Laminar-specific functional connectivity mapping with multi-slice line-scanning fMRI

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37 ABSTRACT

38 Laminar BOLD-fMRI has been applied to better depict the neuronal input and output circuitry and functional
 39 connectivity across cortical layers by measuring local hemodynamic changes. Despite extensive studies detecting
 40 laminar fMRI signals to illustrate the canonical microcircuit, the spatiotemporal characteristics of laminar-specific
 41 information flow across different cortical regions remain to be fully investigated in both evoked and resting states.
 42 Here, we developed a multi-slice line-scanning fMRI (MS-LS) method to detect laminar fMRI signals in adjacent
 43 cortical regions with high spatial (50 μ m) and temporal resolution (100 ms) in anesthetized rats. Across different
 44 scanning trials, we detected both laminar-specific positive and negative BOLD responses in the surrounding cortical
 45 region adjacent to the most activated cortex under evoked condition. Specifically, in contrast to the typical Layer
 46 (L) 4 correlation across different regions due to the thalamocortical projections for trials with positive BOLD, a
 47 strong correlation pattern specific in L2/3 was detected for the trials with negative BOLD in adjacent regions, which
 48 indicate a brain state-dependent laminar-fMRI responses based on corticocortical interaction from different trials.
 49 Also, we acquired the laminar-specific rs-fMRI signals across different cortical regions, of which the high
 50 spatiotemporal resolution allows us to estimate lag times based on the maximal cross-correlation of laminar-specific
 51 rs-fMRI signals. In contrast to the larger variability of lag times in L1 and 6, robust lag time differences in L2/3, 4,
 52 and 5 across multiple cortices represented the low-frequency rs-fMRI signal propagation from the caudal to the
 53 rostral slice. In summary, our work provides a unique laminar fMRI mapping scheme to better characterize trial-
 54 specific intra- and inter-laminar functional connectivity with MS-LS, presenting layer-specific spatiotemporal
 55 variation of both evoked and rs-fMRI signals.

56 INTRODUCTION

57 Resting-state (rs-) fMRI is widely utilized in clinical and cognitive neuroscience for functional connectivity
 58 mapping, attributing to its unique capability to identify low frequency oscillation arising from neuronal oscillation
 59 across different brain states (1-5). The conventional method of rs-fMRI is based on echo-planar-imaging (EPI) to
 60 detect T2*-weighted blood-oxygen-level-dependent (BOLD) signal low-frequency fluctuation across functionally
 61 connected brain regions (6). This mapping method has been increasingly utilized to parcellate functional networks
 62 across the whole brain (7-11). Lately, high field fMRI has also been utilized in the mapping scheme to identify
 63 dynamic BOLD responses and cerebral blood volume (CBV) signals with layer specificity in bottom-up or top-
 64 down tasks (12-18), indicating the possible functional network across different cortical layers. However, the spatial
 65 resolution of fMRI images is often limited by the demanding requirement for high temporal resolution, i.e., fast-
 66 sampling of rs-fMRI oscillation patterns, at sub-second with sufficient signal-to-noise ratio (SNR) (19-21). In
 67 addition, potential aliasing effects from the cardiorespiratory artifacts (22-24) contaminate fMRI signals, leading to
 68 spurious results for rs-fMRI studies without sufficient sampling rates. It remains challenging to achieve high spatial
 69 resolution with a fast sampling rate to acquire rs-fMRI signals with sufficient SNR for brain functional mapping.

70 Previously, Yu et al. have developed a line-scanning fMRI method to substantially improve spatiotemporal
 71 resolution in rat brains, achieving 50- μ m spatial resolution along a line profile which covers different cortical layers
 72 within 50-ms TR (25). Line-scanning fMRI has also been used in combination with fiber-based optogenetic
 73 stimulation (26) or diffusion-sensitizing gradients (27) to study the fast functional onset with high temporal
 74 resolution. Beyond preclinical studies, line-scanning fMRI is also being utilized in human fMRI studies to provide
 75 functional maps of cortical layers in a reduced field-of-view (FOV) with high spatiotemporal resolution (28-30).
 76 Lately, a similar line-scanning strategy has been used to map diffusion signals in the human brain (31). To date,
 77 however, no fMRI measurements have been performed on multiple cortical regions manifesting laminar-specific
 78 functional connectivity with high spatiotemporal resolution comparable to the original line-scanning fMRI method.

79 In this work, we extended the line-scanning fMRI method towards multi-slice acquisition to acquire line profiles
 80 from different cortical regions with high spatial (50 μ m) and temporal (100 ms) resolution. To investigate functional
 81 connectivity driven by the underlying neural network, we analyzed the intra- and inter-laminar correlation features
 82 under both evoked and resting-state conditions in anesthetized rats. Upon electrical stimulation of the forepaw,
 83 adjacent cortical regions to the forepaw somatosensory cortex (FP-S1) showed either positive or negative BOLD
 84 responses with distinct laminar-specific correlation patterns across different fMRI trials. Meanwhile, owing to the
 85 fast sampling rate, we were able to detect laminar-specific lag times of rs-fMRI signals with low-frequency
 86 fluctuation, showing specific signal propagation (< 0.1 Hz) across different cortices. Our work demonstrates the

feasibility of multi-slice line-scanning fMRI (MS-LS) to identify evoked laminar correlation patterns and resting-state signal propagation across different cortical regions.

RESULTS

Mapping evoked laminar-specific BOLD signals with multi-slice line-scanning fMRI

We first developed the MS-LS method (Fig. 1A) to map laminar-specific BOLD responses from different cortical regions covering FP-S1 and adjacent cortices in one hemisphere (25) (details in **Method**). Left forepaw stimulation (Fig. 1B) activated contralateral cortical FP-S1, which has been reliably identified by using the conventional echo-planar-imaging (EPI) method (Fig. 1A) (32-34). As shown in Fig. 1C, three different line-scanning profiles from the corresponding cortical regions (i.e., rostral, middle, and caudal slices) were acquired by switching off phase-encoding gradient and defining the field-of-view (FOV) with two saturation slices to avoid aliasing from regions outside the FOV (Fig. 1A). Evoked BOLD-fMRI signals in three slices were simultaneously recorded as a function of time across the cortical depth (0-2 mm), showing the most salient BOLD responses in the caudal slice with decreased BOLD signals from the middle to the rostral slice as shown in Fig. 1D-F.

Figure 1. Evoked BOLD responses using the MS-LS method. **A.** Schematic illustration of the multi-laminar line-scanning experimental design on EPI-BOLD activation maps of different cortical regions overlaid on anatomical RARE images. Three different regions (white boxes) between two saturation slices (green boxes) were targeted from a rostral (Ro) to a caudal (Cd) region covering FP-S1 of one hemisphere. **B.** Left forepaw electrical stimulation (3 Hz, 4 s, 2.5 mA) following an fMRI design paradigm (1 s off, 4 s on and 15 s off). **C.** Representative three different line-scanning profiles from the corresponding slices. Each colored box indicates the corresponding cortical region. **D-F.** Average results from all data sets ($n = 18$ trials of 4 rats). **D.** Demeaned fMRI time series of raw (black) and filtered (magenta, orange, light purple, bandpass: 0.01-0.1 Hz) data (average of 40 voxels) in the rostral (upper), middle (middle), and caudal (lower) cortical regions under the evoked condition. Red boxes indicate 32 epochs for 10 min 40 sec. **E.** Normalized line profile maps showing the laminar-specific fMRI responses across the cortical depth (0–2 mm) in the three cortical regions (40 voxels with 50 μ m resolution). The time courses

were processed by band-pass filtering (0.01-0.1 Hz). **F.** Average BOLD changes of the individual voxel time courses (mean epoch with 20 s) across the cortical depth in the three cortical regions.

Distinguishing the inter- and intra-laminar functional connectivity across different cortices

Besides the typical positive BOLD signal detected in FP-S1 and adjacent area upon stimulation (25,26,35), negative BOLD signals were also reported in the adjacent cortex of activated regions (36-40). We applied MS-LS to characterize the laminar-specific correlation patterns of varied BOLD signals in cortical areas adjacent to activated FP-S1. As shown in **Fig. 2A**, highly varied BOLD responses in the rostral slice were observed across all trials with left forepaw stimulation. We sorted individual trials into two groups based on BOLD responses of the rostral slice, showing group 1 with positive and group 2 with negative BOLD signals (**Fig. 2A** and **S1**). **Fig. 2B** shows the laminar-specific BOLD responses across the three different slices for both groups. The spreading negative BOLD responses were observed at the superficial layers in the rostral slice and salient undershoot of the BOLD signal was detected in the middle slice in group 2 in contrast to the monophasic positive BOLD responses across the three slices in group 1. This result demonstrates that distinct BOLD responses in adjacent cortices to activated FP-S1 can be specified by MS-LS with laminar specificity.

Next, the intra-and inter-laminar correlation maps (**Fig. 2C**) were calculated to obtain group-averaged results. In the 3×3 matrix (**Fig. 2D** and **2F**), the intra-laminar correlation maps were arranged on the diagonal blocks, while the inter-laminar correlation maps (i.e., rostral-middle, rostral-caudal, and middle-caudal correlation maps) were arranged on the off-diagonal blocks. From the intra-laminar correlation analysis, higher correlation coefficients were observed in superficial layers over deep layers of caudal and middle slices in both groups, but the rostral slice showed stronger correlation at Layer (L) 4 in group 1 versus L2/3 in group 2 (**Fig. 2E** and **2G**). Also, the inter-laminar correlation coefficients in group 1 showed higher values in L4 over other layers, presumably presenting robust BOLD signal correlation of FP-S1 through thalamocortical projections (25,41,42). In contrast, the correlation coefficients for Ro-Mi and Ro-Ca in group 2 showed higher values in L2/3, possibly presenting a negative BOLD signal-dominated correlation through corticocortical projection-mediated lateral inhibition (43-47). These results demonstrate distinct layer-specific correlation features underlying the positive and negative BOLD at the cortical areas adjacent to activated FP-S1, indicating brain state-dependent laminar fMRI responses across different trials.

Mapping the laminar-specific functional connectivity in rs-fMRI

The MS-LS method could also be used to investigate the laminar-specific correlation features of rs-fMRI signals from the different cortical regions. **Fig. 3A** shows representative Z-score normalized time courses (average of 40 voxels) from the rostral, middle, and caudal slices, as well as the 2D line profile rs-fMRI maps as a function of time across the cortical depth, specifically presenting a highly synchronized slow oscillatory pattern. **Fig. 3B** shows the inter- and intra-laminar correlation maps of the rs-fMRI signals, presenting the highly correlated low-frequency

154 signal fluctuation distributed in different cortical layers and across multiple cortical areas. In particular, the power
 155 spectral density (PSD) analysis of the rs-fMRI signal fluctuation shows peaked oscillatory powers at 0.01-0.02 Hz
 156 across different cortical areas (**Fig. 3C**), which is consistent with the previous study in anesthetized rats (48). Based
 157 on intra-laminar cross-correlation analysis, earlier lag times were detected in the middle layers (L2/3, 4, 5, median:
 158 -0.157, -0.207, -0.171 sec), whereas significantly later lag times were detected in the deep layer (L6, median: 0.216
 159 sec) when compared to the mean BOLD signal of all the layers (**Fig. 3D**). To examine how the rs-fMRI signal
 160 propagated across different cortical areas, we calculated inter-laminar lag times by comparing layer-wise BOLD
 161 signals between slices of Ro-Mi, Mi-Ca, and Ro-Ca (**Fig. 3E**). The lag times were not uniform across different
 162 layers. For the Ro-Ca case, it shows clear fMRI signal propagation from the caudal to the rostral slice at L2/3 and
 163 4 (-0.806 ± 0.703 sec, -0.577 ± 0.651 sec). Similarly, the Ro-Mi case shows earlier fMRI signal fluctuation at L2/3
 164 to 6 from the middle to the rostral slice, but opposite at L1 (0.223 ± 0.810 sec), which may be caused by the large
 165 draining veins distributed across the middle slice (**Fig. 3A**). For the Mi-Ca case, the different lag time was only
 166 observed at L6, showing significantly later fMRI signal fluctuation at the caudal slice (0.777 ± 0.616 sec). These
 167 results demonstrate resting-state laminar-specific temporal correlation features across different cortical areas, which
 168 can be attributed to underlying either neuromodulation or vascular distribution across different cortical layers.

169

Figure 3. A. *Left:* Demeaned rs-fMRI time series of raw and filtered (bandpass: 0.01-0.1 Hz) data (average of 40 voxels) in rostral (upper), middle (middle), and caudal (lower) cortical regions from one representative trial (10 min 40 sec). *Right:* Normalized line profile maps showing the laminar-specific responses across the cortical depth (0 – 2 mm, 40 voxels) in the three different regions. The maps were processed by band-pass filtering (0.01-0.1 Hz). **B.** Laminar-specific correlation maps from the same trial in **(A)**. In a 3×3 matrix, diagonal and off-diagonal blocks represent intra- and inter-laminar correlation maps, respectively. The black arrows (**A** and **B**) in the middle region indicates the BOLD effects of large draining vein around the superficial layers (L1 and L2/3). **C.** The PSDs of Z-score normalized fMRI time series (average of 40 voxels) showing the peaked low-frequency power at 0.01-0.02 Hz (purple arrows). **D** and **E.** Average results ($n = 18$ trials of 4 rats) representing layer-wise intra- (**D**) and inter-laminar (**E**) lag times. **D.** The middle layers (L2/3, 4, 5) show earlier lag times whereas L6 shows significantly later lag times. **E.** In Ro-Mi, Mi-Ca, and Ro-Ca lag times, L2/3, 4, 5 had earlier lag times (except L5 in Mi-Ca) whereas L1 and 6 had significantly later (except L1 in Mi-Ca) lag times, clearly showing the caudal to the rostral rs-fMRI signal propagation via L2/3 and L4. All significant tests were performed with one-way ANOVA (post-hoc: p-value <0.05, Bonferroni correction).

181 DISCUSSION

182 In this work, we applied the MS-LS method to identify intra- and inter-laminar correlation patterns of evoked and
183 resting-state fMRI signals with high spatial and temporal resolution across multiple cortical regions. Distinct
184 laminar-specific correlation patterns in the cortex adjacent to the activated FP-S1 were detected based on either
185 positive or negative BOLD responses, which may be regulated by altered brain states across different fMRI trials
186 in anesthetized rats (36,49-51). The MS-LS mapping scheme is also applied to map the laminar correlation pattern
187 of low frequency rs-fMRI signal fluctuation, presenting sub-second lag times of rs-fMRI signal propagation through
188 cortical layers and across different cortical regions given the fast sampling rate.

189 An interesting observation of evoked laminar fMRI signal using the MS-LS method is the negative BOLD responses
190 detected in the rostral region adjacent to FP-S1. Specifically, this temporal feature of the negative BOLD signal is
191 different from the initial dip of positive BOLD as reported previously (52,53). It remains unclear whether the
192 negative BOLD response has vascular or neuronal origins (54-64). As indicated by extensive studies, the BOLD
193 signal relies on the integrated interactions of cerebral blood flow and volume (i.e., CBF and CBV) changes with
194 metabolic rate of oxygen consumption (CMRO₂) which are in principle caused by balanced proportional changes
195 in both excitatory and inhibitory neuronal activity (65-67). Putative mechanisms of negative BOLD related to
196 specific hemodynamic responses suggest two sources: i) the remaining elevation of CBV in contrast to returning to
197 baseline CBF and CMRO₂ (54-57), ii) the decoupling between CBF and CMRO₂ during or after stimulation (57-
198 59). Also, based on the vascular blood supply, a local blood-stealing effect from adjacent cortices to provide more
199 blood to the most activated region is also proposed (60,61). With direct neuronal activity recording, the suppression
200 of neuronal activity in the adjacent cortical regions due to lateral inhibition has also been reported (62-64). The first
201 two mechanisms (i and ii) for signal decrease below baseline following the BOLD activation profile are likely
202 explained by the prolonged post-stimulus undershoot in activated brain regions. As shown in **Fig. 2B**, while the

negative BOLD mainly occurred at the superficial layer where large arteries may contribute to CBV signal changes, robust negative BOLD signal was also detected at the L2/3 (blue color in the color bar). Also, the negative BOLD signal was maintained with longer spreading function than typical positive BOLD responses excluded the only contribution from CBV and CBF effects, suggesting a sustained corticocortical post-synaptic inhibition (**Fig. S1 A**) (47,68). It is also noteworthy that the negative BOLD group (group 2) shows the salient biphasic HRF of the positive BOLD, of which the post-stimulus undershoot may be also caused by the decreased neuronal activity (43).

Using the MS-LS method, we also detected the robust rs-fMRI slow oscillation pattern across cortical layers of three slices with a peak frequency power at 0.01-0.02 Hz. Previous studies with simultaneous fMRI and optical fiber-based Ca^{2+} recording in anesthetized rats have reported that neuronal calcium oscillations underlie the low frequency rs-fMRI signal fluctuation near 0.01-0.04 Hz (48). Also, intrinsic astrocytic Ca^{2+} transients have been reported to mediate global negative BOLD signals contributing to the low-frequency rs-fMRI signal fluctuation (69), suggesting brain state-dependent global neuromodulation of the ultra-slow oscillatory patterns (70-72). Moreover, negative global rs-fMRI during ultra-slow oscillation is also linked with pupil dynamics, showing converged effect of arousal state fluctuation and autonomic regulation (73-77). In contrast to the brain-wide rs-fMRI mapping of previous studies, we revealed laminar-specific signal propagation of the low frequency rs-fMRI signal fluctuation (**Fig. 3E**). Interestingly, the signal propagation direction varied at the different cortical layers. We observed more uniform lag times from L2/3 to L5 between different cortical regions, but largely varied lag times at L1 and L6. It is plausible that rs-fMRI signal oscillation in L2/3 to L5 is primarily driven by the global neuromodulation through subcortical projections (78), but the signal oscillation in L1 and L6 more likely relies on the different vascular density distribution that contributes to dynamic BOLD responses (79-82).

Technical limitations pertaining to MS-LS acquisition should be considered for future work. As a modification to the conventional line-scanning method (25), two saturation RF pulses were followed by three excitation RF pulses to acquire fMRI signals from three slices using MS-LS method. Given a certain set of sequence parameters (e.g., TE, readout bandwidth, etc), imperfect performances of the saturation RF pulses result in contaminating fMRI signals, due to aliasing artifacts that arise from fast T1 relaxation of tissue signals in outside of the FOV. It can be avoided by applying a region-of-interest selective refocusing RF pulse (83-85) instead of saturation RF pulses. Moreover, in the interleaved acquisition of MS-LS, we have longer TR (100 ms in this work compared to 50 ms TR of the conventional method), thus preserving readout bandwidth due to tradeoff between tSNR enhancement and minimum TR. For the future work, implantable inductive coils (86) or wireless amplified NMR detector (87,88) can be applied to enhance tSNR and to achieve a fast sampling rate simultaneously.

METHODS

Animal preparation. The study was performed in accordance with the German Animal Welfare Act (TierSchG) and Animal Welfare Laboratory Animal Ordinance (TierSchVersV), in full compliance with the guidelines of the EU Directive on the protection of animals used for scientific purposes (2010/63/EU). The study was reviewed by the ethics commission (§15 TierSchG) and approved by the state authority (Regierungspräsidium, Tübingen, Baden-Württemberg, Germany). A 12-12 hour on/off lighting cycle was maintained to assure undisturbed circadian rhythm. Food and water were obtainable ad libitum. A total of 4 male Sprague–Dawley rats were used in this study.

Anesthesia was first induced in the animal with 5% isoflurane in the chamber. The anesthetized rat was intubated using a tracheal tube. A mechanical ventilator (SAR-830, CWE, USA) was used to ventilate animals throughout the whole experiment. Femoral arterial and venous catheterization was performed with polyethylene tubing for blood sampling, drug administration, and constant blood pressure measurements. After the surgery, isoflurane was switched off, and a bolus of the anesthetic alpha-chloralose (80 mg/kg) was infused intravenously. After the animal was transferred to the MRI scanner, a mixture of alpha-chloralose (26.5 mg/kg/h) and pancuronium (2 mg/kg/h) was constantly infused to maintain the anesthesia level for reduced motion artifacts.

EPI fMRI acquisition. All data sets from rats were acquired using a 14.1T/26 cm (MagneX, Oxford) horizontal bore magnet with an Avance III console (Bruker, Ettlingen) and a 12-cm diameter gradient system (100 G/cm, 150 μ s rising time). A home-made transceiver surface coil with 6-mm diameter was used on the rat brain in all experiments. For the functional map of BOLD activation (**Fig. 1A**), a 3D gradient-echo EPI sequence was acquired with the following parameters: TR/TE 1500/11.5 ms, FOV $1.92 \times 1.92 \times 1.92$ cm³, matrix size $48 \times 48 \times 48$, spatial resolution $0.4 \times 0.4 \times 0.4$ mm³. A high order (*e.g.*, 2nd or 3rd order) shimming was applied to reduce the main magnetic field (B₀) inhomogeneities at the region-of-interest. For anatomical reference of the activated BOLD map, a RARE sequence was applied to acquire 48 coronal images with the same geometry as that of the EPI images. The fMRI design paradigm for each trial comprised of 200 dummy scans to reach steady-state, 10 pre-stimulation scans, 3 scans during stimulation, and 12 post-stimulation scans with a total of 8 epochs.

MS-LS acquisition. GRE-based MS-LS datasets were acquired in anesthetized rats for evoked and rs-fMRI. The MS-LS method was applied by increasing the slice dimension (1 to 3) to record fMRI signals in three different cortical regions covering FP-S1 (*i.e.*, rostral, middle, and caudal cortices) and using two saturation slices to avoid aliasing artifacts along the phase-encoding direction (**Fig. 1A**). The phase-encoding gradient was turned off to acquire line profiles (**Fig. 1C**). Laminar-specific fMRI responses from the three cortices were acquired along the frequency-encoding direction with 50- μ m spatial resolution. The following acquisition parameters were used: TR/TE 100/9 ms, TA 10 min 40 sec, FA 50°, slice thickness 1.2 mm, slice gap 1.5 mm, FOV 6.4×3.2 mm², and matrix 128×32 . The fMRI design paradigm for each epoch consisted of 1 second pre-stimulation, 4 seconds

stimulation, and 15 seconds post-stimulation for a total of 20 seconds. A total of 6400 lines (i.e., 10 min 40 sec) in each cortex were acquired in every single trial for evoked and rs-fMRI. Evoked BOLD activation was identified by performing electrical stimulation to the left forepaw (300 μ s duration at 2.5 mA repeated at 3 Hz for 4 seconds).

Data Analysis. All signal processing and analyses were implemented in MATLAB software (Mathworks, Natick, MA) and Analysis of Functional NeuroImages software (89) (AFNI, NIH, USA). For evoked fMRI analysis in **Fig. 1A**, the hemodynamic response function (HRF) used was the default of the block function of the linear program 3dDeconvolve in AFNI. BLOCK (L, 1) computes a convolution of a square wave of duration L and makes a peak amplitude of block response = 1, with $g(t) = t^4 e^{-t} / [4^4 e^{-4}]$. Each beta weight represents the peak height of the corresponding BLOCK curve for that class. The HRF model is defined as follows:

$$HRF(t) = \int_0^{\min(t, L)} g(t-s) ds$$

Cortical surfaces were determined based on signal intensities of fMRI line profiles (**Fig. 1C**). The detailed processing procedure was conducted as described in the previous line-scanning study (25). For **Fig. 1D** and **3A**, demeaned fMRI time courses were used as follows: $(x - \mu)$, where x was the original fMRI time courses and μ was the mean of the time courses. The line profile map concatenated with multiple line-scanning fMRI profiles was normalized by its maximum intensity. Average BOLD time series and percentage changes were defined as $(S - S_0) / S_0 \times 100 \%$, where S was the BOLD signal and S_0 was the baseline. S_0 was obtained by averaging the fluctuation signal in the 1-second pre-stimulation window in evoked fMRI that was repeated every 20 seconds with the whole time series (640 sec). The BOLD time series in each ROI were detrended and bandpass filtered (0.01-0.1 Hz, FIR filter) before analyzing line profile maps, correlation coefficients, PSDs, and cross-correlation.

Temporal correlation analysis which is generally accepted as an indicator of corticocortical functional interaction, *e.g.*, increased correlation is thought to reflect increased functional connectivity between two brain sites (90,91). In this study, the correlation analysis was employed as an important indicator of laminar-specific functional connectivity across multiple cortical regions. Laminar-specific fMRI time courses were used and converted to the bandpass filtered time courses. The correlation is defined as follows:

$$Corr_{xy}(a, b) = \frac{\sum_{i=1}^n (x_{a,i} - \bar{x}_a)(y_{b,i} - \bar{y}_b)}{\sqrt{\sum_{i=1}^n (x_{a,i} - \bar{x}_a)^2 \sum_{i=1}^n (y_{b,i} - \bar{y}_b)^2}}$$

where x, y indicates filtered fMRI time series (n time points) and a, b indicates a voxel from one cortical region. The correlation was calculated by ‘corr’ function in MATLAB (Type: Pearson, Rows: pairwise).

For resting-state PSD analysis, fMRI time series was converted to the Z-score normalized time series. Subsequently, the converted time series was used to compare the frequency responses among the different regions avoiding the dependency of the difference in signal amplitudes (**Fig. 3C**). The Z-score normalized time courses were calculated as follows: $(x - \mu) / \sigma$, where x was original fMRI time courses and μ , σ were the mean and the standard deviation of the time courses, respectively (zscore function in Matlab). PSDs were calculated by Welch's estimation method (pwelch function in MATLAB, FFT length: 2000, overlap: 50%).

For lag time calculation in rs-fMRI, voxel-wise lag times were calculated by analyzing cross-correlation coefficients across all cortical layers or across different cortical regions (xcorr function in MATLAB). One laminar-specific lag time was determined as the time point with the maximal cross-correlation coefficient (**Fig. 3D and E**). The boundaries of different cortical layers were determined as provided in the previous line-scanning study (25).

For statistical analysis in evoked and rs-fMRI, one-way ANOVA was performed to compare laminar-specific values using the post-hoc test and to determine whether there were statistically significant differences among the associated population means in different cortical layers or areas. Student t-test was performed to divide all the trials into two groups (**Fig. S1 B**). The p-values < 0.05 were considered statistically significant.

Data availability. The data that support the findings of this study are available from the corresponding authors upon request.

Code availability. The related signal processing codes are available from the corresponding author upon reasonable request.

Competing interests. The authors declare no competing interests.

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526

Supplementary information

527

528 **Figure S1.** Evoked average BOLD time courses in the two groups. **A.** Average BOLD time courses (mean epoch with 20 sec) of average
529 voxel (a total of 40 voxels) representing that group 1 (11 trials) has positive BOLD while group 2 (7 trials) has negative BOLD. **B.** Student
530 t-test result with all the trials showing group 1 and 2 are significantly different (* $p = 1.5055 \times 10^{-6}$).