} \) and exponent \( n \).

that changing orientation scales the curve relating response to contrast (Figure 4B). There seems to be an underestimation of the response to \( 0^\circ \) at 50% contrast, but this underestimation was not systematic.

We let the model fit every pixel in the images independently, and then generated images for the predicted responses (Figure 5). These predicted responses closely resemble the optical responses (Figure 2B). Indeed, for this dataset the model accounts for 85.3% of the overall variance.

Figure 4. Examples of fits of the full model for one pixel (142,56). Curves are predictions of full model, where all five parameters are optimized for this pixel. Error bars represent two SDs. Gray area indicates two SDs of response to blank screen. A. Responses as a function of orientation, for two contrasts (○ 12%, ● 50%). Responses to remaining contrasts are omitted for clarity. B. Responses as a function of contrast for two orientations (○ \( 0^\circ \), ● \( 90^\circ \)). Responses to remaining orientations are omitted for clarity.

Figure 5. Predictions of the full model for the stimuli in Figure 2. These responses are predicted by fitting the response of each pixel independently of other pixels. The model thus allows the dependence of response on contrast to vary from pixel to pixel.

Fit quality was high throughout the region of interest, with the model explaining between 90% and 95% of the variance for the majority of the pixel responses (Figure 6A). Fit quality was lowest near blood vessels, as can be seen by superimposing a trace of the vasculature (Figure 6B) upon the map showing percentage of variance explained by the model. In these regions one can expect the quality of the data to be lowest because of artifacts caused by changes in blood flow and volume. Indeed, these are regions where the SD of the responses is highest (Figure 1D).

Figure 6. Map of fit quality for the full model. A. Percentage of the variance explained by the model for each pixel in the region of interest. B. Same map overlaid by a trace of the vasculature.

The fits of the full model involve leaving all five parameters free to vary from pixel to pixel. These parameters thus correspond to five maps.

Three of these maps correspond to the parameters of the orientation selectivity function \( f(o) \) (Figure 7). The map of preferred orientation (Figure 7A) shows the typical organization in which preferred orientation varies smoothly around singularities or pinwheel centers (Blasdel & Salama, 1986; Bonhoeffer & Grinvald, 1991; Hübener & Bonhoeffer, 2002). The map of maximal response (Figure 7B) is simply an indication of signal strength, and is likely to be related both to the experimental conditions (e.g., changing levels of illumination because of the curvature of the imaged region) and to the functional architecture of visual cortex (e.g., because of preferences for spatial frequency;
Bonhoeffer et al., 1995; Issa, Trepel, & Stryker, 2000). The map of tuning width indicates that selectivity is broad at locations corresponding to pinwheels and fractures. This observation might be explained by optical blurring, caused by close proximity of neurons with widely different orientation preferences, or by genuinely low orientation selectivity (Swindale et al., 2003).

These maps are closely related to others that have appeared widely in the literature. The map of preferred orientation (Figure 7A) is commonly obtained from the angle of the vector sum of responses to different orientations (Figure 1B). We have verified that this method gives results similar to ours (see also Swindale et al., 2003). The maps of maximal response (Figure 7B) and tuning width (Figure 7C), in turn, are related to the map of “orientation tuning strength” obtained from the length of the vector sum of responses to different orientations (see, e.g., Swindale, 1982; Blasdel & Salama, 1986; Weliky, Bosking, & Fitzpatrick, 1996). Our maps are perhaps more informative, because they distinguish between regions where the signal is stronger regardless of visual stimulus and regions where orientation tuning is sharp (see Swindale et al., 2003 for a similar viewpoint).

The remaining two maps concern the two parameters describing the dependence of response on contrast, $g(c)$ (Figure 8A and D). Neither map seems to suggest a large variability across the cortical surface. The map for semisaturation contrast $c_{50}$ (Figure 8A) is rather uniform, with values closely scattered around the median, 32.7% (Figure 8B). The map for the exponent $n$ (Figure 8D) also appears rather uniform, having values that tend to be close to the median ($n = 1.64$), or to be outliers near 10, the upper limit we set in our fit procedure (Figure 8E). The presence of these outliers is typical when one fits the hyperbolic ratio to contrast response curves (Albrecht & Hamilton, 1982; Sclar et al., 1990): The nature of the exponent is such that the difference between values of 10 and 11 is negligible, unlike the difference between values of 1 and 2.

Consistent with the view that semisaturation contrast and exponent appear to be largely uniform is the fact that sites where they deviate most from the respective medians lie under or near the most obvious blood vessels (Figure 8C).
and F). We have seen that these sites correspond to the lowest fit quality (Figure 6), and to the highest variability in the data (Figure 1B).

To investigate possible relationships between maps of model parameters, we computed their pairwise correlations (Table 1). While semisaturation contrast $c_{50}$ and exponent $n$ do not appear to be correlated with the functional map of orientation preference $O_p$ (Figure 7A) or tuning width $O_w$ (Figure 7C), they correlate with the map of maximal response $R_{\text{max}}$ (Figure 7B). We found a strong positive correlation of $R_{\text{max}}$ and $c_{50}$ (correlation coefficient $r = 0.49$) and a strong negative correlation of $R_{\text{max}}$ and $n$ ($r = -0.70$). Other parameters appeared uncorrelated, except for a significant negative correlation between $R_{\text{max}}$ and orientation tuning width $O_w$ ($r = -0.35$). This correlation does not involve parameters of the contrast responses, so we can ignore it for the moment. The high pairwise correlation between contrast response parameters and maximal response $R_{\text{max}}$ might reflect a physiological reality, or—more likely—result from parameter trading. Trading occurs when changes in one parameter can compensate for changes in another parameter. In our case, responses that do not show much saturation at high contrast might be well fitted by choosing a high $n$, but this must be accompanied by a low $R_{\text{max}}$ otherwise predicted responses will be too high. Conversely, choosing a high $c_{50}$ must be accompanied by a high $R_{\text{max}}$ otherwise predicted responses will be too low. Parameter trading is associated with overfitting, which occurs when a model has more free parameters than can be constrained by a dataset.

To investigate whether the maps of the contrast response parameters might contain some spatial structure that is not apparent in Figure 8A and B, we computed their power spectrum (Figure 9). As a control, we first computed the power spectrum of the maps of orientation selectivity parameters. For the map of preferred orientation (Figure 7A), the power spectrum peaks at 1.33 cycles/mm (Figure 9A). This frequency corresponds to a period of 0.75 mm, in line with previous measurements (Muller et al., 2000). Roughly corresponding peaks are observed in the power spectra (Figure 9B and C) of the maps of maximal response and tuning width (Figure 7C and D). These peaks are instead scarcely visible in the power spectra (Figure 9D and E) of the maps of the parameters determining contrast response functions: semisaturation contrast $c_{50}$ and of exponent $n$ (Figure 8A and D). We thus find little evidence for spatial structure in these maps, beyond a simple tendency for nearby points to have similar fit values, which would be expected given the smooth nature of the data. This analysis, thus, supports the qualitative impression that there is little spatial structure to the maps in Figure 8A and B.

Taken together, these results are an indication that five free parameters for pixel responses might be too many. Because of their approximate spatial homogeneity (Figure 8A and D and Figure 8B and C), the parameters in excess are likely to be those describing contrast responses: semisaturation contrast $c_{50}$ and exponent $n$.

### Spatial uniformity of contrast responses

To test whether semisaturation contrast $c_{50}$ and exponent $n$ vary significantly across the cortical surface, we asked whether fixing their values to be constant across the

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<th>$O_p$</th>
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Table 1. Pairwise correlations between model parameters. If pixels are independent samples, all values would be significant to $p < 1 \times 10^{-16}$ ($p < 1 \times 10^{-100}$ for values in bold).
surface would yield a significant loss in fit quality.

As illustrated in Figure 10A and B, the answer is no: the loss in fit quality is minor. Choosing $c_{50} = 30\%$ and $n = 1.4$ for all pixels still explains 81.6\% of the variance, even though the resulting reduced model requires only 3 parameters per pixel. By comparison, the full model explains 85.4\% of the variance, at a cost of 5 parameters per pixel. Considering that for this animal there were 16,675 pixels in the region of interest, going from the full model to the reduced model translates into a savings of 33,350 parameters.

In a tiny portion of the pixels, the reduced model appears to perform better than the full model (bin to the left of 0 in the histogram of Figure 10B). This behavior would be in principle impossible, and occurs simply because the fitting procedure stops trying to improve fits when changes in fit parameters yield only negligible improvements. The procedure can stop at slightly different fit quality levels during fits of two models.

The reduced model can be used to summarize the contrast responses observed in the whole dataset (Figure 10C). To obtain this graph, we used the reduced model (1) to select for each pixel the stimulus orientation that gave the maximal response, and (2) to scale these responses by the maximal predicted response for that orientation. The model predicts that all data thus scaled should fall on the same contrast response curve, and the data support this prediction (Figure 10C).

The fit achieved by the reduced model for the single pixel of Figure 4 is illustrated in Figure 11. With only three parameters the reduced model explains 91.4\% of the variance in this pixel (down from 92.8\% for the full model).

Indeed, for 70\% of the pixels, the reduced model accounts for <3\% of the variance less than the full model (Figure 10B). The regions where moving from the full model to the reduced model entailed a larger loss in fit quality (Figure 12A) are often in proximity to blood vessels, where data are noisier (Figure 12B).

Examples of predicted optical responses based on the reduced model are illustrated in Figure 13. These responses resemble closely those predicted by the full model (Figure 5), and the actual responses of the cell (Figure 2). Comparison by eye, thus, confirms the quantitative similarity of the goodness of fit of the full and reduced models.

Maps of the three free parameters are illustrated in Figure 14. The only parameter that differs visibly in the two models is the maximal response $R_{\text{max}}$ (Figure 14B). Whereas in the fits of the full model (Figure 7B) $R_{\text{max}}$ varied more abruptly in the range between 0.002 and 0.012, in the fits of the reduced model (Figure 14B) it varied more smoothly, and in a restricted range (0.004-0.010). This effect is consistent with the hypothesis that the full model was overfitting, so that this parameter was not appropriately constrained.

Figure 11. Examples of fits of the reduced model for one pixel (142,56). Curves are predictions of reduced model, where the parameters $c_{50}$ and $n$ determining response to different contrasts are constrained to be the same for all pixels. Format as in Figure 4.
As with the full model, in the reduced model there is still some residual correlation between parameters. In particular, higher maximal response \( R_{\text{max}} \) values tend to be found in regions of sharper orientation tuning: negative correlation between \( R_{\text{max}} \) and tuning width \( O_w \) remains strong (\( r = -0.39 \)). This correlation has been observed also by others who have investigated its origins (Swindale et al., 2003). These origins might partly lie in the resolution limits of the intrinsic signal imaging method. Pixels near orientation singularities represent responses of a number of cells with often widely differing orientation preferences (Maldonado et al., 1997). The response at any one orientation will therefore appear lower and the orientation tuning broader than for pixels in iso-orientation domains where all pixels share a similar orientation preference (Swindale et al., 2003).

Up to now we have presented data from a single experiment. Our results, however, were confirmed by experiments in three additional animals. The results of these experiments are summarized in Figure 15A, which shows the fit quality of the reduced model for each case, depending on the choice of values for semisaturation contrast \( c_{50} \) and exponent \( n \). The signal to noise ratio in these experiments was lower: consequently, with all its free parameters the full model could explain only a lower portion of the variance (74.8%, 50.4%, and 35.2%) than in the experiment illustrated in detail above (85.3%). Crucially, the reduced model performed almost as well in each case, explaining 71.7%, 48.1%, and 31.3% of the variance.

Another likely consequence of noisy data, the optimum values for \( c_{50} \) and \( n \) varied considerably between experiments. While for our example dataset we had \( c_{50} = 30\% \) and \( n = 1.4 \), for the remaining datasets we had \( c_{50} = 30, 100, \) and \( 59\% \) and \( n = 1.0, 2.0, \) and \( 2.0 \).

In spite of this variability, our main result is confirmed: the reduction of the model from five to three parameters per pixel barely affected the quality of the fits. This effect is also clear at the level of single pixels: Those pixels that were fitted well by the full model were also fitted well by the reduced model (Figure 15B). Moreover, just as in our example dataset (Figure 15C), for these additional datasets the reduced model summarizes the contrast response of the entire imaged surface (Figure 15C). That such a graph is possible is another indication that contrast responses were largely uniform across the imaged area. In other words, in all four experiments, the parameters describing the pixel contrast responses appear to be unchanging across the cortical surface.
Figure 15. Analysis of three additional datasets. Format is as in Figure 10. A1-C1: Experiment 122099. A2-C2: Experiment 101001. A3-C3: Experiment 031301.

Discussion

We found that contrast responses in cat V1 can be measured with optical imaging, and be described by the same function as is used for single neurons. In particular, orientation tuning and contrast response are separable at the level of individual image pixels. Contrast response parameters appear to be uniform over large regions of cat V1, and to be thus unrelated to functional maps. The data indicate that functional maps, and in particular the orientation preference map, are contrast-invariant.

Contrast response parameters in functional imaging

While the diversity of contrast response parameters across our experiments limits the extent to which we can generalize our results to compare them with single-cell data, there is a clear trend to suggest that semisaturation for many neurons is reached at contrasts below that obtained from our imaging data. Indeed, in cat V1, the average semisaturation contrast \( c_{50} \) of individual neurons is 15.2% ± 1.06% (and the average exponent \( n \) is 2.5 ± 0.12; Albrecht & Hamilton, 1982).

One of the factors that might contribute to the difference between contrast responses measured optically and in single neurons is the lack of spatial resolution. Even though it represents only \((22 \mu m)^2\) of cortical surface, each pixel in our images is likely to report the activity of quite a number of neurons from a larger area, whose optical responses are lumped by optical scattering, by slight motion of the brain between images, and by a spreading of the metabolic demand (reflected in the deoxy- to oxyhemoglobin ratio) from the point of origin. Moreover, the optical response repre-
sents more than just spiking activity: both presynaptic and subthreshold postsynaptic activity contribute to the intrinsic imaging signal, resulting in a much wider point spread function than that obtained for suprathreshold postsynaptic activity alone (Griswold, Lieke, Frostig, & Hildesheim, 1994). As commonly recognized in psychophysics, the sum of the activities of neurons with different contrast responses can have a contrast response that is shallower and has higher semisaturation contrast than many of the individual neurons. Another factor that is likely to play a role is a possible nonlinearity of the transformation from single-cell firing rates to intrinsic optical signals. If this nonlinearity were expansive, it would emphasize large responses to the expense of small responses, and thus distort the apparent contrast responses into having larger semisaturation contrasts.

Contrast invariance of orientation selectivity in functional maps

Precisely because pixel responses reflect the activity of many neurons, our finding that the map of orientation selectivity is contrast-invariant is somewhat surprising. Indeed, contrast invariance in the responses of single neurons does not necessarily imply contrast invariance in the responses of a pixel.

Under which conditions can contrast invariance of pixel orientation tuning be expected?

Consider that there are \( N \) neurons that contribute to the response of a pixel. The pixel response to contrast \( c \) and orientation \( o \) is

\[
R(o, c) = \sum_{i=1}^{N} R_i(o, c) = \sum_{i=1}^{N} f_i(o) g_i(c),
\]

where \( R_i \) is the response of the \( i \)-th neuron. The first equality simply indicates that the pixel summates the output of many neurons. The second equality reflects the fact that responses of individual neurons are contrast-invariant, so they can be written as the product of a function of orientation and one of contrast.

The pixel response will be contrast-invariant if one (or both) of the following two conditions is met:

First, the neurons could have very similar orientation selectivity, \( f_i(o) = f(o) \). Then

\[
R(o, c) = f(o) \sum_{i=1}^{N} g_i(c).
\]

Second, the neurons could have very similar contrast response, \( g_i(c) = g(c) \). Then

\[
R(o, c) = g(c) \sum_{i=1}^{N} f_i(o).
\]

The first alternative, that neurons share similar orientation tuning functions, is very plausible throughout most of V1, but less plausible in pinwheel centers, where nearby neurons tend to have widely different preferred orientations, at least as far as spiking responses are concerned (Maldonado et al., 1997). Neurons near pinwheels, however, have very broadly tuned membrane potential responses (Schummers, Marino, & Sur, 2002) and if these responses contribute to the optical signal they would appear to resemble each other.

The second alternative, that neurons share similar contrast responses, seems implausible because neurons in cat V1 are known to differ widely both in semisaturation contrast \( c_{50} \) and in exponent \( n \) (Albrecht & Hamilton, 1982; Sclar et al., 1990). It becomes plausible, however, if neurons differ in their contrast responses between cortical layers rather than across the V1 surface. In fact, this is in good agreement with what is known about contrast coding in primate V1 (see below). As we could only image responses at a fixed depth, roughly corresponding to layers 2-3, we would have missed variations in contrast response parameters that occur either vertically, across layers, or horizontally within other layers.

Finally, separability of orientation tuning and contrast responses would be in general lost if the transformation between firing rates and intrinsic optical signals were to be arbitrarily nonlinear. There is only one kind of nonlinearity that would retain separability, and this nonlinearity takes the form of a power function (Miller & Troyer, 2002). Our suggestion, then, is that responses at the level of firing rates in local populations are separable, and that the transformation between firing rate and intrinsic optical signal is an expansive power function (i.e., one with a power > 1).

Uniformity of contrast responses in functional maps

Our finding that contrast responses are largely uniform across the surface of cat V1 agrees with studies of single neuron responses in macaque V1 (Hubel & Livingstone, 1990; Edwards et al., 1995) and in a nocturnal primate, the owl monkey (O’Keefe et al., 1998).

However, measurements of 2-deoxy glucose (2DG) uptake in macaque V1 indicate that major variations in contrast threshold occur vertically, across layers (Tootell, Hamilton, & Switkes, 1988). These variations are likely to reflect a partial segregation of magnocellular and parvocellular retinotopic inputs. Layers 4B, 4Ca, and 6 exhibited strongest 2DG uptake with low-contrast stimulation, the same layers that appear to receive preferential magnocellular thalamic input, which has lower contrast threshold (reviewed in Livingstone & Hubel, 1988). Tootell and collaborators (1988) found contrast threshold to vary also horizontally within layers 2-3, being somewhat lower inside cytochrome oxidase (CO) blobs than outside. Subtle variations in contrast threshold occurring only in this layer might have been missed by the studies of single neuron responses (Hubel & Livingstone, 1990; Edwards et al., 1995; O’Keefe et al., 1998).
Sampling bias might also explain the disagreement between our findings and the preliminary report of Schulze et al. (1999). These authors found contrast threshold of single units in cat V1 to be lower in CO blobs than elsewhere. Overall, in the cat visual system, the evidence for two distinct processing streams originating from two classes of retinal ganglion cells is much weaker than in primates (see Scannell, Blakemore, & Young, 1995). While X and Y cells with different physiological properties exist in retina and LGN, it is less clear whether these represent segregated input channels to V1. Laminar segregation of X- and Y-cell geniculate inputs to layer 4 of cat V1 is weak (Humphrey, Sur, Uhlrich, & Sherman, 1985a, 1985b). However, X and Y-cell inputs do not appear to converge on individual cortical neurons (Martin & Whitteridge, 1984). More recently, it has been shown that blob regions of cat V1 (Murphy, Jones, & Van Sluyters, 1995) receive predominantly Y-cell input (Boyd & Matsubara, 1996) and display lower spatial and higher temporal selectivity than interblob regions (Shoham, Hübener, Schulze, Grinvald, & Bonhoeffer, 1997), reminiscent of the magnocellular pathway in monkeys. While our study does not directly address the relationship between blobs, spatial frequency domains, and contrast thresholds, our evidence suggests that beyond these differences in selectivity there is no additional segregation with respect to contrast thresholds.

Notably, the uniformity of semisaturation contrast that we found extends to orientation singularities (“pinwheel centers”). Even though pinwheel centers and CO blobs are not coincident, they both tend to occur in the center of ocular dominance stripes (Bartfeld & Grinvald, 1992). It has been suggested that the location of pinwheel centers might be correlated with the layout of other functional maps in V1 and that neural responses in these locations might be different from those elsewhere. In particular, neurons in pinwheel centers can be expected to be more monocular than elsewhere (Bartfeld & Grinvald, 1992; Hübener, Shoham, Grinvald, & Bonhoeffer, 1997), and broadly tuned for orientation, albeit only in membrane potential (Schummers et al., 2002) and not in firing rate (Maldonado et al., 1997). Moreover, neurons in pinwheel centers appear to be selective for the highest or lowest spatial frequencies (Issa et al., 2000), and may be more strongly affected by visual adaptation (Dragoi, Rivaudulla, & Sur, 2001; but see Sengpiel & Bonhoeffer, 2002). Our results, however, suggest that neurons in pinwheel centers do not play a special role in contrast processing.

In future experiments, it may be possible to obtain better estimates of contrast responses by using a periodic stimulation method and thus placing the contrast-related signal in a different temporal frequency band from much of the noise (Kalatsky & Stryker, 2003). This method is likely to provide a major improvement over our long steady stimulus presentations. Indeed, in our experiments, the signal-to-noise ratio was particularly adversely affected by fluctuations in the overall spatial mean, which we could not remove. The spatial mean is notoriously variable, and fluctuates slowly over periods of seconds or tens of seconds.

As with all negative results, finally, it should be kept in mind that absence of evidence is not evidence of absence. In particular, failure to find functional organization for a given stimulus attribute may not necessarily imply lack of functional organization. For example, it is conceivable that the signal/noise ratios in our experiments would not have been sufficient to reveal maps of attributes such as spatial frequency. The spatial frequency maps demonstrated by Shoham et al. (1997) are harder to observe than orientation domains, and are most evident in kittens and not in adult cats. Moreover, the diversity of contrast response parameters across our experiments, and the difficulty in relating them to responses of single neurons, limit the strength of our evidence.

A conservative summary of our results, thus, is that if there is a functional map in V1 of contrast response attributes, this map must be markedly weaker than the maps of orientation preference, of tuning width, and of maximal response.

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