Principles underlying sensory map topography in primary visual cortex

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The primary visual cortex contains a detailed map of the visual scene, which is represented according to multiple stimulus dimensions including spatial location, ocular dominance and stimulus orientation. The maps for spatial location and ocular dominance arise from the spatial arrangement of thalamic afferent axons in the cortex. However, the origins of the other maps remain unclear. Here we show that the cortical maps for orientation, direction and retinal disparity in the cat (Felis catus) are all strongly related to the organization of the map for spatial location of light (ON) and dark (OFF) stimuli, an organization that we show is OFF-dominated, OFF-centric and runs orthogonal to ocular dominance columns.

Cortical organization of ON–OFF retinotopy

Previous studies have shown that ON and OFF thalamic afferents are clustered in the visual cortex15–18 but their spatial arrangement and relationship with other features of cortical topography are unknown. By measuring ON and OFF retinotopy along cortical horizontal penetrations, we show that ON and OFF cortical domains form interlaced patterns similar to ocular dominance patterns. Figure 2a illustrates a horizontal penetration crossing multiple interlaced ON and OFF domains. In this penetration, the retinotopy remained nearly constant at the peak of each domain and changed by about half a receptive field centre between domains of the same sign (for example, OFF to OFF). The horizontal track illustrated in Fig. 2a ran roughly parallel to a single ocular dominance column for more than 2 mm. Figure 2b illustrates a different horizontal track that crossed ocular dominance columns perpendicularly (see also Extended Data Fig. 1b). As in the previous example, the retinotopy was nearly constant around the peak of each domain and changed by about half a receptive field centre between peaks of the same sign. However, unlike in Fig. 2a, the ON and OFF domains peaked at nearly the same cortical location (around the centre of the ocular dominance column). We did not find a pronounced mismatch in retinotopy between the two eyes at the borders of ocular dominance columns in cats, as has been reported in primates14. Instead, the retinotopy remained well matched in both spatial position and contrast polarity (Extended Data Figs 1b, 2). To quantify the topographic arrangement of ON and OFF domains, we calculated the correlation between normalized ON and OFF responses across cortical distance separately for penetrations that ran parallel or perpendicularly to ocular dominance columns (Fig. 2c; see Extended Data Fig. 1b and Methods for selection criteria). If the ON and OFF response strengths reached their maximum at different cortical locations (as in Fig. 2a), the correlation would approach a value of −1, whereas if they reached their maximum at the same cortical location (as in Fig. 2b), the correlation would approach a value of 1. The average correlation of the ON–OFF cortical
Figure 1 | Recording from the horizontal dimension of visual cortex.

a. Recording configuration. b. Left, receptive fields mapped with light (ON) and dark (OFF) spots and ON–OFF receptive field difference. Right, orientation preference predicted by a 2D fast Fourier transform (FFT) from the ON–OFF receptive field difference. c. Orientation and direction tuning shown as response plot (left) and polar plot (right). d. Changes in orientation and direction preference across horizontal cortical distance.

Figure 2 | Topographic organization of ON and OFF cortical domains.

a. Example of a recording running parallel to an ocular dominance column. Icon on the left illustrates the recording (arrow) relative to the contralateral (C) and ipsilateral (I) columns. From top to bottom, the figure shows orientation tuning (polar and response plots), maximum ON (red) and OFF (blue) responses at each cortical site (line plot) and changes in ON and OFF receptive field position with cortical distance. 

b. Recording running perpendicular to ocular dominance columns (icon on the left) for contralateral (black) and ipsilateral (orange) eyes (continuous and dashed traces, respectively, in line plots). c. Cross-correlation between ON and OFF response profiles (red and blue lines, respectively, in a and b) in penetrations tangential (left) and perpendicular (right) to ocular dominance columns. d. Average correlation between ON and OFF response profiles in tangential (Tang.) penetrations (n = 5 penetrations, n = 5 animals) and perpendicular (Perp.) penetrations (n = 6 penetrations, n = 4 animals). e–g. Averages for spatial scale, half period and full period of ON–OFF correlation (average differences are not significant). All error bars are s.d. Statistical comparisons made with two-sided Wilcoxon tests.

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Figure 3 | Cortical topographic relationships between ON–OFF, retinotopy and orientation preference. a, Topography and retinotopy (Ret.) of two ON domains (receptive fields shown at top). b, OFF domains \((n = 20\) domains, \(n = 12\) animals) are wider than ON domains \((n = 24\) domains, \(n = 12\) animals). c, Domains of the same sign \((n = 16\) domains, \(n = 12\) animals) are separated by twice as much distance as domains of different signs \((n = 31\) domains, \(n = 12\) animals). d, Retinotopy changes more across domains \((\text{dom.})\) of the same sign \((n = 65\) domains, \(n = 20\) animals) than within domains \((n = 125\) domains, \(n = 20\) animals). e, Retinotopy changes more between domains of different \((\text{dif.})\) signs \((n = 31\) pairs of domains, \(n = 12\) animals) than between domains of the same sign \((n = 16\) pairs of domains, \(n = 12\) animals). f, Example recording showing smooth changes in retinotopy with cortical distance \((\text{Cort. dis.})\) at 0.5 receptive fields \((\text{RF})\) per mm \((n = 496\) paired comparisons). g, The OFF pathway anchors the cortical retinotopy of both monocular \((\text{top})\) and binocular \((\text{Binoc.})\) receptive fields \((\text{bottom})\); contralateral, black; ipsilateral, orange). ON responses \((\text{red})\) rotate around OFF responses \((\text{blue})\), as illustrated by individual series of receptive fields \((\text{left})\), receptive fields averaged across cortical distance \((\text{Average})\) and retinotopy of strongest ON and OFF responses \((\text{Retinot.})\). h, Retinotopy changes with cortical distance for ON, OFF and ON–OFF responses \((\text{red}, \text{maximum}; \text{blue}, \text{minimum})\). Dotted lines show 20% of maximum ON responses \((n = 2,603\) paired comparisons, \(n = 8\) animals). i, Retinotopy changes are more restricted for OFF than ON responses \((n = 962\) ON and 962 OFF paired-comparisons, \(n = 23\) animals). j, Binocular retinal disparity is smallest when measured between OFF subregions \((\text{top})\), \(n = 502\) for ON–OFF, 251 for ON–ON and 251 for OFF–OFF subregions, \(n = 28\) animals). k, Retinal disparity changes more than OFF retinal disparity with differences in spatial phase \((\text{bottom})\). l, Periodicity in orientation preference across horizontal cortical distance within a single penetration \((\text{left})\) and across penetrations \((\text{middle})\); middle, \(n = 618\) paired comparisons, \(n = 37\) animals). The orientation periodicity resembles the periodicity of the ON–OFF correlation \((\text{right})\); \(n = 11\) penetrations, \(n = 8\) animals). m, Retinotopy difference between subregions of different signs falls rapidly with cortical distance \((n = 13,416\) paired comparisons, \(n = 23\) animals). All error bars are s.e.m. *\(P < 0.05\), **\(P < 0.001\) with two-sided Wilcoxon tests.

Periodicity was \(+0.65 \pm 0.17\) (mean ± s.d.) in penetrations perpendicular to ocular dominance columns and \(-0.78 \pm 0.20\) in penetrations running tangentially (Fig. 2d, \(P = 0.004\), Wilcoxon test), indicating that ON and OFF domains are interlaced along the main axis of the ocular dominance column but aligned along its perpendicular axis.

The periodicity of ON and OFF domains was similar in penetrations running tangentially and perpendicular to ocular dominance columns. It had a sigma of about 0.3 mm (Fig. 2e; 0.27 ± 0.12 mm and 0.25 ± 0.10 mm for tangential and perpendicular penetrations, respectively), a half period of about 0.6 mm (Fig. 2f; 0.56 ± 0.10 mm and 0.61 ± 0.16 mm) and a period of about 1.1 mm (Fig. 2g; 1.06 ± 0.21 mm and 1.21 ± 0.31 mm). To quantify in more detail the cortical spread and retinotopy change in each cortical domain, we selected penetrations that passed through a sequence of three or more ON and OFF domains (Fig. 3a; only ON domains shown for clarity). Consistent with our previous results, OFF cortical domains were significantly larger than ON cortical domains (Fig. 3b; OFF: 0.65 ± 0.32 mm, ON: 0.49 ± 0.15 mm, \(P = 0.048\), Wilcoxon test) but were separated by similar cortical distances (ON to ON: 0.88 ± 0.23 mm; OFF to OFF: 1.0 ± 0.24 mm, \(P = 0.314\), Wilcoxon test), which was about twice the distance separating domains of different signs (Fig. 3c; 0.9 ± 0.24 mm versus 0.45 ± 0.17 mm, \(P < 0.0001\), Wilcoxon test). The retinotopy change was limited to less than 0.2 receptive field centres within each domain and approached 0.5 receptive field centres between domains of the same sign (Fig. 3d; 0.18 ± 0.12 versus 0.44 ± 0.24 receptive field centres, \(P < 0.0001\), Wilcoxon test). When normalized by cortical distance, the retinotopy moved faster between domains of different signs than domains of the same sign, probably because domains of different signs are less likely to share thalamic afferents (Fig. 3e; 0.57 ± 0.39 versus 0.38 ± 0.21 receptive field centres per mm, \(P = 0.036\), Wilcoxon test).

ON–OFF retinotopy and ocular dominance columns

Retinotopy is thought to change abruptly at the borders of ocular dominance columns in monkeys because of the interruption caused by the cortical representations of the two eyes. Notably, our recordings revealed smooth changes in retinotopy in cats. To quantify these retinotopy changes, we selected tangential penetrations that passed through a sequence of at least three ocular dominance columns (for example, left–right–left, Extended Data Fig. 1b) and then measured how retinotopy changed between the peaks of ocular dominance columns for the same eye. Consistent with previous work, ocular dominance columns had an average width of around 0.5 mm in the cat.
(0.44 ± 0.14 mm, n = 31) and ocular dominance columns for the same eye were separated from each other by around 1 mm (1.02 ± 0.17 mm, n = 13). Similar to the retinotopy changes between ON–OFF domains of the same sign (Fig. 3d), the retinotopy changes between ocular dominance columns of the same eye were about 0.5 receptive field centres (0.55 ± 0.22 receptive field centres, n = 13, data not shown). In fact, some cortical penetrations showed almost a perfect linear relationship between cortical distance and retinotopy with a slope of 0.5 receptive field centres per mm (Fig. 3f).

**OFF responses anchor cortical retinotopy**

Our previous work demonstrated that OFF thalamic afferents cover larger cortical territory and make stronger connections than ON thalamic afferents in cat visual cortex. Because of their larger horizontal extent, retinotopy should change less with cortical distance for OFF than ON cortical responses. We found not only that OFF retinotopy is more precise than ON retinotopy but also that it acts as the anchor of the cortical retinotopic map. This unexpected result, which we previously reported in an abstract, has now been replicated in tree shrew visual cortex and it seems also to be present in primates (Extended Data Fig. 3). In horizontal penetrations through cat visual cortex, we frequently found that ON retinotopy rotated around OFF retinotopy (Fig. 3g), and that the retinotopy scatter was larger for ON than OFF responses (Fig. 3h–i; 0.65 ± 0.79 versus 0.51 ± 0.61 receptive field centres per mm, P < 0.0001, Wilcoxon test). Notably, OFF retinotopy anchored not only the monocular retinotopic map but also the binocular retinal disparity. In binocular receptive fields, the retinotopy changed less for OFF than ON responses and, although OFF retinotopy tended to be spatially aligned between the two eyes, ON retinotopy rotated around OFF (Fig. 3g, bottom; see also Extended Data Fig. 3 for an example in a macaque). Binocular retinal disparity was largest for receptive field subregions of different signs, intermediate for ON–ON subregions and smallest for OFF–OFF subregions (Fig. 3j, top: 0.31 ± 0.18, 0.23 ± 0.20 and 0.14 ± 0.11 receptive field dominated.

**Figure 4 | Changes in retinotopy explain changes in orientation and direction preference throughout the cortex.** a. Horizontal penetration showing a strong relationship between changes in ON–OFF retinotopy and orientation preference. Responses to light stimuli (middle) rotate around responses to dark stimuli (top) as seen in the dark–light difference (bottom). Orientation and direction tuning and ON/OFF retinotopy are shown below the colour panels (small circles in polar plots are orientation predictions based on dark–light receptive fields). b. Predicted and measured comparisons in 109 penetrations (916 recording sites, n = 26 animals) that passed our selection criteria (see Methods; dashed lines mark maximum possible mismatch). c. Normalized count of differences between measurements and predictions (median, 17.3°). d. Horizontal penetration passing through a pinwheel (at 0.5–0.6 mm) that was completely OFF. e. Pinwheel centres (aligned at cortical distance zero) tended to have higher absolute (Abs.) contrast polarity (strong OFF or ON dominance) than their cortical neighbourhoods (n = 19 penetrations, n = 13 animals; P < 0.0001 for difference in orientation (Ori.) selectivity and P = 0.039 for difference in absolute contrast polarity when comparing 0 and ±0.3 mm, one-sided Wilcoxon tests). f. Histogram showing the contrast polarity of the 19 pinwheels from e. g. Horizontal penetration passing through regions with abrupt changes in direction preference (0.1–0.3 mm and 0.6–0.7 mm). Abrupt changes in direction were associated with abrupt changes in retinotopy (arrows at top and line plots at bottom). h. Aligning direction reversals at cortical distance zero (n = 24 penetration sections, n = 10 animals) revealed a strong association between direction and retinotopy changes (RF pos). All error bars are s.e.m.
fields strongly dominated by one contrast polarity, as our methods for selection criteria, and the median prediction error was only how changes in ON–OFF retinotopy result in changes in orientation and direction preference.

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ON–OFF retinotopy and orientation preference

Our previous work showed that the arrangement of OFF and ON thalamic afferents in the visual cortex is closely related to the representation of orientation preference. To quantify this relationship across the horizontal dimension of the visual cortex, we first compared the average periodicity of ON–OFF retinotopy with the periodicity of orientation preference across cortical distance. The periodicity of orientation preference was very pronounced even in single horizontal penetrations (Fig. 3k, left and Extended Data Fig. 4) and, on average, it had a half period of 0.67 mm and a full period of 1.27 mm (Fig. 3k, middle), which closely matched the average periodicity of ON–OFF retinotopy (Fig. 3k, right; average periodicity of ON–OFF retinotopy 0.57/1.02 mm; 0.56/1.06 mm for tangential penetrations and 0.61/1.21 mm for perpendicularly penetrations). The difference in retinotopy between neurons separated by 0.1 mm was 1.6-times larger for subregions of different signs (ON–OFF) than for subregions of the same sign (Fig. 3l). However, the different/same-sign ratio decayed rapidly with cortical distance to 89% at 0.3 mm (Fig. 3l) and 0.3 mm is the approximate size of purely OFF dominated or ON dominated pinwheels. The limited retinotopy changes at the borders of ocular dominance columns seem ideal to generate a smooth and precise map of retinal disparity.

ON–OFF retinotopy and direction preference

Although our previous work predicted that cortical changes in ON–OFF retinotopy should be related to changes in orientation preference, we were surprised to find that changes in ON–OFF retinotopy were also related to changes in direction preference. Because weaker receptive field subregions generate responses with longer response latencies than those of stronger subregions, cortical responses coincide in time and reinforce each other when a stimulus moves from a weak to a strong subregion but not from a strong to a weak subregion. In cortical horizontal penetrations that passed through direction fractures (rapid reversals of direction preference), abrupt changes in the retinotopic position of the strongest receptive field subregion were associated with abrupt changes in direction preference (Fig. 4g). To quantify this relationship, we selected penetrations in which direction preference changed abruptly but orientation remained relatively constant (to avoid rotations or translations in retinotopy that were not related to direction). In 24 penetrations that met this criterion, rapid reversals in direction preference (Fig. 4h, marked as 0 cortical distance) were strongly associated with rapid changes in the retinotopy of the strongest receptive field subregion and both occurred within 0.1 mm of each other.

Discussion

Our findings suggest that the topography of the visual cortex in carnivores and primates is governed by a precise match in the properties of the thalamic afferents that converge at a given cortical point. The afferents are precisely matched in retinotopy, which changes slowly at 0.5 receptive field centres per mm in cats (Fig. 5a). They are also matched in eye input and ON–OFF polarity, which leads to a columnar organization for both ocular dominance and ON–OFF responses in Fig. 5a). In OFF domains, which are mostly prominent, OFF afferents are better matched in retinotopy than ON afferents; the opposite is true in ON domains. In this OFF-dominated and OFF-centric topography, changes in orientation and direction preference are determined by changes in ON–OFF retinotopy. Therefore, orientation preference may show a tendency to remain constant across the border of ocular dominance columns simply because ON–OFF retinotopy also remains constant (Fig. 5a).

It is unclear what developmental mechanisms could generate this precise ON–OFF retinotopic match at each cortical point. However, if OFF domains with precisely matched retinotopy appear first during development, the retinotopy of the ON afferents may have to be displaced within each OFF domain so that ON and OFF afferents can simultaneously drive the same cortical targets (Fig. 5b). This mechanism would make ON receptive fields rotate around OFF receptive fields and, as a consequence, orientation and direction maps would originate (Fig. 5c) in a sensory map that is represented as continuously...
as possible. In the visual cortex, this continuous representation could be accomplished by precisely matching the response properties of ON and OFF thalamic afferents; however, the same principles may apply to other sensory spaces and afferents feeding other cortical areas that have maps for touch, hearing or spatial navigation.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Blasdel, G. G. & Salama, G. Voltage-sensitive dyes reveal a modular organization in monkey striate cortex. Nature 321, 579–585 (1986).
2. Bonhoeffer, T. & Grinvald, A. Iso-orientation domains in cat visual cortex are arranged in pinwheel-like patterns. Nature 353, 429–431 (1991).
3. Ohki, K. et al. Highly ordered arrangement of single neurons in orientation columns. Nature 442, 925–928 (2006).
4. Kaschube, M. et al. Universality in the evolution of orientation columns in the visual cortex. Science 330, 1113–1116 (2010).
5. Nauhaus, I. & Nielsen, K. J. Building maps from maps in primary visual cortex. Curr. Opin. Neurobiol. 24, 1–6 (2014).
6. Levy, M., Lu, Z., Dion, G. & Kara, P. The shape of dendritic arbors in different functional domains of the cortical orientation map. J. Neurosci. 34, 3231–3236 (2014).
7. Cossetti, L. et al. Functional organization of excitatory synaptic strength in primary visual cortex. Nature 518, 399–403 (2015).
8. Miller, K. D. A model for the development of simple cell receptive fields and the ordered arrangement of orientation columns through activity-dependent competition between ON- and OFF-center inputs. J. Neurosci. 14, 409–441 (1994).
9. Jin, J., Wang, Y., Swadlow, H. A. & Alonso, J. M. Population receptive fields of ON and OFF thalamic afferents; however, the same principles may apply to other sensory spaces and afferents feeding other cortical areas that have maps for touch, hearing or spatial navigation.
10. Wang, Y. et al. Columnar organization of spatial phase in visual cortex. Nature Neurosci. 18, 97–103 (2015).
11. Chapman, B. & Godecke, I. Cortical cell orientation selectivity fails to develop in the absence of ON-center retinal ganglion cell activity. J. Neurosci. 20, 1922–1930 (2000).
12. Chapman, B., Zahs, K. R. & Stryker, M. P. Relation of cortical cell orientation selectivity to alignment of receptive fields of the geniculocortical afferents that arborize within a single orientation column in ferret visual cortex. J. Neurosci. 11, 1347–1358 (1991).
13. Paik, S. B. & Ringach, D. L. Retinal origin of orientation maps in visual cortex. Nature Neurosci. 14, 919–925 (2011).
14. Hubel, D. H. & Wiesel, T. N. Ferrier lecture. Functional architecture of macaque monkey visual cortex. Proc. R. Soc. Lond. B Biol. Sci. 198, 1–59 (1977).
15. Jin, J. Z. et al. On and off domains of geniculate afferents in cat primary visual cortex. Nature Neurosci. 11, 88–94 (2008).
16. Zahs, K. R. & Stryker, M. P. Segregation of ON and OFF afferents to ferret visual cortex. J. Neurophysiol. 59, 1410–1429 (1988).
17. McConnell, S. K. & LeVay, S. Segregation of on- and off-center afferents in mink visual cortex. Proc. Natl Acad. Sci. USA 81, 1590–1593 (1984).
18. Norton, T. T., Rager, G. & Kretz, R. ON and OFF regions in layer IV of striate cortex. Brain Res. 327, 319–323 (1985).
19. Kaschube, M. et al. The pattern of ocular dominance columns in cat visual cortex: intra- and interindividual variability of column spacing and its dependence on genetic background. Eur. J. Neurosci. 18, 3251–3266 (2003).
20. Kremkow, J. et al. Asymmetries in ON and OFF cortical retinotopy: are OFF receptive fields the anchors of cortical retinotopic maps? Soc. Neurosci. abstr. 639.09 (2013).
21. Lee, K.-S., Hu, X., Cynx, J. & Fitzpatrick, D. Specificity in the spatial organization of receptive fields supporting multiple functional maps in tree shrew visual cortex. Soc. Neurosci. abstr. 232.13 (2015).
22. Samaika, R., Wang, B. S. & Cang, J. Experience-dependent and independent binocular correspondence of receptive field subregions in mouse visual cortex. Cereb. Cortex 24, 1658–1670 (2014).
23. Karam, P. A. & Boyd, J. D. A micro-architecture for binocular disparity and ocular dominance in visual cortex. Nature 458, 627–631 (2009).
24. Sharma, J., Angelucci, A. & Sur, M. Induction of visual orientation modules in auditory cortex. Nature 404, 841–847 (2000).
25. Humphrey, A. L., Sur, M., Uhlirch, D. J. & Sherman, S. M. Projection patterns of individual X- and Y-cell axons from the lateral geniculate nucleus to cortical area 17 in the cat. J. Comp. Neurol. 233, 159–189 (1985).
26. Reid, R. C., Soodak, R. E. & Shapley, R. M. Linear mechanisms of directional selectivity in simple cells of cat striate cortex. Proc. Natl Acad. Sci. USA 84, 8740–8744 (1987).
27. Jagadeesh, B., Wheat, H. S. & Ferster, D. Linearity of summation of synaptic potentials underlying direction selectivity in simple cells of the cat visual cortex. Science 262, 1901–1904 (1993).
28. Tolhurst, D. J. & Dean, A. F. Scalable model of direction selectivity in simple cells of the cat's striate cortex. Vis. Neurosci. 6, 421–428 (1991).
29. Albrecht, D. G. & Geisler, W. S. Motion selectivity and the contrast-response function of simple cells in the visual cortex. Vis. Neurosci. 7, 531–546 (1991).
30. McLean, J., Raab, S. & Palmer, L. A. Contribution of linear mechanisms to the specification of local motion by simple cells in areas 17 and 18 of the cat. Vis. Neurosci. 11, 271–294 (1994).
31. Livingstone, M. S. Mechanisms of direction selectivity in macaque V1. Neuron 20, 509–526 (1998).
32. Smith, G. B., Whitney, D. E. & Fitzpatrick, D. Modular representation of luminance polarity in the superficial layers of primary visual cortex. Neuron 88, 805–818 (2015).
33. Blasdel, G. G. Orientation selectivity, preference, and continuity in monkey striate cortex. J. Neurosci. 12, 3139–3161 (1992).
34. Albos, K. & Wolf, W. Early post-natal development of neuronal function in the kitten's visual cortex: a laminar analysis. J. Physiol. (Lond.) 348, 153–185 (1984).
35. Swindale, N. V., Shoham, D., Grinvald, A., Bonhoeffer, T. & Hubener, M. Visual cortex maps are optimized for uniform coverage. Nature Neurosci. 3, 822–826 (2000).
36. Woolsey, T. A. & Van der Loos, H. The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. Brain Res. 17, 205–242 (1970).
37. Friedman, R. M., Chen, L. M. & Roe, A. W. Modality maps within primate somatosensory cortex. Phil. Trans. R. Soc. Lond. B Biol. Sci. 301, 12724–12729 (2004).
38. Miller, L. M., Escabi, M. A., Read, H. L. & Schreiner, C. E. Functional convergence of response properties in the auditory thalamocortical system. Neuron 32, 151–160 (2001).
39. Hafting, T., Fyhn, M., Molden, S., Moser, M. B. & Moser, E. I. Microstructure of a spatial map in the entorhinal cortex. Nature 436, 801–806 (2005).
40. Peyrache, A., Lacroix, M. M., Petersen, P. C. & Buzsaki, G. Internally organized mechanisms of the head direction sense. Nature Neurosci. 18, 569–575 (2015).
**METHODS**

All procedures were performed in accordance with the guidelines of the US Department of Agriculture and approved by the Institutional Animal Care and Use Committee at the State University of New York, State College of Optometry.

**Surgery and preparation.** Adult male cats (aged 6–12 months, 
$n = 40$) were tranquilized with acemprozine (0.2 mg kg$^{-1}$, intramuscularly) and initially anaesthetized with ketamine (10 mg kg$^{-1}$, intramuscularly). An intravenous catheter was inserted into each hind limb to allow continuous infusions of propofol (5–10 mg kg$^{-1}$, intravenous infusion) for anaesthesia, vecuronium bromide (0.2 mg kg$^{-1}$, intravenous) for muscle paralysis, and saline (1–3 ml h$^{-1}$) for hydration. All vital signs were closely monitored and carefully maintained within normal physiological limits. The nictitating membranes were retracted with 2% neosynephrine and the pupils dilated with 1% atropine sulphate. Contact lenses were used to protect the corneas and focus visual stimuli on the retina. The positions of the optic disc and the area centralis were plotted on a screen in front of the animal using a fibre optic light source. Details of the surgical procedures have been described previously$^{15}$. We also performed recordings in one male rhesus macaque (age, 8.5 years; 10 kg) using similar procedures to those described above. The macaque was anaesthetized with ketamine (10 mg kg$^{-1}$, intramuscularly) and diazepam (0.75 mg kg$^{-1}$, intravenously) followed by propofol (1.8 mg kg$^{-1}$, intravenously) and a continuous infusion of sufentanil citrate that was maintained throughout the experiment (6–20 μg kg$^{-1}$ h$^{-1}$, intravenous). The animal was paralysed after finishing the surgery with vecuronium bromide (0.1 mg kg$^{-1}$, intravenous).

**Electrophysiological recordings and data acquisition.** We used linear 32-channel multielectrode arrays (inter-electrode distance, 0.1 mm; Neuronexus) to record multi-unit neuronal activity along the horizontal dimension of primary visual cortex (Fig. 1a). The signals from the recording electrodes were amplified, filtered, and collected by a computer running Raspunit (Plexon), as previously described$^{15}$. The multielectrode arrays were inserted with a small angle nearly parallel to the cortical surface (< 5°), parallel to the anteroposterior axis in the middle of the posterior lateral gyrus and centred in layer 4. The centring of the recordings in layer 4 was estimated from cortical depth, local field potentials and the presence of simple receptive fields measured with white noise, which are mostly restricted to layers 4 and 6 in cat visual cortex$^{14,15}$. Sample size was chosen to be the largest possible for each analysis performed. All comparisons were evaluated for statistical significance using two-sided Wilcoxon tests (signed-rank for paired data and rank-sum for non-paired), except for that shown in Fig. 4e (one-sided Wilcoxon test). Data distributions are described in the main text by their mean and s.d. (median for Fig. 4c) while the figures show either s.d. or s.e.m. (see figure legends). No randomization was used to determine how samples or animals were allocated to experimental groups and no blinding approach was used for sample selection.

**Visual stimulation.** Visual stimuli were generated in Matlab (The MathWorks) using the Psychophysics Toolbox extensions$^{46}$ and presented on a calibrated CRT monitor (refresh rate 120 Hz, mean luminance 61 cd m$^{-2}$). The monitor was positioned so that the receptive fields of all recorded channels were covered by the visual stimulus. We used light and dark moving bars (16 directions, 8 orientations) to measure orientation tuning (Fig. 1c) and receptive fields were mapped using sparse noise stimuli. The frames of the sparse noise were updated at a rate of 30 Hz (monitor refresh rate 120 Hz) and the sparse noise targets were either light (120 cd m$^{-2}$) or dark (< 2 cd m$^{-2}$). Light targets were presented on a dark background and dark targets on a light background (Extended Data Fig. 1a). We used large targets (1–2° width) to drive responses from weak receptive field flanks. The use of large stimuli greatly overestimates the size of the receptive fields but provides large targets (1–2° width) to drive responses from weak receptive field flanks. The ground and dark targets on a light background (Extended Data Fig. 1a). We used ground and dark targets on a light background (Extended Data Fig. 1a).

**Data analysis.** All data analysis was performed in Matlab using customized analysis routines as described below for each major set of measurements.

**Oriental for simple receptive fields.** Simple receptive field tuning was measured with moving bars (16 directions of motion) and fitted with a von Mises function$^{44}$. The orientation or direction preference and selectivity were extracted from the fits as previously described$^{44}$. To precisely estimate the spatial ON and OFF receptive fields of each recording site, we calculated the peri-stimulus-time histogram (PSTH) at a temporal resolution of 1 ms for each stimulus pixel. This analysis resulted in a 3D array (x-space, y-space, time) representing the neuronal response in space and time. We then estimated the spatial receptive field by integrating all spikes caused by the stimulus onset (Extended Data Fig. 1a, grey shaded area in the PSTH) after smoothing the temporal response with a Gaussian window (sigma, 10 ms). The ON receptive fields were calculated from the response onset to the light targets and the OFF receptive fields from the onset to dark targets. This analysis resulted in four receptive field measurements for each cortical site (contra eye: ONc and OFFc; ipsi eye: ONi and OFFi). Each receptive field was then normalized by subtracting its mean and dividing by its maximum. The normalized ON and OFF receptive fields were then used to calculate the ON–OFF receptive fields by subtracting OFF from ON. When showing receptive fields to compare changes in ON–OFF retinotopy across the cortex (Fig. 2a), we normalized by the maximum response to ON or OFF, whichever was greater (normalization for contrast polarity). When showing binocular receptive fields to compare changes in ocular dominance (Fig. 2b), we normalized by the maximum response of both eyes, whichever was greater (normalization for ocular dominance). The receptive field integration time was 50 ms to measure ON–OFF retinotopy (Fig. 2), 200 ms to measure contralateral/ipsilateral retinotopy (Extended Data Fig. 1b) and variable to predict ON–OFF retinotopy (same procedure as explained in ‘binocular organization of ON–OFF’ below). Receptive field similarity across recording sites was estimated by calculating the correlation coefficient between the ON–OFF receptive fields.

**Binocular alignment of receptive fields.** To measure the binocular organization of ON and OFF retinotopy, we first had to align the monocular receptive fields because the eyes were misaligned by the muscle paralysis in our preparation. To achieve unbiased eye alignment, we made use of the high number of simultaneously measured receptive fields (32 recording positions), using an approach that was very successful at revealing cortical maps for retinal disparity$^{23}$. To that end, we calculated the retinotopic receptive field (Rr) by summing the ON and OFF receptive fields of all channels, separately for the ipsilateral (Rri) and contralateral eye (Rrc). We then performed a 2D cross-correlation analysis between Rri and Rrc to estimate the horizontal and vertical shift between the two eyes and used this measurement to align both eyes.

**Cortical domains for ON–OFF.** To calculate the cortical ON–OFF domains, we analysed the neuronal responses to light and dark sparse noise stimuli. For each cortical site we calculated the spatial receptive fields (ONc, ONi, OFFc, OFFi) at the peak of the response onset (the temporal response was smoothed with a Gaussian window; sigma, 10 ms). To extract the relative strength between ON–OFF and ipsilateral–contralateral responses, we normalized the amplitude of the receptive fields by the strongest response at each cortical site. A small Gaussian window (sigma, 1 recording channel) was used to smooth the responses across cortex. This analysis resulted in a 3D array for each stimulus condition (ONc, ONi, OFFc, OFFi), representing x and y of the visual field (retinotopy) and the 32 recording channels (cortical distance). From this 3D representation of the ON–OFF cortical domains, we calculated the 1D cortical activation profiles (Fig. 2a, b; red and blue traces) by using the value of the maximum response at each cortical site. This analysis resulted in 1D activation profiles for ONc, ONi, OFFc and OFFi that represented the relative strength of ON–OFF and ipsilateral–contralateral responses at each cortical position. To estimate the correlation, spatial scale and periodicity of the ON–OFF responses across cortical distance, we calculated the cross-correlation between the ON and OFF cortical activation profiles (Fig. 2a, b; red and blue traces). We used the correlation coefficient between ON and OFF as the measure for the overall correlation between ON–OFF domains. The spatial spread was estimated as the standard deviation of a Gaussian function fitted to the central part of the cross-correlogram (Fig. 2c). The half period was taken as the first reversal in the cross-correlogram and the full period as the second peak (Fig. 2c). To compare the cortical widths of ON and OFF domains, we selected horizontal cortical penetrations that crossed at least three ON or OFF cortical domains (Fig. 3a). We then measured the width of each domain as the number of contiguous recording sites that generated responses with high signal-to-noise ratio (SNR > 5) and averaged the widths separately for ON and OFF domains (Fig. 3b). The cortical distance between domains was measured between the most central recording sites within each domain (Fig. 3c). The retinotopy change was measured as the difference in retinotopy between two recording sites, using the larger receptive field diameter as the unit (Fig. 3d, e).

To compare the ON–OFF arrangement to ocular dominance columns, we selected our longest horizontal recording tracks that either remained monocular for the entire track or alternated between left and right eyes along the track length. We assumed that a horizontal track that remained monocular for the same eye for more than 1.2 mm was running roughly tangentially to an ocular dominance column and that a track that showed multiple alternating monocular responses for left and right eyes was running roughly perpendicular. Following this strict criteria, five horizontal tracks were classified as tangential to an ocular dominance column (average track length and range: 1.74 ± 0.5 mm, 1.2–2.6 mm; average and range of ON/OF domain number: 3.2 ± 0.97, 2–5) and six tracks were classified as perpendicular (average track length and range: 2.23 ± 0.51 mm, 1.4–2.9 mm; average and range of ocular domain number: 4.3–1.52, 2–5).

**Cortical domains for ocular dominance.** We selected horizontal cortical penetrations that passed through at least three different ocular dominance domains. The width of each domain was measured as the number of contiguous recording sites that generated responses with high SNR (SNR > 5). The cortical distance between the peaks of ocular dominance domains was measured as the distance between the
central recording sites within each domain. The retinotopy change was measured as the difference in retinotopy between receptive fields located between the peaks of ocular dominance columns for different eyes using the larger receptive field diameter as the unit.

**Retinotopy change of ON–OFF responses.** To estimate the retinotopy change across the horizontal dimension of cortex, we measured the centre of the strongest peak receptive field subregion by calculating the centre of mass around the peak response (using a receptive field threshold at 70–80% of maximum response). We then calculated the Euclidian distances between the receptive field centres of paired recording sites and normalized this distance by the diameter of the larger receptive field. The receptive field diameter was approximated from the area of the receptive field with a response above 20% of the maximum response (assuming a circular receptive field). To maximize the accuracy of our measurements, the population analysis included only cortical sites with SNR > 10.

**Binocular organization of ON–OFF retinotopy.** To study the binocular organization of the ON–OFF retinotopy, we fitted a 2D Gabor function to the ON–OFF receptive fields (ON–OFFc, ONi–OFFi). We then extracted the spatial phase difference from the Gabor fits and measured binocular disparity as the retinotopic distance between the positions of the subregions from the ON–OFF receptive fields. We calculated the ON–OFF receptive field by optimizing ON–OFF segregation, as this resulted in better and more reliable fits to the 2D Gabor function. To achieve this, we used a sliding window of 50 ms and calculated the ON–OFF receptive field with a range of starting positions (0–100 ms). From this ensemble of ON–OFF receptive fields, we selected the one that had the highest SNR and most balanced ON–OFF receptive field. ON–OFF balance was calculated as the absolute value of contrast polarity, where contrast polarity is \((\text{max(ON)} - \text{max(OFF)})/ (\text{max(ON)} + \text{max(OFF)})\). If the absolute contrast polarity equals 0, ON and OFF responses are equally strong; if it equals 1, responses are completely dominated by either OFF or ON. Because the spatial phase can vary over the time course of the spatiotemporal receptive field, we always used the same time point to calculate the ON–OFF receptive fields in both eyes. To maximize the accuracy of the measurements, the population analysis included only sites with ON–OFF receptive fields that had SNR > 6 and were well fit by the Gabor function (goodness of fit > 0.5).

**Orientation and ON–OFF periodicity.** To study the orientation periodicity, we extracted the orientation preference from the fitted tuning curves (see above) and then calculated the orientation difference as a function of cortical distance. We measured the orientation difference between all possible pairs on our 32-channel recording array (n = 496 per recording array). We repeated this analysis across our entire data set and calculated the median orientation difference for each cortical distance (Fig. 3k, middle). To ensure that the measurement was precise, we included only pairs with excellent fits in orientation tuning (goodness of fit > 0.9), pronounced orientation selectivity (orientation selectivity index > 0.5) and responses with high SNR (SNR > 4), resulting in 20,672 pairs across all possible cortical distances (orientation selectivity was defined as the ratio between the response at the preferred orientation and the response at the orthogonal orientation). We then estimated the half period from the first reversal of the average orientation difference across cortical distance and the full period from the second minimum (Fig. 3k, middle). To characterize the periodicity of ON–OFF responses across cortical distance, we averaged all cross-correlation measurements from ON–OFF cortical domains (Fig. 2c). Because ON–OFF domains are anti-correlated in recordings tangential to the ocular dominance bands but correlated in recordings perpendicular to ocular dominance bands (Fig. 2d), we multiplied the cross-correlograms of the recordings perpendicular to ocular dominance columns by −1 before averaging. We then obtained periodicity measures from the average normalized ON–OFF correlation for both the half period and the full period (Fig. 3k, right).

**Predicting orientation preference from the receptive field.** To predict the orientation preference from the ON–OFF receptive fields, we first calculated the ON–OFF receptive field difference using the sliding window approach described above (see Binocular organization of ON–OFF retinotopy). We then used the 2D discrete FFT (2D-FFT) of the ON–OFF receptive field to estimate the predicted preferred orientation preference (Fig. 1b, right). This population analysis included only horizontal cortical penetrations that had at least five recording sites with receptive fields showing clear ON–OFF segregation (SNR of ON–OFF receptive field > 8) and good orientation selectivity measured with moving bars (orientation selectivity > 0.5; goodness of fit for orientation tuning > 0.6). The peaks in the 2D-FFT also had to be distant from the origin, as otherwise the preferred orientation extracted from the 2D-FFT would be ambiguous.

**Orientation pinwheels and direction fractures.** To investigate a possible relationship between ON–OFF dominance and orientation selectivity at pinwheel centres, we selected horizontal recordings in which orientation changed abruptly. To make our sample of orientation discontinuities as homogeneous as possible, we selected only cortical regions that were completely monocular, responded strongly to all stimulus orientations and had responses with high SNR (SNR > 5). We then measured changes in both orientation selectivity and absolute contrast polarity (OFF or ON dominance) as a function of cortical distance from the region with lowest orientation selectivity (Fig. 4e). To investigate a possible relationship between ON–OFF dominance and abrupt changes in direction preference, we selected sections of horizontal cortical penetrations in which orientation preference changed by < 45° but direction preference changed abruptly within < 0.2 mm (receptive field SNR > 5). We then marked the abrupt changes in direction preference as cortical distance 0 and measured changes in direction preference and spatial location of the strongest subregion within the receptive field as a function of cortical distance. To measure the changes in retinotopic position with the maximum accuracy possible, we did not subtract responses to different stimuli and made all the measurements directly from responses to light stimuli.

41. Martinez, L. M. et al. Receptive field structure varies with layer in the primary visual cortex. *Nature Neurosci.* 8, 372–379 (2005).
42. Brainard, D. H. The psychophysics toolbox. *Spat. Vis.* 10, 433–436 (1997).
43. Swindale, N. V., Grinvald, A. & Shmuel, A. The spatial pattern of response magnitude and selectivity for orientation and direction in cat visual cortex. *Cereb. Cortex* 13, 225–238 (2003).
44. Lashgari, R. et al. Response properties of local field potentials and neighboring single neurons in awake primary visual cortex. *J. Neurosci.* 32, 11396–11413, (2012).
45. DeAngelis, G. C., Ghose, G. M., Ohzawa, I. & Freeman, R. D. Functional micro-organization of primary visual cortex: receptive field analysis of nearby neurons. *J. Neurosci.* 19, 4046–4064 (1999).
Extended Data Figure 1 | Measurements of ON–OFF responses and ocular dominance columns. **a**, ON and OFF receptive fields were mapped with light (ON) and dark (OFF) sparse noise and calculated from the response to the stimulus onset (grey shaded area). **b**, Horizontal penetrations that ran for more than 1.2 mm through a monocular band were assumed to be nearly parallel to ocular dominance columns (top) and those that alternated between monocular responses for left and right eyes were assumed to be nearly orthogonal to ocular dominance columns (bottom). Receptive fields normalized for ocular dominance. Icons on the left illustrate ocular dominance columns for contralateral (C) and ipsilateral (I) eyes (arrow illustrates horizontal penetration). Each receptive field box has a side of 27°.
Extended Data Figure 2 | ON–OFF domains are matched across eyes.
a. Integrating the ON–OFF receptive fields over 0.7 mm of horizontal cortical distance reveals ON and OFF receptive field subregions that are segregated in visual space and well matched between eyes. Notice the excellent binocular match of the receptive field subregions measured with light spots (left, two subregions displaced vertically in both eyes), and dark spots (middle left, one central subregion in both eyes). The ON–OFF receptive field difference also shows an excellent binocular match (middle right), so the ON–OFF segregation can still be seen after combining the receptive fields of the two eyes (right). b. Integrating the ON–OFF receptive fields over a much longer distance (1.6 mm of cortex, different horizontal penetration) still reveals separate receptive field subregions with excellent binocular match. The 1.6-mm-average receptive fields of the left and right eyes have both two ON subregions that are displaced diagonally and retinotopically matched (left). They also have two OFF subregions that are also displaced diagonally and retinotopically matched between the two eyes (middle left). A hint of the ON subregions can still be seen in the ON–OFF receptive field difference (middle right) and receptive field of both eyes combined (right), even if the receptive fields were averaged over 1.6 mm of cortex. Each square box framing a receptive field has a side of 16.2°.
Extended Data Figure 3 | The OFF pathway might also anchor retinotopy in the primary visual cortex of the macaque. ON–OFF retinotopy measured along 0.3 mm of horizontal cortical distance in macaque primary visual cortex (n = 1 monkey). As in the cat, changes in OFF retinotopy are more restricted than changes in ON retinotopy in the receptive fields of both eyes. Panels labelled ‘average’ show receptive fields averaged across cortical distance separately for each eye and both eyes. Plots labelled ‘retinotopy’ show the retinotopy of the receptive field pixel that generated the strongest ON (red) or OFF (blue) response, shown separately for each eye and both eyes. Each square box framing a receptive field has a side of 12°.
Extended Data Figure 4 | Periodic changes in orientation preference.

a, Colour map showing normalized frequency of orientation difference between paired recordings measured at different cortical distances within a single horizontal penetration (same as Fig. 3k left). b, Difference in orientation preference between all possible paired recordings measured within the same horizontal penetration as in a (n = 496 paired comparisons, n = 1 animal). c, Same as a but for multiple recording sites obtained from multiple penetrations (n = 20,672 paired comparisons, n = 36 animals).
Extended Data Figure 5 | Additional examples of horizontal recordings showing a correlation between changes in ON–OFF retinotopy and orientation preference. a, Horizontal recording through 0.9 mm of cortex. From top to bottom, the first three panel rows show series of OFF, ON and ON–OFF receptive fields (left) and receptive fields averaged across horizontal cortical distance (right). The bottom row shows the orientation or direction tuning (left) and the retinotopy (Retinot.) of the strongest response within each receptive field (right; ON, red; OFF, blue). The small circles in the orientation plots illustrate the preferred orientation predicted from the ON–OFF receptive field. b, c, Horizontal recordings through binocular regions of length 0.5 mm (b) and 0.7 mm (c). Notice the accurate binocular match in ON–OFF retinotopy between the two eyes and also the striking binocular similarity in orientation preference, direction preference and orientation and direction selectivity. Each receptive field box has a side of 27° (a), 23° (b) or 23.6° (c).
Extended Data Figure 6 | Example of a horizontal penetration in which we recorded from several single neurons separated from each other by 0.1 mm. Format is similar to Fig. 4a and Extended Data Fig. 5a. The only difference is that the receptive fields and orientation plots were obtained from single neurons instead of multiunit activity. The last row shows spike waveforms from each single neuron (average and s.d.). Each square box framing a receptive field has a side of 23°.
Extended Data Figure 7 | Example of a cortical region in which OFF retinotopy rotates around ON retinotopy. The figure shows a series of receptive fields mapped with dark (OFF) and light stimuli (ON) and the ON–OFF receptive field difference. The last receptive field on the right for each row shows the average of all receptive fields across 0.8 mm of cortical distance. The plot on the right shows the retinotopy of the ON (red) and OFF (blue) receptive fields. Cortical regions where OFF retinotopy rotated around ON retinotopy were more difficult to find than regions where ON retinotopy rotated around OFF retinotopy. To estimate the relative frequency of ON and OFF retinotopy rotations, we measured the distance between the retinotopic centre of mass of single horizontal penetrations for each ON or OFF receptive field (81 penetrations with receptive field measurements from at least five recording sites per penetration). We then calculated a ratio of the average distances, as (ON – OFF)/(ON + OFF), and used a ratio of 0.5 as an arbitrary threshold to classify a penetration as OFF-anchored (ON rotates around OFF) or ON-anchored (OFF rotates around ON). Based on this criterion, there were 3.75 more OFF-anchored than ON-anchored penetrations (15 versus 4 penetrations, respectively; \( n = 17 \) animals). Each square box framing a receptive field has a side of 19.4°.