

Original Research Article

Functional organization of spatial frequency tuning in macaque V1 revealed with two-photon calcium imaging

Shu-Chen Guan^{a,1}, Nian-Sheng Ju^{b,1}, Louis Tao^b, Shi-Ming Tang^{a,b,c,*}, Cong Yu^{a,c,d,*}

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ARTICLE INFO

Primary visual cortex
Spatial frequency
Functional map
Two-photon imaging
Macaque

ABSTRACT

V1 neurons are functionally organized in orientation columns in primates. Whether spatial frequency (SF) columns also exist is less clear because mixed results have been reported. A definitive solution would be SF functional maps at single-neuron resolution. Here we used two-photon calcium imaging to construct first cellular SF maps in V1 superficial layers of five awake fixating macaques, and studied SF functional organization properties and neuronal tuning characteristics. The SF maps ($850 \times 850 \mu\text{m}^2$) showed weak horizontal SF clustering (median clustering index = 1.43 vs. unity baseline), about one sixth as strong as orientation clustering in the same sets of neurons, which argues against a meaningful orthogonal relationship between orientation and SF functional maps. These maps also displayed nearly absent vertical SF clustering between two cortical depths (150 & 300 μm), indicating a lack of SF columnar structures within the superficial layers. The underlying causes might be that most neurons were tuned to a narrow two-octave range of medium frequencies, and many neurons with different SF preferences were often spatially mixed, which disallowed finer grouping of SF tuning. In addition, individual SF tuning functions were often asymmetric, having wider lower frequency branches, which may help encode low SF information for later decoding.

1. Introduction

V1 neurons are often functionally organized in orientation columns that group neurons with similar orientation preferences (Hubel and Wiesel, 1962, 1968), likely a result of wiring optimization (Chklovskii and Koulakov, 2004) or self-organization during evolution (Kaschube et al., 2010). Although the roles of functional columns have been questioned (Horton and Adams, 2005), the local structures of orientation maps are found to be correlated to the tuning properties of neurons (Callaway, 1998; McLaughlin et al., 2003; Nauhaus et al., 2008; Li et al., 2019), which suggests that functional organizations may be related to different aspects of neural computation.

What has been less clear is the functional organization of V1 spatial frequency (SF) tuning. V1 neurons are also selective to stimulus SF (Campbell et al., 1969; Maffei and Fiorentini, 1977; De Valois et al., 1982). However, whether and how the SF tuning of V1 neurons are functionally organized remain debated (reviewed by Issa et al., 2008;

Nauhaus et al., 2012). Results from single-unit recordings on SF tuning clustering are mixed. In cat area 17, DeAngelis et al. (1999) reported significant but weak SF clustering that was about 1/5 as strong as orientation clustering, and Tolhurst and Thompson (1982) and Molotchnikoff et al. (2007) found no clear SF clustering evidence. In Monkey V1, Silverman et al. (1989) discovered that neurons' different peak frequencies are distributed continuously and smoothly, consistent with their earlier 2-DG uptake results for SF columns (Tootell et al., 1988). Yet Edwards et al. (1995) described nearly even distributions of SF tuning from 1 to 6 cpd in blob to interblob cells, except some additional (~20 %) higher SF tuning in interblob cells, which would not support significant SF clustering. Intrinsic signal optical imaging (ISOI) studies, which target large-scale functional organizations of neural selectivities but lack single-neuron resolution, reveal either low and high SF preference regions (Hubener et al., 1997; Shoham et al., 1997; Kim et al., 1999), or more continuous clusters of SF tuning (Everson et al., 1998; Issa et al., 2000; Xu et al., 2007). However, it has been questioned

* Corresponding authors at: PKU-Tsinghua Center for Life Sciences, Peking University, Beijing, China.
tangshm@pku.edu.cn (S.-M. Tang), yucong@pku.edu.cn (C. Yu).

¹ Equal contribution co-first authors.

that ISOI evidence for SF clusters could be spurious because of the influence of vascular artifacts (Sirovich and Uglesich, 2004).

Apparently a full understanding of the SF functional organization in V1 would benefit greatly from SF maps at single-neuron resolution, which is technically feasible with two-photon calcium imaging. A recent two-photon imaging study with calcium indicator dye Oregon Green BAPTA-1AM (OGB-1) revealed horizontal SF clustering and vertical SF columns in macaque V1 (Nauhaus et al., 2012), but the functional maps from large-scale imaging of this study were pixel-based with no cellular information (cellular details were only available with small-scale imaging in $200 \times 200 \mu\text{m}^2$ areas). To amend this issue, here we used long-term two-photon imaging with genetically encoded calcium indicators (GCaMP5) to construct first SF functional maps at single-neuron resolution in 5 awake macaque monkeys, using the methodology originally reported in Li et al. (2017). Our results revealed SF clustering in macaque V1 that was too weak to support a key conclusion of Nauhaus et al. (2012) that SF and orientation functional maps overlap orthogonally.

2. Materials and methods

The SF tuning data were collected from five rhesus monkeys () aged at 5–8 years. The same monkeys’ orientation tuning data, which were collected in the same sessions, have been reported in Ju et al. (2021). The details of materials and methods are repeated here for convenience. The only exception is the selection of SF tuned neurons, which replaced the selection of orientation tuned neurons in Ju et al. (2021).

Each monkey was prepared with two sequential surgeries under general anesthesia and strictly sterile conditions. In the first surgery, a 20-mm diameter craniotomy was performed on the skull over V1. The dura was opened and multiple tracks of 100–150 nl AAV1.hSynap. GCaMP5G.WPRE.SV40 (AV-1-PV2478, titer 2.37×10^{13} (GC/mL), Penn Vector Core) were pressure-injected at a depth of $\sim 350 \mu\text{m}$. The dura was then sutured, the skull cap was re-attached with three titanium lugs and six screws, and the scalp was sewn up. After the surgery, the animal was returned to the cage, treated with injectable antibiotics (Ceftriaxone sodium, Youcare Pharmaceutical Group, China) for one week. Postop analgesia was also administered. The second surgery was performed 45 days later. A T-shaped steel frame was installed for head stabilization, and an optical window was inserted onto the cortical surface. More details of

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Data were analyzed with customized MATLAB codes. A normalized cross-correlation based translation algorithm was used to correct motion artifacts (Li et al., 2017). After the correction, fluorescence changes were associated with corresponding visual stimuli through the time sequence information recorded by Neural Signal Processor (Cerebus system, Blackrock Microsystem). By subtracting the mean of the 4 frames before stimuli onset () from the average of the 6th-9th frames after stimuli onset () across 5 or 6 repeated trials for the same stimulus condition (same orientation, spatial frequency, size, and drifting direction), the differential image ($\Delta =$) was obtained.

The regions of interest (ROIs) or possible cell bodies were decided through sequential analysis of 216 differential images in the order of SF

Neurons in each SF map appeared to mostly prefer a limited range of SFs (Fig. 2A). In Maps A1, A2, C1, and C2 at both depths, neurons were mainly tuned to medium SFs, and those in Maps B, D, and E at both depths were mainly tuned to higher SFs. The percentage distribution plots suggest that most neurons in each SF map were tuned to a 2-octave SF range (Fig. 3A), consistent with electrophysiological estimates (De Valois et al., 1982). Take A1 for example, 84.0 % neurons' preferred SFs fell into a 2-octave range (0.99–3.97 cpd) at 150 μ m, and 94.1 % at 300 μ m (1.04–4.17 cpd). On average 86.1 % of neurons from all maps were tuned to a 2-octave range of SFs. These limited ranges of SF preference, plus frequently mingled positions of neurons tuned to different SFs (Fig. 2A), often made it difficult for neurons' SF preferences to be further divided into distinct and finer SF clusters.

The SF preferences appeared to be stable beyond the limited areas of FOVs in the same animals. Fig. 3B compared preferred SFs in FOVs A1 and A2 in Monkey A that were slightly overlapping at one corner, and C1 and C2 in Monkey C that were separated by approximately 300 μ m (Fig. S1). In both animals, the median preferred SFs differed by 1 octave or less between two adjacent FOVs at 150 μ m, and were virtually unchanged at 300 μ m (around 2 cpd). These limited results suggested that, besides the limited ranges of SF tuning within FOVs, I

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depths, which is difficult with current two-photon imaging technology.

Our results are largely consistent with several electrophysiological reports that SF clustering is weak or insignificant in cat area 17 and macaque V1 (Tolhurst and Thompson, 1982; Edwards et al., 1995; DeAngelis et al., 1999; Molotchnikoff et al., 2007). However, they are in contrary to the report of distinct 2-DG uptakes with low and high SF stimuli in respective V1 blob and interblob regions by Tootell et al. (1988), and to their subsequent single-unit findings of continuous and smooth transitions of preferred SFs over a cortical distance of 0.6–0.7 mm (Silverman et al., 1989). It is unclear why our two-photon results are consistent with some early reports but not the ones from the De Valois group. Nevertheless, our two-photon imaging study and De Valois et al. (1982) both revealed that most parafoveal neurons in macaque V1 prefer a 2-octave range of medium SFs. As discussed above, the limited range of SF tuning may have contributed to weak SF clustering.

Our results also differed from those in an earlier two-photon imaging study

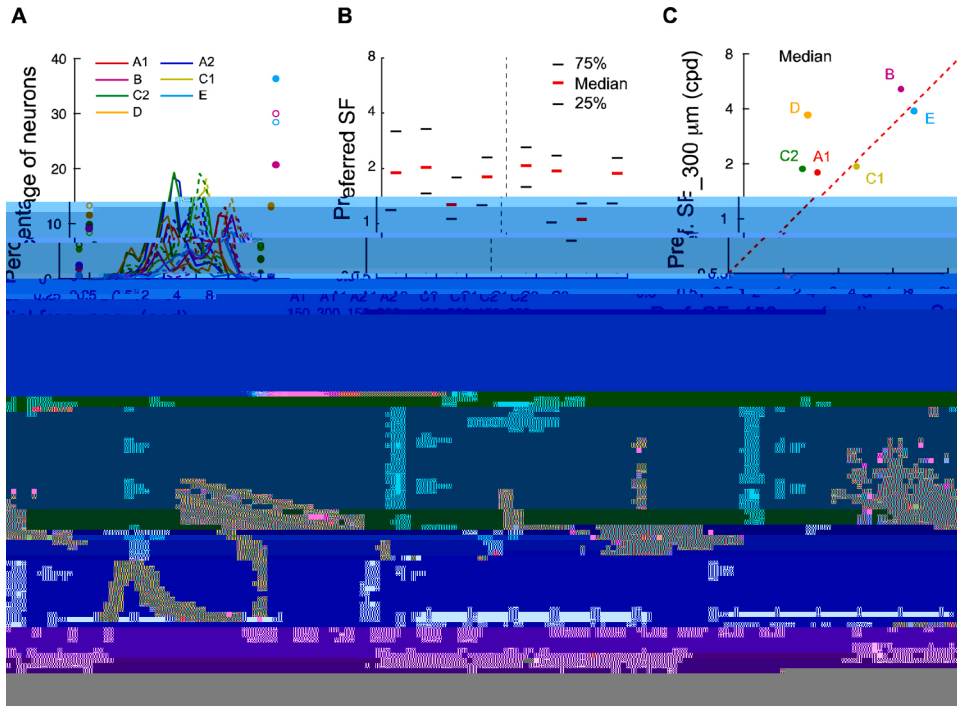


Fig. 3. SF tuning properties in V1 superficial-layer neurons. **A.** The percentage distribution of neurons against preferred SF in each map. Isolated filled and open circles indicate the percentages of low-pass (left side) and high-pass (right side) neurons on the basis of data fitting at 150 and 300 μm cortical depths, respectively. **B.** SF preference distributions of adjacent SF maps A1 and A2, and of C1 and C2, at 150 and 300 μm cortical depths, respectively. **C.** The median preferred SFs at 150 μm vs. 300 μm in each SF map (A2 excluded due to misalignment). **D.** The percentage distribution of neurons against SF tuning bandwidth in each map. Isolated filled and open circles on the right side indicate the summed percentages of low-pass and high-pass neurons at 150 and 300 μm cortical depths, respectively. **E.** SF tuning bandwidth vs. preferred SF. The left panel shows individual neurons' data and the loglinear regression of Map A1_150. The right panel shows loglinear regression lines of all maps.

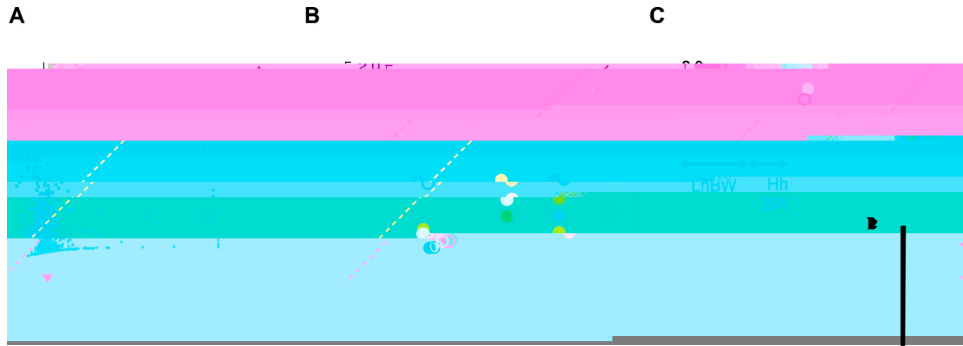


Fig. 4. Asymmetric SF tuning functions. **A.** Illustrations of lower- and higher-half bandwidths at half height (LhBW & HhBW). **B.** LhBW vs. HhBW of each neuron in Map A1_150. Red arrows indicate medians. **C.** Median LhBW vs. HhBW of each map.

appearance of SF clustering. Our Fig. S2 demonstrates this trend of inflated SF clustering in some pixel-based SF maps constructed with the same method and imaging resolution of Nauhaus et al. (2012). Second, Nauhaus et al. (2012) presented different stimulus conditions with zero time gaps, likely because of the time constraints posed by the use of toxic calcium indicator dye. As a result, the slow calcium signals from preceding trials inevitably interfered with those of later trials, which would smear the SF preferences of neighboring neurons and enhance SF clustering to certain degree. Third, the SF clustering indices in Nauhaus et al. (2012) might be biased by relatively small numbers of neurons pooled from multiple images. In contrast, our estimates are likely less biased on the basis of 14 SF maps and a total of 10k neurons.

The exact SF range varied among maps (Fig. 2A). These variations were unrelated to retinal eccentricity, as FOVs in Monkeys B, D and E, which showed higher preferred SFs, were not closer to the fovea than those in Monkeys A and C. Other possibilities included individual differences of SF preferences among monkeys, sampling biases in the same monkey, and SF clustering in a grander scale than orientation tuning. Fig. 3B showed similar SF tuning across adjacent FOVs A1 and A2, and across C1 and C2, in two macaques, which was inconsistent with sampling bias and grander SF clustering. Emergent large-FOV two-photon

imaging technology (e.g., Ota et al., 2021) would provide more definitive solutions to these issues.

Hubel and Wiesel first suggested that orientation, eye of origin, and retinal location can be uniformly represented with optimal (orthogonal) overlay of orientation and ocular dominance maps (Hubel and Wiesel, 1977). This uniform representation becomes more challenging when additional functional maps such as direction and disparity maps are considered (Swindale, 2000; Horton and Adams, 2005). One implication of our findings is that orientation and SF maps can hardly maintain a meaningful orthogonal relationship (Nauhaus et al., 2012) since there is not much SF clustering to begin with. As a result, including SF to the uniform representation of stimulus features does not add further burden to the already complex superpositions of functional maps.

Neurons in our SF maps were seldomly tuned to low SFs or low pass, even if macaques, like humans, are sensitive to low SF stimuli (De Valois et al., 1974). Differential neural responses to bright and dark regions of low-SF stimuli may represent the low-SF information (Benvenuti et al., 2018). Alternatively, the wider low-half bandwidth (LhBW) of SF tuning functions in most neurons (Fig. 4) suggests that low SF information may be picked up by medium-frequency neurons. This possibility is supported by psychophysical adaptation and masking results that a lowpass

SF filter handles all SF information at the filter's preferred and lower SFs (Blakemore and Campbell, 1969; Wilson et al., 1983). The low SF information may then be decoded by later V2 neurons whose preferred SFs are 2-octave lower than those of V1 neurons (Foster et al., 1985). Imagine that a low-SF stimulus is responded by two V1 neurons tuned to the same medium SF, and their SF tuning functions are identical in HhBW but differ in LhBW, a subtraction of two tuning functions by a V2 neuron would produce a low-SF peak.

Ac no ledgments

This study was supported by Natural Science Foundation of China grants 31230030 and 31730109, and funds from Peking-Tsinghua Center for Life Sciences, Peking University. We thank Geoff Ghose, Stanley Klein, Haidong Lu, Guy Orban, Adam Reeves, and Rufin Vogels for their very helpful comments and suggestions.

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The Peer Review Overview and Supplementary data associated with this article can be found in the online version, at doi:<https://doi.org/10.1016/j.pneurobio.2021.102120>.

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