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The organization of spatial frequency maps measured by cortical flavoprotein autofluorescence

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ABSTRACT

To determine the organization of spatial frequency (SF) preference within cat Area 17, we imaged responses to stimuli with different SFs using optical intrinsic signals (ISI) and flavoprotein autofluorescence (AFI). Previous studies have suggested that neurons cluster based on SF preference, but a recent report argued that SF maps measured with ISI were artifacts of the vascular bed. Because AFI derives from a non-hemodynamic signal, it is less contaminated by vasculature. The two independent imaging methods produced similar SF preference maps in the same animals, suggesting that the patchy organization of SF preference is a genuine feature of Area 17.

1. Introduction

Neurons within primary visual cortex of visual mammals are selective for the spatial frequency (SF) of a stimulus (Campbell, Cooper, & Enroth-Cugell, 1969; Movshon, Thompson, & Tolhurst, 1978; Tolhurst & Thompson, 1982) and neurons with similar SF preference cluster together, as has been shown for other response properties like orientation preference and ocular dominance (Hubel & Wiesel, 1962). Electrophysiological studies have shown that neighboring neurons are more likely than chance to prefer similar SFs (DeAngelis, Ghose, Ohzawa, & Freeman, 1999; Maffei & Fiorentini, 1977; Tolhurst & Thompson, 1982), and on a larger scale, maps of SF preference measured using intrinsic signal imaging (ISI) have suggested that there is an ordered map of SF preference across the surface of cortical Area 17 in the cat (Everson et al., 1998; Hubener, Shoham, Grinvald, & Bonhoeffer, 1997; Issa, Trepel, & Stryker, 2000; Shoham, Hubener, Schulze, Grinvald, & Bonhoeffer, 1997a). However, a recent reanalysis of ISI data has called into question the maps of SF preference, suggesting that these maps represent the vascular structure of visual cortex and not spatial modulation of SF preference (Sirovich & Uglesich, 2004). Based on an additional data set, the authors also suggested that SF preference does not vary in an organized fashion across the tangential extent of Area 17 (Sirovich & Uglesich, 2004).

To determine if SF preference varies systematically across the cortical surface we reinvestigated its organization using flavoprotein autofluorescence imaging (AFI). AFI is a non-hemodynamic measure of cellular metabolism derived from the fluorescence of flavoproteins associated with the electron transport chain in mitochondria (Foster, Galeffi, Gerich, Turner, & Muller, 2006; Reinert, Dunbar, Gao, Chen, & Ebner, 2004; Tohmi, Kitaura, Komagata, Kudoh, & Shibuki, 2006; Turner, Foster, Galeffi, & Somjen, 2007). We have recently shown that AFI produces high-quality images of cortical organization in the cat, with improved spatial and temporal resolution compared to intrinsic signal imaging (Husson, Mallik, Zhang, & Issa, 2007). Because AFI does not rely on blood oxygenation or blood flow changes, it has far fewer vascular artifacts than does ISI (Husson et al., 2007).

AFI maps suggest that SF preference has a clustered organization that varies across the cortical surface of cat Area 17, consistent with previous ISI studies (Everson et al., 1998; Hubener et al., 1997; Issa et al., 2000; Shoham et al., 1997a). Furthermore, the SF preference maps and tuning curves measured by AFI are statistically similar to those produced by ISI in the same animals, suggesting that tangential variation of SF preference is a genuine feature of the organization of primary visual cortex.

2. Materials and methods

All experimental procedures were approved by the University of Chicago Institutional Animal Care and Use Committee. Results from four female cats aged 14 weeks or older are reported. For two of the animals data from different experiments

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were reported in (Husson et al., 2007). The surgical procedures are described in detail in Husson et al. (2007), and are briefly summarized here. Cats were anesthetized with thiopental (20–30 mg/kg IV loading dose, 2–10 mg/kg IV, PRN for maintenance). Ophthalmic phenylephrine (10%) and atropine (1%) were instilled in the eyes, and the eyes were focused at 40 cm with contact lenses. Portions of areas 17 and 18 were exposed through a craniotomy and the brain was stabilized with 3% agarose in sterile saline and then covered with a glass coverslip.

2.1. Visual stimuli

Visual stimuli were created using the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997) in MATLAB (The MathWorks, Inc, Natick, MA), and were presented on a 21″ gamma-corrected CRT display (Dell P1230, Round Rock, TX). Data from two of the four animals were collected using an uncorrected monitor, resulting in the inclusion of low amplitude harmonics (a Fourier transform of the stimulus shows a second-harmonic with an amplitude of 12% that of the fundamental's amplitude, and a third harmonic with an amplitude of 4%); results from these two animals are reported separately in supplementary figures. The stimuli were viewed binocularly from 40 cm. To generate SF maps, sine wave gratings were presented at four orientations (0°, 45°, 90°, 135°) and six spatial frequencies (0.1, 0.25, 0.5, 0.75, 1.0, 1.5 c/°) in pseudo-random order. All gratings were presented at 80% contrast, and drifted across the central 60° of visual space at a temporal frequency of 2 c/s. Each stimulus was initially stationary for 6 s, and then drifted for 6 s. Drift direction reversed every 2 s. Each stimulus set included four randomly interleaved mean-luminance gray stimuli; the responses averaged over these four stimuli were used to produce a "blank" response image. Images were collected over the last 5.5 s of each stimulus presentation, and were averaged over 16 or 32 presentations of each stimulus.

2.2. Optical imaging

Images were obtained using a Dalsa 1M30 camera (Dalsa Corp, Waterloo, Ontario, Canada) mounted on a macroscope (Bonhoeffer & Grinvald, 1996) with 50 mm lenses (1.4 f, Nikon, Melville, NY) and controlled by a custom LabVIEW (National Instruments, Austin, TX) interface. The cortex was illuminated with two 12 W light sources (Oriel, Richmond, CA) through fiber optic cables. For ISI, the cortex was illuminated with 610 ± 10 nm light with a matched filter (Newport, Stratford, CT) in front of the CCD camera and images were acquired at 30 frames per second, temporally averaged over four images, and spatially binned after acquisition (2 × 2 bins). For AFI, fluorescence was excited with 420–490 nm light and the emitted light was long-pass filtered above 515 nm (Chroma, Rockingham, VT). Images were acquired at 5–10 fps with no temporal or software binning, but with 2 × 2 on-chip spatial binning.

2.3. Image analysis

AFI and ISI images were analyzed using custom software in the IDL environment (ITT Visual Information Solutions, Boulder, CO). To produce single-condition images, we first normalized the average response for a given condition to the response to a blank screen ("blank normalization"). Both AFI and ISI images were then spatially high-pass filtered by first generating a smoothed image with a moving window (1680 × 1680 μm) and then subtracting the smoothed image from the original image to remove low frequency spatial components. Response amplitudes were measured in restricted regions (templates) of the imaged field to exclude areas that were either non-responsive or contaminated by large vascular patterns or other artifacts.

Orientation and SF preference maps were constructed as described previously (Issa et al., 2000). Orientation preference maps (angle maps) were calculated using the standard vector-averaging method (Blasdel & Salama, 1986). SF preference was measured at each pixel's preferred orientation (selected from four orientations: 0°, 45°, 90°, and 135°). An SF tuning curve was generated for each pixel from the responses at its preferred orientation. The 'average SF tuning curve' for a large region of cortex was calculated by averaging SF tuning curves from all the pixels in that region. For average AFI and ISI tuning curves the Pearson correlation coefficient was calculated using measured values ($N = 6$ SFs) and p-values are reported for the null hypothesis that the R^2 value for the correlation equals zero.

To compare maps of SF preference generated by ISI and AFI procedures, we first aligned the AFI and ISI orientation maps for each experiment. This was necessary because of small shifts that occur between imaging runs. Alignment was carried out by maximizing the correlation between ISI and AFI orientation preference maps over a 10 × 10 pixel window corresponding to 240 × 240 μm of cortex (orientation maps for all experiments are shown in Supplementary figure 1). Note that this alignment procedure is independent of the SF maps, and as such would not bias any relationships between them.

To determine if the maps generated by ISI and AFI are statistically similar within templated regions, we estimated the preferred SF of a pixel by interpolating responses near the frequency that best activated the pixel [as in (Issa et al., 2000)]. We then calculated the difference in SF preference between each pixel of the two measured maps ("Measured Difference"). This distribution was then compared to the distribution expected if the spatial organization of the SF maps were different but the maps shared the same range of spatial frequencies ("the null hypothesis"). The distribution for the null hypothesis was generated by shuffling the location of pixels in the ISI SF map and calculating the difference between the shuffled ISI map and the AFI SF map ("Shuffled Difference"; the shuffled difference was calculated from the average of 100 pseudo-random shuffles). This procedure maintains the overall distribution of SF preferences, but assumes they are not spatially organized.

We used the Kolmogorov–Smirnov non-parametric test to determine if the "Measured Difference" and "Shuffled Difference" were drawn from the same distribution. If the difference between the measured AFI and ISI maps was significantly smaller than the difference between the measured AFI map and the shuffled ISI map then we concluded that the measured maps were more similar than expected by chance.

A similar Kolmogorov–Smirnov analysis was performed to compare SF tuning curves measured using the two techniques. Pearson correlation coefficients were first calculated for AFI and ISI tuning curves measured at each pixel in the templated area. The distribution of measured correlation coefficients was compared to the distribution for the null hypothesis that the correlation between AFI and ISI tuning curves is due to similar average SF tuning curves over the entire region, not due to the spatial structure of the SF maps. The distribution of the null hypothesis was determined by calculating correlation coefficients after shuffling pixel locations in the ISI images.

The signal-to-noise ratio (SNR) of a data set was estimated as follows. For a given spatial frequency, the signal strength was defined as the variance of pixel intensity in response to the stimuli (averaged over four orientations). The noise level is estimated from the variance in response to a stimulus that produced minimal modulation of cortical activity (we used the response to 1.5 c/° gratings averaged over four orientations, which had the lowest variance in all experiments). The SNR is calculated as the ratio of the signal at the optimal spatial frequency to the noise.

Because we were testing the similarity of maps across modalities within a single imaged field, rather than similarity of maps across animals, statistical tests of similarities were performed on each pair of maps (ISI and AFI maps). The number of samples (N) in the Kolmogorov–Smirnov tests is therefore equal to the number of pixels in a templated field.

3. Results

We used two independent techniques to map SF preference in cat Area 17 by measuring optical responses to sinusoidal gratings with different spatial frequencies. Fig. 1 shows an example of responses to a single grating using AFI and ISI. In these images, bright areas represent patches of cortex that are activated by the stimulus, while dark areas are inactive (Fig. 1B and C). As has been observed in previous intrinsic signal imaging studies (Everson

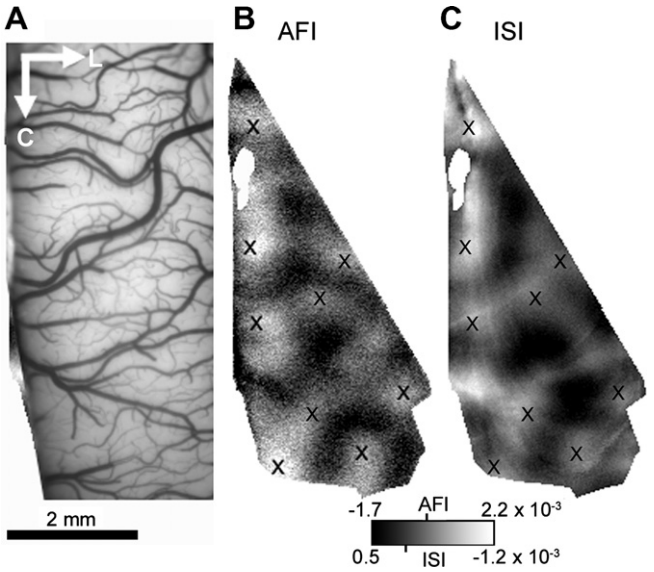

Fig. 1. AFI and ISI responses to a sinusoidal grating. (A) The lateral gyrus imaged under green light to highlight the vascular pattern. (B) Response to a 0.5 c/°, horizontal sinusoidal grating imaged with flavoprotein autofluorescence; active regions appear bright. (C) Response to the same stimulus imaged with intrinsic signal imaging. For comparison with (B), the image intensities have been inverted to make bright areas represent stronger cortical activity. "X"s mark the same locations in (B) and (C). The grayscale bar shows the amplitude of responses ($\Delta F/F$ for AFI and $\Delta R/R$ for ISI), with the 0-points marked by short vertical lines.