Current Biology

Laminar processing of numerosity supports a canonical cortical microcircuit in human parietal cortex

Highlights

- Neurons in parietal cortex are tuned to numerosity, i.e., set size of a group of items
- Numerosity tuning width increases with preferred numerosity for all cortical depths
- Tuning width decreases away from central layers, i.e., becomes more specific
- Parietal laminar processing parallels early visual cortex and processing hierarchy

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In brief

Van Dijk et al. use ultra-high field fMRI and population receptive field-based modeling to investigate numerosity processing across cortical depth in the parietal cortex. They find that numerosity responses become increasingly specific moving away from the central layers, suggesting a laminar processing scheme similar to early visual cortex.

Report

Laminar processing of numerosity supports a canonical cortical microcircuit in human parietal cortex

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SUMMARY

As neural signals travel through the visual hierarchy, spatial precision decreases and specificity for stimulus features increases.^{1–4} A similar hierarchy has been found for laminar processing in V1, where information from the thalamus predominantly targets the central layers, while spatial precision decreases and feature specificity increases toward superficial and deeper layers.^{5–17} This laminar processing scheme is proposed to represent a canonical cortical microcircuit that is similar across the cortex.^{11,18–21} Here, we go beyond early visual cortex and investigate whether processing of numerosity (the set size of a group of items) across cortical depth in the parietal association cortex follows this hypothesis. Numerosity processing is implicated in many tasks such as multiple object tracking,²² mathematics,^{23–25} decision making,²⁶ and dividing attention.²⁷ Neurons in the parietal association cortex are tuned to numerosity, with both a preferred numerosity tuning and tuning width (i.e., specificity).^{28–30} We quantified preferred numerosity responses across cortical depth in the parietal association cortex with ultra-high field fMRI and population receptive field-based numerosity modeling.^{1,28,31} We find that numerosity responses sharpen, i.e., become increasingly specific, moving away from the central layers. This suggests that the laminar processing scheme for numerosity processing in the parietal cortex is similar to primary visual cortex, providing support for the canonical cortical microcircuit hypothesis beyond primary visual cortex.

RESULTS AND DISCUSSION

We use ultra-high field functional magnetic resonance imaging (fMRI) to investigate whether processing of numerosity across cortical depth in parietal cortex fits the canonical cortical microcircuit hypothesis. Ultra-high field MRI allows us to measure both anatomical and functional cortical depth-dependent signals that reflect information processing across cortical depth (for reviews, see De Martino et al.,¹² Lawrence et al.,¹³ Stephan et al.,¹⁴ Self et al.,¹⁷ Petridou and Siero,³² and Self and Roelfsema³³).

Studies using fMRI have revealed a network of cortical areas that are involved in numerosity processing.^{27,29,34–38} Recent fMRI studies have shown that a number of cortical locations involved in numerosity processing are topographically organized, with numerosity preference gradually changing along the cortical surface.^{28,31,39} Neuronal populations in these topographic regions show numerosity tuning, with large responses for specific, preferred numerosity and increasingly smaller responses when the numerosity is further away from the preferred numerosity of that neuronal population. A cluster of numerosity topographic maps is located in the parietal association cortex.

Participants ($n = 7$) viewed stimuli consisting of groups of circles that systematically increased and decreased in numerosity over time²⁸ (see inset in [Figures 1C and 1E](#)). Meanwhile, we collected fMRI data (0.93 mm isotropic resolution; [Figure 1B](#)). We segmented gray matter and obtained a continuous measure of cortical depth, normalized with respect to cortical thickness⁴⁰ (range = 0–1, <https://nighres.readthedocs.io/en/latest/>, with 0 representing the white matter boundary and 1 the gray matter/cerebrospinal fluid boundary; [Figure 1A](#)). This equi-volume model provides a coordinate system of cortical depth that is independent from local cortical folding. We sampled across 9 cortical depth bins, deriving single profiles ([STAR Methods](#)).

It is important to note that an acquisition resolution of 0.93 mm isotropic would at best allow us to measure 4 different compartments, assuming a 3–4 mm cortical thickness.⁴¹ However, we obtain 9 separate estimates along normalized cortical depth. We can obtain these estimates thanks to the folded nature of cortical surface. When measuring MRI signal along cortical depth, we are using a regular grid (the field of view of the acquisition resolution, 0.93 mm isotropic), while we sample signal from an irregular (folded) portion of the cortex. Thus, the center of a

Figure 1. Processing steps and model fits

(A) Volume-preserving cortical depth map on a partial, sagittal anatomical slice. Inset: whole-brain anatomical slice with the location of the partial slice (red box).
 (B) Mean susceptibility distortion-corrected functional data registered to the anatomy, with gray matter-cerebrospinal fluid (CSF) (red) and gray-white matter (green) borders.
 (C) Preferred numerosity results at all depths. Threshold: cross-validated model $R^2 = 8\%$. Insets: cut-outs of the central portion of example stimulus displays with numerosity 1, 4, and 7. Stimuli all fell within a 0.75° visual angle radius from the center of fixation.
 (D) Preferred numerosity at the middle depth bin projected on a partial mesh corresponding to the coverage in (A)–(C), coronal view. Threshold: model $R^2 = 8\%$.
 (E) Time series of an example voxel (dashed line with blue discrete time points) overlaid with best-fitting predicted fMRI response (solid green line). Goodness of fit (model R^2) = 76%, preferred numerosity = 3, tuning width = 3.8. Inset top right: tuning model for the example voxel. Inset below x axis: stimulus sequence. Additional examples can be found in Figure S2.
 (F) Model cross-validated explained variance generally increases toward the cortical surface (cortical surface = 1). Blue shaded regions denote the SEM for separate participants. Red shaded region denotes the SEM across participants.
 See also Figures S1A and S2.

voxel on the regular grid could be located close to the cerebrospinal fluid surface, close to the white matter surface, or anywhere between the two, thus allowing a denser sampling rate along cortical depth (STAR Methods).

Next, we modeled the numerosity responses,²⁸ resulting in a preferred numerosity and tuning width estimate (specificity) for each cortical location, cortical depth, and participant (Figures 1C–1E). We selected all profiles within the field of view of the functional data for which model fits across all depths exceeded an individual cross-validated explained variance threshold for further analysis. These individual thresholds were set to the 99th percentile of the cross-validated model explained variance in white matter for each participant; i.e., the threshold was set

at $p = 0.01$, uncorrected as derived from an empirical null distribution ($M = 8\%$, $SD = 2\%$, range = 5%–12%). As expected,¹⁵ cross-validated model explained variance generally increased toward the cortical surface (Figure 1F; see also Figure S1A for BOLD amplitude across depth, which follows the same pattern).

Tuning width increases with preferred numerosity

For every cortical depth bin, we performed a linear fit on the median tuning width across preferred numerosity. The tuning width increased systematically with preferred numerosity across participants: $M_{slope} = 1.40$, $SD_{slope} = 0.98$, and $range_{slope} = 0.07$ –4.18 (see Figure 2 for three example cortical depth bins for a participant

Figure 2. Preferred numerosity and tuning width for three example cortical depths for an example participant

The cross-validated model explained variance threshold (thr) for each sampled depth was 12%. Each panel shows the preferred numerosity versus tuning width. Average normalized depth: 0.18 (deep/infragranular, A), 0.52 (approx. middle/granular, B), and 0.83 (superficial/supragranular, C). Blue circles and lines represent median preferred tuning width and 95% confidence intervals. Red lines and shaded regions represent the least-squares median fits and their 95% confidence intervals. Overall fit slopes were $M_{slope} = 1.40$, $SD_{slope} = 0.98$, and $range_{slope} = 0.07$ – 4.18 across all cortical depths and participants. Tuning width consistently increased with increasing numerosity for each cortical depth. See also [Figures S1C](#) and [S1D](#).

and [Figures S1C](#) and [S1D](#) for the average fit slope and intercept values across cortical depth for each participant).

Response specificity sharpens toward deeper and superficial layers

To quantify the change in response specificity across cortical depth, we sampled tuning width estimates at numerosity 2 and 3. These numerosities were selected as they were most prevalent in the data (37% and 23%, respectively; [Figure S1B](#)), hence resulting in the most stable estimates. To reduce inter-participant variability, the sampled tuning width estimates for each participant were Z scored. For both numerosities, the tuning width profiles showed an inverted U-shape across cortical depth, with tuning width being largest around the middle cortical depth and decreasing toward both the deep and superficial cortical depth portions ([Figure 3](#)). t tests on the parameters of individual second-degree polynomial fits on the Z scored tuning width profiles revealed that the inverted U-shape of the tuning curve was significant for both sampled numerosities, with parameter values for preferred numerosity 2 ([Figures 3A](#) and [3B](#)) of slope = 8.1, quadratic component = -7.73 , $t(6)_{slope} = 4.01$, $p = 0.007$, $t(6)_{quadratic} = -5.52$, and $p = 0.002$. For preferred numerosity 3 ([Figures 3C](#) and [3D](#)), these values were slope = 6.58, quadratic component = -6.48 , $t(6)_{slope} = 3.05$, $p = 0.022$, $t(6)_{quadratic} = -4.03$, and $p = 0.007$. Fitting only a straight line, forgoing the quadratic component, resulted in non-significant results for all model parameters and numerosities. The results were highly similar using restrictions in maximum tuning width, removing the outer depth bins, using different thresholds ([Figure S3](#)), and using the median tuning width, rather than sampling at a specific numerosity ([Figure S4A](#)). Preferred numerosity does not vary significantly across cortical depth ([Figures S4B](#) and [S4C](#)).

Laminar processing of numerosity supports a canonical cortical microcircuit in parietal cortex

We tested whether the cortical microcircuit hypothesis holds for numerosity processing in human parietal cortex. This

hypothesis states that cortical information processing follows universal principles that are shared across the cortex.^{18–21} To assess the feasibility for this hypothesis for numerosity processing in the parietal cortex, we evaluated response specificity (tuning width) profiles across cortical depth, elicited by viewing numerosity stimuli. We find that tuning width increases with preferred numerosity at individual cortical depths. This increase is consistent with previous findings across preferred numerosity at lower resolutions.^{28,31} Moreover, we show that specificity for numerosity increases toward both superficial and deeper cortical depth portions, as tuning width is largest in the central portion and sharpens in the direction of both the superficial and deeper portions of cortical depth. This processing hierarchy is similar to that of primary visual cortex.¹⁶ Therefore, we suggest that our results provide evidence that the laminar processing hierarchy is preserved across the parietal cortex, thus supporting the hypothesis that the increased specificity for stimulus dimensions along the laminar hierarchy may represent a canonical cortical microcircuit that may be similar across the cortex.^{11,18–21}

The infra- and supragranular layers receive feedback from regions higher in the hierarchy.⁶ Thus, this feedback also likely contributes to the neural tuning across cortical depth. Therefore, the response sharpening we observe across cortical depth may be an effect of a combination of feedback connections from regions higher in the numerosity processing hierarchy and local processing.

Potential vasculature confounds

Cortical vasculature varies systematically across cortical depth.⁴² Could the tuning width profile thus be driven by the vasculature rather than neural effects? We think that this is unlikely. First, due to the local vasculature, signal amplitudes increase monotonically toward the cortical surface.^{43,44} In particular, vessel diameters—to which gradient echo BOLD is particularly sensitive—increase toward the surface, resulting in a steady increase in signal amplitude in that direction.^{43,45–48}

Figure 3. Tuning width profiles show response sharpening with increased processing toward deep (cortical depth < 0.5) and superficial (cortical depth > 0.5) cortical depth portions

(A and C) Z scored tuning width, sampled at preferred numerosity 2 and 3 from the median fits for each depth bin (red lines in Figure 2). Shaded regions represent the 95% between-participant confidence interval of the fit. (A) Fit parameters: slope = 8.1, quadratic component = -7.73 , $t(6)_{\text{slope}} = 4.01$, $p = 0.007$, $t(6)_{\text{quadratic}} = -5.52$, and $p = 0.002$. (C) Fit parameters: slope = 6.58, quadratic component = -6.48 , $t(6)_{\text{slope}} = 3.05$, $p = 0.022$, $t(6)_{\text{quadratic}} = -4.03$, and $p = 0.007$.

(B and D) Tuning width, sampled at preferred numerosity 2 and 3. Polynomial fit (red line) and confidence interval (shaded region) transformed from Z scored data (A).

For all panels: gray lines represent individual participants. Red lines represent the best second-degree polynomial fit. See also Figures S1B, S3, and S4.

Additionally, we assert that larger signal amplitudes are related to increased variance explained (although the physiological noise levels do scale with signal amplitude^{49–51}). Therefore, if the results were purely driven by the vasculature, we would expect tuning width to show a monotonous decrease or increase toward the cortical surface. Second, previous work using fMRI across cortical depth in early visual cortex¹⁵ has shown that the pattern of spatial pooling of visual responses is not a direct reflection of the cortical vascular organization, and closely resembles neurophysiological measurements in primates.¹⁷ All in all, it is unlikely that the presented results are merely an artifact of the inherent properties of the fMRI signal, and we believe that these results reflect signals of neural origin.

In conclusion, we provide evidence that laminar processing for numerosity in the parietal association cortex follows the same pattern as laminar processing in early visual cortex, with response specificity increasing away from the central portion of cortical depth toward both the deeper and more superficial depths. This processing parallels the increasing feature

specificity across the visual processing hierarchy. Thus, we suggest that this finding expands support for the canonical microcircuit hypothesis stating that laminar processing is organized similarly across the cortex.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cub.2021.07.082>.

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AUTHOR CONTRIBUTIONS

J.A.v.D. and S.O.D. designed the experiment; J.A.v.D. collected the data; J.A.v.D. and A.F. analyzed the data; all authors contributed to and reviewed the manuscript text; J.A.v.D. prepared all figures; and S.O.D., N.P., and A.F. provided general supervision and guidance throughout.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw and analyzed data	This paper; Open Science Framework	https://osf.io/ay87b/
Software and algorithms		
MATLAB (Mathworks, 2015b)	http://www.mathworks.com/products/matlab/	RRID: SCR_001622
R/studio (3.4.4)	http://www.r-project.org/	RRID: SCR_001905
Python (3.7.4/2.7.18 for AFNI)	http://www.python.org/	RRID: SCR_008394
AFNI	http://afni.nimh.nih.gov/afni/	RRID: SCR_005927
Nighres package for Python	https://nighres.readthedocs.io/en/latest/	https://doi.org/10.1093/gigascience/giy082
Analysis code	This paper; Open Science Framework	https://osf.io/ay87b/
MIPAV	http://mipav.cit.nih.gov/	RRID: SCR_007371
Freesurfer	http://surfer.nmr.mgh.harvard.edu/	RRID: SCR_001847
Vistasoft	http://white.stanford.edu/software/	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests should be directed to and will be fulfilled by the lead contact, Jelle A. van Dijk (j.van.dijk@spinozacentr.nl)

Materials availability

This study did not generate reagents.

Data and code availability

- Raw data reported in this paper will be shared by the lead contact upon request. Processed data are publicly available as of the date of publication and can be found at <https://osf.io/ay87b/>.
- All original code has been deposited at <https://osf.io/ay87b/> and is publicly available as of the date of publication. DOIs are listed in the [key resources table](#).
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Eight participants (1 female, mean age: 31.4 years, age range 22–45 years old) participated in this study. All participants were familiar with the MRI environment and had participated in previous experiments. They had normal or corrected-to-normal visual acuity. All participants signed informed consent. All experimental procedures were approved by the medical ethics committee of the University Medical Center Utrecht and adhered to the guidelines of the Declaration of Helsinki. One participant was excluded due to excessive motion between runs.

METHOD DETAILS

Stimuli

Stimuli were presented on a 32-inch LCD screen, specifically designed for use in an MRI environment.⁵² The screen resolution was 1920 × 1080 pixels, with a screen size of 69.8 × 39.3 cm, a refresh rate of 120 Hz, and a built-in linear luminance look-up table. The display was positioned at the far end of the bore and was viewed via a mirror positioned in the head coil of the MRI scanner. The total viewing distance was 220 cm. The total visible vertical extent of the screen subtended 10.2 degrees visual angle (deg).

All stimuli were generated in MATLAB (Mathworks; 2018a) using the Psychophysics toolbox.^{53,54} A large cross consisting of two thin (1 pixel) red diagonal lines starting at the corners of a 10.2 × 10.2 degree invisible square and intersecting in the center of the screen was presented at all times, with a mean luminance gray background that covered the entire screen. Stimuli consisted of

groups of circles, randomly distributed to be presented entirely within an invisible circle with a 0.75-degree radius around the center of the screen to minimize eye movements. Each group of circles contained 1 to 7 or 20 circles, with 20 functioning as a baseline. Numerosity 20 was chosen to this end as it is expected to be well outside the response range of neuronal populations responding to numerosities 1 to 7, while neuronal populations responding to contrast energy would be expected to respond most strongly to this energy-rich condition.⁵⁵ The total surface area (702 pixels) of each group of circles was constant between different numerosities. This resulted in a decreased size per circle with increasing numerosity (see inset Figure 1C). A previous study controlled for dot size, crowding, total circumference, shape, and total area of the numerosity stimuli. The authors observed that there is little difference between estimates resulting from different stimuli.²⁸ In the current study we adopted a constant total area as it equates global stimulus energy (contrast) among different numerosities. On regular trials, the circles were black. However, groups of white circles with the same numerosity were randomly presented instead of black ones on approximately 9% of the trials. Participants were asked to respond to these presentations by means of a button press and foveate the middle of the fixation cross during the entire experiment. No numerosity-related judgments were required. Average task performance was 97.4% correct (range 69.8 - 100% for individual runs).

Stimuli were presented for 350 ms, followed by 250 ms of mean luminance gray. Numerosities 1 to 7 were displayed sequentially, first in ascending order and then in descending order. Each numerosity was presented six times (3600 ms including mean luminance gray presentations) before the next numerosity was presented. Numerosity 20 was presented after each ascending or descending sweep. These presentations of numerosity 20 lasted 14400 ms (24 presentations). The combination of the ascending and descending sweep interleaved with numerosity 20 was repeated 4 times for each run. Each run started with 20 presentations of numerosity 20 (12000 ms). The total presentation time for one run was 5 min 28.8 s. The stimulus sequence and displays were near-identical to those previously used by Harvey and colleagues.^{28,31} Participants completed 7 (1 participant), 8 (2 participants) or 9 (5 participants) runs.

MRI and fMRI acquisition

High resolution anatomical and functional data were acquired using a Philips Achieva 7 T scanner with a maximum gradient strength of 40 mT/m and a slew rate of 200 T/m/s (Philips, Best, the Netherlands). A dual-channel volume transmit head coil was used for all scans (Nova Medical, MA, USA). A 32-channel receive coil (Nova Medical, MA, USA) was used for all anatomical scans, and two custom-built 16-channel high-density surface receive arrays were used for all functional scans⁵⁶ (MRCoils BV). Positioning of these receive arrays was performed outside the scanner. First, participants put on a cloth cap to prevent sliding of the surface arrays on their hair and facilitate accurate targeting of the region of interest. Next, the receive arrays were placed on the head, with their centers at approximately the height of electrode location CPz (following the extended 10-20 system for electrode positioning), aligned with the midline of the head of the participant. The receive arrays touched each other but did not overlap. The exact positioning was varied per participant, as the process was ultimately guided by the personalized location of the most prominent topographic numerosity map in parietal cortex (NPC1), based on previous numerosity fMRI studies for all but one participant. After positioning, the receive arrays were fixed in place using PowerFlex self-adherent tape. The participant was then carefully transferred to the scanner environment. The participant was positioned such that the middle of the receive arrays (in the volume transmit coil) was approximately in the isocenter.

T1-weighted anatomical data for four participants were acquired using an MP2RAGE sequence.⁵⁷ Sequence parameters were: T11 = 800 ms, T12 = 2700 ms, TR_{MP2RAGE} = 5500 ms, TR/TE = 6.2/2.3 ms, flip angle $\alpha_1 = 7^\circ$, and $\alpha_2 = 5^\circ$, bandwidth = 403.7 Hz/pixel, acceleration factor using SENSE encoding = 3.5×1.3 (RL and AP respectively), resolution = 0.65 mm isotropic, whole-brain coverage, total scan time 9 min 57 s. T1-weighted anatomical data for the other three participants was acquired using an MP2RAGE sequence combined with a proton-density (PD)-weighted sequence. The PD-weighted acquisition was used to correct for image intensity inhomogeneities. Sequence parameters for the MP2RAGE acquisition were: TR/TE = 7/2.8 ms, flip angle $\alpha = 8^\circ$, resolution = 0.8 mm isotropic, whole-brain coverage, total scan time 4 min 56 s. Sequence parameters for the PD acquisition were: TR/TE = 5.8/2.8 ms, flip angle $\alpha = 1^\circ$, resolution = 0.8 mm isotropic, whole-brain coverage, total scan time 1 min 16 s. The field of view, orientation, and location of the PD scan was identical to the MP2RAGE. T1-weighted anatomical data for one participant were acquired on a 3T MRI scanner. Sequence parameters were: TR/TE = 10/4.6 ms, flip angle $\alpha = 8^\circ$, resolution = $0.80 \times 0.75 \times 0.75$ mm, whole-brain coverage.

Functional data were acquired using a T2*-weighted 3-dimensional multi-shot EPI (3D-EPI, two shots per slice, 24 slices, 48 shots overall). The sequence parameters were: TR/TE = 60/28.9 ms, flip angle: 20° , acceleration factor using SENSE encoding: 3.5 (right-left) $\times 1.3$ (anterior-posterior), echo planar factor: 37, voxel size = 0.93 mm isotropic, FOV = 119 (right-left) $\times 119$ (feet-head) $\times 22.32$ (anterior-posterior) mm, 24 coronal slices, and 10% oversampling in the slice direction. The volume repetition time was 1200 ms. Each functional run was 274 time frames in duration. The acquisition volume was rotated approximately 40° in the sagittal plane to assure maximum coverage of the region of interest (Figure 1B). The exact angulation differed between participants, depending on their orientation in the scanner and location of the region of interest.

For each functional run, we acquired an additional five volumes with reversed right-left phase-encoding. Reversing the phase-encoding direction ensured that these so-called 'topup' runs had the opposite geometrical distortions to the original volumes.⁵⁸

Pre-processing: anatomical data

Gray/white matter classifications of the MP2RAGE anatomical data (four participants) were carried out using MIPAV (<http://mipav.cit.nih.gov/>) with the CBS-tools plugin⁵⁹ (<https://www.nitrc.org/projects/cbs-tools/>). For the MP2RAGE anatomical data (four

participants), gray/white matter classification was performed using Freesurfer (<http://surfer.nmr.mgh.harvard.edu/>). All classifications were manually optimized. After registration to the functional data (see below), these classifications were used to compute volume-preserving distance maps between the gray-white matter (GM/WM) border and the gray matter-cerebrospinal fluid (GM/CSF).⁶⁰ These were computed using the Nighres processing tools.⁴⁰

Pre-processing: functional data

Data processing was performed using AFNI⁶¹ and MATLAB (Mathworks; 2015b). First, a warp field to correct for susceptibility distortions was calculated using a nonlinear transformation, with the last five de-obliques volumes of each functional run and the first five de-obliques volumes of each topup run as input. Subsequently, motion parameters within and between runs were estimated by aligning the volumes of each run to the last volume of the respective run (within run motion), and aligning the last volume of each run to the last volume of the first run (between run motion) using Fourier interpolation, and regressed out. Next, the first 10 volumes of each run were removed to ensure that the signal had reached a steady state. All runs were despiked using the AFNI function 3dDespike. This step was implemented to remove spurious large fluctuations in signal amplitude between two time points. These spikes were replaced with the average of the closest two non-spike time points. Then, each run was divided into four equal parts of 66 volumes each, corresponding with the four stimulus cycles. These parts were then scaled, converting the time-series information to percentage signal change by dividing the signal of each voxel by its temporal mean, multiplying that signal with 100, and subsequently subtracting 100 to ensure that the temporal mean of that voxel was zero percent signal change. First-degree polynomial detrending was then performed on all scaled partial runs to remove linear in- or decreases in the signal, using the AFNI function 3dDetrend. All detrended, scaled partial runs were then averaged and the between partial-run average temporal mean of each voxel was added to create the mean time-series. This time-series volume was then used as input for the numerosity modeling (see below under 'Numerosity modeling'). The mean time-series and resulting model estimate volumes were subsequently de-obliques, and the previously computed warp field to correct for susceptibility distortions was applied.

Next, the mean EPI image was computed by taking the temporal mean of the mean time-series volume. This EPI mean image was initially registered to the anatomy using a multi-step procedure. The resulting transformation matrices from this procedure were combined and subsequently applied to the anatomical data to avoid resampling of the functional data. First, the anatomy was restricted to roughly the parietal lobe using the AFNI function @clip_volume. Next the EPI mean image was de-obliques using the AFNI function 3dWarp. Then, the de-obliques EPI mean image and anatomy were brought into the same space by aligning the center of mass of the anatomy to the de-obliques EPI mean image. Then, the 'Nudge dataset' AFNI plugin was used to manually shift and rotate the de-obliques EPI mean image to provide a good starting point for two automated registration steps. These registration steps consisted of affine transformations to further optimize the registration, using local Pearson correlation as cost function.⁶² The first transformation allowed for a maximum rotation and/or shift of 3 mm in any direction, while the second transformation allowed for a maximum rotation and/or shift of 1 mm. These affine transformations were then applied to the manually moved, mean de-obliques EPI image. The transformation matrices of the manual step and the two affine transformations were combined into a single affine matrix. This matrix was then inverted and applied to the restricted anatomy to register it to the de-obliques EPI mean image. The registered anatomy was then resampled to the 0.93 mm isotropic resolution and grid of the EPI mean image, and subsequently inspected visually for registration quality. The same procedure was then applied to the gray-white matter classifications.

Lastly, using the anatomy volume-preserving distance map volumes (see 'Pre-processing: anatomical data'), the warped model estimate volumes (cross-validated explained variance, tuning width, and preferred numerosity) were sampled across 9 cortical depths using the laminar profile sampling function in Nighres, resulting in computational 'columns' (see 'Cortical depth analysis' below for further details).

QUANTIFICATION AND STATISTICAL ANALYSIS

Numerosity modeling

Numerosity tuning for each voxel was estimated from the mean time-series data and the stimulus time course, using methods very similar to population receptive field (pRF) mapping.¹ These methods have previously been described by Harvey and colleagues.²⁸ This analysis was performed using MATLAB, the Vistasoft software package (<http://white.stanford.edu/software/>) and custom numerosity-modeling code from Harvey and colleagues.^{28,31}

In short, we estimated the aggregate numerosity tuning of the hemodynamic consequences of the activity of neuronal populations in each voxel. This numerosity tuning consisted of a preferred numerosity, and a tuning width for each (numerosity-selective) voxel. A forward model was used to predict the aggregate population response based on the overlap of the presented numerosity at each time point in time and the numerosity tuning. This model estimated preferred numerosity and tuning width in logarithmic space as the mean and standard deviation of a Gaussian, respectively. Next, the predicted population response was convolved with a canonical two-gamma hemodynamic response function (HRF⁶³) to make a predicted fMRI time-series. This process was repeated to create a set of predicted fMRI time-series for all combinations of a large range of preferred numerosity (67 equal steps between 0 and 2 (corresponding to 1–7.4 in linear space)) and tuning width (400 equal steps between 0.03 and 3) parameters. Note that this results in a maximum tuning width of 148 for the largest preferred numerosities. The predicted time-series were then compared to the fMRI time-series of each voxel, and the best fitting parameters for each voxel were chosen by minimizing the sum of squared errors between the predicted and measured time-series (Figures 1C and 1D). The best-fitting preferred numerosity and tuning width were

then converted to linear space, resulting in an asymmetric Gaussian with a peak at the preferred numerosity and an tuning width estimate represented by its full width at half maximum (FWHM,²⁸ inset [Figure 1E](#)). These procedures were performed for all voxels within the field of view of the functional scans for each participant, including the white matter. This resulted in preferred numerosity and tuning width estimates, along with the explained variance of these estimates for each voxel. Next, the fitting procedure was repeated using only the odd and even runs. The parameter estimates from the odd runs and the timeseries data of the even runs and vice versa were then used to estimate the cross-validated model explained variance. The two cross-validated explained variance values for each voxel were then averaged. All reported explained variance/ R^2 values are cross-validated.

Cortical depth analysis

Nine levelsets⁴⁰ were generated from white matter to the gray matter surface. A cortical depth profile was generated by iteratively growing the normal vectors from the middle levelset to intersect the closest levelset toward the gray matter surface, and storing the corresponding coordinates. The same procedure was adopted to grow the profile toward white matter from the middle levelset. The two sets of coordinates were merged to form the coordinate set of a single cortical depth profile. The value assigned to each point along the profile was obtained by linear interpolation (e.g.: the profiles storing variance explained information were obtained via linear interpolation of the variance explained volume along each profile's cortical depth coordinates). One profile was assigned to each voxel within gray matter, based on the nearest obtained profile coordinate. The previous calculations were performed using Nighres. All subsequent calculations were performed using MATLAB, unless stated otherwise. Profiles from neighboring voxels sample from similar cortical locations, thus are very similar and provide redundant information. To mitigate these redundancies, we selected profiles from voxels at a given cortical depth (between 0.15 and 0.25) for further analysis. Changing the selection criteria to a different range across cortical depth did not significantly change the results (for results using a cortical depth between 0.75 and 0.85, see [Figures S3C](#) and [S3F](#)). Next, only those profiles for which the cross-validated model explained variance at each of the nine depths was equal to or higher than the 99th percentile cross-validated R^2 in the white matter voxels included in the field of view of the functional scans for each participant, were included in the subsequent processing steps. The average number of selected profiles was $M = 217$ ($SD = 117$, range = 90 - 392), while the average individual cross-validated R^2 threshold was $M = 8\%$ ($SD = 2$ range = 5%–12%). Next, we estimated a bootstrapped median fit on the preferred numerosity and tuning width for each cortical depth bin using R. These median fits were subsequently sampled at numerosity 2 and 3 to obtain the fitted tuning width. These numerosities were chosen as they were the most frequently present values on average ([Figure S1B](#)). These samples were Z-transformed for each sampled numerosity and participant separately, resulting in Z-transformed median tuning width profiles across cortical depth for each participant for numerosity 2 and 3. Next, a quadratic fit was performed for each profile, and the predictor estimates (intercept, slope, and quadratic component) of all participants were tested against zero using a one-sample t test. For a direct comparison, we repeated this procedure, using only a linear fit (intercept and slope). For visualization purposes, a quadratic fit was performed on the aggregate Z-transformed data of all participants together, separately for each numerosity of interest ([Figures 3A](#) and [3C](#)). A reverse Z-transform was then performed for this fit ([Figures 3B](#) and [3D](#)).

As mentioned at the beginning of the results sections, due to the cortical folding of the cerebral cortex, the center of a voxel on the regular grid (isotropic acquisition, 0.93mm) could be located close to the cerebro-spinal fluid surface, close to the white matter surface or anywhere between the two, thus allowing a denser sampling rate along cortical depth. This comes at the cost of partial volume effects that can especially influence voxels located at the edge of the gray matter (close to the cerebro-spinal fluid surface, close to the white matter surface), we acknowledge this limitation. We mitigate this limitation by re-analyzing our data, excluding the outermost depth bins (close to cerebrospinal fluid surface) to reduce the potential partial volume effects. These results can be found in [Figures S3B](#) and [S3D](#), confirming our main findings, showing that these are unlikely to be the result of partial volume effects.