Contextual responses drive a unique laminar signature in human V1

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# SUMMARY

Neuronal populations in visual cortex balance stimulus-driven retinal input and context-related input from neighboring neurons. These types of input are transmitted by cortical connections terminating in different layers of the cortex, thus providing a window into the balance of stimulus-driven versus context-related computations. Here, we combined line-scanning fMRI with a targeting approach to record responses across cortical depth in unprecedented detail in humans. Signals were recorded from a specific patch of cortex of each individual participant and custom-designed stimuli to the population receptive field (pRF) properties of this cortical patch. Stimulus-driven responses were localized across the cortical depth with amplitudes increasing toward the pial surface. In contrast, contextual stimuli elicited responses at superficial and deeper cortical depths; termination sites of descending connections. These findings draw links between invasive animal neurophysiological studies and human neuroimaging, and open the door to cognitive manipulation in humans due to the non-invasive nature.

# KEYWORDS

line-scanning; pRF; laminar; ultra-high field MRI; 7T; BOLD fMRI, context, divisive normalization

# INTRODUCTION

Individual neurons in the early visual cortex respond to specific parts of the visual field, referred to as the receptive field (RF)1,2. The responses of these neurons are not solely reliant on stimulus-driven input; rather, they are complemented by context-related responses integrated from neighboring neurons3–6 (Figure 1, *top panels*). These responses can be modeled using surround-suppression or divisive normalization (DN). Specifically, DN has been shown to unify disparate responses including surround-suppression and compression6,7. In DN, the responses of neurons are modeled as the ratio of the *activation* and *normalization pool*8,9. The activation pool refers to the population of neurons predominantly driven by direct, feedforward input through ascending connections to the classical RF (blue arrows). The normalization pool consists of neurons that integrate contextual information from surrounding areas and contribute to balancing the responses of the activation pool via divisive normalization processes. While the activation pool primarily reflects direct input, the normalization pool mediates surround suppression and other forms of contextual modulation6,8,9. From a biological perspective, contextual computations cannot be explained by ascending connections alone. Such computations require descending and lateral connections between neurons and neighboring areas4,5,10–13.

Ascending and descending connections terminate in specific layers of the cortex (Figure 1, *bottom panels*)14,15. Invasive neurophysiology studies in macaques have shown that stimuli within the classical RF elicit the earliest responses in layer 4 of primary visual cortex (V1)4,10,16–19. These neurons primarily receive excitatory input from ascending connections that originate in the lateral geniculate nucleus (blue projections)2,17,20,21. As stimuli extend beyond the classical RF, the visual system integrates contextual information from surrounding areas. These inputs are conveyed via descending (from neighboring neurons or areas to superficial and deeper layers of V14,5; red projections) and lateral connections (present across the cortical column)13 corresponding to the normalization pool. Lateral connections primarily integrate information from nearby stimuli, contributing to near-surround modulation. In contrast, descending connections relay higher-order contextual information from distance regions, influencing far-surround processing3,22,23. Therefore, altering stimuli such that they target the activation (classical RF) and normalization (surround) pool should engage different laminar connections (Figure 1, middle panels) and thus inform us how context-related computations are processed in the cortical layers of human V1.

High-resolution functional MRI (fMRI) permits access to different layers of the cortex24,25. This cortical depth-resolved fMRI enables researchers to study the flow of information through the cortex26,27. Contemporary fMRI acquisitions typically sample the cortex with ~0.8 millimeter isotropic resolutions24,25,28–31, which is still large compared to the spatial dimensions of the cortical layers: at this resolution, the entire cortical depth is covered by about 2–3 data points (voxels). To infer underlying laminar specific responses, data from a region-of-interest (ROI) is typically upsampled28,32,33. This method requires extremely precise segmentation of the cortical sheet and accurate co-registration of anatomical and functional data34. It also assumes that the depth-dependent fMRI signal across cortical depth (i.e., the tangential direction) remains consistent within the ROI33; blood vessels oriented 90° with respect to the B0-field can cause significant signal reduction35–37. Given the highly complex folding pattern of the cortex38, this results in high variability across the ROI. These issues translate to data with high statistical dependency and low variability across cortical depth and require averaging over a large ROI32–34. Subpopulations of this ROI might exhibit variability in functional properties (e.g., RFs or center-surround configurations), reducing the specificity of the employed paradigm or acquisition scheme39,40. To improve spatial and functional precision across cortical depth, acquisitions with higher resolutions are required. This can be achieved by using anisotropic voxel dimensions and/or reducing spatial coverage33, while ensuring that the ROI is sampled at high resolution perpendicular to the cortical sheet41–43.

Initially developed in rodents44, line-scanning fMRI has recently been adopted to investigate neuroscientific questions in humans43,45,46. Line-scanning uses a single slice (2.5 millimeter thick) where the signal outside the region of interest is suppressed using 2 saturation pulses (4 millimeter gap)44,45 (Figure 1, purple beam). Thus, spatial coverage is sacrificed in order to sample responses from a specific patch of cortex with ultra-high spatial resolution (250 µm along cortical depth)44–46. The high spatial resolution allows the cortical depth of a specific patch to be covered by 6-10 data points, greatly reducing the statistical dependence between data points33,34. Similar to animal neurophysiology, experiments can then be designed to target the functional properties of this cortical patch10,16. Rather than measuring activity from single neurons, fMRI samples from large groups of neurons (20.000 to 30.000 neurons per cubic millimeter in cerebral cortex47,48). Similar to single neurons, these groups (or populations) respond preferentially to specific parts of the visual field as well49,50, referred to as the population receptive field (pRF)51. Using our established selection-targeting method43, we generated stimuli specifically tailored to the functional properties derived from computational modeling of participants' targeted patches of the visual cortex. These stimuli targeted different populations of neurons facilitating stimulus-driven or contextual processes. Combining ultra-high spatial resolution with our functional targeting approach uniquely positions us to measure cortical depth-resolved responses and bridge human fMRI with non-human primate electrophysiology.

# RESULTS

## Cortical-patch specific stimuli evoke differential responses

Each participant was presented with a unique set of stimuli designed based on the functional organization of the target patch (see Figure 2A, Figure S1). We deduced the locations of the zero-crossing and full-width half-max for each target pRF in visual space to highlight the stimulus configurations relative to its spatial profile (Figure 2B). We then derived size-tuning curves through simulations by passing stimuli of increasing sizes ranging from 0 to 10 degrees of visual angle (dva) to the estimates of the pRFs6,52–55. Based on the size-tuning curve, we derived three stimuli centered on the pRF (Figure 2C) differentially eliciting stimulus-driven and contextual processes (Figure 1, *middle panels*). These stimuli indeed generated distinct responses collapsed over cortical depth, suggesting differential engagement of stimulus-driven and contextual processes (Figure 2D):

(1) *center* stimulus (green): This stimulus consisted of a radial checkerboard stimulus with the size that elicited the maximum response according to the size-tuning curve (i.e., maximally targeting stimulus-driven processes); (2) *large annulus* (blue): This stimulus consisted of a concentric ring subtending 2 dva, stimulating as much of the surround of the pRF as permitted by the physical dimensions of the screen. From the location of the target pRF in visual space, we drew the largest possible, non-occluded stimulus. The shortest distance served as the radius of the stimulus; (3) *medium annulus* (orange): Similar to the large annulus, this stimulus consisted of a 2 dva-wide concentric ring with a radius halfway between the center and large annulus stimuli, preferably without spatial overlap with the other stimuli. All stimuli had 2 radial cycles per degree of stimulus size and 1 angular cycle per degree of stimulus size. In cases where parts of the screen were obstructed by the MR setup (e.g., transmit boxes, eye-tracker, MRI bore), we iteratively adjusted the stimuli based on verbal feedback from participants to ensure the stimuli were not occluded (Figure 2C).

To verify that responses localized to the intended target locations, we first averaged all the responses from data points across cortical depth and runs with identical timing (see Methods details: Experimental setup). Response profiles were extracted from a time window starting 2 seconds prior to stimulus onset to 14 seconds after. This resulted in 2 (effective runs with different event timings) × 5 (events per run) profiles for each stimulus for each participant. The stimulus eliciting the largest response in the target pRF, according to the computational model, indeed resulted in the largest response of all stimuli (Figure S2). To formally test the response magnitude across events, we averaged the response over a time period around the peak of the response to the *center* stimulus (Figure S2, insets) and entered this into a linear mixed-effects model (Table S1). The model demonstrated significant differences between stimulus types (*p* < 0.001). Using Tukey HSD pairwise comparisons, we found that the estimated mean response for the center stimulus (1.48 ± 0.094) was significantly higher than the response to the medium annulus (-1.22 ± 0.087; Δ = 1.22, 95% CI = 1.00–1.44, *p* < 0.001) and large annulus (-1.36 ± 0.087; Δ = 1.36, 95% CI = 1.14–1.58, *p* < 0.001). The difference in mean response between the medium and large annulus was not significant (Δ = -0.14, 95% CI = -0.36–0.07, *p* = 0.27). This confirms that the line-scanning approach was positioned in the cortical patch with the selected pRF.

## Context drives responses at superficial and deeper depths

The number of data points across the cortical depth of the target patch varied across participants due to differences in cortical thickness, ranging from 6 to 10 data points. To standardize the depth profiles for analysis, we regridded the data so that 20 evenly spaced points covered the cortical depth for all participants. Because of the increased resolution in the laminar direction, this study has reduced statistical dependency across depth compared to previous studies that sampled 2-3 data points to 20 cortical depths28,32,33,56–58. As we are not measuring individual layers of the cytoarchitecture, we discuss the results in terms of the three compartment model (superficial, middle, and deep depths)14. We then produced depth-by-time plots44, colored by magnitude, to illustrate the evolution of responses across time and depth (Figure 3, left and middle column). The center stimulus (eliciting stimulus-driven processes) elicited a clear positive BOLD response across all cortical depths. In contrast, the response to the large annulus (eliciting contextual processes) was markedly different (Figure 3; bottom row). A negative deflection was observed at middle depths, flanked by positive peaks at superficial and deeper depths (Figure 3; bottom row, middle column).

To visualize this effect more clearly, we collapsed the responses across depth over time using the group response to the center stimulus averaged across depth as a template. This approach is favored over using a time window (as used in Figure S11) due to variability in response latency across participants. We calculated a weighted average to quantify how well the participants’ depth-dependent response profiles for other stimuli matches the profile for the group-averaged response to the center stimulus. This was achieved using the expression:

where hrfdepth represents the cortical depth-dependent response profiles for the other stimuli, and hrftemplate corresponds to the profile of the center stimulus (averaged over participants and cortical depth). The result provides a scaling factor that indicates how closely the depth-dependent responses of other stimuli align with the characteristic shape of the center stimulus profile (Figure S2, green profile in bottom-right panel labeled as “average”). A high value was assigned if the average profile was strongly represented in the profile of individual depths, and a low value if the opposite was true. To reduce noise, we normalized the profiles by subtracting each participant's individual mean from the profiles and adding back the mean across participants.

For the response to the center stimulus, responses near the superficial surface (*pial*) were more strongly represented by the average profile compared to deeper depths (*wm*), a pattern often observed in the laminar fMRI literature27,36,43,59–63. Additionally, a small peak can be observed in middle cortical depths (see also ref37), which could be putatively linked to the termination site of ascending feedforward projections14,15. In contrast, the response profile to the large annulus exhibited peaks in response magnitude at the termination sites of descending feedback projections4,5, as well as a deflection at middle depths (see Figure S3A for participant-specific profiles). This was not an artifact of voxel selection (Figure S3B, Figure S11), normalization strategy (Figure S3C), weighting method (Figure S3D), or interpolation (Figure S4).

## Modeling the laminar profiles for large annuli

To quantify this effect, we defined a model with two Gaussian distributions representing the peaks at superficial (peak at 25%) and deep (peak at 75%) cortical depths (Figure 4AB). This model was based on (1) the observation that descending connections carrying context-related information from higher-order areas are received in superficial and deeper layers of V114,64, and (2) findings from an earlier animal electrophysiological experiment that used a strikingly similar setup in which these layers demonstrated responses to a large annulus16. Derivative components were added to each Gaussian distribution to account for individual differences in the anatomy of the target patch, allowing the peaks to slightly shift (Figure 4B). To account for carry-over effects from deeper into superficial depths37,65–68, we included a linear term due to the limited data points and ease-of-use36. We then summed the beta values from the context-related model components (𝛽context:double peak+derivatives, excluding the linear term, Figure 4B), which enabled us to quantify the extent to which a response reflected stimulus-driven or contextual processes (Figure 4CD, Figure S5).

A one-way ANOVA revealed a significant main effect of stimulus type (*F*1,20 = 15.34, *p* < 0.001, partial η2 = 0.43), wherein the large annulus stimulus had higher t-stat values for the contextual component of the model (1.63 ± 0.61) compared to the center stimulus (-1.18 ± 0.38), *t*10 = 4.01, *p* = 0.002, Cohen's *d* = 1.37. We evaluated the impact of draining vasculature in two ways. First, instead of using a linear component to account for the draining vein effect, we used a negative exponential component increasing towards the surface - better reflecting the macrovascular contribution (Figure S6). Second, we deconvolved the profiles across cortical depth using the vascular model from Markuerkiaga and colleagues65,69 (Figure S7). Based on histological data70 and vascular modeling67, they derived how each layer affects subsequent layers (see Table 1 in Marquardt, et al.69 or Figure 3F in Markuerkiaga, et al.65). The deconvolved profiles were then entered in the same model as described above. Neither of these vascular correction methods altered the observed activation differences or conclusions. This suggests that stimuli eliciting predominantly contextual processes resulted in responses at sites where descending feedback connections terminate, whereas stimuli eliciting stimulus-driven processes resulted in a strong BOLD response across cortical depth with a putative peak in middle depths where ascending feedforward projections terminate.

## Medium annulus produced mixed response

In the previous section, we examined responses to stimuli that were designed to maximally elicit either stimulus-driven (center) or contextual (large annulus) processes. Presenting a stimulus that lies somewhere in between these extremes should result in a response pattern that is intermediate (medium annulus). Indeed, the response profile deviated from the other two stimuli. The time courses are more noisy and the profile across depth less specific (Figure 5, first and second panel). This could be due to vascular carry-over effects obscuring true responses (Figure S7) or because the stimulus configuration differed across participants (Figure S1, Figure S8); for some participants, the medium annulus may have hit stimulus-driven populations, whereas for others it was fully in the surround, driving contextual processes. The response profile of the medium annulus showed widespread activation with peaks closer to middle depths compared to the large annulus (Figure 5, third panel), potentially reflecting lateral processing.

Even though the mixed nature of the stimulus renders interpretation difficult, we subjected the laminar profiles elicited by the medium annulus to the same model described earlier (Figure 5, Figure S9). A one-way ANOVA across all stimulus types revealed a significant main effect of stimulus type on the beta values from the context-related model components (*F*2,30 = 7.11, *p* = 0.003, partial *η*2 = 0.32). Post-hoc analysis with Holm's correction (Figure 5, last panel) showed that the context-related component was significantly higher for the medium annulus (0.75 ± 0.38) compared to the center stimulus (-1.18 ± 0.38; *t*10 = 3.15, *p* = 0.03, Cohen's *d* = 1.51). The difference between the large and medium annulus was not significant (*p* = 0.22). Together, these results show that stimuli designed to elicit contextual processes result in responses that differ from stimuli eliciting stimulus-driven processes through ascending feedforward connections.

# DISCUSSION

## Probing contextual responses with line-scanning fMRI

In this work, we used divisive normalization (DN) to probe the laminar signature of stimulus-driven and contextual processes. DN is observed across the brain in multiple systems8,9,71–73 and is therefore often considered a canonical computational operation that the brain employs for various purposes9. Central to DN is the principle that the output of a given neuron depends not only on its direct stimulation (activation) component but also on the integration of signals from nearby neurons (normalization component). These processes provide insights into the nature of feedforward and feedback mechanisms: the activation component is driven by ascending bottom-up connections terminating in the middle layers of the cortex2,17,20,21, while contextual integration from neighboring neurons or areas arises from descending feedback connections terminating in the superficial and deeper layers of the cortex4,5,14,15. Thus, altering the relative contribution of stimulus-drive and context can reveal processing circuits within the layers of the cortex. In this study, we applied our selection and targeting framework to investigate these responses in humans using ultra-high-resolution line-scanning fMRI43. Specifically, we targeted a patch of cortex with defined functional properties and presented stimuli designed through computational modeling to probe the laminar signatures of stimulus-driven or contextual processing. Our results demonstrate that cortical depth-dependent responses varied based on the preference to elicit stimulus-driven or contextual processes.

## Distinct context-dependent laminar signature

Stimuli designed to maximally elicit stimulus-driven processes (*center*) resulted in responses across the cortical depth with a putative peak in the middle depths, whereas contextual stimuli (*large annulus*) elicited positive responses in the superficial and deeper cortical depths and a negative response in middle layers (Figure 3). Many studies have attempted to separate feedforward from feedback processing, but direct comparison is rendered complicated due to different experimental paradigms. Such paradigms include texture-segmentations10, low-spatial frequency stimuli18, high-contrast drifting gratings74, and line segments19. Differences in experimental setup will target different neuronal populations across cortical depth, often resulting in differences in the laminar profile of spiking75.

Yet, Bijanzadeh and colleagues16 performed a study with many similarities to the experimental setup presented in this work: In an attempt to study surround suppression, a special category of divisive normalization, they recorded responses across cortical depth to a similar set of stimuli: a stimulus inside the classical RF, an annulus stimulating the near surround, and an annulus stimulating the far surround. Similar to our work, they found that responses to stimuli in the surround were constrained to superficial and deeper layers of the cortex; layers where descending connections carrying context-related information from neighboring regions terminate14,15. They also observed the earliest activation in the middle layers in response to a stimulus in the center of the RF. In the current study, we found a small peak in the middle layers as well, which became clearer after accounting for the carry-over effects (see next section)37. One important distinction is, however, that Bijanzadeh, et al.16 mainly reported differences in latencies between stimulus-driven and contextual processes, whereas the current study reports on amplitude across cortical depth. The speed with which these signals are transmitted makes it complicated to capture using BOLD fMRI10,18,75, though faster acquisitions with more trials may be able to reveal such effects more robustly25,44,76,77. Nevertheless, the similarities in experimental design and outcome (pattern of activation across cortical depth) highlight the possibility to link animal electrophysiological experiments with non-invasive human fMRI experiments.

While the observed effects align with the idea of DN, alternative explanations may also account for, or contribute to the observed responses. Similar to the studies previously discussed3,5,16, the results could be interpreted in many context-dependent processes such as lateral inhibition, of surround-suppression or differences in temporal dynamics between the stimuli. For example, t he large annulus might engage lateral inhibitory circuits that suppress activity in the middle layers, while enhancing processing in superficial and deep layers4,10,78. Alternatively, the large annulus might recruit distinct populations of excitatory and inhibitory neurons across layers, with inhibition dominating in the middle layers and excitation driving responses in superficial and deep layers79,80. The large annulus might also elicit non-linear response patterns, where neurons in the middle layer are less responsive due to saturation or competitive suppression5,9. From a vascular perspective, the BOLD signal in superficial and deep layers may be amplified due to their proximity to larger veins and the influence of feedback inputs. These factors, combined with potential suppression of middle-layer activity (layer 4) by the large annulus, could account for the observed depth-specific BOLD response patterns57,81–83. However, different frameworks are unified by the specific circuits that underlie them: ascending, descending, and horizontal projections. Complementary approaches perturbing these processes may inform us further about how the cortex resolves them using the same circuit architecture75,84.

## BOLD response reflects metabolic needs

The BOLD effect arises from a change in relative amounts of oxygenated and deoxygenated blood. It is therefore an indirect measure of neuronal activity, linked to particular neuronal firing patterns such as local field potentials (LFP)85–89, or spiking activity (in case of pRFs)90. Upon neuronal activation, metabolic demands trigger extra delivery of blood towards the site of activation91. Deoxygenated blood, which distorts the magnetic field due to unpaired iron atoms, is pushed away, resulting in increased signal92,93. Within the cortex, penetrating arteries branch off the pial network to supply the parenchyma of nutrients68,83, while veins drain the deoxygenated paramagnetic blood unidirectionally towards the pial surface94–96. The corollary of this process is twofold: 1) BOLD signal changes are larger at superficial cortical depths where the deoxygenated blood is pooled97–102 and 2) signals from deeper depths influence the signal at superficial depths, which has been referred to as the “draining vein effect”, “carry-over effects”, or “leakage problem”37,65–68. Strategies have since been developed to mitigate the effect of large pial veins, including acquisition103–106, analysis37,65,97,107–110, and experimental design51,111,112 (for further details, see these laminar fMRI reviews28,32,34,75,84,113–116).

Why, then, is the carry-over effect less visible for contextual stimuli? The center stimulus is designed to stimulate the center of the pRF, maximally eliciting the stimulus-driven processes. Such processes drive feedforward inputs into the middle layers of V110,14–16. The transmission of these inputs involves dense excitatory synapses and high-frequency spiking activity to propagate sensory input89,91,117. The metabolically demanding processes increase local oxygen consumption and blood flow, producing a robust BOLD response89,91,118. In contrast, the large annulus primarily engages lateral and feedback inputs facilitating contextual processes119. Contextual processes are modulatory, rather than driving, i.e., modulate existing activity rather than generate new action potentials4,5,120–123. These factors are thought to be metabolically less demanding compared to ascending feedforward inputs120,122,124,125. This difference in metabolic demand between these inputs might explain the presence of the draining vein effect in the stimulus-driven condition but not the contextual condition as well as overall magnitude differences.

## Limitations of this study

The effective resolution of line-scanning is influenced by several factors, including the curvature of the targeted cortical sheet126–128, participant motion129,130, the quality of saturation slabs45, targeting success43, and positioning relative to the surface coils used for MR signal detection131,132. The significant reduction in the field-of-view during line-scanning imposes limitations on its applicability for examining larger-scale processes, such as between-area communication. This setup is further complicated by the use of circular, flickering checkerboard stimuli designed based on pRFs estimated using a bar-sweep configuration24,51, which were acquired using a different sequence24, on different days133–136, and with varying levels of thermal noise46,137,138. These factors collectively affect the neuronal population (and therefore the pRF) that is ultimately targeted24,134,139. Additionally, we modeled the responses using the DN-model, but given the focus on the surround in early visual cortex, these could have been modeled using the difference-of-gaussian model as well140. Regardless of model choice, the pRF stimulus with bar configurations is primarily a spatial design that does not account for the time dimension. In the current setup, stimuli perturb neuronal populations for much shorter durations (2 seconds) compared to the bar configuration (15–20 seconds). It remains unclear how such changes in the temporal characteristics of stimuli influence processing dynamics across cortical depth. Lastly, future work could develop more advanced definitions of contextual processing. This work operationalized this by using a biphasic model representing termination sites of feedback projections. While this model is relatively simple and allows for some degree of interpretation, it does not provide a mechanistic account.

## Bridging neurophysiology and fMRI

In this study, we applied the selection and targeting framework of line-scanning to investigate contextual processing across cortical depth. This strategy mimics invasive electrophysiological setups in which a known target is probed across depth with electrodes. Using existing pRF data, we designed stimuli tailored uniquely for each participant to maximally elicit stimulus-driven and contextual processes. The advantages of this approach are twofold: (i) cortical depth is sampled by significantly more data points (6–10 vs. 2–3), reducing partial voluming and minimizing the effects of large veins; and (ii) a specific patch of cortex can be targeted, improving the specificity of the experimental paradigm. We demonstrated that the stimulus eliciting stimulus-driven processes (center stimulus) resulted in strong responses across all cortical depths, with particularly strong responses near the cortical surface and a small peak in the middle depths—the site where ascending feedforward connections from LGN terminate. In contrast, stimuli eliciting contextual processes produced responses more constrained to superficial and deeper depths—sites where descending context-related connections from neighboring areas terminate. These findings align with evidence from animal studies and computational models, highlighting the potential to establish direct links between animal methodologies and human research. The non-invasive nature of this experimental setup offers new opportunities to explore cognitive manipulations in humans.

# RESOURCE AVAILABILITY

## Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Jurjen Heij ([j.heij@herseninstituut.knaw.nl](mailto:j.heij@herseninstituut.knaw.nl)).

## Materials availability

This study did not generate new unique agents

## Data and code availability

* Data in BIDS-format141 will be made available on request in compliance with GDPR regulations
* The code for this paper is available in the following repositories: Preprocessing of fMRI, anatomical pipeline, and handling of line-scanning data: <https://github.com/gjheij/linescanning>; Line-scanning experiment: <https://github.com/gjheij/LineExps/tree/main/ActNorm3>; Analysis: <https://github.com/spinoza-centre/holeresponse>.
* Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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# AUTHOR CONTRIBUTIONS

Conceptualization, T.K. & S.O.D.; methodology, J.H., T.K. & S.O.D.; investigation, J.H.; writing – original draft, J.H.; writing – review & editing, J.H., L.R., J.C.W.S., W.Z., T.K., & S.O.D.; funding acquisition, J.C.W.S., W.Z., T.K., & S.O.D.; resources, W.Z., T.K., & S.O.D.

# DECLARATION OF INTEREST

The authors declare no competing interests

# SUPPLEMENTAL INFORMATION

Figures S1-S11, Table S1, and their legends in a PDF

# FIGURE LEGENDS

**Figure 1. Laminar distribution of ascending feedforward and descending feedback projections.**

Stimuli falling within the classical receptive field (RF) primarily elicit stimulus-driven processes mediated by ascending feedforward (FF) connections (red). These signals are then propagated through connections spanning multiple cortical layers (interlaminar) toward the surface and to neighboring regions. In contrast, stimuli on the flanks of the classical RF predominantly drive contextual processes, where information from neighboring areas is integrated via lateral and descending feedback (FB) connections (blue) to the superficial and deep layers. Cortical layers thus provide a unique window into the interplay between stimulus-driven and context-related computations. Using line-scanning fMRI (purple rectangle), we can capture depth-resolved responses to custom-designed stimuli weighted differentially towards stimulus-drive or context with unprecedented detail.

**Figure 2. Participant-specific stimulus design procedure.**

(A) Example stimuli as presented on the screen, targeting the location in visual space that is encoded by the target cortex patch.

(B) Spatial response profile of target pRF with full-width at half-maximum (FWHM) denoted with dark gray bands and zero-crossings in light gray.

(C) From the location of the target pRF (x, y) in visual space, we determined the smallest distance to the edge of the screen (xvisual field or yvisual field) behind the MRI bore in order to present the largest stimulus possible without occlusion. The radius for the large stimulus (blue) was set to this distance (orange line + blue line + black line). The radius for the medium stimulus (orange) was set to be halfway of the distance between the center stimulus and large annulus. (D) Response profiles for the different stimuli averaged across the cortical depth for a representative participant (see Figure S2 for all participants).

**Figure 3**. **Response evolution for** **the center (green) and large annulus (blue)** **stimuli**.

The left column represents the response profiles as extracted from the time courses.

The middle column represents depth-by-time plots colored by magnitude.

The right column is generated by taking the response profile to the center stimulus averaged across participants and cortical depth (hrftemplate) as template to generate cortical depth-dependent weights. This effectively collapsed the profiles over time, allowing more insights in the cortical profile. Using this weighting, the center stimulus elicted responses that increased towards the pial surface (pial) with a small peak at the middle depths. The large annulus elicited positive responses more reserved to superficial and deeper depths with negative responses at middle depths. These results are in line with electrophysiological and anatomical predictions about the laminar distribution of contextual integration. Shaded error represents 95% confidence intervals.

**Figure 4. Modeling contextual integration across cortical depth for the large annulus.**

(A) Based on invasive anatomical and functional findings, we expected strong responses in superficial (peak at 25%) and deep depths (peak at 75%) (red shaded boxes). We modeled these using a single Gaussian distribution for each termination site (gray profiles). We added derivative terms to each distribution to allow for participant variability in the exact location of the positive peaks.

(B) From the full design matrix, we summed the beta values over the last 4 regressors representing the contextual component (𝛽context).

(C) Model fit of a representative participant’s response to the large annulus.

(D) Comparison of summed beta-estimates from the contextual component (𝛽context) of the model for center and large annulus stimuli. \*\**p* < .01.

**Figure 5**. **Medium annulus produces mixed response.**

The first panel represents the response profiles as extracted from the time courses.

The second panel represents depth-by-time plots colored by magnitude.

The third column represents the medium annulus response weighed by the response to the center stimulus. Whereas the large annulus elicited responses close to superficial depths (~15% away from pial surface, Figure 3, bottom right panel), the medium annulus elicited responses in multiple sites across cortical depth.

The last column represents the model outcome of all stimulus events. The medium annulus fell in between the center stimulus and large annulus (center and large annulus stimuli taken from Figure 4D). Shaded error represents 95% confidence intervals.

# References

1. Hubel, D. H. & Wiesel, T. N. Receptive fields of single neurones in the cat’s striate cortex. *J. Physiol.* **148**, 574–591 (1959) doi: 10.1113/jphysiol.1959.sp006308.

2. Hubel, D. H. & Wiesel, T. N. Receptive fields, binocular interaction and functional architecture in the cat’s visual cortex. *J. Physiol.* **160**, 106–154 (1962) doi: 10.1113/jphysiol.1962.sp006837.

3. Angelucci, A. *et al.* Circuits for local and global signal integration in primary visual cortex. *J. Neurosci.* **22**, 8633–8646 (2002) doi: 10.1523/jneurosci.22-19-08633.2002.

4. Angelucci, A. & Sainsbury, K. Contribution of feedforward thalamic afferents and corticogeniculate feedback to the spatial summation area of macaque V1 and LGN. *J. Comp. Neurol.* **498**, 330–351 (2006) doi: 10.1002/cne.21060.

5. Nurminen, L., Merlin, S., Bijanzadeh, M., Federer, F. & Angelucci, A. Top-down feedback controls spatial summation and response amplitude in primate visual cortex. *Nat. Commun.* **9**, (2018) doi: 10.1038/S41467-018-04500-5.

6. Aqil, M., Knapen, T. & Dumoulin, S. O. Divisive normalization unifies disparate response signatures throughout the human visual hierarchy. *PNAS* **118**, (2021) doi: 10.1073/pnas.2108713118.

7. Aqil, M., Knapen, T. & Dumoulin, S. O. Computational model links normalization to chemoarchitecture in the human visual system. *Sci. Adv.* **10**, eadj6102 (2024) doi: 10.1126/sciadv.adj6102.

8. Heeger, D. J. Normalization of cell responses in cat striate cortex. *Vis. Neurosci.* **9**, 181–197 (1992) doi: 10.1017/S0952523800009640.

9. Carandini, M. & Heeger, D. J. Normalization as a canonical neural computation. *Nat. Rev. Neurosci.* **13**, 51–62 (2012) doi: 10.1038/nrn3136.

10. Self, M. W., van Kerkoerle, T., Supèr, H. & Roelfsema, P. R. Distinct Roles of the Cortical Layers of Area V1 in Figure-Ground Segregation. *Curr. Biol.* **23**, 2121–2129 (2013) doi: 10.1016/j.cub.2013.09.013.

11. Lamme, V. A. F. & Roelfsema, P. R. The distinct modes of vision offered by feedforward and recurrent processing. *Trends Neurosci.* **23**, 571–579 (2000) doi: 10.1016/S0166-2236(00)01657-X.

12. Lamme, V. A. The neurophysiology of figure-ground segregation in primary visual cortex. *J. Neurosci.* **15**, 1605–1615 (1995) doi: 10.1523/JNEUROSCI.15-02-01605.1995.

13. Bolz, J. & Gilbert, C. D. Generation of end-inhibition in the visual cortex via interlaminar connections. *Nature* **320**, 362–365 (1986) doi: 10.1038/320362a0.

14. Felleman, D. J. & van Essen, D. C. Distributed hierarchical processing in the primate cerebral cortex. *Cereb. Cortex* **1**, 1–47 (1991) doi: 10.1093/cercor/1.1.1.

15. Hubel, D. H. & Wiesel, T. N. Laminar and columnar distribution of geniculo‐cortical fibers in the macaque monkey. *Journal of Comparative Neurology* **146**, 421–450 (1972) doi: 10.1002/cne.901460402.

16. Bijanzadeh, M., Nurminen, L., Merlin, S., Clark, A. M. & Angelucci, A. Distinct Laminar Processing of Local and Global Context in Primate Primary Visual Cortex. *Neuron* **100**, 259-274.e4 (2018) doi: 10.1016/j.neuron.2018.08.020.

17. Fitzpatrick, D., Lund, J. S. & Blasdel, G. G. Intrinsic connections of macaque striate cortex: afferent and efferent connections of lamina 4C. *J. Neurosci.* **5**, 3329–3349 (1985) doi: 10.1523/JNEUROSCI.05-12-03329.1985.

18. Schroeder, C. E., Mehta, A. D. & Givre, S. J. A spatiotemporal profile of visual system activation revealed by current source density analysis in the awake macaque. *Cereb. Cortex* **8**, 575–592 (1998) doi: 10.1093/cercor/8.7.575.

19. van Kerkoerle, T., Self, M. W. & Roelfsema, P. R. Layer-specificity in the effects of attention and working memory on activity in primary visual cortex. *Nat. Commun.* **8**, 13804 (2017) doi: 10.1038/ncomms13804.

20. Reid, R. C. & Alonso, J.-M. Specificity of monosynaptic connections from thalamus to visual cortex. *Nature* **378**, 281–284 (1995) doi: 10.1038/378281a0.

21. Callaway, E. M. Local circuits in primary visual cortex of the macaque monkey. *Annu. Rev. Neurosci.* **21**, 47–74 (1998) doi: 10.1146/annurev.neuro.21.1.47.

22. Angelucci, A. & Shushruth, S. Beyond the classical receptive field: surround modulation in primary visual cortex. in *The New Visual Neurosciences* (eds. Werner, J., S. & Chalupa, L., M.) 425–444 (The MIT Press, 2013).

23. Angelucci, A. *et al.* Circuits and Mechanisms for Surround Modulation in Visual Cortex. *Annu. Rev. Neurosci.* **40**, 425–451 (2017) doi: 10.1146/annurev-neuro-072116-031418.

24. Dumoulin, S. O., Fracasso, A., van der Zwaag, W., Siero, J. C. W. & Petridou, N. Ultra-high field MRI: Advancing systems neuroscience towards mesoscopic human brain function. *NeuroImage* **168**, 345–357 (2018) doi: 10.1016/j.neuroimage.2017.01.028.

25. Petridou, N. & Siero, J. C. W. Laminar fMRI: What can the time domain tell us? *NeuroImage* **197**, 761–771 (2019) doi: 10.1016/j.neuroimage.2017.07.040.

26. Kok, P., Bains, L. J., van Mourik, T., Norris, D. G. & de Lange, F. P. Selective Activation of the Deep Layers of the Human Primary Visual Cortex by Top-Down Feedback. *Curr. Biol.* **26**, 371–376 (2016) doi: 10.1016/j.cub.2015.12.038.

27. de Hollander, G., van der Zwaag, W., Qian, C., Zhang, P. & Knapen, T. Ultra-high field fMRI reveals origins of feedforward and feedback activity within laminae of human ocular dominance columns. *NeuroImage* **228**, 117683 (2021) doi: 10.1016/j.neuroimage.2020.117683.

28. Huber, L. *et al.* Layer-dependent functional connectivity methods. *Prog. Neurobiol.* **207**, 101835 (2021) doi: 10.1016/j.pneurobio.2020.101835.

29. Raimondo, L. *et al.* Advances in resting state fMRI acquisitions for functional connectomics. *NeuroImage* **243**, (2021) doi: 10.1016/j.neuroimage.2021.118503.

30. Oliveira, I. A. F. *et al.* Comparing BOLD and VASO-CBV population receptive field estimates in human visual cortex. *NeuroImage* **248**, 118868 (2022) doi: 10.1016/j.neuroimage.2021.118868.

31. Oliveira, I. A. F., Siero, J. C. W., Dumoulin, S. O. & van der Zwaag, W. Improved Selectivity in 7 T Digit Mapping Using VASO-CBV. *Brain Topogr.* **36**, 23–31 (2023) doi: 10.1007/s10548-022-00932-x.

32. Polimeni, J. R., Renvall, V., Zaretskaya, N. & Fischl, B. Analysis strategies for high-resolution UHF-fMRI data. *NeuroImage* **168**, 296–320 (2018) doi: 10.1016/j.neuroimage.2017.04.053.

33. Kashyap, S. *et al.* Resolving laminar activation in human V1 using ultra-high spatial resolution fMRI at 7T. *Sci. Rep.* **8**, 17063 (2018) doi: 10.1038/s41598-018-35333-3.

34. Kashyap, S., Ivanov, D., Havlicek, M., Poser, B. A. & Uludağ, K. Impact of acquisition and analysis strategies on cortical depth-dependent fMRI. *NeuroImage* **168**, 332–344 (2018) doi: 10.1016/j.neuroimage.2017.05.022.

35. Gagnon, L. *et al.* Quantifying the Microvascular Origin of BOLD-fMRI from First Principles with Two-Photon Microscopy and an Oxygen-Sensitive Nanoprobe. *J. Neurosci.* **35**, 3663–3675 (2015) doi: 10.1523/JNEUROSCI.3555-14.2015.

36. Fracasso, A., Luijten, P. R., Dumoulin, S. O. & Petridou, N. Laminar imaging of positive and negative BOLD in human visual cortex at 7 T. *NeuroImage* **164**, 100–111 (2018) doi: 10.1016/j.neuroimage.2017.02.038.

37. Havlicek, M. & Uludağ, K. A dynamical model of the laminar BOLD response. *NeuroImage* **204**, 1–44 (2020) doi: 10.1016/j.neuroimage.2019.116209.

38. van Essen, D. C. *et al.* Cerebral cortical folding, parcellation, and connectivity in humans, nonhuman primates, and mice. *PNAS* **116**, 26173–26180 (2019) doi: 10.1073/pnas.1902299116.

39. Bonin, V., Histed, M. H., Yurgenson, S. & Reid, R. C. Local Diversity and Fine-Scale Organization of Receptive Fields in Mouse Visual Cortex. *J. Neurosci.* **31**, 18506–18521 (2011) doi: 10.1523/JNEUROSCI.2974-11.2011.

40. Qubad, M. *et al.* Improved correspondence of fMRI visual field localizer data after cortex-based macroanatomical alignment. *Sci. Rep.* **12**, 14310 (2022) doi: 10.1038/s41598-022-17909-2.

41. Balasubramanian, M., Mulkern, R. V., Neil, J. J., Maier, S. E. & Polimeni, J. R. Probing in vivo cortical myeloarchitecture in humans via line-scan diffusion acquisitions at 7 T with 250-500 micron radial resolution. *Magn. Reson. Med.* **85**, 390–403 (2021) doi: 10.1002/mrm.28419.

42. Balasubramanian, M., Mulkern, R. V. & Polimeni, J. R. In vivo irreversible and reversible transverse relaxation rates in human cerebral cortex via line scans at 7 T with 250 micron resolution perpendicular to the cortical surface. *Magn. Reson. Imaging* **90**, 44–52 (2022) doi: 10.1016/j.mri.2022.04.001.

43. Heij, J. *et al.* A selection and targeting framework of cortical locations for line-scanning fMRI. *Hum. Brain Mapp.* **44**, 5471–5484 (2023) doi: 10.1002/hbm.26459.

44. Yu, X., Qian, C., Chen, D. Y., Dodd, S. J. & Koretsky, A. P. Deciphering laminar-specific neural inputs with line-scanning fMRI. *Nat. Methods* **11**, 55–58 (2014) doi: 10.1038/nmeth.2730.

45. Raimondo, L. *et al.* A line through the brain: implementation of human line-scanning at 7T for ultra-high spatiotemporal resolution fMRI. *J. Cereb. Blood Flow Metab.* **41**, 2831–2843 (2021) doi: 10.1177/0271678X211037266.

46. Raimondo, L. *et al.* Robust high spatio-temporal line-scanning fMRI in humans at 7T using multi-echo readouts, denoising and prospective motion correction. *J. Neurosci. Methods* **384**, 109746 (2023) doi: 10.1016/J.JNEUMETH.2022.109746.

47. Azevedo, F. A. C. *et al.* Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J. Comp. Neurol.* **513**, 532–541 (2009) doi: 10.1002/cne.21974.

48. Herculano-Houzel, S. The human brain in numbers: a linearly scaled-up primate brain. *Front. Hum. Neurosci.* **3**, (2009) doi: 10.3389/neuro.09.031.2009.

49. Victor, J. D., Purpura, K., Katz, E. & Mao, B. Population encoding of spatial frequency, orientation, and color in macaque V1. *J. Neurophysiol.* **72**, 2151–2166 (1994) doi: 10.1152/jn.1994.72.5.2151.

50. Brewer, A. A., Liu, J., Wade, A. R. & Wandell, B. A. Visual field maps and stimulus selectivity in human ventral occipital cortex. *Nat. Neurosci.* **8**, 1102–1109 (2005) doi: 10.1038/nn1507.

51. Dumoulin, S. O. & Wandell, B. A. Population receptive field estimates in human visual cortex. *NeuroImage* **39**, 647–660 (2008) doi: 10.1016/j.neuroimage.2007.09.034.

52. Jones, H. E., Grieve, K. L., Wang, W. & Sillito, A. M. Surround Suppression in Primate V1. *J. Neurophysiol.* **86**, 2011–2028 (2001) doi: 10.1152/jn.2001.86.4.2011.

53. Wang, T. *et al.* Laminar Subnetworks of Response Suppression in Macaque Primary Visual Cortex. *J. Neurosci.* **40**, 7436–7450 (2020) doi: 10.1523/JNEUROSCI.1129-20.2020.

54. Self, M. W. *et al.* Orientation-Tuned Surround Suppression in Mouse Visual Cortex. *J. Neurosci.* **34**, 9290–9304 (2014) doi: 10.1523/JNEUROSCI.5051-13.2014.

55. Nassi, J. J., Lomber, S. G. & Born, R. T. Corticocortical Feedback Contributes to Surround Suppression in V1 of the Alert Primate. *J. Neurosci.* **33**, 8504–8517 (2013) doi: 10.1523/JNEUROSCI.5124-12.2013.

56. Huber, L. *et al.* Ultra-high resolution blood volume fMRI and BOLD fMRI in humans at 9.4 T: Capabilities and challenges. *NeuroImage* **178**, 769–779 (2018) doi: 10.1016/j.neuroimage.2018.06.025.

57. Huber, L. *et al.* High-Resolution CBV-fMRI Allows Mapping of Laminar Activity and Connectivity of Cortical Input and Output in Human M1. *Neuron* **96**, 1253-1263.e7 (2017) doi: 10.1016/j.neuron.2017.11.005.

58. Huber, L. *et al.* Sub-millimeter fMRI reveals multiple topographical digit representations that form action maps in human motor cortex. *NeuroImage* **208**, 116463 (2020) doi: 10.1016/j.neuroimage.2019.116463.

59. Koopmans, P. J., Barth, M., Orzada, S. & Norris, D. G. Multi-echo fMRI of the cortical laminae in humans at 7T. *NeuroImage* **56**, 1276–1285 (2011) doi: 10.1016/j.neuroimage.2011.02.042.

60. Polimeni, J. R., Fischl, B., Greve, D. N. & Wald, L. L. Laminar analysis of 7T BOLD using an imposed spatial activation pattern in human V1. *NeuroImage* **52**, 1334–1346 (2010) doi: 10.1016/j.neuroimage.2010.05.005.

61. Siero, J. C. W., Petridou, N., Hoogduin, H., Luijten, P. R. & Ramsey, N. F. Cortical depth-dependent temporal dynamics of the BOLD response in the human brain. *J. Cereb. Blood Flow Metab.* **31**, 1999–2008 (2011) doi: 10.1038/jcbfm.2011.57.

62. van der Zwaag, W. *et al.* fMRI at 1.5, 3 and 7 T: Characterising BOLD signal changes. *NeuroImage* **47**, 1425–1434 (2009) doi: 10.1016/j.neuroimage.2009.05.015.

63. van Dijk, J. A., Fracasso, A., Petridou, N. & Dumoulin, S. O. Linear systems analysis for laminar fMRI: Evaluating BOLD amplitude scaling for luminance contrast manipulations. *Sci. Rep.* **10**, 5462 (2020) doi: 10.1038/s41598-020-62165-x.

64. Rockland, K. S. & Pandya, D. N. Laminar origins and terminations of cortical connections of the occipital lobe in the rhesus monkey. *Brain Res.* **179**, 3–20 (1979) doi: 10.1016/0006-8993(79)90485-2.

65. Markuerkiaga, I., Barth, M. & Norris, D. G. A cortical vascular model for examining the specificity of the laminar BOLD signal. *NeuroImage* **132**, 491–498 (2016) doi: 10.1016/j.neuroimage.2016.02.073.

66. Markuerkiaga, I., Marques, J. P., Gallagher, T. E. & Norris, D. G. Estimation of laminar BOLD activation profiles using deconvolution with a physiological point spread function. *J. Neurosci. Methods* **353**, 109095 (2021) doi: 10.1016/j.jneumeth.2021.109095.

67. Uludaǧ, K., Müller-Bierl, B. & Uğurbil, K. An integrative model for neuronal activity-induced signal changes for gradient and spin echo functional imaging. *NeuroImage* **48**, 150–165 (2009) doi: 10.1016/j.neuroimage.2009.05.051.

68. Uludağ, K. & Blinder, P. Linking brain vascular physiology to hemodynamic response in ultra-high field MRI. *NeuroImage* **168**, 279–295 (2018) doi: 10.1016/j.neuroimage.2017.02.063.

69. Marquardt, I., Schneider, M., Gulban, O. F., Ivanov, D. & Uludağ, K. Cortical depth profiles of luminance contrast responses in human V1 and V2 using 7 T fMRI. *Hum. Brain Mapp.* **39**, 2812–2827 (2018) doi: 10.1002/hbm.24042.

70. Boas, D. A., Jones, S. R., Devor, A., Huppert, T. J. & Dale, A. M. A vascular anatomical network model of the spatio-temporal response to brain activation. *NeuroImage* **40**, 1116–1129 (2008) doi: 10.1016/j.neuroimage.2007.12.061.

71. Olsen, S. R., Bhandawat, V. & Wilson, R. I. Divisive Normalization in Olfactory Population Codes. *Neuron* **66**, 287–299 (2010) doi: 10.1016/j.neuron.2010.04.009.

72. Reynolds, J. H. & Heeger, D. J. The Normalization Model of Attention. *Neuron* **61**, 168–185 (2009) doi: 10.1016/j.neuron.2009.01.002.

73. Rabinowitz, N. C., Willmore, B. D. B., Schnupp, J. W. H. & King, A. J. Contrast Gain Control in Auditory Cortex. *Neuron* **70**, 1178–1191 (2011) doi: 10.1016/j.neuron.2011.04.030.

74. Xing, D., Yeh, C.-I., Burns, S. & Shapley, R. M. Laminar analysis of visually evoked activity in the primary visual cortex. *PNAS* **109**, 13871–13876 (2012) doi: 10.1073/pnas.1201478109.

75. Self, M. W., van Kerkoerle, T., Goebel, R. & Roelfsema, P. R. Benchmarking laminar fMRI: Neuronal spiking and synaptic activity during top-down and bottom-up processing in the different layers of cortex. *NeuroImage* **197**, 806–817 (2019) doi: 10.1016/j.neuroimage.2017.06.045.

76. Silva, A. C., Lee, S.-P., Iadecola, C. & Kim, S.-G. Early Temporal Characteristics of Cerebral Blood Flow and Deoxyhemoglobin Changes during Somatosensory Stimulation. *J. Cereb. Blood Flow Metab.* **20**, 201–206 (2000) doi: 10.1097/00004647-200001000-00025.

77. Silva, A. C. & Koretsky, A. P. Laminar specificity of functional MRI onset times during somatosensory stimulation in rat. *Proceedings of the National Academy of Sciences* **99**, 15182–15187 (2002) doi: 10.1073/pnas.222561899.

78. Blakemore, C. & Tobin, ElisabethA. Lateral inhibition between orientation detectors in the cat’s visual cortex. *Exp. Brain Res.* **15**, (1972) doi: 10.1007/BF00234129.

79. Isaacson, J. S. & Scanziani, M. How Inhibition Shapes Cortical Activity. *Neuron* **72**, 231–243 (2011) doi: 10.1016/j.neuron.2011.09.027.

80. Keller, A. J. *et al.* A Disinhibitory Circuit for Contextual Modulation in Primary Visual Cortex. *Neuron* **108**, 1181-1193.e8 (2020) doi: 10.1016/j.neuron.2020.11.013.

81. Goense, J. B. M. & Logothetis, N. K. Laminar specificity in monkey V1 using high-resolution SE-fMRI. *Magn. Reson. Imaging* **24**, 381–392 (2006) doi: 10.1016/j.mri.2005.12.032.

82. Duvernoy, H. M., Delon, S. & Vannson, J. L. Cortical blood vessels of the human brain. *Brain Res. Bull.* **7**, 519–579 (1981) doi: 10.1016/0361-9230(81)90007-1.

83. Blinder, P. *et al.* The cortical angiome: an interconnected vascular network with noncolumnar patterns of blood flow. *Nat. Neurosci.* **16**, 889–897 (2013) doi: 10.1038/nn.3426.

84. Yang, J., Huber, L., Yu, Y. & Bandettini, P. A. Linking cortical circuit models to human cognition with laminar fMRI. *Neurosci. Biobehav. Rev.* **128**, 467–478 (2021) doi: 10.1016/j.neubiorev.2021.07.005.

85. Ogawa, S., Lee, T. M., Kay, A. R. & Tank, D. W. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *PNAS* **87**, 9868–9872 (1990) doi: 10.1073/pnas.87.24.9868.

86. Ogawa, S. *et al.* Functional brain mapping by blood oxygenation level-dependent contrast magnetic resonance imaging. A comparison of signal characteristics with a biophysical model. *Biophys. J.* **64**, 803–812 (1993) doi: 10.1016/S0006-3495(93)81441-3.

87. Logothetis, N. K. The Underpinnings of the BOLD Functional Magnetic Resonance Imaging Signal. *J. Neurosci.* **23**, 3963–3971 (2003) doi: 10.1523/JNEUROSCI.23-10-03963.2003.

88. Logothetis, N. K. What we can do and what we cannot do with fMRI. *Nature* **453**, 869–878 (2008) doi: 10.1038/nature06976.

89. Logothetis, N. K., Pauls, J., Augath, M., Trinath, T. & Oeltermann, A. Neurophysiological investigation of the basis of the fMRI signal. *Nature* **412**, 150–157 (2001) doi: 10.1038/35084005.

90. Klink, P. C., Chen, X., Vanduffel, W. & Roelfsema, P. R. Population receptive fields in nonhuman primates from whole-brain fMRI and large-scale neurophysiology in visual cortex. *eLife* **10**, e67304 (2021) doi: 10.7554/eLife.67304.

91. Theriault, J. E. *et al.* A functional account of stimulation-based aerobic glycolysis and its role in interpreting BOLD signal intensity increases in neuroimaging experiments. *Neurosci. Biobehav. Rev.* **153**, 105373 (2023) doi: 10.1016/j.neubiorev.2023.105373.

92. Filo, S. *et al.* Non-invasive assessment of normal and impaired iron homeostasis in the brain. *Nat. Commun.* **14**, 5467 (2023) doi: 10.1038/s41467-023-40999-z.

93. Pauling, L. & Coryell, C. D. The Magnetic Properties and Structure of Hemoglobin, Oxyhemoglobin and Carbonmonoxyhemoglobin. *PNAS* **22**, 210–216 (1936) doi: 10.1073/pnas.22.4.210.

94. An, H. & Lin, W. Impact of intravascular signal on quantitative measures of cerebral oxygen extraction and blood volume under normo- and hypercapnic conditions using an asymmetric spin echo approach. *Magn. Reson. Med.* **50**, 708–716 (2003) doi: 10.1002/mrm.10576.

95. Thulborn, K. R., Waterton, J. C., Matthews, P. M. & Radda, G. K. Oxygenation dependence of the transverse relaxation time of water protons in whole blood at high field. *BBA* **714**, 265–270 (1982) doi: 10.1016/0304-4165(82)90333-6.

96. Baranovicova, E. *et al.* Thalamic paramagnetic iron by T2\* relaxometry correlates with severity of multiple sclerosis. *J. Biomed. Res.* **31**, 301–305 (2017) doi: 10.7555/JBR.31.20160023.

97. Panchuelo, R. M. S., Schluppeck, D., Harmer, J., Bowtell, R. & Francis, S. Assessing the Spatial Precision of SE and GE-BOLD Contrast at 7 Tesla. *Brain Topogr.* **28**, 62–65 (2015) doi: 10.1007/s10548-014-0420-4.

98. Turner, R. How much cortex can a vein drain? Downstream dilution of activation-related cerebral blood oxygenation changes. *NeuroImage* **16**, 1062–1067 (2002) doi: 10.1006/nimg.2002.1082.

99. Bianciardi, M., Fukunaga, M., van Gelderen, P., De Zwart, J. A. & Duyn, J. H. Negative BOLD-fMRI Signals in Large Cerebral Veins. *J. Cereb. Blood Flow Metab.* **31**, 401–412 (2011) doi: 10.1038/jcbfm.2010.164.

100. Kay, K. *et al.* A critical assessment of data quality and venous effects in sub-millimeter fMRI. *NeuroImage* **189**, 847–869 (2019) doi: 10.1016/j.neuroimage.2019.02.006.

101. Olman, C. A., Inati, S. & Heeger, D. J. The effect of large veins on spatial localization with GE BOLD at 3 T: Displacement, not blurring. *NeuroImage* **34**, 1126–1135 (2007) doi: 10.1016/j.neuroimage.2006.08.045.

102. Shmuel, A., Yacoub, E., Chaimow, D., Logothetis, N. K. & Uğurbil, K. Spatio-temporal point-spread function of fMRI signal in human gray matter at 7 Tesla. *NeuroImage* **35**, 539–552 (2007) doi: 10.1016/j.neuroimage.2006.12.030.

103. Huber, L. *et al.* Cortical lamina-dependent blood volume changes in human brain at 7T. *NeuroImage* **107**, 23–33 (2015) doi: 10.1016/j.neuroimage.2014.11.046.

104. Oliveira, I. A. F., Schnabel, R., van Osch, M. J. P., van der Zwaag, W. & Hirschler, L. Advancing 7T perfusion imaging by pulsed arterial spin labeling: Using a parallel transmit coil for enhanced labeling robustness and temporal SNR. *PLoS One* **19**, e0309204 (2024) doi: 10.1371/journal.pone.0309204.

105. Ivanov, D. *et al.* Comparison of 3T and 7T ASL techniques for concurrent functional perfusion and BOLD studies. *NeuroImage* **156**, 363–376 (2017) doi: 10.1016/j.neuroimage.2017.05.038.

106. Oshio, K. & Feinberg, D. A. GRASE (Gradient-and Spin-Echo) imaging: A novel fast MRI technique. *Magn. Reson. Med.* **20**, 344–349 (1991) doi: 10.1002/mrm.1910200219.

107. Chen, G., Wang, F., Gore, J. C. & Roe, A. W. Layer-specific BOLD activation in awake monkey V1 revealed by ultra-high spatial resolution functional magnetic resonance imaging. *NeuroImage* **64**, 147–155 (2013) doi: 10.1016/j.neuroimage.2012.08.060.

108. Olman, C. A. *et al.* Layer-Specific fMRI Reflects Different Neuronal Computations at Different Depths in Human V1. *PLoS One* **7**, e32536 (2012) doi: 10.1371/journal.pone.0032536.

109. Yu, X. *et al.* Direct imaging of macrovascular and microvascular contributions to BOLD fMRI in layers IV–V of the rat whisker–barrel cortex. *NeuroImage* **59**, 1451–1460 (2012) doi: 10.1016/j.neuroimage.2011.08.001.

110. Kay, K., Jamison, K. W., Zhang, R. Y. & Uğurbil, K. A temporal decomposition method for identifying venous effects in task-based fMRI. *Nat. Methods* **17**, 1033–1039 (2020) doi: 10.1038/s41592-020-0941-6.

111. Muckli, L. *et al.* Contextual Feedback to Superficial Layers of V1. *Curr. Biol.* **25**, 2690–2695 (2015) doi: 10.1016/J.CUB.2015.08.057.

112. Fracasso, A., Petridou, N. & Dumoulin, S. O. Systematic variation of population receptive field properties across cortical depth in human visual cortex. *NeuroImage* **139**, 427–438 (2016) doi: 10.1016/j.neuroimage.2016.06.048.

113. Pais-Roldán, P., Yun, S. D. & Shah, N. J. Pre-processing of Sub-millimeter GE-BOLD fMRI Data for Laminar Applications. *Front. Neuroimaging* **1**, (2022) doi: 10.3389/fnimg.2022.869454.

114. Demirayak, P., Deshpande, G. & Visscher, K. Laminar functional magnetic resonance imaging in vision research. *Front. Neurosci.* **16**, (2022) doi: 10.3389/fnins.2022.910443.

115. Chai, Y. & Zhang, R.-Y. Exploring methodological frontiers in laminar fMRI. *Psychoradiology* **4**, kkae027 (2024) doi: 10.1093/psyrad/kkae027.

116. Chai, Y. *et al.* Improving laminar fMRI specificity by reducing macrovascular bias revealed by respiration effects. *Imaging Neurosci.* **2**, 1–16 (2024) doi: 10.1162/imag\_a\_00249.

117. Harris, J. J., Jolivet, R. & Attwell, D. Synaptic Energy Use and Supply. *Neuron* **75**, 762–777 (2012) doi: 10.1016/j.neuron.2012.08.019.

118. Douglas, R. J. & Martin, K. A. C. Neuronal Circuits of the Neocortex. *Annu. Rev. Neurosci.* **27**, 419–451 (2004) doi: 10.1146/annurev.neuro.27.070203.144152.

119. Weber, B., Keller, A. L., Reichold, J. & Logothetis, N. K. The Microvascular System of the Striate and Extrastriate Visual Cortex of the Macaque. *Cereb. Cortex* **18**, 2318–2330 (2008) doi: 10.1093/cercor/bhm259.

120. Douglas, R. J. & Martin, K. A. C. Recurrent neuronal circuits in the neocortex. *Curr. Biol.* **17**, R496–R500 (2007) doi: 10.1016/j.cub.2007.04.024.

121. Crick, F. & Koch, C. Constraints on cortical and thalamic projections: the no-strong-loops hypothesis. *Nature* **391**, 245–250 (1998) doi: 10.1038/34584.

122. Sherman, S. M. & Guillery, R. W. On the actions that one nerve cell can have on another: Distinguishing “drivers” from “modulators”. *PNAS* **95**, 7121–7126 (1998) doi: 10.1073/pnas.95.12.7121.

123. Self, M. W., Kooijmans, R. N., Supèr, H., Lamme, V. A. & Roelfsema, P. R. Different glutamate receptors convey feedforward and recurrent processing in macaque V1. *PNAS* **109**, 11031–11036 (2012) doi: 10.1073/pnas.1119527109.

124. Lauritzen, M. Reading vascular changes in brain imaging: is dendritic calcium the key? *Nat. Rev. Neurosci.* **6**, 77–85 (2005) doi: 10.1038/nrn1589.

125. Hupé, J.-M. *et al.* Feedback Connections Act on the Early Part of the Responses in Monkey Visual Cortex. *J. Neurophysiol.* **85**, 134–145 (2001) doi: 10.1152/jn.2001.85.1.134.

126. Leprince, Y. *et al.* Combined Laplacian-equivolumic model for studying cortical lamination with ultra high field MRI (7 T). in *IEEE International Symposium on Biomedical Imaging (ISBI)* 580–583 (IEEE, 2015). doi:10.1109/ISBI.2015.7163940 doi: 10.1109/ISBI.2015.7163940.

127. Shamir, I. *et al.* A framework for cortical laminar composition analysis using low-resolution T1 MRI images. *Brain Struct. Funct.* **224**, 1457–1467 (2019) doi: 10.1007/s00429-019-01848-2.

128. Trampel, R., Bazin, P.-L., Pine, K. & Weiskopf, N. In-vivo magnetic resonance imaging (MRI) of laminae in the human cortex. *NeuroImage* **197**, 707–715 (2019) doi: 10.1016/j.neuroimage.2017.09.037.

129. Godenschweger, F. *et al.* Motion correction in MRI of the brain. *Phys. Med. Biol.* **61**, R32–R56 (2016) doi: 10.1088/0031-9155/61/5/R32.

130. Zaitsev, M., Maclaren, J. & Herbst, M. Motion artifacts in MRI: A complex problem with many partial solutions. *J. Magn. Reson. Imaging* **42**, 887–901 (2015) doi: 10.1002/jmri.24850.

131. Priovoulos, N. *et al.* A local multi-transmit coil combined with a high-density receive array for cerebellar fMRI at 7T. *NMR Biomed.* **34**, e4586 (2021) doi: 10.1002/NBM.4586.

132. Petridou, N. *et al.* Pushing the limits of high-resolution functional MRI using a simple high-density multi-element coil design. *NMR Biomed.* **26**, 65–73 (2013) doi: 10.1002/nbm.2820.

133. Alvarez, I., De Haas, B., Clark, C., Rees, G. & Schwarzkopf, D. Comparing different stimulus configurations for population receptive field mapping in human fMRI. *Front. Hum. Neurosci.* **9**, (2015) doi: 10.3389/fnhum.2015.00096.

134. Lage-Castellanos, A., Valente, G., Senden, M. & De Martino, F. Investigating the Reliability of Population Receptive Field Size Estimates Using fMRI. *Front. Neurosci.* **14**, (2020) doi: 10.3389/fnins.2020.00825.

135. Senden, M., Reithler, J., Gijsen, S. & Goebel, R. Evaluating Population Receptive Field Estimation Frameworks in Terms of Robustness and Reproducibility. *PLoS One* **9**, e114054 (2014) doi: 10.1371/journal.pone.0114054.

136. van Dijk, J. A., de Haas, B., Moutsiana, C. & Schwarzkopf, D. S. Intersession reliability of population receptive field estimates. *NeuroImage* **143**, 293–303 (2016) doi: 10.1016/j.neuroimage.2016.09.013.

137. Bianciardi, M. *et al.* Sources of functional magnetic resonance imaging signal fluctuations in the human brain at rest: a 7 T study. *Magn. Reson. Imaging* **27**, 1019–1029 (2009) doi: 10.1016/j.mri.2009.02.004.

138. Triantafyllou, C. *et al.* Comparison of physiological noise at 1.5 T, 3 T and 7 T and optimization of fMRI acquisition parameters. *NeuroImage* **26**, 243–250 (2005) doi: 10.1016/j.neuroimage.2005.01.007.

139. Benson, N. C. *et al.* The Human Connectome Project 7 Tesla retinotopy dataset: Description and population receptive field analysis. *J. Vis.* **18**, 23 (2018) doi: 10.1167/18.13.23.

140. Zuiderbaan, W., Harvey, B. M. & Dumoulin, S. O. Modeling center-surround configurations in population: Receptive fields using fMRI. *J. Vis.* **12**, 1–15 (2012) doi: 10.1167/12.3.10.

141. Gorgolewski, K. J. *et al.* The brain imaging data structure, a format for organizing and describing outputs of neuroimaging experiments. *Sci. Data.* **3**, 160044 (2016) doi: 10.1038/sdata.2016.44.

142. Peirce, J. W. PsychoPy—Psychophysics software in Python. *J. Neurosci. Methods* **162**, 8–13 (2007) doi: 10.1016/J.JNEUMETH.2006.11.017.

143. Dale, A. M. Optimal experimental design for event-related fMRI. *Hum. Brain Mapp.* **8**, 109–114 (1999) doi: 10.1002/(SICI)1097-0193(1999)8:2/3<109::AID-HBM7>3.0.CO;2-W.

144. Mumford, J. A., Poline, J.-B. & Poldrack, R. A. Orthogonalization of Regressors in fMRI Models. *PLoS One* **10**, e0126255 (2015) doi: 10.1371/journal.pone.0126255.

145. Chen, G. *et al.* BOLD Response is more than just magnitude: Improving detection sensitivity through capturing hemodynamic profiles. *NeuroImage* **277**, 120224 (2023) doi: 10.1016/j.neuroimage.2023.120224.

146. Gao, J. S., Huth, A. G., Lescroart, M. D. & Gallant, J. L. Pycortex: an interactive surface visualizer for fMRI. *Front. Neuroinform.* **9**, (2015) doi: 10.3389/fninf.2015.00023.

147. Savitzky, Abraham. & Golay, M. J. E. Smoothing and Differentiation of Data by Simplified Least Squares Procedures. *Anal. Chem.* **36**, 1627–1639 (1964) doi: 10.1021/ac60214a047.

148. Vallat, R. Pingouin: statistics in Python. *JOSS* **3**, 1026 (2018) doi: 10.21105/joss.01026.

149. Seabold, S. & Perktold, J. Statsmodels: Econometric and Statistical Modeling with Python. (2010).

# STAR★METHODS

## Key resource table

A KRT has been included as a file called **KRT.docx**.

## Experimental model and study participant details

13 participants (ages 23–50 years, 5 female) participated in this study. All participants had normal or corrected-to normal visual acuity, were screened prior to the experiments to ensure MR compatibility, and provided written informed consent as approved by the ethics committee of the Vrije Universiteit Amsterdam. Some participants were scanned twice targeting a different pRF, resulting in a total of 18 individually sampled cortical patches.

## Method details

### Experimental setup

The visual stimuli were generated using the Psychopy package142, wrapped in exptools2 [https://github.com/gjheij/exptools2](https://github.com/gjheij/exptools2%7D). Stimuli were displayed on an MRI-compatible screen located outside the bore (Cambridge Research Systems 32" LCD widescreen, 1920×1080 resolution, 120Hz refresh rate) and viewed by participants through front-silvered mirrors (example stimuli shown in Figure 2A). Each stimulus presentation consisted of an 8Hz flickering stimulus displayed for 2 seconds. The experiment started with a blank screen of mean luminance for the duration of the dummy scan (~42s) and baseline (30s) before the stimuli started to appear. The inter-stimulus intervals (ISIs) were jittered following a negative exponential decay to reduce collinearity between subsequent events143–145 and were spaced far apart to enable epoching strategies. ISImin/ISImax/ISImean values were 14s/24s/18s, resulting in five stimulus presentations per stimulus per run (7 minutes). To maximize signal-to-noise ratio (SNR) while limiting predictability, we simulated two sets of stimulus presentation orders and intervals. These two variations were randomly presented to participants during the session. To ensure engagement, a small fixation dot was presented in the center of the stimulus, which changed color (red to green) at intervals following a negative exponential decay (ISImin/ISImax/ISImean = 4s/8s/6s). Eye movements were monitored using an EyeLink 1000 eye-tracker system at 1000Hz (<https://www.sr-research.com>), and participants were instructed to report color changes via a button press.

### Vertex selection

Similar to our previous approach43, vertex selection was performed using surface processing procedures from pycortex146. We aimed to identify a vertex within the primary visual cortex (manually delineated based on polar angle maps) that met the following criteria: located in the eccentricity band subtending 1.5–3 dva, at least 1 dva away from the vertical meridian, with sufficient variance explained (r2>0.55) and reasonable pRF sizes (σ1>0.50 dva). A binary mask representing the surviving vertices was visually inspected using FreeSurfer's FreeView. From this mask, we selected a vertex within a blob that was favorably positioned with respect to curvature and neighboring vertices. This approach ensured that responses from similarly behaving pRFs would be accurately projected into the line.

### Data acquisition

The workflow includes two separate scan sessions typically acquired on different days. The first session is dedicated to the acquisition of anatomical information and whole-brain population receptive field (pRF) estimation (see ref43 for acquisition and experimental paradigm). In the second session, we perform our functional line-scanning experiment, targeting a specific location on the cortical surface. All acquisitions were performed on a Philips Achieva 7T MRI system.

The line-scanning functional acquisition used a modified multi-echo 2D gradient-echo sequence where the phase-encoding gradients are removed and two OVS bands are used to suppress signals outside the line45,46. With this sequence, 94.3 ± 1.3% of undesired signals outside the region of interest is suppressed45,46. A gap of 4 mm between the two OVS bands was used, resulting in a nominal resolution for the line of 4×2.5×0.25 mm3, with 0.25 mm in the laminar direction. Other parameters were: TR/TE1-5 = 105 ms/6 ms, 14 ms, 22 ms, 30 ms, 38 ms, readout bandwidth = 131.4 Hz/pixel, FA = 16°46. Data were acquired using two custom-built high-density 16-channel surface coil arrays (total 32 channels) for signal reception131,132 and the NOVA coil for transmission (Nova Medical, Wilmington, MA). The gradient coil has a maximum amplitude of 40 mT/m and a 200 T/m/s maximum slew rate.

For registration, a 4-min whole-brain T1-weighted scan was acquired using the two-channel transmit coil to receive (Nova Medical, Wilmington, MA), at a resolution of 1.5 mm isotropic (FOV = 245×245×184 mm3 matrix = 164×163×184, TR/TE = 6.2 ms/3 ms, FA1/FA2 = 5°/7°, TRMP2RAGE/TI1/TI2 = 5500 ms/800 ms/2700 ms). Two short additional scans accompanied the line-scanning acquisition: for the nominal line representation, a slice image with phase encoding but without OVS bands was acquired. For line coil sensitivity maps used in reconstructing line-scanning data, a slice image with phase encoding and OVS bands was acquired.

### Data reconstruction and quality assessment

The reconstruction of the line-scanning data was performed offline using MATLAB Gyrotools. Multichannel coil data were combined using a temporal signal-to-noise ratio (tSNR) and coil sensitivity-weighted sum-of-squares (SoS) scheme per echo45,46 . Multi-echo data were subsequently combined using a sum-of-squares operation to maximize signal stability and contrast-to-noise (CNR)46. To minimize habituation effects, stimuli were presented in two different orders. High-frequency noise was addressed by applying a Savitzky-Golay filter147 (31 samples, 3rd order) before averaging runs with the same stimulus order.

Line-scanning fMRI is particularly sensitive to movement due to its limited coverage. To mitigate this, our participant pool consisted of highly experienced individuals, and movement was further restricted by securing the chin to the transmit coil with tape. The target area of interest was manually delineated on a run-to-run basis by identifying the CSF/gray matter and gray/white matter boundaries using the anatomical reference slice. For each event, we extracted and averaged the time period from 2 seconds before stimulus onset to 14 seconds after (Figure S10AC). This process resulted in an average response to each stimulus for each participant across the different stimulus orders (Figure 10B). Based on the responses across the entire line, we confirmed that the stimuli evoked the most specific responses in the target area (Figure S10D).

Cortical locations were included if they met two criteria (Figure S11). First, the response to the center stimulus had to be larger than the responses to the other stimuli. Second, this stimulus had to exhibit the draining vein effect across cortical depth. To verify responsiveness, we used a general linear model (GLM) with a canonical HRF, where the prediction based on center stimulus events was entered as a regressor. This analysis yielded variance explained across the line, allowing us to confirm that the largest responses occurred near the intended target location. For depth profiles, we estimated the magnitude evolution within a 5–7 second window after stimulus onset. Since cortical depth was covered by a varying number of data points across participants, the data were regridded so that cortical depth was uniformly covered by 20 data points28,56–58.

In cases where discrepancies occurred between the selected data points and the variance explained, an additional shift was applied to voxel selection. For some participants, this adjustment resulted in cleaner responses to the center stimulus and an improved draining vein profile (black profiles). From the 18 individually sampled pRF responses, 11 (defined as *n*) were included in the final analysis. It is important to note that this procedure enhanced the precision of the results by increasing statistical power (higher *n*), but the findings remained consistent even without this adjustment (Figure S3B).

### Quantification and statistical analysis

Significance testing was performed using the Python packages *Pingouin*148 and *statsmodels*149. For each comparison, tests for normality and homogeneity of variance were conducted, and the appropriate statistical test (parametric or non-parametric) was selected based on the results. For within-participant comparisons of stimulus responses, paired-samples t-tests were applied. Group comparisons were conducted using ANOVA, followed by Holm-corrected post-hoc tests when applicable. The significance level was set to α = 0.05. Unless otherwise specified, 95% confidence interval (SEM\*1.96) was used to quantify precision. The specific statistical tests, significance levels, and precision measurements are reported in the text and/or figure captions.