SUPPLEMENTARY DISCUSSION

We used large sine grating stimuli with varying relative interocular spatial phase disparity, as was used previously in numerous electrophysiological studies in anesthetized cats and monkeys (Refs 18-21). These stimuli overcome numerous limitations of single-bar disparity stimuli. Monocular artifacts from single-bar disparity stimuli have been reviewed by Cumming & DeAngelis (Ref 31, pg. 205-206). In our study, response tuning curves to sine grating stimuli were fitted with sine functions whereby coefficients of determination and accuracy of data-to-fit were very high (see our Supplementary Fig. S6). Previous electrophysiological studies in anesthetized cats, anesthetized monkeys, and awake behaving monkeys that used sine grating disparity stimuli have also fitted their data with sinusoids (see Refs 19-21, 32). Our disparity tuning curves with calcium imaging were as sharp as those previously reported with single-unit recordings. These data suggest that response saturation via calcium indicator fluorescence was not a factor in our experiments. We provide more direct evidence that response saturation was unlikely by (i) examining the sensitivity of the fluorescence signal to extremely small changes in stimulus orientation and (ii) simultaneous recording of spikes with calcium indicator fluorescence (Supplementary Fig. S2).

In our experiments with sine grating stimuli, the stability of trial-by-trial, single-cell responses to disparity stimulation (Fig. 1) and the stability of the disparity maps over several hours (Fig. 4), suggested that the position of the two eyes was stable within each experiment (also see Supplementary Fig. S5). Even if the eyes are slightly misaligned, as long as the positions of the eyes are fixed and the receptive fields are near centrally located on the stimulus display, disparity selectivity and sensitivity can be appropriately measured. Any fixed vergence angle simply provides the exact same phase offset to all simultaneously recorded cells. Since eye position might vary slightly among animals because of anesthesia and paralysis, it is difficult to pool the preferred spatial phase disparity data for all cells across different animals, unless eye position is accurately determined (see next paragraph below). Nevertheless, within a single experiment when more than 100 cells are simultaneously recorded, any disparity phase differences amongst those simultaneously recorded cells cannot be contaminated by eye misalignment. Thus, (i) the existence of disparity maps, (ii) correlations (or lack thereof) with other features such as ocular dominance at the level of single cells, and (iii) map gradient interactions are all valid. However, it would still be useful to know the range of absolute disparities that are represented.

Since we recorded from more than 100 cells simultaneously at each site, monocular retinotopic positions could provide an accurate estimate of eye position, but only if the retinotopic sampling is finer than the receptive field size. In three cats, we obtained accurate estimates of vergence angle with this method and one example is shown in Supplementary Fig. S5. However, in four other cats used for disparity
experiments, Monte Carlo standard error estimations from Gaussian fitting of the retinotopic time course showed that the vergence angle could not be accurately extracted based on confidence intervals. The responses were sufficiently large enough to show that the receptive fields of both eyes were located on the stimulus display. However, the retinotopic interval was too coarse or monocular responses from one of the two eyes were not strong enough to provide a reliable estimate of eye position. To facilitate the pooling of preferred spatial phase disparity data for every recorded cell across different animals requires that—at each site—cells respond to monocular stimulation of each eye. As evident in Fig. 1g, this may be difficult to achieve. Even if monocular stimulation of each eye produces a significant response to the retinotopic stimulus, each cell needs to respond at 2–3 adjacent positions of the retinotopic stimulus, not just a single position, or else there may be a small (but significant) vergence angle that will be undetected.

We again emphasize that the lack of being able to estimate vergence angle in some experiments does not diminish our conclusions. Because the map for preferred spatial phase disparity was often orthogonal to the map for ocular dominance and robust disparity maps were obtained in different ocular dominance domains, this suggests that spatial phase disparity is unrelated to ocular dominance. The sensitivity to disparity (F1/F0) is independent of vergence angle, so it was straightforward to pool the data across all animals (Fig. 3).

In none of the imaged sites did we observe a correlation between preferred disparity phase and F1/F0. In one of sixteen 300 x 300 μm imaged areas, we did observe a significant inverse correlation between F1/F0 and ocular dominance.

It is expected that any relationship between the relative gradient direction of two maps will become weaker for progressively smaller gradient amplitudes. The extreme case being a zero gradient for either map, at which point the relative gradient direction of the two maps cannot be determined. To test this hypothesis, we calculated the median of the scalar product of two gradients (calculated pixel-by-pixel). The median scalar product was correlated with both vector-based VMratio and Bin ratio measures: VMratio vs. Median Scalar Product ($R = 0.53; P < 0.05$) and Bin ratio vs. Median Scalar Product ($R = 0.55; P < 0.05$). Thus, two maps such as disparity phase and ocular dominance with a small median product of gradients demonstrated a wide distribution of relative gradient directions with no significant map interaction ($VMratio < 2$), and two maps with a large median product of gradients had a narrower distribution of relative gradient directions with a significant map interaction ($VMratio > 3$).

Although we have demonstrated a fine-scale topographic mapping for disparity in the striate cortex, this need not necessarily represent a map for horizontal disparity—usually thought to be more directly responsible for the sensation of depth in central vision. Because our grating stimuli were one-dimensional, a map for vertical disparity could also explain our data obtained with obliquely oriented gratings (both imaged sites in Fig. 1). However, since several of the disparity maps were obtained with vertically
oriented gratings (e.g., Fig. 2), it is clear that there is at least a map for horizontal disparity. When using full-field gratings that are not horizontal, any horizontal disparity can be exactly produced by the appropriate phase disparity. Consequently, a map for horizontal disparity could explain all of our results, but we cannot exclude the possibility that there is also a map for vertical disparity.

Additional references

31. Cumming, B. G. & DeAngelis, G.C. The physiology of stereopsis. *Annu. Rev. Neurosci.* **24**, 203-238 (2001).
32. Prince, S. J. D., Cumming, B. G. & Parker, A. J. Range and mechanism of encoding of horizontal disparity in macaque V1. *J. Neurophysiol.* **87**, 209-221 (2002).

SUPPLEMENTARY FIGURES AND LEGENDS begin on the next page.
Supplementary Figure S1. Stability of ocular dominance with repeated imaging.

a–b, Cell-based and pixel-based direction maps show a uniform iso-direction domain at this imaged site. c, Time course of four direction-selective cells, two repeats. d, Anatomical images (left) and cell-based ocular dominance maps (right) at five depths show stable maps with repeated imaging. e, Ocular dominance histograms from two depths separated by 21 μm show similar distributions. For all experiments, data for population statistics were only considered if the imaging depth (Z interval) was separated by at least 20 μm to ensure the sampling of different cells at each depth. Here, we recorded data at a finer Z interval only to demonstrate the stability of ocular dominance maps over time (no plasticity) and the spherical nature of the calcium indicator loading. Scale bars, 100 μm.
Supplementary Figure S2. Sensitivity of calcium imaging and its relationship with action potentials in cat visual cortex.

a, Calcium imaging from a 300 x 300 μm site 272 μm below the pia. This region shows rapid but smooth changes in preferred orientation. Such sites were not used for disparity experiments, as it was impractical to present the disparity stimuli at all orientations and directions. 

b, Calcium indicator response time course for four cells (from the site shown in a) to twenty-four changes in stimulus direction (15° steps). Around the peak response, which is at a different preferred direction for each cell, responses get gradually weaker rather than show an abrupt decay to baseline. Such graded responses from neighboring neurons with different preferred stimulus directions provide good circumstantial support that OGB-1 AM can reliably track small changes in tuning preferences in the cat visual cortex.

c, To examine the relationship between changes in OGB-1 AM fluorescence in neuronal cell bodies and action potentials more directly, we performed electrophysiological recordings from sites loaded with the calcium indicator. Site shown is 300 x 300 μm, 198 μm below the pia. Green channel represents staining pattern of cells loaded with OGB-1 AM and red channel shows the electrophysiological recording pipette filled with Alexa 594.

d, Post-stimulus time histogram for electrophysiological response (red) and time course change in calcium indicator fluorescence (black) are overlaid. The electrophysiological response was from a single unit and the calcium indicator fluorescence signal was averaged from 23 neuronal cell bodies immediately surrounding the tip of the recording pipette. Increasing visual stimulus contrast produced similar graded increases in spiking activity and calcium indicator fluorescence. An earlier study by Ohki et al (2005) demonstrated matched orientation tuning between calcium indicator fluorescence and spiking in cat visual cortex for firing rates up to ~30 spikes/s (see Fig. 5b in Ref. 22). Scale bars, 100 μm.
Supplementary Figure S3. Relationship between maps for binocular disparity phase, ocular dominance, and spatial frequency.
All data are from a single experiment, same site as shown in Fig. 4, but here a larger 600 x 600 μm region was imaged. Depth = 232 μm below the pia. Data in each row are for a pair of maps, shown as smooth pixel maps, contour plots, and gradient direction difference histograms. a–d, Relationship between binocular disparity phase and ocular dominance gradients was orthogonal. e–h, Relationship between disparity and spatial frequency gradients. A relatively weak spatial frequency gradient was present but this gradient was also orthogonal to the disparity gradient. Thus, the disparity gradient was not an artifact of spatial frequency changing across the imaged site. To avoid artifacts, edges (105 μm on each side) were excluded from the analysis. Scale bar, 100 μm.
Supplementary Figure S4. Binocular disparity phase vs. ocular dominance contour plots and gradient direction difference histograms for five additional imaged sites.

a–c, Three sites, each from a different cat, showed orthogonal relationships with $VMratio > 3$.

b–d, Two sites, each from a different cat, where no interaction was observed ($VMratio < 2$).

Scale bar, 100 $\mu$m.
Supplementary Figure S5. Preferred disparity was unrelated to preferred orientation and F1/F0; and stable vergence was evident in monocular retinotopic responses.

a, Disparity phase map. b, Preferred orientation map for same cortical site shown in a. c, F1/F0 map. For checking the stability of eye position in the same experiment, monocular retinotopic stimuli (see Methods Summary) were interleaved and flashed at 10 positions across the receptive fields of each eye and calcium indicator fluorescence was simultaneously recorded from 140 cells. d, Retinotopic tuning curve for a single cell from contralateral eye stimulation. e, Average tuning curve for all 120 cells that showed selective responses to retinotopic stimulation of the contralateral eye (P < 0.05, ANOVA across 10 retinotopic positions). f, Histogram showing the distribution of the peak response in the receptive field for all cells to contralateral eye stimulation. g–i, Tuning curves and histogram for ipsilateral eye stimulation (91 cells showed selective responses to stimulation of the ipsilateral eye, P < 0.05, ANOVA across 10 retinotopic positions). Scale bar, 100 µm.
Supplementary Figure S6. Reliability of binocular disparity sensitivity estimation. Figure legend continues on next page.
Supplementary Figure S6 legend continued from previous page:

a, Location of cells from a single imaged site, same as Fig. 1h. The 114 cells that were significantly tuned for binocular disparity phase (white) and 10 cells not tuned (grey) are shown. Scale bar, 100 µm.

b, Average time course (blue) and tuning curve (red, black) for all 114 tuned cells. Response time courses are to monocular stimulation and eight spatial phase disparities, as described in Fig. 1. In time course responses, the Y axis (∆F/F) was scaled identically for all cells. Tuning curves are for disparity only and derived from the data for each cell (red), mean ± s.e.m. Sine fits are shown in black. For tuning curves, the Y axis (∆F/F) was auto scaled for each cell to highlight the relationship with sine fits. Small error bars across all cells suggest reliable trial-by-trial responses (see Fig. 1g). The median coefficient of determination ($R^2$) across all cells was 0.89, suggesting that a sine function provided a good analytical model for the data. The accuracy of the sine fits were determined from using Monte Carlo simulations so that the mean and standard deviation (s.d.) of $F1/F0$ were calculated—see c and Methods.

c, Scatter plot of mean $F1/F0$ (black) and standard deviation (red) for all tuned cell, numbered 1–114. Cell numbers in a, b, and c are in correspondence. Mean $F1/F0$ was at least twice as large as the standard deviation for all cells. Together, these data show a high fidelity of the $F1/F0$ metric for disparity sensitivity.
Supplementary Figure S7. Control to show that response onset transients were not responsible for single-cell disparity tuning preference and overall map structure.

Data are shown from two different animals. Disparity maps for the first animal are shown in a-b and for the second animal are shown in c-d. Since our disparity stimuli were 8 s long and 2 Hz temporal frequency, we had 16 cycles of grating stimulation. In analyzing the data, we normally used all 5 imaging frames (1.6 s each) that corresponded to the presentation of the visual stimulus. As a control to determine whether the response onset dominated disparity tuning, we reexamined the data after removing the response to the first three cycles of grating stimulation – using only the last 4 of 5 imaging frames. Maps using all 5 frames are shown in a and c. Maps using the last 4 frames are shown in b and d. The preferred tuning and overall map structure were indistinguishable. Scale bar, 100 µm.
Supplementary Figure S8. Controls to show that brain movements were negligible.
While each of our imaging frames had a slow acquisition rate, with our standard 512 x 512 pixel laser scanning, each of the 512 lines was 2-3 ms in duration and each pixel was collected within a few μs. Thus, brain movements can be detected as a blur of certain structures in the image. a, Maximum projection Z stack from the surface of the brain through 450 μm depth from a kitten while it was alive with a healthy heart rate and normal ventilation rate. Blood vessels were labeled with Sulforhodamine 101 via the femoral vein and cells were labeled with Alexa 594 through intracortical injection. Vessels from the surface through the depth of the imaged site were well focused. If there were axial movements, this image would be distorted. b-c, As an additional control, we eroded the cell body masks used for quantitative analysis of single-cell responses and reexamined the tuning from a smaller/central area of the cell mask. The color-coded cell map from a cortical region that has neighboring cells with different preferred disparities was identical whether the full (b) or the eroded (c) mask was used. Moreover, the tuning curves with the full mask (d, f, h) vs. eroded mask (e, g, i) were very similar. This further suggests that we had no artifacts from brain movements.
Supplementary Figure S9. Edge effects.
Our two-photon images have cellular resolution so microscopic structural boundaries were visible. For capturing the global relationship between two functional maps, it was therefore necessary to smooth each map with a filter that was larger than the distance between two adjacent cells (see Methods).

a, Model map with no smoothing.
b, Model map after smoothing.
Small edge artifacts were introduced from smoothing. Also, edges of some imaged cortical sites may not be stained with the calcium indicator. For these reasons, it was necessary to exclude the edges of the map after smoothing (the area outside the black square in b) to ensure that the analysis of map interaction (e.g., Fig. 2g) was accurate.