Supplementary Materials & Methods

fMRI study of cat visual cortex.

The experimental methods used here have been fully described in a previous publication (Moon et al., 2007). Animal use was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Experimental design and stimuli. Anesthetized and paralyzed cats (N=2) were presented with high-contrast, full-field drifting square-wave gratings (spatial frequency, 0.15 cycles/degree; temporal frequency, 2 cycles/s; motion direction reversing every 0.5 s), displayed at one of 8 equally-spaced orientations (0, 22.5, …., 157.5 degrees). Each orientation was shown for 10 seconds, after which the next counterclockwise orientation was presented. Thus, a full cycle through all 8 orientations required 80 seconds to complete. Each experimental run consisted of 11 cycles for a total duration of 880 seconds.

MRI scanning procedures. Animals were scanned using a 9.4 Tesla Varian (Palo Alto, CA) MR system, with a custom-built surface coil (1.6 cm diameter) positioned over the primary visual cortex. Functional imaging was performed using a 2D gradient echo (GE) echo-planar imaging (EPI) sequence (TE 18ms, TR 500ms, FOV 2 × 2 cm, slice thickness 1mm, in-plane resolution 0.3125 × 0.3125mm). The imaging slice was positioned with the aid of a venographic MR image to avoid large superficial veins and to include as much visual cortical surface area as possible. Three functional runs were completed for each of the 2 cats analyzed here.

Data analysis. Brain voxels were first identified as those with average intensities greater than 80% of the mean intensity of the volume, then a mean trend timecourse was determined by averaging together all brain voxels at each time point. The time series of each voxel was then orthogonalized with respect to this global trend by subtracting the best fit of the trend timecourse from each voxel's time series. Spatial filtering and multivariate pattern analysis were performed as for the human data, described below.

Univariate statistical analysis used analytic methods similar to those previously developed for continuous phase-encoded retinotopic mapping (Sereno et al., 1995). Briefly, the amplitude and phase of modulation at the stimulus periodicity (80 seconds/cycle) were determined by taking the Fourier transform of the time series at each voxel. Under the assumption of temporally white noise and the null hypothesis of no stimulus-driven activity, the ratio of the power of modulation at the stimulus frequency to the average power at all other "noise" frequencies for a time course of N time points is distributed as an *F*-statistic with 2 and N-2 degrees of freedom (Tootell et al., 1998). Voxels were considered reliably orientation-selective if their *F*-statistic exceeded a Bonferroni-corrected significance level of $P < 0.05$. These voxels were rendered on the cortical surface in colors according to the phase of their response, reflecting the preferred orientation (**Fig. 1**).

fMRI study of human visual cortex.

Subjects. Four right handed males, ages 27-37, participated in this study after providing written informed consent. All had normal or corrected to normal vision. The study was approved by the Vanderbilt University Institutional Review Board.

Experimental design and stimuli. Participants were asked to discriminate letters presented at fixation while oriented gratings (0°, 45°, 90°, or 135°) were displayed in the left and right visual fields (**Fig. 2A**). The orientation stimuli consisted of two flickering square-wave gratings (mean luminance, 6.5 cd/m^2; contrast, 100%; spatial frequency, 1.4 cycles per degree with randomized phase; flicker rate, 125 ms on/125 ms off) presented in semi-annular apertures (0.8–7.6° eccentricity) in the left and right visual fields. The orientation stimuli in each hemifield covered 160º of polar angle around the central letter stream, with 20º gaps inserted along the vertical meridian to ensure that V1 responses to each grating would be confined exclusively to the contralateral hemisphere. The left and right apertures were separated by 0.8° of visual angle at their innermost point and by 2.6° at their outermost point.

Eye movements were discouraged by requiring subjects to perform a letter discrimination task at fixation (letter size ~0.5°) throughout each fMRI run. A series of letters were centrally presented at a rate of 5 items per second. The subject's task was to report whenever a target letter ('J' or 'K') appeared by pressing corresponding buttons on a response box with the index or middle fingers of the right hand. Targets appeared at randomly selected intervals, with exactly one target appearing in each 4.5-second scanner acquisition interval. Mean accuracy for this task was 85% correct.

Each experimental run consisted of eleven 18s blocks. A run began with a block of letters only, followed by 4 blocks of both letters and gratings, a middle block of letters only, 4 more blocks of letters and gratings, and a final block of letters. The peripheral gratings, when present, were irrelevant to the subjects' primary letter discrimination task at fixation. Orientations were counterbalanced so that each of the 4 possible orientations appeared in both the left and right visual fields during each set of 4 blocks. Each subject completed 11 or 12 experimental runs within a scanning session.

The subjects wore prism glasses in order to view stimuli on a rear-projection screen positioned above the chest, which was illuminated by an Avotec (Stuart, FL) LCD projector. The stimulus background was a medium gray (6.5 cd/m^2) in an otherwise dark room.

MRI scanning procedures. Functional images were acquired on a Philips Achieva 7 Tesla MRI scanner at the Vanderbilt University Institute of Imaging Science (VUIIS), using a volume transmit coil and a 16-channel receive-array head coil (Nova Medical, Wilmington MA). A 3D fast field echo (FFE) sequence was used for functional imaging (3D-FFE parameters: acquisition time, 4.5 s per volume; TE 25 ms; TR 36 ms; flip angle 17°; 13 echo EPI readout train; sensitivity encoding (SENSE) acceleration factor 3.0). The field of view (FOV) was 128 × 128 × 33mm, with an isotropic spatial resolution of 1 × 1 × 1mm. The imaging slab was centered over the occipital pole and oriented roughly perpendicular to the calcarine sulcus.

Anatomical images used for cortical reconstructions (acquired at 1 mm^3 resolution) and retinotopic mapping data were collected in separate imaging sessions on a 3 Tesla Philips Intera Achieva scanner.

Data analysis. Functional images were aligned using FSL's MCFLIRT (Jenkinson et al., 2002) for motion correction (6 degrees of freedom, sinc interpolation). The first functional volume was discarded.

For univariate analyses, the voxelwise time series were fit by a general linear model, using boxcar regressors convolved with a gamma function (delta, 2.25s; tau, 1.25s) for each experimental condition. Additional regressors were included to model polynomial drift terms up to second order. The statistical significance of planned contrasts was assessed according to a fixed-effects model in each individual subject.

The borders of area V1 were identified on the reconstructed cortical mesh based on retinotopic mapping data acquired in separate scan sessions. These surface-based regions of interest (ROIs) were then projected back into the functional volume space, identifying the functional voxels that intersected the gray matter of area V1. Separate volumetric ROIs were generated for the left and right hemispheres of each subject.

Human orientation preference maps (**Fig. 2**) were generated from the estimated amplitude of the responses to each of the four orientations. A vector-valued response estimate was

calculated for each voxel by taking the difference between response amplitudes for the cardinal orientations (0º minus 90º) and the oblique orientations (45º minus 135º), and interpreting these values as the real and imaginary components, respectively, of a complex number. These complex values were projected onto the cortical surface and color-coded according to their phase, showing only those voxels that displayed statistically significant responses in the unfiltered contrast of all orientations versus fixation ($P < 0.05$). This vector-averaging method is likely adequate for broad, unimodal tuning curves (Swindale, 1998).

Spatial filtering. Three different types of spatial filters were used, including 3D volumetric filtering with both ideal and Gaussian kernels, as well as an iterative surface-based smoothing method.

Ideal lowpass filtering was performed in the frequency domain by taking the spatial Fourier transform of each 3D volume, setting all frequency components above the desired cutoff to zero, and inverting the Fourier transform. This is equivalent to convolution in the spatial domain with a circularly-symmetric sinc kernel. However, ideal filtering can lead to pronounced Gibbs ringing artifacts in the spatial domain, due to the prominent sidelobes of the sinc kernel.

3D Gaussian smoothing was performed using FSL's fslmaths utility. Gaussian kernels do not cause ringing artifacts in the spatial domain, but have a comparatively slow transition between passband and stopband in the frequency domain. Although Gaussian smoothing

substantially attenuates high spatial frequency components, it does not eliminate them entirely (Kamitani and Sawahata, 2009). The effects of this slow passband-stopband transition are most evident in the classification of highpassed data with small kernel sizes (**Supplementary Fig. 1**), where the accuracy of classification of Gaussian filtered data is notably better than that of ideally filtered data. As the Gaussian filters do not provide perfect frequency isolation, patterns on scales substantially more coarse than that suggested by the nominal kernel size can contribute greatly to the classification accuracy in this regime.

Surface-based smoothing was performed using an iterative method described by Hagler et al. (2006), as implemented in Freesurfer's mri_surf2surf utility. Functional volumes were carefully manually aligned to a reference T1-weighted anatomical scan, which was used to reconstruct the cortical surface of each hemisphere as a triangular mesh (Dale et al., 1999; Fischl et al., 1999). The functional data were then projected onto this reconstructed cortical mesh by taking the intensity values of voxels at 75% of the distance from the gray matter/white matter boundary to the pial surface, and mapping them onto the corresponding mesh vertices. The projected functional intensity values were then spatially smoothed by repeated nearest-neighbor averaging along the reconstructed cortical mesh, which is approximately equivalent to smoothing by a surface-based Gaussian kernel with a full width at half maximum (FWHM) proportional to the square root of the number of iterative smoothing steps (Hagler et al., 2006). The surface based smoothing method thus has frequency isolation characteristics comparable to that of the volumetric Gaussian filter.

Both Gaussian and surface-based smoothing were performed at 1mm FWHM increments. Approximately equivalent ideal filtering was performed for each level of Gaussian smoothing, taking the equivalent cutoff frequency of the Gaussian to be the spatial frequency at which signals were attenuated to half their original power (approximately -3dB). Highpass filtered data were generated simply by subtracting the lowpassed data from the original images.

Multivariate pattern analysis. For pattern classification analysis, after spatial filtering, all timecourses were first z-transformed (normalized by the standard deviation of the time course). Response amplitudes for each individual stimulus block (10s in cat data, 18s in human) were then estimated by a general linear model, as described above. The linear classifier we used consisted of linear support vector machines (SVMs), implemented by liblinear (Fan et al., 2008). A classifier was trained on block amplitude data from all but one experimental run, leaving the remaining run held out as independent test data. This leave-one-run-out procedure was repeated for all N runs, to obtain a measure of orientation classification accuracy for activity patterns in V1.

For the cat data, SVMs were trained on all brain voxels (those exceeding 80% of the mean image intensity, as above), giving 1497 voxels for one cat and 1156 voxels for the other. In the human data, voxels used for training were those in left or right V1 that exceeded a significance threshold of $P < 0.01$ in the contrast of all orientations versus letters only, leading to an average of 2070 voxels included per hemisphere in the

volumetric analysis and 1519 voxels per hemisphere for the surface-based analysis. (Qualitatively similar results, not shown, were obtained for thresholds of $P < 0.05$, 0.001, 0.0001, and 0.00001.) Separate classifiers were trained to predict the contralaterally presented orientation in left and right V1.

The feature selection contrast of all orientations versus baseline (letters only) is orthogonal to the between-orientations contrasts of interest, and served merely to identify those voxels corresponding to the regions of retinotopic cortex that were driven by the visual stimulus. To ensure that test and training data were independent (Kriegeskorte et al., 2009), the feature selection contrast was calculated separately for each stage of the classifier cross-validation, including only those runs used as training data at each step. However, the orthogonality of the feature selection contrast with respect to all contrasts between orientations indicates that such rigorous separation of test and training data is not necessary to obtain unbiased classification results (Friston et al., 2006). Repeating this analysis with all runs included in a single feature selection contrast gives results effectively indistinguishable from those obtained by cross-validated feature selection (**Supplementary Fig. 6**).

Additional support vector machines were trained and tested on the spatially averaged activity of all significantly active ($P < 0.01$) voxels within each V1 ROI, and on the global trend time course data for the cat visual cortex (rightmost data points, **Figures 1B** and **2D**).

Supplementary methods - references.

Dale AM, Fischl B, Sereno MI (1999) Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage 9:179-94

Fan R, Chang K, Hsieh C, Wang X, Lin C (2008) LIBLINEAR: A Library for Large Linear Classification. J. Mach. Learn. Res. 9:1871-1874

Fischl B, Sereno MI, Dale AM (1999) Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. Neuroimage 9:195-207

Friston KJ, Rotshtein P, Geng JJ, Sterzer P, Henson RN (2006) A critique of functional localisers. Neuroimage 30:1077-1087

Hagler DJ, Saygin AP, Sereno MI (2006) Smoothing and cluster thresholding for cortical surface-based group analysis of fMRI data. Neuroimage 33:1093-103

Jenkinson M, Bannister P, Brady M, Smith S (2002) Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 17:825-41

Kamitani Y, Sawahata Y Spatial smoothing hurts localization but not information: Pitfalls for brain mappers. NeuroImage In Press.

Kriegeskorte N, Simmons WK, Bellgowan PSF, Baker CI (2009) Circular analysis in systems neuroscience: the dangers of double dipping. Nat. Neurosci 12:535-540

Sereno MI, Dale AM, Reppas JB, Kwong KK, Belliveau JW, Brady TJ, Rosen BR, Tootell RB (1995) Borders of multiple visual areas in humans revealed by functional magnetic resonance imaging. Science 268:889-93

Swindale NV (1998) Orientation tuning curves: empirical description and estimation of parameters. Biol Cybern 78:45-56

Tootell RB, Hadjikhani N, Hall EK, Marrett S, Vanduffel W, Vaughan JT, Dale AM (1998) The retinotopy of visual spatial attention. Neuron 21:1409-22