Hierarchical Representation for Chromatic Processing across Macaque V1, V2, and V4

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Neuron

Hierarchical Representation for Chromatic Processing across Macaque V1, V2 and V4 --Manuscript Draft--

Manuscript Number:	NEURON-D-19-02131R2
Full Title:	Hierarchical Representation for Chromatic Processing across Macaque V1, V2 and V4
Article Type:	Research Article
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Abstract:	The percept of color is an internal label for the inferred spectral reflectance of visible surfaces. To study how spectral representation is transformed through modular subsystems of successive cortical areas we undertook simultaneous optical imaging of intrinsic-signals in macaque V1, V2 and V4, supplemented by higher resolution electrophysiology and two-photon imaging in awake macaques. We find a progressive evolution in the scale and precision of chromotopic maps, expressed by a uniform blob-like architecture of hue responses within each area. Two-photon imaging reveals enhanced hue-specific cell clustering in V2 compared with V1. A phenomenon of endspectral (red and blue) responses that is clear in V1, recedes in V2 and is virtually absent in V4. The increase in mid- and extra-spectral hue representations through V2 and V4 reflects the nature of hierarchical processing as higher areas read out locations in chromatic space from progressive integration of signals relayed by V1.
Suggested Reviewers:	Bevil Conway, Doctor NIH bevil@nih.gov Bevil Conway is a famous neurophysiologist studying color perception using both imaging and electrophysiology. Gregory Horwitz, Doctor University of Washington ghorwitz@uw.edu Gregory Horwitz is an outstanding neuroscientist studying color perception using electrophysiology.

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Opposed Reviewers:	Haidong Lu, Doctor Beijing Normal University haidong@bnu.edu.cn Conflict of interest.
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Dr. Ulrich Schridde
Neuron Scientific Editor,

July 07, 2020

Dear Dr. Schridde

We would like to thank the Editorial team at Neuron for continuing to assist us in piloting NEURON-D-19-02131R2 through the review process with a *“minor revision”* decision.

We were happy to note that reviewer 1 stated that ***“This already strong paper is much improved”***, expressing that ***“I do not want to stand in the way of the dissemination of this important work”***. They suggested a new regression model, although they ***“realized this is a lot to ask”***. We have nevertheless implemented this to allay their remaining concerns. Reviewer 2 is fully satisfied with our work, and in the previous round remarked that ***“This is an outstanding work - both in terms of the techniques combination and their application in alerts monkeys, the high quality data obtained from several visual areas and the important questions”***.

It was heartening to see that reviewer 3 recognized that ***“The work they report is a tour-de-force of several state-of-the-art methods, and I am sure the results will be very useful to anybody investigating the cortical representation of color”***. Of concern to us however, the third referee now provides a novel set of more extensive, purportedly ‘fault-finding’ commentary. *Much of this could and should have been raised in the first review.* Several points betray selective reading of the manuscript, and are refuted simply by citing the existing text (and/or existing literature). The first round of review judged the logic of our study as predicated on the assumption that color and form are separately encoded in V1. We were obliged to add **Figure S7** in the last revision to address this misconception. However, the follow-up review gave no justification for this major criticism. The second round focused on the broad nature of color processing, yet appear agnostic to the unique value of the current study comparing across three successive visual areas of the same preparation (a point borne out by the strong support obtained from Reviewers 1 and 2). We have nonetheless taken all actionable suggestions constructively (for example, adding a new CIELAB analysis into **Figures 5CD and 6B**), and ensured that we carefully disambiguate any text where improvements can be made (please see our reply to the Reviewers appended to this letter).

We would like to re-emphasize that we believe there is no precedent in the literature for such a thorough investigation of cortical functional organization along the visual hierarchy of V1, V2, and V4 for color representation. The color vision system progressively removes the V1 color bias along the visual pathway and achieves spectral uniformity better reflecting color perception. We have implemented whatever improvements we can, to meet the reviews’ expectation for publication of this manuscript. We would also like to thank you very much for your time and support and are looking forward to hearing from you.

With best wishes as always,

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Hierarchical Representation for Chromatic Processing across Macaque V1, V2 and V4

Subtitle: Hue representations across the ventral visual hierarchy

Response to reviewers:

We thank the reviewers for their additional expert comments and supportive remarks to our work. We believe that our paper has now been further improved through integrating the reviewers' latest suggestions into our revised manuscript. The first reviewer asked us to further improve our regression analysis, and we have done it. The third reviewer provided a novel set of more extensive comments separate from their first-round review. We have taken all actionable suggestions constructively, and ensure we have tried to carefully disambiguate any text where improvements could be made. Please find below our point-by-point replies to their comments, along with the modifications we have made following the reviewer's suggestions. The original comments of the reviewers are shown in *blue italics*, and our replies in normal typeface. We would be delighted if this paper now meets with our reviewers' expectation for publication.

Reviewer #1:

This already strong paper is much improved. The analysis of linear vs. circular chromotopic organization remains a weak point, however.

Major comments:

The fact that "the hue circle was linearized by a discontinuity at whichever location yielded the maximal regression coefficient" invalidates the regression p-values. As I understand it, the permutation test avoids this problem (I assume that the discontinuity was customized for each permuted data set). Can the p-values from the linear regressions be eliminated?

Author reply: Thank you for further comments on the regression analysis. The previous analysis was performed on two sets of data, one based on the hue circle without a discontinuity, and the other by a discontinuity operation generating a linearized color metric. For the linearized model test ($d(\theta) = k \cdot \theta + b$) of hue maps, the discontinuity cut points were based on the number of hues in a hue map, i.e. a hue map with 4 hues has 4 potential discontinuity points while a hue map of 7 hues has 7 discontinuity points. For the regression analysis, we first found the maximal regression coefficient regardless of the discontinuity location. In the analysis, the maximal regression coefficient always generates the smallest p-value. We agree that the p-value needs to be corrected by the number of discontinuity points (e.g. Bonferroni correction). After the p-value correction for regression, 41.8% (51/122) hue maps in V1, 54.2% (39/72) in V2, and 58.5% (38/65) in V4 satisfy the statistical criteria (please see **Reply Fig.1A**), all three areas contain relatively high percentages of hue maps showing a sequential organization of adjoining hues activating neighboring cortical regions. However, our overarching aim is to show that cortical hue mapping evolves toward something that is recognizably closer to a perceptual hue map in V4 than it is in V1. A linear hue metric (as reviewer 3 correctly pointed out) does not align with perception. Therefore, we focus our reporting on planar color space (see the next reply to your circular analysis suggestion), and move the result of our analysis using the linearized color metric into supplementary information in the newly revised manuscript. We have also revised the corresponding methods.

In the new circular hue metric regression, cortical distances between peak points were modeled as a linear function of hue angle from 0 to 180°. This is odd: if the color peak points were arranged perfectly on a circle, their pairwise distances would not be a linear function of hue angle. The aforementioned complication introduced by the ad hoc discontinuity of hue angle applies to this analysis too.

Ideally, the data would be fit by a pair of models, one that assumes that cortical distance is a linear function of hue angle, and one that assumes that cortical distance is a periodic function of hue angle. The positions of peak points on the cortex, not the distances between them, would be assumed to be subject to independent errors. Hue angle would be parameterized as sine and cosine components to avoid the need to introduce a discontinuity to the hue axis. The quality of the model fits would be compared using rigorous, established techniques. I realize this is a lot to ask, and I do not want to stand in the way of the dissemination of this important work. I do, however, think that the circular hue metric analysis, at least as I understand it, is potentially more misleading than illuminating.

Author reply: Thank you for your comments on the circular hue metric analysis. We agree that, in perfectly arranged color circle (radius= r), the direct distance (d) between pairwise response peak points of two hues (with an angular difference of θ from the center of the circle) would be $d(\theta)=2*r*\sin(\theta/2)$. For the circularity test of hue maps, as you point out, we should have used a sin / cosine function.

Reply Fig.1 Percentage of chromotopic maps in V1, V2 and V4 satisfying statistical criteria tested with a linearized or circular color metric. **A**, Percentage of chromotopic maps passed significance criteria ($P < 0.05$ for regression analysis, randomization and permutation tests) using a linearized color metric showing a sequential organization of adjoining hues activating neighboring cortical regions. **B**, Percentage of chromotopic maps passed significance criteria showing approximate spatial organization as the perceptual hue circle. Chi-square test with Bonferroni Correction, *: $p < 0.05$, **: $p < 0.01$, n.s.: not significant.

By using a model specified by $d(\theta)=2*r*\sin(\theta/2)$, and utilizing the same regression criteria and statistical validation we used previously, there are 25.4% (31/122) hue maps in V1, 43.1% (31/72) in V2 and 52.3% (34/65) in V4, that satisfy the criteria. The percentages in V2 and V4 are substantially increased when compared with that in V1 (**Reply Fig.1B**). Thank you for your comments that have improved the rigor of our regression models. We have added **Reply Fig.1B** into main **Figure 5D** and revised the text in our methods and results.

Minor comments:

The responses to reviewers states: "In 2-photon imaging, we recorded: 3608 visual responsive V1 neurons (one-way ANOVA, $p < 0.05$) and 6202 visual responsive V2 neurons from a second monkey. We have added this information in the corresponding figure legend in the revised manuscript." I was unable to find this information in the legend to Figure 4, which I assume is the correct location.

Author reply: The cell number information was in the legend of **Figure S5** in the last revised manuscript. Now we have moved this information to the main text of this revised manuscript (Page 9).

The phrase, "targeted electrophysiology", in a paper that uses intrinsic optical imaging to map color domains could be taken to mean that electrode penetrations were targeted to color domains discovered on the basis of imaging. To avoid this confusion I suggest deleting the word "targeted" from "targeted electrophysiology".

Author reply: We have deleted the word "targeted" from the corresponding sentences. Thank you!

Figure 3 legend: "The uniformity increases monotonically from V1 to V2 and V4... but not within each area". This phrasing implies a test for a monotonic increase in uniformity within areas.

Author reply: We are sorry for this confusion. For clarity, we have revised the corresponding sentence to read: “the response power ratios varies among the seven hues within each area (V1 and V2, $P < 10^{-6}$; V4, $P = 0.0165$; bootstrap, details in STAR Methods), but the uniformity of the overall distribution of color responses increases monotonically from V1 to V2 and V4 ($P < 10^{-6}$ for all comparisons, bootstrap)”.

The terms "hue power" and "response power" appear to mean the same thing.

Author reply: Yes, the two terms “hue power” and “response power” mean the same thing. We have unified these descriptions as “response power” in our revised manuscript. Thank you!

Reviewer #2:

The authors fully addressed my concerns.

Author reply: Thank you for your time and attention.

Reviewer #3:

The authors have thoroughly revised their manuscript. The work they report is a tour-de-force of several state-of-the-art methods, and I am sure the results will be very useful to anybody investigating the cortical representation of color. However, I think in terms of analysis it falls short of making a contribution worthy of a high-impact journal.

Author reply: Thank you for taking the time to rereview our manuscript. We would like to re-emphasize that we believe there is no precedent in the literature for such a thorough investigation of cortical functional organization simultaneously across V1, V2, and V4. We have done our best to combine several challenging methods in anaesthetized and awake macaques, with analytic methods much improved from previous studies, we for the first time demonstrate that hue representations are **progressively** transformed along the ventral visual pathway.

I will go through the 4 highlights given by the authors:

Highlight 3:

End-spectral hues dominate V1 whereas chromatic uniformity increases in V2 and V4

All the hue preferences are measured using a single amplitude for each color direction, and the magnitude of stimulation in the different directions is vastly different. The authors acknowledge this in their response letter and in the manuscript. Everyone agrees that it is obviously non-meaningful to speak of an end-spectral bias in color tuning, when the end-spectral bias is caused by a bias in the stimulation in the face of a known and well-characterized linear chromatic response in V1 (Lennie et al, 1990). Why then do they keep using this term?

Author reply: We agree that the term “endspectral bias” does not distinguish the origin of the bias. **As you mention**, we do acknowledge in the discussion how maximally saturated computer graphics stimuli are expected to differentially drive the two cone-opponent (P & K) afferent systems and cells in V1 blobs combine P and K inputs linearly. The endspectral responses we see are consistent with previous studies including the latest 2-photon imaging study of V1 using similar standard color stimuli (Garg et al., 2019; Tootell et al., 1988; Valverde Salzmann et al., 2012; Xiao et al., 2007). Whatever your views are on these

studies, the critical difference of our work is these endspectral responses in V1 recede in V2 and are nearly absent in V4. In other words, with the same set of stimuli, what is important is that the color vision system progressively removes the bias **along the visual pathway**. Given that single units sampled at a still higher level (PIT cortex) do appear to provide a more uniform representation of perceptual color space (Bohon et al 2016), this is a valuable finding only obtainable by a comparative study across cortical areas in the same animals. These results are analogous to what was proposed in the ending sentence of Lennie et al 1990: “Thus, while the putative achromatic mechanism seems to be reasonably well developed in striate cortex, there are only modest signs of the red-green and yellow-blue mechanisms whose existence is so firmly established by psychophysics. For these we apparently must look to regions beyond striate cortex”.

As we agree the terminology itself is imprecise, we have endeavoured to rectify this. We have revised the manuscript throughout to consistently refer to endspectral responses in V1 as a hue response bias, not a hue representation bias. In other words, it is appropriate to report it as an empirical result (as have other recent studies e.g. Nasr and Tootell, 2018; Garg et al., 2019), not as an intrinsic property of V1. This distinction is awkward to convey with limited characters as a highlight, so we have omitted reference to endspectral bias in highlight 3. Elsewhere in the text all references to ‘endspectral bias’ in V1 have been amended to ‘endspectral response’. Nevertheless, the previous highlight 3 has been deleted and replaced with: “Chromotopic organization achieves greater spectral uniformity from V1 to V2 to V4” as highlight 4 in the revised manuscript.

It is of interest that this stimulation bias is no longer visible in V4, but what is the explanation for this? Do V4 neurons adapt their responses to the stimulus set? Little is offered in the manuscript what computations could underlie this transformation of color signals.

Author reply: The “*little we offer*” is constrained by the data presented; our study was not designed to address the nature of cellular computation. Discussing specifically the cellular data in V2, we mention first the idea of ‘contrast tuning’, then defer that in favour of sensitivity to a specific ratio of parvo and konio signal intensities. Both might qualify, loosely, as ‘computations’ – but for such speculation to be tested requires new experiments that are specifically designed to answer this. The remit of this study is to demonstrate in the same preparation that such a transformation does occur **progressively** from V1 to V4, we don’t claim that we solve how it is done, and it seems entirely reasonable that this is a fruitful avenue for future study.

Highlight 4:

Color representation is hierarchically refined to better reflect color perception

The authors argue that ideally "the spatial organization of cortical hue-response loci approximates the hue-metric of perceptual color space". However, for this they equate "color perception" with angles in the RGB chromaticity diagram that do not have any metric properties. Why is the distance from red to cyan 180 deg for the authors and the distance from red to green 120 deg? This makes a tremendous difference for the regression analyses on pages 12ff. The x-axes of all these regressions does simply not reflect perception on an interval scale, and probably not even an ordinal scale. Is red-cyan really larger than red-green?

There is no color space that perfectly represents human color perception. For many practical purposes, the CIELAB space is used and its DE-metric could be considered state-of-the-art. It would certainly be a better approach than rgb angles.

The whole issue of linear versus circular representations is a bit strange. A linear representation doesn't make any sense from the viewpoint of perception, where the hue circle is firmly established. It also doesn't make sense if one looks at firing rates, because for example in V1 responses to equal chromatic contrasts are equal,

which indicates that there must be some kind of polar or cylindrical organization. My suspicion is that the evidence for linear representations come from the fact that the "blue" half of color space was underrepresented in these experiments, that the maximum regression coefficient was searched for in the linearization, and that the residuals of the regression were not really considered. For the last point, look at Figure 5C (V4-a), where small and far distances are systematically underestimated by the regression. Also, looking at the the corresponding Figure 5B, I would say that it is difficult to imagine a better example of a circular hue representation.

Author reply: To address, first, the issue of *"linear versus circular representations"*: we accept, of course, that a linear hue metric is at odds with the planar nature of perceptual hue space. However, many hue maps in all three areas return significant chromotopy as assessed by this metric, and we thought this worth reporting. Nevertheless, in view of the other issues raised here (and by reviewer 1), we have changed the strategy of reporting in this part of the manuscript in favour of planar hue metrics (e.g. the linear metric regression charts are now relegated to supplemental materials).

Concerning our phrase *"the spatial organization of cortical hue-response loci approximates the hue-metric of perceptual color space"* we draw attention to the wording: **'approximates'** does not mean **'replicates'**. We (as a community) might expect some relationship between cortical and perceptual organization, but our physiological understanding is too weak to predict the exact fidelity of the relationship. Clearly, the hue circle provides a closer approximation of perceptual color space than the linearized metric derived from it. We accept all the comments on the nature of the hue circle, but the circular metric (now based on geometric length, as advised by reviewer 1) does capture a hierarchical progression across area V1-V2-V4. We see this as the most illuminating aspect of our chromotopic analysis, and have revised the presentation to focus upon it (e.g. Fig 5C of the main text now illustrates the chord metric regression charts). The reviewer comments that *"There is no color space that perfectly represents human color perception"*; but we have adopted the suggestion to examine DE metrics from CIELAB color space, that come closer to this goal. We now report the outcome of testing the CIELAB DE76 metric (see **Reply Fig.2**): this again shows a significant enhancement of chromotopy in V4 with respect to V1, but it also reveals fewer significant hue maps overall in comparison to the simpler hue circle. Nevertheless, we have added this result into **Figure 5C&D** and **Figure 6B** of the revised manuscript.

Reply Fig.2 Percentage of chromotopic maps in V1, V2 and V4 matching the organization of CIELAB color space. Regression analysis was performed between the cortical distance of hue responses within hue map and color distances (DE76) in CIELAB color space. The histogram shows the percentages of chromotopic maps satisfying the criteria of $P < 0.05$ for regression analysis, randomization test and permutation test. Chi-square test with Bonferroni Correction, *: $p < 0.05$, ***: $p < 0.001$, n.s.: not significant.

The reformulation of the chromotopic analysis presentation in the Results therefore reflects a trade-off between a hue-metric's capacity to capture an element of cortical organisation, and its relative fidelity to human color space. We now encapsulate our findings in the corresponding result section with the following, low-key summary statement:

"...it seems fair to conclude that whilst, upto the level we studied, a typical cortical hue map may lack a high-fidelity relationship to the organization of perceptual color space, there is nonetheless a demonstrable enhancement in the chromotopic precision from V1 to V2 to V4 that is recognizably 'perceptual' in character".

Finally, to avoid any confusion or misunderstanding highlight 4 has been deleted.

Highlight 2:

Blobs evolve progressively in spatial scale and separation of hue representation

This is presumably reflected in Figure 7, where distance to red is analyzed. The most prominent feature of Figure 7 is that the distance between red and purple is smaller than red-cyan or red-green, which speaks clearly for a circular representation in all three areas.

Author reply: Thank you for the comments. However, the smaller red-purple distance is consistent with circularity but not proof of circularity; analysis of all hue-pair distances against a circular hue metric (as discussed in previous reply) is required to reliably diagnose a circular conformation. The elementary ‘twin-spot’ chromotopy of hue maps in V1, as we now refer to it (e.g., PROY vs. GCB foci, as seen in hue map V1-c in **Figure 5B**) is evidently not circular, but does have a RP distance smaller than RC or RG.

Highlight 1:

Color-response blobs comprise a uniform architecture across V1, V2, and V4

To me, it seems unclear what aspect of color processing the blob architecture reflects. The imaging approach and methods used here (and elsewhere) target neuronal responses for which the color response is larger than the luminance response. Neurons responding equally well to both kinds of stimulation that would still show a strong color tuning are thereby avoided, even though 50% of V1 neurons might belong to this groups according to Shapley and colleagues.

Author reply: We think “*neuronal responses for which the color response is larger than the luminance response*” specifically applies to the Red/Green (& Blue/Yellow) versus Black/White gratings reported solely in the first sub-section of Results.

The “50% of V1 neurons” referred by the reviewer are the cell class originally described by Shapley’s group as ‘color-luminance’ cells, and later as ‘double-opponent’ cells (Johnson et al., 2001, 2008). Some of these cells have a balanced response to isoluminant color and achromatic luminance stimuli, others show a mild preference for one or the other. Therefore, a substantial fraction of this population should contribute, either positively or negatively, to the differential population response maps resulting from the Red/Green (& Blue/Yellow) versus Black/White contrast.

Other issues:

The reference to "pointillism" in the title is misleading and unjustified and should be removed. Similarly the first paragraph of the introduction and the concluding remarks would better be deleted.

Author reply: We think that an engaging title, especially considering the relevance of representations of color in both physiology and art, enhances the impact of a paper. Reviewers 1 and 2 seem satisfied with our changes following the first round of review: the opening paragraph sets out the relevant context justifying the use of the term Pointillism (removing in our opinion any potential for being ‘misled’), and the final paragraph added speculation as to the parallels of cortical hierarchical processing with the perceptual phenomenon of color assimilation. Consulting with the editorial team, we have nevertheless deleted “Cortical Pointillism” in the title, as well as the first paragraph of the introduction and the concluding remarks.

Page 3, line 4: reference Lennie et al, 1990.

Author reply: Thank you for your suggestion, and we have added this reference into Introduction as well.

Figure 1A: The wavelengths at the red end are about twice as long as at the blue end (400 versus 700 nm).

Whatever is drawn in the spectrum picture is wrong.

Author reply: Thank you for your attention. This scheme of visible electromagnetic spectrum is for illustration only, however, we have deleted this sub-figure from Figure 1 accordingly.

Figure 1B: This is the kind of diagram I criticized in my first review. There is ample of evidence for joint representations of color and form in visual cortex.

Author reply: We absolutely agree with the reviewer on the joint representations of color and form in visual cortex as raised in the first review. This comment informed a number of changes in the last manuscript revision, including the addition of **Figure S7**. Here we would point out that nothing in the **Fig 1B** diagram indicates that color and form fail to interact, and we have reiterated this point in the revised legend as “**physiologically, color and orientation information are not mutually exclusively encoded**”. Generally, the labels of the CO defined modules are accurate: hue maps are unique to blobs in V1, and similarly with respect to thin stripes in V2.

Figure 4F: Cluster index seems to be 1 for V1 color. What does that mean? No clustering?

Author reply: As stated in the manuscript (page 11), the cluster index is close to 1 for a pair of color responses in V1 at a distance range exceeding 150 μm , which suggests an absence of clustering of neurons preferring similar hues beyond this range. However, within the distance range of 150 μm , the cluster index is significantly larger than 1, which suggests the neurons with similar hue preference are clustered.

Page 12: "precision increase from V1 to V2 to V4" The heading suggests such a precision index, the last line of the first paragraph on page 13 states that the "sample size is too small for statistical analysis of any enhancement in precision".

Author reply: The increase in precision indicated in the sub-heading is justified by the increase in the proportion of color response sites showing significant hue mapping from V1 to V2 to V4 – as measured by intrinsic signal optical imaging of the three successive areas.

The line cited by the reviewer refers specifically and explicitly to the smaller sample obtained by 2-photon imaging, which has been stated previously as “**sample size is too small for statistical analysis of any enhancement in precision**”. However, we have reworded this to “the limited sample size of the 2-photon dataset precluded statistical comparison of the frequency of significant hue maps in V1 and V2 ” in the revised manuscript.

Page 16: "We found that the average cortical distance between blue and green is shorter than that between red and green in both V1 and V2, but not in V4" is a good example of a sentence that is absolutely meaningless with respect to perception. Is blue more different from green than it is from red? I don't know of any empirical measurements.

Author reply: As noted above, the paper provides various forms of evidence that the hue map organization in V4 is recognisably closer to a perceptual organisation than it is in V1. We nowhere claim to have privileged knowledge of the precise perceptual separation of red, green and blue that, as the reviewer reminds us, has not been empirically established. The paragraph immediately preceding the passage cited by the reviewer establishes that, in V1, green is effectively colocalised with blue. In V4 it is not (it moves closer to red, as we state). Even if we do not know the relative perceptual distances, we do know that green is not blue: the two hues are distinct. Therefore, we submit that the hue map change from V1 to V4 is a perceptually meaningful observation.

Figure 8: this figure is not supported by the data and should be removed.

Author reply: We take this comment to refer to the depiction of hue maps (as the anatomical data is well-verified). The relative size and overlap of specific hue-response loci are response averages directly from our data. We believe this schematic diagram to be a fair and simple depiction of the typical (average) conformation of hue maps in each area as a general summary. We have revised the head of the figure legend to illustrate the color response domains or modules only: ***“Schematic of the Averaged Chromotopic Maps in V1, V2 and V4”***. Again, here we repeatedly make no claim as that orientation and color information is not jointly processed in visual cortex (**Figure S7** links to this Figure), but illustrating only the evolution of hue maps toward something closer to a ‘perceptual’ organization of color spaces (with both hue circle and CIELAB spaces) along the visual hierarchy. Thank you for the above suggestion on CIELAB space analysis.

Page 18: "The functional operations associated with each hierarchical stage of color processing are not well established". The current manuscript doesn't say anything about function either.

Author reply: The manuscript interprets the various observations across V1, V2 and V4 as indicative of a progressive integration of parvo and konio signals as higher areas read out discrete chromatic loci from the signals delivered to V1: unmistakably, a functional synopsis of hierarchy.

Page 19: "differential activation achieved by adding chromatic contrast to an achromatic square-wave grating matched in luminance contrast and spatial frequency; this activity should also include a component generated by oriented, hue-selective cells".

This differential activation will not include cells responding to both color and luminance, such as most double-opponent cells (see Shapley and colleagues).

Author reply: The sentence following the one cited above indeed refers to Shapley’s work (Johnson et al 2001, 2004, 2008). The ‘color-luminance’ class of cell (as initially defined by Johnson et al, 2001; later redefined as a double-opponent cell by Johnson et al, 2008) is most frequent in layer 2/3, the layer responsible for the bulk of the optical signal; hence we took careful note of its reported properties, which include orientation selectivity. The class comprises cells with response ratios between chromatic and luminant stimuli falling within the range 0.5 to 2.0. Thus, the addition of chromatic contrast to our spatially matched achromatic gratings (i.e. in the 7-hue test set) should certainly have elicited a greater response from at least half of the population of color-luminance cells, those with a ratio > 1. This response must contribute to the differential activation that we analysed.

Page 22: "color is less a simple sensation, and more of a cognitive construct". I don't see what leads to this conclusion. Nothing in the manuscript has to do with "cognition".

Author reply: The boundary between color perception and color cognition is not well determined, certainly not physiologically. For example, we do not know at what stage in the hierarchy is redness equated with ripeness. Because of the title change, the concluding remarks have been deleted in the revised manuscript.

Reference :

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Hierarchical Representation for Chromatic Processing across Macaque V1, V2 and V4

Subtitle: Hue representations across the ventral visual hierarchy

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SUMMARY

The percept of color is an internal label for the inferred spectral reflectance of visible surfaces. To study how spectral representation is transformed through modular subsystems of successive cortical areas we undertook simultaneous optical imaging of intrinsic-signals in macaque V1, V2 and V4, supplemented by higher resolution electrophysiology and two-photon imaging in awake macaques. We find a progressive evolution in the scale and precision of chromotopic maps, expressed by a uniform blob-like architecture of hue responses within each area. Two-photon imaging reveals enhanced hue-specific cell clustering in V2 compared with V1. A phenomenon of endspectral (red

and blue) responses that is clear in V1, recedes in V2 and is virtually absent in V4. The increase in mid- and extra-spectral hue representations through V2 and V4 reflects the nature of hierarchical processing as higher areas read out locations in chromatic space from progressive integration of signals relayed by V1.

Highlights

1. Color-response blobs comprise a uniform architecture across V1, V2 and V4
2. Blobs evolve progressively in spatial scale and separation of hue representation
3. Two-photon imaging shows greater hue-specific cell clustering in V2 than in V1
4. Chromotopic organization achieves greater spectral uniformity from V1 to V2 to V4

INTRODUCTION

Understanding how the spectral reflectance of a surface ultimately results in the perception of a color has been the remit of both scientists and artists over the centuries (Conway, 2012; Conway et al., 2010; Mollon, 2003). Human and macaque perceptual color space is three-dimensional, inherent in the trichromatic sampling of the spectrum by long-, medium-, and short-wavelength (L, M, and S) sensitive cones in the retina. The chromatic plane of color space (**Figure 1A**) derives from two forms of cone opponency: L versus M, and S versus L+M, that are transmitted from retinal ganglion cells through parvocellular and koniocellular neurons in the lateral geniculate nucleus (LGN) before entering the early visual cortex for further processing (Chatterjee and Callaway, 2002; Hendry and Reid, 2000; Lennie et al., 1990; Martin et al., 1997; Roy et al., 2009). Cells in early visual cortex can only be tuned to discrete loci in color space by integrating signals from the parvo and konio channels (Conway, 2001; Conway et al., 2002; Conway and Livingstone, 2006; Horwitz and Hass, 2012; Hubel and Livingstone, 1987; Johnson et al., 2001, 2004, 2008; Kiper et al., 1997; Solomon and Lennie, 2005). The cytochrome oxidase (CO) blobs of V1 are the first cortical site of parvo and konio

convergence. Single-unit electrophysiological studies of CO blobs have reported cell classes sensitive to either red/green or blue/yellow color contrast, the latter in a minority (Livingstone and Hubel, 1984), with some suggestion that individual blobs are dominated by one or the other (Landisman and Ts'o, 2002a; Ts'o and Gilbert, 1988). The canonical color pathway (**Figure 1B**) then relays from the CO blobs of V1 through CO thin stripes of V2 to color-specialized domains of V4 and posterior inferotemporal cortex (PIT) referred to as 'blobs' (Chen et al., 2008; Conway et al., 2010; Conway et al., 2007; Conway and Tsao, 2006; Harada et al., 2009; Landisman and Ts'o, 2002b; Li et al., 2014; Livingstone and Hubel, 1984; Lu and Roe, 2008; Sincich and Horton, 2005a; Tanigawa et al., 2010; Xiao et al., 2007; Xiao et al., 2003), and further anterior patches (Chang et al., 2017). How this integration of chromatic information is subserved by anatomical color pathways has yet to be fully resolved.

Figure 1. Schematics of Color Space, the Ventral Visual Stream and Visual Stimulus Sets

- (A) Hue wheel (upper) is one dimension shared by both HSL and HSV color spaces. The hues of red (R), orange (O), yellow (Y), green (G), cyan (C), blue (B) and purple (P) in this study are defined as 0°, 30°, 60°, 120°, 180°, 240°, and 300°, respectively in the hue wheel. Tested hues are replotted in Macleod-Boynton color space (lower) to illustrate their differential cone excitations.
- (B) Anatomical modular substructure of parallel pathways within the V1-V2-V4-IT hierarchy; physiologically, color and orientation information are not mutually exclusively encoded.
- (C) Achromatic and dual-hue stimuli used to map orientation and color domains.
- (D) Stimuli of isoluminant square-wave color gratings used for chromotopic mapping.

Hue is a quantitative perceptual attribute of color commonly used to describe the stimulus position within the visible spectrum (**Figure 1A**). As 'color processing', broadly defined, can incorporate a

contribution to cue-invariant form vision, we refer more narrowly to spectral hue processing that, empirically, we attribute to modules better activated by chromatic contrast than achromatic luminance contrast. Previous optical imaging of intrinsic signal (OIS) studies adopting this criterion have confirmed the co-localization of chromatic response domains with CO blobs in V1 and thin CO stripes in V2 (Landisman and Ts'o, 2002b; Lu and Roe, 2008; Xiao et al., 2003) and the segregation of such modules in V4 (Tanigawa et al., 2010). Hue maps have been separately recorded by OIS in V1 blobs (Xiao et al., 2007), V2 thin stripes (Lim et al., 2009; Xiao et al., 2003), and V4 color domains (Li et al., 2014; Tanigawa et al., 2010). These hue maps appear to be organized in a chromotopically structured manner, i.e. similar hues activate neighboring cortical positions, but how they reflect perceptual color space is unknown. In addition, the response of intrinsic signals are heavily biased in favor of red and blue hues, particularly in V1 (Valverde Salzmänn et al., 2012; Xiao et al., 2007). This endspectral response within V1 and its CO blobs, firstly reported utilizing the 2DG technique even after equating relative saturation (Tootell et al., 1988), was also reported in human fMRI (Nasr and Tootell, 2018). A recent study using 2-photon calcium imaging confirms the preponderance of cells preferring red and blue hues in V1, whilst failing to identify chromatic heterogeneity amongst blobs (Garg et al., 2019).

We may conceive of an idealized cortical hue map as isometrically organized, whereby hue responses are equally weighted and the spatial organization of cortical hue-response loci approximates the planar metric of perceptual color space. What remains unclear is how hue maps within the anatomical color modules evolve with hierarchical processing from V1 to V4 – given that single units sampled at a still higher level (PIT cortex) do appear to provide a more uniform representation of perceptual color space (Bohon et al., 2016). Does the chromotopic pattern of response in V1 persist or transform into something closer to the idealized isometric organization defined above? To address these questions, we probed spectrally specific responses with various chromatic stimuli (**Figure 1A, CD**), performing simultaneous intrinsic optical imaging of V1, V2 and V4 in the same anesthetized macaque preparation, allied to electrophysiological recordings (V1, V2 and V4) and 2-photon imaging (V1 and V2) in awake macaques. Deploying multiple techniques across these cortical areas enables us to quantify the similarities and differences at both cellular and population levels, examining how spectral responses develops progressively along the primate ventral visual pathway.

RESULTS

Mapping Color Response Domains across V1, V2, and V4

We first used either red/green (RG) or blue/yellow (BY) gratings matched to achromatic gratings (Landisman and Ts'o, 2002b; Li et al., 2014; Lu and Roe, 2008; Tanigawa et al., 2010; Xiao et al., 2007) to reveal chromatic response domains (**Figure 2A-C, Figure S1**; identified as isolated dark patches in differential maps of cortical population responses). **Figure S1A** shows an example where RG stimulation strongly activated blob shaped color-response domains in V1, V2 thin stripes, and V4, as identified in previous imaging studies (An et al., 2012; Lu and Roe, 2008; Tanigawa et al., 2010). Comparison to the differential map of orthogonal orientations shows that orientation-tuned domains in V1, V2 and V4 avoid direct, concentric overlap with the color-response domains. Similar segregated activations in V2 and V4 are shown for a second example with the orientation responses displayed as a polar orientation map (some fainter orientation responses are visible within the color-domains; **Figure S1B**). In summary, our results are consistent with earlier single-unit recordings and optical imaging data (Landisman and Ts'o, 2002b; Livingstone and Hubel, 1984; Lu and Roe, 2008; Tanigawa et al., 2010; Xiao et al., 1999), in demonstrating modular subsystems for processing color across macaque V1, V2, and V4.

A Uniform Blob-like Architecture of Color Responses across V1, V2, and V4

The color-response domains concurrently activated by RG gratings are blob-like, notably larger and more widely spaced in V2 than in V1 (**Figure 2A**). Likewise, **Figure 2B** shows co-activated color-response blobs across V1, V2, and V4. The domain size of unprocessed V4 color responses (**Figure 2C**) was substantially larger, roughly equating in scale to the color 'globs' measured at several millimeters in diameter by fMRI (Conway et al., 2007). The smaller foci that we refer to as color-response blobs emerged after further processing. This finding of the color-response blobs within each area was common to all animals examined (**Figure S2AB**).

Figure 2. Color-Response Blobs in V1, V2 and V4

- (A) Color-response blobs evoked by RG gratings, circled by red dotted lines, in V1 and V2. A: anterior; L: lateral.
- (B) Simultaneous recording of color-response blobs evoked by red stimulus in V1, V2 and V4. LS: lunate sulcus.
- (C) Color-response blobs (middle, after standard spatial filtering) within a V4 glob (left, unprocessed map). The outline of the glob transposed to an orientation polar map of the same V4 region (right) demonstrates the weaker orientation responses in color-response domains. STS: superior temporal sulcus.
- (D) Differential orientation and color responses maps, and histological CO staining of the corresponding region. A thin stripe is outlined by the white dashed square. Scale bar: 1mm.
- (E) Box plots of sizes of CO staining blobs and color-response blobs in V1 from 3 hemispheres (n.s., $P > 0.05$, Wilcoxon rank-sum test, n: blob numbers).
- (F) Comparison of response blob sizes in V1, V2 and V4 from 7 hemispheres (Wilcoxon rank sum test with Bonferroni correction, ***, $P < 0.001$).

In seeking a quantitative measure of color blob size, we were concerned that, as previously described for V1 (Landisman and Ts'o, 2002b), color-response domains were found to be more reliably mapped by RG gratings than by blue/yellow gratings. We reasoned that the sum activation achieved by our set of seven single-hue stimuli, introduced for the purpose of hue mapping, should provide a better index of color-response domain size than the partial activation revealed by RG gratings alone. These single-hue stimuli (red, orange, yellow, green, cyan, blue and purple) were square-wave gratings, coupled to an achromatic grating of matching luminance contrast and spatial frequency (SFs; **Figure 1D**) aiming to elicit maximal optical signals from the surface of cortex containing single- and double-opponent color sensitive cells (Friedman et al., 2003; Johnson et al., 2001, 2004, 2008; Kiper et al., 1997; Lennie et al., 1990). The sizes of color-response blobs were compiled from the envelope of the

response domains elicited by single-hue stimuli, where all pixels exceeded a threshold response level at three standard deviations (SD) above the baseline (see **STAR Methods**).

This criterion of physiological measurements was verified for V1 in conjunction with V1 histological CO-staining from three hemispheres (an example case is shown in **Figure 2D**). CO blob boundaries were determined by standard image processing methods, designating blob territory as the darkest staining 33% of the region examined (An et al., 2012; Sincich and Horton, 2005b). The average diameter of blobs was 292 ± 5 microns (mean $\pm$ s.e.), consistent with previous reports (Farias et al., 1997; Solomon, 2002). The image alignment gave an average shift of 91 ± 36 μ m (mean $\pm$ s.d.) between a CO blob and the corresponding color response blob (attributable to differential histological shrinkage) – comparable to a previous study using the same method (Valverde Salzmänn et al., 2012). After correcting for histological shrinkage of the imaged tissue, CO blobs were similar in size to color response blobs set at the 3-SD criterion. **Figure 2E** shows that there was no statistical difference in their mean area size (CO blob: 0.109 ± 0.003 mm², V1 response blob: 0.105 ± 0.004 mm², mean $\pm$ s.e), empirically validating our procedure for measurement of color-response blob size in V1. Applying the same criterion, we found that the sizes of color blobs in V2 (0.268 ± 0.013 mm²) and V4 (0.235 ± 0.014 mm²) were comparable, and that both were significantly larger than those in V1 (**Figure 2F**). We note, in addition, that the cortical organization of orientation preference domains obeys a similar size principle. The sizes of concurrently recorded orientation domains are equivalent in V2 and V4, but significantly larger than those in V1 (**Figure S2C**). In summary, the functional architecture of color-response blobs in V1 is retained hierarchically across V2 and in V4, with a larger blob size in both areas.

The Evolution of Hue-Representation from V1 to V4

A major aim of this study was to investigate how color signals are transformed across three successive visual areas. Previous studies have demonstrated endspectral response bias in V1 (Garg et al., 2019; Tootell et al., 1988; Valverde Salzmänn et al., 2012; Xiao et al., 2007; Yoshioka and Dow, 1996), so we asked if this response asymmetry extends to V2 and V4. Examining the same population of color-response blobs identified by the procedures described above (151 blobs in V1, 80 in V2 and 86 in V4), we observed that both the size and the strength of intrinsic signals modulate with stimulus

hue. Thus, we defined the individual hue response power as the product of the strength and the size of response to each hue (i.e. the mean pixel signal strength multiplied by the number of pixels activated by each hue); this represents the power of the activation to each hue within each color-response blob. We then computed the power ratio of each hue as the response power for that hue divided by the sum of all the response powers within the same color-response blob. Finally, averaging across all color-response blobs within each area, across monkeys, we obtained mean response power ratios for V1, V2 and V4, shown as a rose plot in **Figure 3A**. As expected, in V1 the priority of red, followed by blue is obvious, echoing previous reports of the spectral preferences of V1 blobs measured by diverse techniques across species. However, this endspectral response lessens in V2, and is further diminished in V4 where the hue response profile is statistically more uniform than V1 and V2. Box plots verify that the mean response power ratio is significantly greater to red than to other hues in both V1 and V2, and that this precedence wanes in V4 (**Figure 3B**). The priority of blue to the five hues other than red is notable in V1, but absent from V2 and V4 (**Figure S3A**).

Intrigued by this outcome, we added a complementary analysis of spectral variation of LFP responses recorded in a separate study of alert animals. **Figures 3C** and **S3C** show average rectified current traces obtained in LFP recordings from supragranular layers in V1, V2 and V4. Statistical comparison (**Figure 3D**) of current amplitude between red and other hues shows the current amplitude to red is largest in V1 and V2, but attains parity with most other hues in V4. Similar to intrinsic signals, in V1 we find that blue is inferior to red whilst ranking above the other hues, but loses this status in V2 and V4 (**Figure S3BD**). A sample of single unit data from the same awake monkey recordings (**Figure S4**) reflects the same pattern observed in population recordings from both intrinsic signals and LFP responses, demonstrating that the population hue response changes from one of endspectral dominance in V1 to a markedly more uniform distribution in V4.

Figure 3. Comparison of Population Responses between Red and Other Hues in V1, V2 and V4

(A) Rose plots of response power ratio (mean $\pm$ s.e.) of hues. The response power ratios varies among the seven hues within each area (V1 and V2, $P < 10^{-6}$; V4, $P = 0.0165$; bootstrap, details in STAR Methods), but the uniformity of the overall distribution of color responses increases monotonically from V1 to V2 and V4 ($P < 10^{-6}$ for all comparisons, bootstrap).

(B) Comparison of response power ratio between red and other hues (***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, n.s.: $P > 0.05$, Wilcoxon rank sum test with Bonferroni correction).

(C) The traces of average rectified currents to single-hue patch stimuli. Gray dashed line: stimulus onset; Shaded regions: s.e. of current across channels. Data from 51, 36, and 24 recording channels in supragranular layers from each area.

(D) Comparison of rectified current amplitudes between red and other hues (Wilcoxon signed-rank test with Bonferroni correction). The current amplitudes are Z-score normalized within each recording channel.

How Do Hue Signals Map onto Individual Cells in V1 and V2?

LFPs and intrinsic signals are both low resolution and indirect indices of neuronal spiking behavior. We therefore sought to establish the cellular basis for the concordance of spectral tuning between these two signals in larger neuronal populations while retaining the ability to perform subsequent spatial analyses. We applied 2-photon calcium imaging in V1 and V2 of two alert monkeys. Experiments were conducted using the identical set of seven test hues, here taking the form of small, static patches isoluminant to the achromatic background. In 2-photon imaging, we recorded 3608 visual responsive V1 neurons (one-way ANOVA, $P < 0.05$) and 233 visual responsive V2 neurons from

one monkey; and 6202 visual responsive V2 neurons from a second monkey. **Figure 4A** illustrates the calcium responses of six cells sampled from a color blob in either area, showing various forms of spectral tuning. The population of cells recorded at each site, coded by preferred hue, is shown in **Figure 4B**. Cell counts of preferred hues across all sampled sites in either area show that the frequency of cells preferring red and blue is larger than for all other hues (**Figure S5A**). For V1, these results are consistent with our findings above and previous population (Tootell et al., 1988; Valverde Salzmann et al., 2012; Xiao et al., 2007) and single-cell studies (Garg et al., 2019). For V2, the excess of blue cells in V2 is less prominent than in V1 (χ^2 test, $P < 0.001$) whilst the representation of midspectral hues is correspondingly enhanced.

Figure 4. Hue Preferences of V1 and V2 Cell Clusters in 2-Photon Calcium Imaging

(A) Fluorescence of a sample window (left) with blank stimulus, and fluorescence traces (right) of 6 sample cells in each area. A: anterior; L: lateral; Scale bar: 200 μm .

- (B) Cell distribution maps in V1 and V2 from the same frames in (A) illustrating cell clustering according to their preferred hues. Cells recorded across cortical depths of 100~500 μm (V1), or 100~325 μm (V2).
  - (C) 3D Plots of Color Cell Density Maps in V1 and V2 shown in (B). The 2D cell distribution maps are filtered by a square window of $0.125 \times 0.125 \text{ mm}^2$ (step size: 1 pixel, 1.5625 μm). The z axis represents the density ($\times 10^3/\text{mm}^2$) of cells preferring each hue in the x-y coordinates.
  - (D) Peak density of cells in each of the hue-specific cell clusters shown in (B).
  - (E) 2-photon orientation preference map in V1. 1012 orientation selective cells were collected across cortical depths of 100~500 μm .
  - (F) Cluster index comparison for hue and orientation preference cells in (B) and (E). Clustering indices significantly larger than 1 within distance of 250, 150 and 300 μm for V1 orientation, V1 color and V2 color cells, respectively ($P < 0.001$, bootstrap with 1000 trials of resampling).
  - (G) Hue preference map over a larger scale of V2. The hue preference map in the lower left white box is illustrated in (B).
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While the endspectral bias is modestly diminished in the V2 sample with respect to V1, the topography of hue clustering is much enhanced (**Figure 4B**). In V1 loose red and blue clusters are visible, while the remaining hues are distributed more sporadically. By contrast, in V2, the subsets of cells preferring the minority hues (particularly orange, green and purple) occupy clearly defined sub-zones, with some interspersions at their boundaries. One numerical indication of enhanced clustering in V2 compared to V1 is given by the increase in peak density for cells preferring minority hues, particularly green, as shown in **Figure 4CD**. For more rigorous quantification of this change in chromatic organization from V1 to V2 we adopted a ‘cluster index’, initially introduced to examine the organization of orientation and SF maps obtained by 2-photon imaging of macaque V1 (Nauhaus et al., 2012). The cluster index measures the spatial scale of congregation of similarly tuned cells by charting the fall-off in correlation of tuning properties between cell pairs located increasingly far apart. The index is normalized by the average correlation of randomly selected cell pairs within the inspected field (i.e. as delimited in **Figure 4B**). A cluster index equal to 1 suggests that neurons with different preferences are randomly scattered in a salt and pepper manner. Thus, the decline of the index toward a value of 1.0 quantifies how hue preferences change from more similar to less similar across the cortical surface. The orientation preference map of V1 is regarded as being a highly ordered map showing smooth transitions of orientation preferences across the brain surface. So as a comparison, we first computed the cluster index for V1 orientation responses in the same recording chamber as the color responses (**Figure 4E**). The orientation cluster index (**Figure 4F**) is consistent with the previous report (Nauhaus et al., 2012), demonstrating systematic change of orientation

preferences along the cortical surface. In contrast to orientation responses, the V1 color responses have much lower indices. However, the cluster index for V2 chromotopic map is in a similar range as that for V1 orientation preference map, demonstrating greater hue-specific cell clustering in V2 than in V1. To better visualize this change of hue preferences, we generated a larger field of view constructed from four adjacent sampling windows in V2 (**Figure 4G**). The increase in the orderliness of hue representation in V2 leads us to examine the general issue of precision in chromotopic mapping within the color-response domains of each area.

Characteristics of Hue Maps within V1, V2, and V4

Endspectral responses evidently distorts the metrics of hue-mapping, and the lessening of this bias through V2 and V4, noted above, could be taken to signify an orderly synthesis of perceptual color space. To quantify the changes in precision of chromotopic organization more rigorously we examined color-response domains in V1, V2 and V4 co-activated by single hue stimuli, seeking to measure the relationship between hue cortical positions and corresponding hues in perceptual color space (**Figure 1A**).

1. Chromotopic precision and scale increase from V1 to V2 and V4. We charted chromotopic maps within color-response blobs of V1, V2, and V4 by OIS of seven hemispheres and by 2-photon imaging of V1 and V2 from two hemispheres. Using identical procedures to previous studies (Li et al., 2014; Xiao et al., 2007; Xiao et al., 2003), the locus of peak response for each hue was defined as the contour at 75% of peak signal strength (**Figure 5A**), to generate hue maps by their superimposition (**Figure 5B**). By summing the number of hue regions per domain, the mean values were very close: V1: 4.93 ± 0.13 (n=151); V2: 5.45 ± 0.16 (n=80); V4: 5.15 ± 0.18 (n=86). Note that some hue maps encode all seven hues tested while others are empirically incomplete (e.g. due to occlusion by blood vessels), as also observed in previous studies of V1, V2 and V4 (Li et al., 2014; Tanigawa et al., 2010; Xiao et al., 2007; Xiao et al., 2003). Confirming previous reports, we also noticed that many hue maps across all three areas are rainbow-like in their sequence of hue responses. To better relate our results to previous studies we first sought to quantify this chromotopic organization with respect to a linear hue metric, performing a regression analysis of cortical distance against hue distance for all possible pairs of co-activated hues (omitting maps with fewer than four different hue loci). The hue metric

was generated from the hue circle (**Figure 1A**) linearized by a discontinuity at whichever location yielded the maximal regression coefficient (see **Figure S5BCD** for methodology and examples). Across areas we found that a rising fraction of hue maps – 42% (51/122), 54% (39/72) and 59% (38/65) in V1, V2 and V4, respectively – passed threshold criteria ($P < 0.05$ for regression analysis, randomization and permutation tests), but these proportions did not demonstrate a significant difference (**Figure S5E**).

However, perceptual color space is known to be planar and so we performed a similar regression analysis using a simple circular hue metric: specifically the geometric distance between a pair of hue loci, as if the hue circle of **Figure 1A** were perfectly transcribed upon the cortical surface. As shown in **Figure 5C** (the left panel), this metric also met with mixed success in predicting the configuration of cortical hue maps. Overall, 25% (31/122), 43% (31/72) and 52% (34/65) of intrinsic-signal hue maps in V1, V2 and V4, respectively, passed the threshold for significance ($P < 0.05$ for regression analysis, randomization and permutation tests) with the proportion of significant hue maps in V2 and V4 statistically exceeding that in V1 (**Figure 5D**, the left panel). Whereas only a small fraction of maps (such as ‘V4-a’ in **Figure 5**) are fully circular upon casual inspection, this analysis demonstrates a hierarchical increase in ‘circularity’ as a component of chromotopic organization. Finally, for a third measure, we resorted to CIELab (**Table S1**), a planar color space in which hue separation is more regularized in respect of hue discriminability. Using hue distances specified by the DE76 metric of CIELab space (**Figure 5C**, the right panel), we found that 4.1% (5/122), 5.6% (4/72) and 23.1% (15/65) of hue maps in V1, V2 and V4, respectively, passed the threshold level ($P < 0.05$ for regression analysis, randomization and permutation tests). Whilst, under this criterion, the overall frequency of significant hue maps has diminished, the proportion in V4 remains significantly higher than that in the antecedent areas (**Figure 5D**, the right panel). In summary it seems fair to conclude that whilst, upto the level we studied, a typical cortical hue map may lack a high-fidelity relationship to the organization of perceptual color space, there is nonetheless a demonstrable enhancement in the chromotopic precision from V1 to V2 to V4 that is recognizably ‘perceptual’ in character.

Figure 5. OSI Hue Maps in V1, V2 and V4

- (A)** Loci of peak activity areas evoked by single-hue stimuli.
- (B)** Hue maps combining the peak activity loci of V1, V2 and V4, respectively illustrated in **(A)**. The white dotted contours envelope all color responses at the criterion of 3SD above baseline. Scale bar: 0.5 mm.
- (C)** Regression analysis of cortical hue distances in color blobs against hue distances in a circular color metric (left) and CIE Lab DE76 metric (right) for V1-a, V2-a, and V4-a in **(B)**. Note that the curvature of regression with circular color metric reflects the sinusoidal function of geometric length subtending a given angular hue distance. n , R^2 and P represent sample number, coefficient of determination and P value of the regression analysis, respectively.
- (D)** Percentage of hue maps V1, V2 and V4 satisfying criteria in respect of the circular hue metric (left) and CIE Lab DE76 metric (right). ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., not significant; χ^2 test with Bonferroni correction.

We derived equivalent hue maps in V1 and V2 from 2-photon imaging; **Figure 6A** illustrates two examples from each area. Applying the identical set of regression analyses, we found that all four hue maps from these areas passed the statistical threshold in respect of the linearized color metric, but only the hue maps in V2 did so for the two planar color metrics (**Figure 6B**, **Figure S5F**). We therefore note good consistency between the intrinsic-signal and 2-photon data, although the

limited sample size of the 2-photon dataset precluded statistical comparison of the frequency of significant hue maps in V1 and V2 as visualized by this form of imaging.

Finally, we used the regression slope of hue maps as an index of the scale of chromotopic organization. For the hue-circle metric, we found that hue response loci were spaced further apart in V2 and V4, as reflected by significantly larger regression slopes: V1, $241 \pm 11 \mu\text{m}$; V2, $364 \pm 23 \mu\text{m}$; V4, $362 \pm 28 \mu\text{m}$; Wilcoxon rank-sum test with Bonferroni correction, V1 versus V2 $P < 1.4 \times 10^{-5}$; V1 versus V4: $P < 1.3 \times 10^{-3}$; V2 versus V4: $P = 0.79$). The larger scale of the hue maps is consistent with the larger color-response blob size we measured in V2 and V4. Taken together, this data demonstrates a hierarchical development in both the precision and scale of chromotopic mapping from V1 to V4.

A Hue Maps in 2-Photon Imaging

B With a Circular Color Metric

With a CIELab DE76 Metric

Figure 6. 2-Photon Calcium Imaging Hue Maps in V1 and V2

(A) Loci of 75% peak cell density areas in V1 and V2 evoked by single-hue patch stimuli. Scale bar: $200 \mu\text{m}$.

(B) Scatter plots illustrate the regression analysis for the maps in (A) using a circular color metric (upper) and CIELab DE76 metric (lower).

2. Chromotopic isometry improves across V1, V2, and V4. We have previously described how the relative weighting of hue responses approaches uniformity as endspectral responses diminish from V1 to V4; here we outline a parallel reduction in the anisometric spatial configuration of hue maps from V1 onward, as demonstrated by the intrinsic-signal data. An analysis of the area of overlap between all pairs of hue responses in the color blobs (expressed as overlap ratios – see **STAR Methods**) proves most instructive (**Figure S6A**). It demonstrates that in area V1, red, orange and yellow (ROY) hue-responses have high mutual overlap, as do green, cyan and blue (GCB), the two groupings being largely separate from one another. The seventh hue, purple, generally overlaps ROY, as indicated in **Figure S6A** but, alternatively, can associate with GCB (as seen, respectively, in hue maps V1-b and V1-c versus V1-a of **Figure 5B**). **Figure S6B** shows how these overlap ratios change from V1 to V2 and to V4. Firstly, purple dissociates from the ROY group, particularly in V4. Secondly, the ROY and GCB groupings themselves partially desegregate, more markedly in the case of GCB. Green, in dissociating from cyan and blue, actually increases its overlap with red, orange and yellow.

Figure 7. The Peak-to-Peak Distance between Hue Response Contours within Color Blobs in V1, V2, and V4

(A) The distances between red and other hues. Data is from 125, 69, and 63 chromotopic maps containing responses of red and at least one other color in V1, V2 and V4, respectively.

- (B) Comparison of red-green and blue-green distances (***: $P < 0.001$, **: $P < 0.01$, n.s.: $P > 0.05$, Wilcoxon signed-rank test).
- (C) Comparison of distance difference ratios as $(GB-GR)/(GB+GR)$ (*: $P < 0.05$, Wilcoxon rank sum test with Bonferroni correction). Data of (B) and (C) is from 87, 46, and 41 chromotopic maps containing red, blue and green responses in V1, V2 and V4, respectively.
- (D) Comparison between red-purple and blue-purple distances (Wilcoxon signed-rank test).
- (E) Comparison of distance ratios as $(PB-PR)/(PB+PR)$. Data in (D) and (E) is from 67, 38, and 35 chromotopic maps containing red, blue and purple responses in V1, V2 and V4, respectively.

How do these changes in overlapping hue-response territories affect the spatial uniformity – i.e. the isometry – of the chromotopic gradient? We computed the peak-to-peak distances of hue contours within hue maps to define the separation of hues. The outcome (**Figure 7A**) shows that, whilst red and cyan or blue peak loci are maximally spaced apart in all three areas, the relative locations of the other hues undergo systematic shifts. Taking end spectral red and blue (rather than the less frequent cyan) responses as robust markers for opposite poles of a hue map, we evaluated the relative distances of green and purple response loci from these poles. We found that the average cortical distance between blue and green is shorter than that between red and green in both V1 and V2, but not in V4 (**Figure 7B**). We normalized the difference between green-blue distance (GB) and green-red distance (GR) as a ratio of $(GB-GR)/(GB+GR)$, whereby a ratio near to zero indicates near equality between GB and GR. The ratio in V4 (median: 0.07) is closer to zero than that in V1 (median: -0.33) and V2 (median: -0.22) (**Figure 7C**). In other words, in V4 the green locus adopts an equi-distant position in cortical space, much as it does in planar hue space. The outcome of analogous tests with purple was different: although gaining a more autonomous response territory (**Figure S6B**), overall, purple maintains a relative proximity to red rather than blue (**Figure 7D**) within all three successive areas, with no significant change across V1, V2 and V4 (**Figure 7E**). Notably, however, the purple locus appears to ‘oscillate’ between the red and blue poles, as reflected by the large variance in the box plots of **Figure 7D**.

In summary, and particularly with reference to midspectral green hues, we infer that the spatial parameters of chromotopic maps regularize in the transition from V1 to V2 to V4 (**Figure 8**), more closely approximating an isometric organization mirroring perceptual color space.

Figure 8. Schematic of the Averaged Chromotopic Maps in V1, V2 and V4

The color-response blob is a conserved functional unit enlarging in scale from V1, V2 to V4. The figurative hue maps depict hierarchical evolution from V1 toward a more isometric hue map in V2 and V4. The size of each colored disk quantifies the response power ratio of each corresponding hue. The configuration of hue maps is the averaged peak-to-peak distance between hue responses within each area.

DISCUSSION

The functional operations associated with each hierarchical stage of color processing are not well established. Using two complementary optical techniques – the global view afforded by optical imaging of intrinsic-signal allied to the cellular resolution of 2-photon calcium imaging – we examined the topography of chromatic processing across areas V1, V2 and V4. We confirm the presence of hue maps and describe systematic changes in their scale, precision and composition, as depicted in **Figure 8**, and reiterated below. This schematic, integrating our findings with many previous studies (Chen et al., 2008; Conway et al., 2007; Li et al., 2014; Livingstone and Hubel, 1988; Lu and Roe, 2008; Sincich and Horton, 2005a; Tanigawa et al., 2010; Xiao et al., 2007; Xiao et al., 2003) reflects a classic ‘systems engineering’ mindset for understanding brain function; namely, one in which parallel processing is implemented through discrete modules and serial processing operations traverse distinct hierarchical stages. Growing experience with deep neural networks suggests that the character of neural processing is not so uniformly lawful (Flachot and Gegenfurtner, 2018). Whilst interpreting the hierarchical development of hue mapping as a progressive construction of perceptual color space, we accept that this proposal describes the main current, if not the eddies, in a flow of processing towards downstream sites beyond V4.

Architecture of Chromatic Processing across V1, V2, and V4

Exploiting intrinsic optical imaging to simultaneously record large regions of adjacent cortices, we confirmed the segregation of color-responsive domains in V1, V2 and V4, as depicted in summary schematics **Figures 1B** and **8**. Commonly known as blobs, thin stripes, and “globs” (Conway et al., 2010) they comprise a uniform modular (or blob-like) architecture for color processing, differing across areas chiefly in scale. Although the sizes of color-response blobs in V2 and V4 are comparable, they are on average much larger than those in V1 (**Figures 2, S1, and S2**). Our V4 globs match in size anatomically-defined foci in a similar caudal sector of V4 receiving inputs from V2 thin stripes (Felleman et al., 1997; Xiao et al., 1999), and they are also commensurate with color-response regions identified in V4 and PIT by fMRI studies in macaques (Conway et al., 2007; Conway and Tsao, 2006; Harada et al., 2009; Lafer-Sousa and Conway, 2013; Rosenthal et al., 2018; Wade et al., 2008). This ‘size principle’ of cortical functional organization applies equally to orientation (**Figures S1 and S2.C**), suggesting functional organization and connections in extra-striate visual cortices may be significantly different from V1. We note that the size of some larger color-response blobs in V1 encoding six or seven hues was about twice the average CO blob size, in potential correspondence to so-called ‘blob-bridges’ (Landisman and Ts'o, 2002a; Ts'o and Gilbert, 1988).

The definition of a ‘color-response’ is necessarily empirical, as the functional division of labor between parallel streams remains incompletely understood. For the 2-photon experiments we defined it as selective response to hue; a substantial proportion of V1 cells combine this property with orientation selectivity (see **Figure S7**), consistent with a previous study (Garg et al., 2019). In the intrinsic-signal experiments a ‘color-response’ is the differential activation achieved by adding chromatic contrast to an achromatic square-wave grating matched in luminance contrast and spatial frequency; this activity should also include a component generated by oriented, hue-selective cells. Oriented and non-oriented hue-selective cells likely correspond to the double- and single-opponent classes described by single-unit studies (Johnson et al., 2001, 2004, 2008). Single-opponent cells should be activated by the chromatic phase of our square-wave gratings and double-opponent cells by the border contrast (Friedman et al., 2003); the latter response can exceed the former, at least as demonstrated by voltage sensitive dye imaging in V1 (Zweig et al., 2015). Hence, at least in V1, we can attribute color responses (both 2-photon and intrinsic-signal) to combined activity of double-

and single-opponent cells; higher areas so far lack a similar level of consensus regarding color cell classification.

Inferences from the Evolution of Hue Maps across V1, V2 and V4: Cortical

Construction of Color Space

The chromatic plane of color space is defined by polar coordinates of hue and saturation (with the orthogonal axis corresponding to lightness, or ‘value’). Or, more physiologically, by cartesian (‘cardinal’) axes of chromaticity linked to the peripheral konio- and parvocellular systems, whose conjoint activity must later be read out by cortical cells signaling discrete chromatic loci. Does this read-out commence in the CO blobs of V1 where, as noted above, parvo and konio inputs first converge?

We found a surfeit of red and blue hue responses. This was seen in both our intrinsic-signal and 2-photon imaging data, the former echoing previous studies in macaque (Xiao et al., 2007; Xiao et al., 2011), and marmoset (Valverde Salzmänn et al., 2012), and the latter the recent report of Garg et al (2019), also in macaques. Endspectral response bias in CO blobs was first detected by 2-DG uptake experiments (Tootell et al., 1988), and later by electrophysiological recordings (Yoshioka and Dow, 1996). Significantly, all these studies employed computer monitors for color stimulation. Given the spectral emission characteristics of R, G and B phosphors, endspectral bias looks to be the consequence of the fact that R and B phosphors elicit maximal cone-opponent responses (Mollon, 2009; Tootell et al., 1988): R in respect of L-M (parvo), and B in respect of M-L (parvo) and S-(L+M) (konio) (**Figure 1A**). This account of endspectral bias is corroborated by Valverde Salzman et al. (2012), who correlated the level of blob activation obtained by intrinsic-signal imaging with the L/M and S/(L+M) cone contrast produced by their test hues: they found that L/M contrast alone was a significant predictor of blob activity, and that it was their blue, rather than green stimulus that maximized M-L cone contrast.

The simplest model to account for the known segregation of red and blue responses – and moreover for the segregation of pooled purple, red, orange, and yellow responses from green, cyan and blue, that we now report in V1 (**Table S1, Figure S6**) – is separate terminal foci for parvo afferents transmitting L-M and M-L signals. Exactly how konio signals contribute to this picture is uncertain.

Valverde Salzman et al. (2012) were unable to observe blob activation driven by S cone contrast (i.e. konio signals) alone. We might infer that konio input contributes to our minority foci pooling purple with blue responses, but have little hard evidence for integration of parvo and konio signals in V1; by contrast, the hue maps that we describe in V2 and V4 defy explanation without such integration.

Both forms of optical imaging confirmed that the pattern of endspectral dominance, characteristic of V1, receded in V2 and was virtually absent in V4 (**Figure 3**). The growth of midspectral and extraspectral hue representations in V2 and V4 implies functional convergence of parvo and konio signals, as arguable from closer consideration of the cellular hue map in V2 (**Figure 4G**). A cell receiving solely L-M parvo input that has a preferential response to an orange hue, rather than red, is conceivably ‘contrast-tuned’ for this input – analogous to a class of cell found in V4 that is similarly tuned for achromatic contrast (Sani et al., 2013). However, the purple and orange hues generate near identical levels of L-M cone contrast (see **Figure 1A**): contrast-tuning for a sole L-M cone input should produce intermixed scattering of orange and purple hue preferring cells, on account of physiological noise, much as we observe in V1. The systematic, tight hue clustering seen in V2 precludes such an explanation based on sole channel contrast-tuning; we infer, instead, that such narrow hue preferences depend upon dual input from the parvo and konio channels, and moreover sensitivity to a specific *ratio* of signal intensities. It is both the spectral, and the spatial properties of the cellular hue map that lead to this conclusion.

Complementary analysis of the intrinsic signal imaging showed that the hue maps in V2 and V4 are more isometric than in V1; they have greater uniformity in both the weighting and the spacing of separate hue responses. This is demonstrated by the centralizing shift of the green locus (**Figure 7B**). Even with random scatter, the ‘twin-spot’ configuration of the hue responses in V1 (i.e. PROY vs. GCB foci, as noted above) would be sufficient to register a baseline incidence of significant hue mapping in respect of all three metrics that we tested. Notably, it was the two planar hue-metrics (as opposed to the linearized one) that better demonstrated a rising prevalence of chromotopic organization through V2 to V4. In effect, hue maps develop from V1 to achieve a recognizably closer affinity to perceptual hue space in V4. This indicates that functional integration of the parvo and konio channels is a progressive process – one that might initiate to a minor extent in V1, and not yet be perfected at the level of V4. As presaged above, our systems engineer might stipulate (1) an initial

stage registering activity in cardinal axes of chromatic space that is retinally-specific (given that blobs are heavily monocular); (2) a second stage achieving a cyclopean representation of color contrast, and (3) a third stage at which surface hues (or spectral reflectances) are computed from the conjoint activity in the cardinal axes. Our observations suggest that color processing is not so rigidly implemented in a stage-wise, serial fashion. Nevertheless, the rationale for cortical maps of any description (e.g. visual, somatic or acoustic) appeals to a minimization of wiring length to facilitate interactions between units tuned to nearby parameters of the mapped space and, on that basis, the evolution of cortical maps reflecting planar hue space acts as an index of perceptual color space undergoing hierarchical construction. This process is certain to extend beyond the zone of caudal V4 that we examined, since cells with narrow representations of hue and saturation continue to develop luminance-invariant and shape-invariant properties at stages located more anterior in area V4A, or IT cortex proper (Bohon et al., 2016; Chang et al., 2017; Namima et al., 2014; Sanada et al., 2016).

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AUTHOR CONTRIBUTIONS

W.W. designed research; Y.L., M.L., X.Z., Y.L.L., H.G., J.Y., Z.C., L.Q., N.M., S.T. and W.W. performed research; Y.Y., S.S., and I.M.A. contributed analytic tools; Y.L., M.L., Y.L.L., J.Y., S.S., S.T. and W.W. analyzed data; W.W., Y.L., S.S., I.M.A., M.L. and N.M. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR Methods

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Wei Wang (w.wang@ion.ac.cn).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The complete dataset and MATLAB analysis code are freely available are freely-available from the Lead Contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Fifteen adult rhesus macaques (*Macaca mulatta*, eleven males and four females, 3~7 years old, weighing 4.0–10.9 kg) were used in this study. All experimental procedures were approved by both Animal Committee of the Institute of Neuroscience, Chinese Academy of Sciences and Peking University Animal Care and Use Committee. All experimental procedures were also in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

METHOD DETAILS

Optical Imaging of Intrinsic signal

All experimental procedures for primate research were approved by the Animal Care and Use Committee of the Institute of Neuroscience and by the local ethical review committee of the Shanghai Institutes for Biological Sciences. Eleven adult rhesus macaques (*Macaca mulatta*, seven

males and four females, 4~7 years old, weighing 4.0~9.4 kg) were prepared and maintained for simultaneous optical imaging of intrinsic-signal (OIS) in V1, V2, and V4 as previously described (An et al., 2012; Pan et al., 2012). The optical recording chamber was centered over the lunate sulcus (LS), allowing for concurrent recording of optical signals from a field of view encompassing opercular V1, the full width of V2 that is exposed between V1 and the LS, and a hemicircular field of V4 extending across the prelunate gyrus (Pan et al., 2012; Tanigawa et al., 2010).

A gamma-corrected monitor (Sony G520, 1280x3960 pixels, 100 Hz, luminance range from 0.1 to 80 cd/m²) was placed 57 cm from the eyes to subtend 40°×30°. The monitor was calibrated by SpectroCAL MKII Spectroradiometer (Cambridge Research Systems). The fovea and other retinal landmarks for all subjects were back projected to the screen center using a reversing ophthalmoscope (Jones et al., 2001). Full-screen visual stimuli were generated with PSYCHTOOLBOX-3 running in MATLAB. For mapping the orientation response domains, sinusoidal luminance gratings with orientation 0°, 45°, 90°, or 135° were presented at varied spatial frequency (0.125~6.0 cycles/degree, c/d) and temporal frequency (2~5 cycles/second, c/s). For the ocular dominance test identifying the V1/V2 border we presented the same gratings monocularly, to each eye in turn (An et al., 2012; Lu et al., 2018). In conformity with the stimulus protocols of previous studies (Landisman and Ts'o, 2002b; Li et al., 2014; Lu and Roe, 2008; Tanigawa et al., 2010; Xiao et al., 2007) we used dual color red/green and blue/yellow square-wave gratings to map the gross extent of chromatic response domains, each chromatic grating being contrasted with an achromatic grating of the same spatial frequency (SF) at 0.25 or 0.5 c/d (**Figure 1C**). To map the intrinsic hue-topography of chromatic response domains ("hue maps"), we contrasted seven single-color square-wave gratings with a similarly configured achromatic (gray/black) grating of matching luminance contrast and spatial frequency. The seven test colors and the gray phase of the achromatic grating were isoluminant (~11.20 cd/m²) (**Figure 1D**). The seven hues comprising the test-set were red, orange, yellow, green, cyan, blue and purple, respectively (**Figure 1A**). These are the hues respectively specified by the angles 0°, 30°, 60°, 120°, 180°, 240°, and 300° in the chromatic plane common to the Hue-Saturation-Lightness (HSL) and Hue-Saturation-Value (HSV) perceptual color spaces. To generate isoluminant hue stimuli and take the monitor gamut into consideration, we used a spectroradiometer to ensure all the colors were isoluminant with proper RGB ratio (Evans et al.,

1954). We further converted these colors into CIE 1931 color space for comparison with other studies (see details in **Table S1**); the cone excitations (in the MacLeod-Boynton chromaticity diagram) and cone contrasts of each all tested hue are calculated using 2-deg Smith & Pokorny cone fundamentals (Smith and Pokorny, 1975), and presented in **Figure 1A and Table S1**. The colors shown in the article figures are adjusted using pseudo-colors for better visualization.

Electrophysiological Recordings

We trained two rhesus macaques (*Macaca mulatta*, two males, 6~7 years old, 6.9 and 10.9 kg) to do passive fixation tasks for the awake electrophysiology. The electrophysiological recording procedure using 16-channel multisite linear electrodes (U-probes with intercontact spacing of 150 μm) and Omniplex Recording System (Plexon) was described in our previous study (Lu et al., 2018). Stimuli were generated with PSYCHTOOLBOX-3 running in MATLAB on a gamma-corrected CRT monitor (Vision Master Pro-514, Iiyama; 1600 $\times$ 1200 pixels at 100 Hz) placed 114 cm from eyes. An infra-red eye tracking system (Eyelink 1000, SR Research) was used to track the eye position at 500 Hz whilst the animal maintained fixation within a 1° window. Animals had to fixate on an isoluminant blank screen for 800 ms to initiate a trial and keep fixation for 1000~1500 ms during stimulus presentation. After the stimulus was turned off, the animal had to maintain fixation for another 500 ms. The raw electrophysiological data were sampled at 40 kHz. The data were later filtered with a 300 Hz high-pass Bessel 4 pole filter and multi-unit spikes were detected at ± 3.6 standard deviations using Offline Sorter software (Plexon). The LFP was filtered between 1 and 300 Hz. The data was further analyzed by Fieldtrip Toolbox (Oostenveld et al., 2011). The visual stimuli used were achromatic drifting sinusoidal gratings, static color patches and drifting sinusoidal color gratings, all either full-screen or sized to match the response fields of the recording sites.

As the response fields of neurons within a cortical column are similar in position and size (Nandy et al., 2017), we only took the response field of the most responsive channel to represent each penetration. We used either hand-mapping or computer-automated mapping with a grating patch to depict the response field of the penetration (Chen et al., 2014; Gallant et al., 1996; Lu et al., 2018; Mazer et al., 2002).

U-probes were inserted perpendicular to the cortical surface in areas V1, V2 and V4, in search of color domains where neural responses demonstrated predominant tuning to color stimuli. A neuron was deemed to be color selective if it had a differential response to the 7 hues tested ($P < 0.05$, ANOVA). We calculated the laminar current source density (CSD) with the second spatial derivative of the LFP response across layers in response to a full screen flash stimulus (100 ms of the test hue, or white, followed by 900 ms black in each trial) (Hansen et al., 2011; Lu et al., 2018; Schroeder et al., 1998). We then full-wave rectified the CSD signals to color stimuli in the supragranular layers to obtain the rectified current (Chen et al., 2007; Schroeder et al., 1998). Due to the variance in the magnitude of currents in different cortical depths and penetration sites, we normalized the CSD amplitudes (in the period of 0~150ms after stimulus onset) within each recording channel to eliminate the variation among recording channels. The z-score normalized current amplitude were then compared among different color stimulation conditions. The orientation selectivity index (OSI) of the recording cell is defined as $OSI = (R_{\text{preferred}} - R_{\text{orthogonal}}) / R_{\text{preferred}}$, where R is the cell's response (firing rate) to the preferred orientation or the orthogonal orientation. The color preference index (CPI) is defined as $(R_{\text{color}} - R_{\text{achromatic}}) / (R_{\text{color}} + R_{\text{achromatic}})$, where R is the cell's response to the preferred color stimulus or preferred achromatic orientation.

Histological Cytochrome-Oxidase Staining

Three animals were perfused for conventional histological processing upon termination of optical-imaging. Tangentially oriented sections were cut through regions of the brain underlying the optical recording chamber. These were stained for cytochrome-oxidase (CO) to reveal blob and stripe compartments in areas V1 and V2, respectively, as performed in previous studies (An et al., 2012; Sincich and Horton, 2005b). The histological sections were co-registered with the optical images using as landmarks both the marked boundary of the cortical surface underlying the recording chamber and the location of the LS (An et al., 2012).

2-Photon Imaging

Two adult (3~5 years old, 4~9kg) rhesus macaques were used in the 2-photon imaging experiment. These experimental protocols were approved by the Peking University Animal Care and Use

Committee. Detailed procedures of surgery were described in previous article (Li et al., 2017) . Briefly, the animals were anesthetized and craniotomy was performed in the target brain area. The dura was opened, and an AAV vector (AAV1.hSynap.GCaMP5g.WPRE.SV40, Penn Vector Core or pAAV.Syn.GCaMP6s.WPRE.SV40, Addgene) was injected at multiple cortical sites. The dura was then sutured, bone replaced and nailed, and skin sutured. About 1~2 months later, the cranial window was reopened and the dura was removed. The edge of a glass window was sealed beneath the dura, the bones and edge of the glass window being covered by dental cement. Finally, 3 head posts were mounted on the head (two in front and one in back), and a T-shape brace was connected to the 3 head posts to ensure structural integrity.

After a recovery period of 10 days from the second surgery, the animals were trained to maintain eye-fixation. 2-photon imaging was performed using a Prairie Ultima IV (In Vivo) 2-photon microscope (Bruker Nano, FMBU, formerly Prairie Technologies) with a Ti: Sapphire laser (Mai Tai eHP, Spectra Physics). The wavelength of the laser was set at 1000 nm. With a 16X objective (0.8-N.A., Nikon), an area of 850 μ mX850 μ m was imaged. The fast-resonant scan (up to 32 frames per second) was used to obtain images of neuron activity (8 fps by averaging each 4 raw frames).

During the task, the animal was seated in a primate chair with head restraint. The animal had to fix its eyes on a small white spot (0.1°) within a window of 1° for a minimum of 2 seconds to obtain a juice reward. Eye positions were monitored with an infrared eye-tracking system (ISCAN) at 120 Hz. Visual stimuli were generated by ViSaGe system (Cambridge Research Systems, UK) and a 21" CRT monitor (Sony G520, 80Hz refresh rate, maximum luminance, 105 cd/m²). The CRT was positioned 51cm from the eyes. We estimated the RF sizes and positions of the imaged neurons with small gratings and bars presented at different locations. We used two types of visual stimulus: a static closed circle (0.8° in diameter for V1, and V2) of a specific hue for testing hue preference, or an achromatic grating (size: 0.8° in diameter, spatial frequency: 2.5 c/d, temporal frequency: 2.2 c/s) for testing the cello.8° for V1) of varied orientation (0°, 30°, 60°, 90°, 120°, and 150°) for testing orientation preference. The visual stimulus was presented in the receptive field for 1 second after 1 second blank within the 2 second fixation period. For the regions of interests, we first search for visual responsive domains under low magnification lens (4× objective) in response to our test stimuli.

After we found the region with strong responses, we switched into high magnification lens (16× objective) for detailed recording and mapping.

All data analyses were performed using customized MATLAB software (The MathWorks, Natick, MA). The images from each session were first realigned to a template image (the average image of 1000 frames in the middle of an imaging session) using a normalized cross-correlation-based translation algorithm, to correct the X-Y offset of images caused by the motion between the objective and the cortex. The 8 (125msec) frames preceding stimulus onset, and 8 subsequent frames were separately averaged to form the respective OFF and ON frames of a stimulus. All ON and OFF frames of a stimulus were filtered by a small Gaussian filter ($\delta=2\mu\text{m}$) to remove very high frequency noise. All subsequent processing was based on these ON and OFF frames. Cells were extracted from an (ON-OFF)/OFF image of each stimulus condition by difference of gaussian (DOG) filters, and all isolated pixels of a cell were averaged to calculate that cell's response. Each cell if significantly tuned (one-way ANOVA, $P<0.05$) to one or more color in the testing seven hues was assigned as a hue selective cell. A cell if significantly tuned (one-way ANOVA, $P<0.05$) to one or more orientation in the testing six orientations was assigned as an orientation selective cell. The preferred (maximum response) color (or orientation) is displayed on the cell distribution map. To generate density map of cells preferring each hue across the $850\mu\text{m}$ square recording window, the locations of cells were firstly denoted by white dots (value=1) against a black background (value=0), then this map was submitted to an 2D averaging filter with square window of $0.125\times 0.125\text{ mm}^2$ (step size: 1 pixel, $1.5625\mu\text{m}$). For each hue the cell number within the sliding window when centered on each pixel, divided by the area of the sliding window, represents the hue-specific cell density at that pixel.

We then used the hue-specific cell density maps to generate chromotopic hue maps from the 2-photon data comparable to those obtained from the intrinsic-signal data. First we removed cell noise: for each hue, we found the average cell density of all pixels with non-zero density, and discarded all pixels below a threshold set at 25% of the average density. Hue response loci were then delimited by contours of 75% peak cell density around each peak. Following this procedure, individual hues could return multiple loci; the final hue map comprised loci with the highest hue-specific peak cell density.

To test the spatial scale of cell-clustering according to feature preference in 2-photon imaging data, we computed the cluster index (Nauhaus et al., 2012) for orientation cells in V1 and color cells in V1 and V2. The cluster index is defined as $\text{Cluster index (d)} = E[1 - r_{\text{resampled}}] / E[1 - r_{\text{actual}} | d]$, where d denotes the cortical distance between a pair of cells, r is the correlation coefficient between the tuning properties (either orientation or color feature) of a cell pair and E is the expected value. The cell resampling was performed 1000 times. Values of the cluster index above 1 or below 1 suggest, respectively, that the feature preferences of cell pairs at the prescribed distance are more similar or dissimilar than those of randomly paired neurons in the imaging window. The OSI and CPI are in the same definition as that in the electrophysiology, but cell's response is measured as the change in fluorescence (Garg et al., 2019).

Data Analysis on Optical Imaging of Intrinsic signal

To generate a response map of intrinsic signal, single-condition maps were generated first. We averaged, across trials, a response frame due to the visual stimulation (R_1 , a period of 2~6 s from stimulus onset), and a blank frame (R_0 , the interval from 1.0 to 0.5 s before stimulus onset) to generate a single-condition map of reflectance change $\Delta R/R$ as $(R_1 - R_0)/R_0$. A differential map was then generated by pixelwise subtraction of a contrasting pair of single-condition maps, such as orthogonal orientations (e.g. 0°-90°) or chromatic versus achromatic gratings (e.g. red/green – black/white) (Bonhoeffer and Grinvald, 1996). Polar orientation preference maps were constructed using a vector summation algorithm. For visualization, the images were high-pass filtered (1~1.1 mm in diameter) and smoothed (170 μm in diameter) by circular averaging filters to suppress low- and high-frequency noise while avoiding signal distortion (An et al., 2012; Pan et al., 2012). A variability map was obtained to find pixels with large cross-trial variability (e.g., blood vessels and other noisy regions) and a mask was generated, based on an objectively chosen threshold (An et al., 2012; Lu et al., 2018; Pan et al., 2012).

For quantitative measurement of size, the perimeter of a response domain (as visualized in the differential map) was set at a criterion of 3 standard deviations above baseline. Baseline activity refers to the 'response' in the second half of the blank frame, after equivalent processing through single condition and differential maps, its sampling distribution being determined across pixels in

the differential map subsequent to spatial filtering and masking. The local cortical territory responsive to chromatic/achromatic contrast – that we term a ‘color-response blob’ – was defined as the sum (or envelope) of the response domains pertaining to each of our seven single-hue stimuli under the criterion of mean+3SD.

The size measurement of CO blobs in V1 was based on a previously reported method (Sincich and Horton, 2005b) with two modifications. Firstly, to minimize artifacts, we identified pixels with density level darker than darkest blob (stray lymphocytes) or brighter than brightest interblobs (blood vessels) in the CO stained image, and replaced these pixels with their average surrounding density. Secondly, we used a triangular filter (65 μm width at half-height) instead of Gaussian filter in the original method - to smooth the blobs, that we found better maintains blob shape. Finally, blob territory was designated as the darkest 33% of the region under examination. The measured blob sizes were multiplied by an areal correction index of 1.23 to compensate for tissue shrinkage during histology (Solomon, 2002). The diameters of CO blobs are also measured for comparison although the shape of blobs in V1 are not in perfect circles, we computed the diameter of a CO blob by averaging the length major and minor axes of the blob.

To quantify the accuracy of co-registration between images of CO staining and intrinsic optical imaging, we aimed to measure the average distortion distance of corresponding blobs in CO staining and optical imaging (Valverde Salzmänn et al., 2012). In detail, we measured the position of peak density for each CO blob, and generated the position coordinates of response blobs by averaging the peak response positions across all the hue stimuli we tested. Due to tissue shrinkage during stepwise histological processing, it is not applicable to directly compare the blob positions in CO staining with those obtained by intrinsic optical imaging. Thus, we calibrated the CO position matrix by linear fitting to the position matrix from intrinsic optical imaging so as to quantify the differences between these two matrices. We discarded blobs along blood vessels due to the serious distortion of their positions and generated a final averaged distortion distance between corresponding blobs in CO staining and intrinsic imaging.

To assess the relative level of response to each hue we defined a quantity termed hue ‘response power’ as the product of the response strength and size for each single-hue response domain (or ‘hue-spot’). As before, the size of the hue-spot was determined by the number of pixels above the

mean+3SD threshold, and strength is the mean response strength of those pixels. The ‘power ratio’ of a particular hue within a given color-response blob was defined as that hue’s response power divided by the sum of all hue response powers within the blob. This ratio is frequently zero, for hues that fail to register a super-threshold response in a particular blob; all such null instances were included in calculating the global, mean power ratios that we report for each area. We tested the uniformity of response power distribution in each area, and compared the uniformity between areas by a bootstrap method, as follows. For testing the uniformity of hue response distribution in an individual cortical area: (i) we randomized the response powers for each blob, i.e. reassigning hue labels by randomly rotating through 1-7 positions, to preserve the circularity of color space; (ii) we averaged across blobs and found the variance of the averaged, randomized response powers within an area; (iii) we did this 10^6 times in order to rank the actual empirical variance for that area within the randomized outcomes, and hence calculate P. If hue bias within an individual blob were due to random noise, we would expect it to cancel out in the average across all blobs within an area. For testing differences in the uniformity of response power distribution between two areas: we followed steps (i) and (ii) as above for each area separately, yielding a difference in variance between areas for each iteration. Multiple iterations (10^6) gave a population of variance differences, within which we ranked the actual difference between areas obtained with the empirical data.

To examine hue topography, we systematically measured the proportional overlap between all possible pairings of our seven single-color test-hues. Again, counting only super-threshold pixels (mean+3SD), we calculated the overlap ratio as the proportion of pixels dually responsive to specific hue pairs within a single hue-spot: i.e. the territory of each hue-spot was assigned to a fraction uniquely activated by that hue, and upto six other partially overlapping fractions jointly active with another hue. To better visualize the difference of overlap ratios between a pair of areas, we directly subtracted the overlap ratios of corresponding hue pairs and applied a color scale to the outcome. For graphical presentation of hue topography we drew contours around “peak response zones”, namely pixels thresholded at 0.75 times the maximum strength of each particular hue response within the host color blob, and also above the criterion of mean+3SD (Li et al., 2014; Xiao et al., 2007; Xiao et al., 2003).

For a quantitative assessment of hue topography (i.e. the spatial parameters of chromotopic organization, **Figure S5B**), we used regression analysis of cortical distance against hue distance. Cortical distance was measured between all pairings of the peak points activated by each hue within a given color-response blob (affording regression of 21 data points for a complete map of 7 hues). Regression of each cortical hue map was measured in respect to three color metrics: (1) a linear hue metric, with hue angles 30° - 330° , formed by a linearized hue circle (**Figure S5C**, upper). The function relating peak-to-peak cortical distance (y) to hue angle (x) is $y=a+b*x$. Regression analysis was performed for all possible positions of the discontinuity within the hue circle; the position generating the maximal coefficient of determination (R^2) was selected, and the corresponding P value was adjusted by Bonferroni correction for multiple comparisons (depending on the empirical number of hues in the hue map under examination). (2) a circular hue metric, with hue-angles 30° - 180° (**Figure S5C**, lower). This metric assessed correspondence to geometric length between loci on the hue circle (a sinusoidal function of hue angle) and the function relating peak-to-peak cortical distance to hue angle is $y=a+b*\sin(x/2)$. (3) a metric derived from CIELab color space, specifically planar hue distances as specified by the DE76 metric (Hunt and Pointer, 2011) ; the function relating peak-to-peak cortical distance to hue distance is $y=a+b*x$. The regression coefficient, and slope provided indices of chromotopic precision, and scale, respectively. Note that hue maps with less than four different hue-spots were excluded from analysis due to inadequate sample size (Li et al., 2014).

For an overall measure of the significance of chromotopic organization instanced in each hue map, we combined the p-value obtained from regression with further bootstrap statistics, termed a randomization test and a permutation test, as described in a previous study (Li et al., 2014). For the randomization test, we resampled the cortical position of each hue response within a 1000×1000 matrix, and performed linear regression analysis for the distances of resampled positions (x) against the empirical hue distances (y). For the permutation test, the hue tags of peak points were randomly shuffled and the empirical cortical distances were regressed against the resampled hue distances. Each process repeated for 1000 times generating 1000 values of resampled coefficients of determination (R^2). The empirical R^2 was then ranked within each population of resampled R^2 values, to yield two additional p-values. For the purpose of inter-area comparison, hue maps were accepted as displaying significant chromotopic organization if they attained a threshold level of significance

across all three tests ($P < 0.05$ for regression, randomization test and permutation test). Finally, the proportions of significant chromotopic maps was compared among areas using a χ^2 test.

QUANTIFICATION AND STATISTICAL ANALYSIS

The n numbers are reported explicitly in the main text or figure legends. The statistical tests used were described in detail in the Methods Details subsection for each assay and in the relevant figure legends.

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KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
Rhesus macaque (<i>Macaca mulatta</i>)	Beijing Institute of Xieerxin Biology Resource	http://www.xexbio.com/
Rhesus macaque (<i>Macaca mulatta</i>)	Beijing Prima Biotech	http://www.primasbio.com/cn/Default
Recombinant DNA		
AAV1.hSynap.GCaMP5g.WPRE.SV40	Penn Vector Core	V6270S
pAAV.Syn.GCaMP6s.WPRE.SV40	Addgene	100843-AAV1
Software and Algorithms		
MATLAB	Mathworks	RRID:SCR_001622
Psychophysics Toolbox	http://psychtoolbox.org/	RRID:SCR_002881
Offline Sorter Spike Sortng Software	Plexon	RRID:SCR_000012
Fieldtrip Toolbox	http://www.fieldtriptoolbox.org	RID:SCR_004849
Other		
Omniplex Recording System	Plexon	RRID:SCR_014803

Table S1. The Parameters of Stimulus Colors in Multiple Color Spaces. Related to Figure 1A.

	Hue (°)	RGB	CIE1931 (x, y, Y)	CIELab (L, a, b)	Cone Contrast (L, M, S)
Red	0	(124, 0, 0)	(0.63, 0.34, 11.21)	(39.9, 58.7, 53.4)	(0.28, -0.51, -0.93)
Orange	30	(48, 24, 0)	(0.46, 0.47, 11.13)	(39.8, 3.4, 47.0)	(0.07, -0.16, -0.90)
Yellow	60	(28, 28, 0)	(0.40, 0.52, 11.18)	(39.9, -16.6, 45.5)	(0.02, -0.06, -0.89)
Green	120	(0, 36, 0)	(0.28, 0.61, 11.12)	(40.4, -46.6, 44.1)	(-0.06, 0.07, -0.88)
Cyan	180	(0, 31, 31)	(0.20, 0.29, 11.19)	(40.5, -19.8, -15.8)	(-0.08, 0.12, 0.22)
Blue	240	(0, 0, 255)	(0.15, 0.07, 11.21)	(37.2, 64.3, -99.6)	(-0.22, 0.37, 6.76)
Purple	300	(79, 0, 79)	(0.27, 0.14, 11.26)	(40.0, 63.5, -54.0)	(0.10, -0.18, 1.87)
Gray	-	(25, 25, 25)	(0.28, 0.30, 11.28)	(40.1, -2.4, -9.3)	-

Figure S1. Cortical Response of Orientation and Color in V1, V2 and V4. Related to Figures 1B and 2.

(A) Surface blood vessel map, and differential response maps for RG color and orientation for V1, V2 and V4 from right hemisphere of M080928020. White dashed line marks the border of V1, V2, and V4. Red

dashed circles marked blobs in V1. Red dashed quadrangles marked the inferred locations of the thin stripes in V2 and color domains in V4. A: anterior; L: lateral; Scale bar: 1mm.

- (B)** Surface blood vessel map, differential response maps for RG color and orientation polar map for V2 and V4 from right hemisphere of M09092631. Color in orientation polar map represents orientation preference and brightness represents the response strength.

Figure S3. Additional Comparisons of Response Power Ratio and Rectified Current Amplitude among Hues in V1, V2 and V4. Related to Figure 3.

- (A)** Comparison of response power ratio between blue and other hues in V1, V2 and V4 (***: $P < 0.001$, **: $P < 0.01$, n.s.: $P > 0.05$, Wilcoxon rank sum test with Bonferroni correction). Data from 151 color-response blobs in V1, 80 in V2 and 86 in V4, respectively.
- (B)** Comparison of rectified current amplitude between blue and other hues in V1, V2 and V4. (*: $P < 0.05$, Wilcoxon signed-rank test with Bonferroni correction). Data from 51, 36, and 24 recording channels in supragranular layers of V1, V2 and V4, respectively.

- (C) Traces of average rectified currents to color grating stimuli with stimulus size matching the receptive field in V1, V2 and V4. The gray dashed line indicates the onset of visual stimulation. Shaded regions indicate standard error of current across channels. Data from 37, 36, and 24 recording channels in supragranular layers of V1, V2 and V4, respectively.
- (D) Comparison of Z-score normalized rectified current amplitudes between red/blue and other hues in V1, V2 and V4 (Wilcoxon signed-rank test with Bonferroni correction). The current amplitudes are normalized using Z-score normalization within each recording channel.

Figure S4. Hue Responses at Single-Cell Levels of Electrophysiology. Related to Figure 3.

- (A) Color tuning curves of example single-units in electrophysiology. The horizontal dotted lines represent the level of resting responses.
- (B) Percentage of cells preferring each tested hue by electrophysiological recording. The visual stimulus is either a color flash or a color grating presented in a full-screen size or a size matching the neuron receptive field. Some cells were tested with multiple stimuli, and the number of cells counted basing on their response to at least one of the stimuli we used (but cell is counted only once). The number of cells preferring red, orange, yellow, green, cyan, blue and purple are 28, 1, 0, 2, 0, 12, and 2, respectively in V1, 16, 1, 1, 4, 0, 8, and 2, respectively in V2 and 18, 8, 7, 10, 7, 14, and 11, respectively in V4.

Figure S5. Spatial Organization of Hue Responses. Related to Figure 4, 5 and 6.

- (A)** Percentage and absolute number of cells preferring each test hue across all color-response blobs sampled from in V1 and V2 recorded by 2-photon imaging. Total sample sizes are 2164 and 6202 in V1 and V2 respectively.
- (B)** Schematic of a sample cortical hue map.
- (C)** Comparison of linear and circular hue metrics and (idealized) cortical hue maps. The linear metric is a linearized hue circle (e.g. here with a discontinuity between red and purple) in which hue distances range from 30-330°. The distance apart (d) of hue loci in a cortical hue map is theorized to follow a linear relationship with hue difference (x): $d(x)=a+b*x$, where a and b are constants. In the circular metric hue distances range from 30-180° and cortical hue distance is theorized to obey a trigonometric function: $d(x)=a+b*\sin(x/2)$, where a and b are constants. RG, GB, RB are the pairwise distances between sample hues red (R), green (G) and blue (B), in cortical hue maps.
- (D)** Regression analysis of cortical distance against a linear hue metric for hue maps V1-a, V2-a, and V4-a in **Figure 5B**. n, R² and P represent sample number, coefficient of determination and Bonferroni-adjusted P value of regression analysis, respectively.

- (E) Percentage of hue maps (as obtained by optical imaging of intrinsic signal) in V1, V2 and V4 satisfying criteria of $P < 0.05$ for regression analysis, randomization test and permutation test, in respect of a linear hue metric. n.s., not significant; χ^2 test with Bonferroni correction.
- (F) Scatter plots illustrate the regression analysis for the 2-photon maps in **Figure 6A** using a linear color metric.

Figure S6. Overlap Ratio Analysis on Hue Maps from Optical Imaging of Intrinsic Signal. Related to Figure 7.

- (A) Charts showing the overlap ratio between color-response domains in hue maps of V1, V2 and V4. The overlap ratio is defined as: Overlap ratio = overlapping area of two hues / response size of one hue; each row shows the fractional overlap for the row-specified hue with all other hues, including the fractional non-overlapped area shown in the 'sole' column. The grey-scale map clearly shows the segregated representation of reddish hues and blueish hues in V1, V2 and V4. Data from 151, 80 and 86 chromotopic maps in V1, V2 and V4, respectively.
- (B) Charts showing the change of overlap ratio across areas. The differential overlap ratios were obtained by subtraction of overlap ratios between pairwise cortical areas.

A Intrinsic Optical Imaging

B 2-photon Imaging

C Electrophysiological Recording

Figure S7. Joint Processing of Color and Orientation in V1 Color Domains. Related to Figures 4, 5 and 8.

- (A)** Color and orientation responses in V1 recorded by optical imaging of intrinsic signal. The left panel illustrates the orientation polar maps in V1 from **Figure 5B** (upper). The white dashed lines indicate the response blobs defined under the criterion of 3S.D. above the baseline response. The right panel plots the response strength to preferred color and preferred orientation of every pixel inside the blobs. The orientation and color responses are normalized to the maximum orientation and color responses in the region of interest (ROI), respectively. Scale bar: 0.5 mm.
- (B)** Orientation selectivity and color preference of V1 cells recorded by 2-photon imaging. The orientation selectivity index (OSI) is defined as $OSI = (R_{\text{preferred}} - R_{\text{orthogonal}}) / R_{\text{preferred}}$, where R is the cell's response to the preferred orientation or the orthogonal orientation. The color preference index (CPI) is defined as $(R_{\text{color}} - R_{\text{achromatic}}) / (R_{\text{color}} + R_{\text{achromatic}})$, where R is the cell's response to the preferred color stimulus or preferred achromatic orientation (Achro).
- (C)** Orientation selectivity and color preference of V1 cells recorded by electrophysiology. The left panel plots the OSIs and CPIs of 37 V1 cells recorded using orientation grating and color grating with stimulus size matching the receptive field. The middle and right panels illustrate the color and orientation tuning curves of cell a and b marked in the left panel.