Supporting Online Material for

Reversal of Interlaminar Signal Between Sensory and Memory Processing in Monkey Temporal Cortex

Daigo Takeuchi, Toshiyuki Hirabayashi, Keita Tamura, Yasushi Miyashita*

*To whom correspondence should be addressed. E-mail: yasushi_miyashita@m.u-tokyo.ac.jp

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Subjects

All animal procedures complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Review Committee of the University of Tokyo School of Medicine. The subjects were two adult macaque monkeys (*Macaca mulatta* and *Macaca fuscata*, weighing 10-12 kg). A head holder and a recording chamber for electrophysiological recordings were implanted to the skull under standard aseptic, anesthetic and postoperative treatment protocols (anesthetic: sodium pentobarbital, 3 mg/kg/h, i.v., and xylazine, 2 mg/kg, i.m., supplemented as needed; analgesic: acetaminophen, 20 mg/kg/day, p.o.; prophylactic antibiotic: ampicillin, 100 mg/kg/day, i.m.) (1-2). Blood pressure, heart rate and oxygen saturation were monitored continuously during the surgery.

Using a 4.7-T magnetic resonance imaging (MRI) scanner and 100 mT/m actively shielded gradient coils (1), we obtained high-resolution structural images of the brain for each monkey (in-plane resolutions, 200×200 μm² and 300×300 μm² for monkeys P and E, respectively; slice thickness, 1mm).

Behavioral task

The procedure of the pair-association (PA) task was as described in detail previously (3-5). Briefly, monkeys were trained to perform a pair-association memory task using a set of 6 pairs of visual stimuli (12 monochrome Fourier descriptors) extending approximately 5° x 5° (Fig. 1A). Monkeys were trained for several months to learn all the pairings of stimuli. In each trial, following the presentation of a fixation point (0.2° in size) on the center of a computer screen for 500 ms, a cue stimulus (one of the 12 visual stimuli) was presented for 500 ms. After a delay period of 2000 ms, two stimuli were presented, one of which was the paired-associate of the cue stimulus, and the other was a distractor. The monkey obtained fruit juice as a reward for correctly touching the paired associate within 1500 ms. Throughout the recording sessions after training, the monkeys’ performance was > 90% correct. Eye movements were monitored with a PC-based CCD camera system (3-6), and if the eye position deviated more than 1.2°-1.5° (monkey P) or 3°-3.5° (monkey E) from the fixation point, the trial was automatically terminated.
Electrophysiological recordings

Extracellular recordings were conducted using linear-array multicontact electrodes (7-9) containing 16/24 recording contacts (impedance, 0.3 - 0.5 MΩ at 1 kHz) with an intercontact spacing of 150/100 μm to obtain unit activities (single- and multi-units) and local field potentials (LFPs). Neuronal activity was measured against a local reference that was close to the electrode contacts (a stainless guide tube or the hypodermic metallic shaft surrounding the multicontact electrode) to minimize far-field contributions to LFPs (9).

A linear-array multicontact electrode was inserted through a guide tube, lowered through the dura mater into the brain using a hydraulic microdrive manipulator, and positioned at area 36 (A36) (AP, 17 – 22 mm; ML, 13.5 – 15 mm) (Fig. 1B) (3-6). After coarsely positioning the electrode at the target area, we calculated current source-density (CSD) profiles (7-13) from the stimulus-evoked LFPs of the initial 50-100 trials of the task (for calculation of CSD profiles, see ‘Estimation of cortical layers using CSDs’ in Data Analysis section of the SOM), and obtained a finer estimation for the depth of the electrode. Then, the electrode position was adjusted so that neuronal signals were recorded from all cortical layers. Further adjustment of the electrode position was performed before obtaining the data so that at least one single-unit was isolated with as high a signal-to-noise (S/N) ratio as possible. Note that all the data in the present study including the CSD profiles were obtained after the final adjustment of the electrode position. The isolated single-unit activity was monitored online using the standard window discrimination technique throughout the recording (2-6, 14-15).

Neuronal signals recorded at each contact of the electrode were first fed into unity-gain operational amplifiers, and then fed into a second-stage amplifier. Each signal was then separated into two channels with different band-pass analog filters, with one channel designed to record higher-frequency spiking activities (250 Hz - 8 kHz), (‘spike-channel’), and the other designed to record lower frequency field potentials (0.7 - 170 Hz), (‘field-channel’) (16). For both the spike- and field-channels, a 1-pole Butterworth high-pass filter and a 4-pole Butterworth low-pass filter were used. These signals were digitized through a digital acquisition card, and were stored in a PC with a sampling rate of 31-40 kHz for later off-line analysis. At the end of each recording session, the anteroposterior and lateromedial coordinates of the electrode track were measured by x-ray imaging (2-6). In six penetrations (three penetrations in monkey E
and three penetrations in monkey P), multiple electrolytic lesions were made (1-2) by passing a direct current (10-20 μA for 10-30 s) at pre-selected contacts in each penetration. The lesion marks were identified by histological examinations following all the recordings for each monkey (Table S1).

Histological analyses

Histological analysis in the present study followed our standard protocols (1-2, 5). In brief, at the end of all the electrophysiological recordings, monkeys were deeply anesthetized with sodium pentobarbital (50-60 mg/kg, intravenous injection) and perfused with 4% paraformaldehyde in phosphate buffer (pH 7.4). The brains were cut into 40-μm-thick coronal sections, parallel to the plane of electrode penetrations. Serial sections were stained with cresyl violet for Nissl staining. For some sections, we additionally conducted Gallyas staining (17). The cytoarchitectonic border between A36 and area TE or area 35 was determined according to the criteria described in previous studies (18-19). Histological sections were photographed using standard bright-field optics in BZ-9000 microscope.

For penetrations with electrolytic lesion marks, we estimated the center of the lesion mark by fitting an eclipse on it, and utilized the multiple lesion marks on the histological section as references to estimate the laminar position of the electrode contact at which the earliest current sink was observed (see ‘Estimation of cortical layers using CSDs’ in the ‘Data analysis’ section) (13, 20-22). On the histological sections, we measured the distance between the estimated position of the earliest-sink contact and the center of the granular layer ($D_{ESC-GLC}$, Table S1). The thickness of the granular layer ($W_{GL}$, Table S1) was measured in histological sections containing lesion marks, and then the proportion of $|D_{ESC-GLC}|$ to $W_{GL}$ was calculated. In estimating $D_{ESC-GLC}$ and $W_{GL}$, tissue shrinkage was corrected (1-2, 5) using the distance between within-penetration lesion marks.

Estimation of penetration angle to the cortex

In electrophysiological recordings, we targeted the portions of A36 where the electrode can be inserted vertically into the cortex with the aid of MR images (1, 8-9, 13). For penetrations with lesion marks, the penetration angle to the cortex was more precisely estimated on histological sections by directly measuring the angle between the vertical axis of the cortex and the electrode path which was reconstructed from
within-penetration lesion marks (Table S1). The angles of the remaining penetrations without lesion marks were estimated by overlying the electrode paths into the corresponding histological sections on the basis of the x-ray images of the electrode tracks (2, 4-5). Penetrations where the estimated angle between the electrode path and the direction perpendicular to the cortex exceeded 20˚ were not included in the present study (23-25).

**Database for the cross-correlation analysis**

We recorded both single-units (SUs) and multi-units (MUs), and constructed the database of unit-pairs for the cross-correlation analysis as follows.

To obtain SUs in the off-line analysis, the continuous neuronal signals recorded at ‘spike-channels’ were high-pass (cut-off frequency: 250-420 Hz) filtered digitally and were thresholded for spike detection. Cluster analysis was then conducted using principal components of waveform features (26-29), and a cluster with the largest spike amplitude was isolated using Offline Sorter software (28-29). Only a unit with a refractory period (> 2 ms) in the auto-correlogram was accepted as an SU (14, 26). In addition, a cross-correlogram (CCG) of SU pairs recorded from two contacts with distances of < 0.3 mm (‘near contacts’) was examined for an artifactual peak at the center bin and symmetrical gaps in the surrounding bins (26-27). The peak and gaps (common refractoriness), if present, indicated that the SU pair originated from an identical neuron and therefore, one of the SU with a lower S/N ratio was not included.

To obtain MUs, the continuous neuronal signals recorded at ‘spike channels’ were band-pass filtered (420 Hz to 8 kHz) digitally, and MU spikes were detected by thresholding the continuous signals (30-33) at 4 SDs above the baseline level (33). MU activities recorded at ‘near contacts’ likely contained spikes that originated from an identical neuron (31, 34), and thus a unit-pair recorded at ‘near contacts’ was excluded from the present analysis. Similarly, when each of two MUs recorded at ‘near contacts’ exhibited a significant CCG peak with a unit (SU or MU) in another contact, a unit-pair with higher CCG peak was included in the database. Note that, when each of two SUs recorded at ‘near contacts’ showed a significant CCG peak with a unit (SU or MU) recorded at another contact, we included both of the unit-pairs in the database.

A given recorded unit was defined as cue-responsive if the firing rate during the cue-period (80-580 ms from cue onset) was significantly larger than that during the fix-period at least for one of the cue stimuli (paired t test, \( P < 0.05 \)) (14-15), and was defined as delay-responsive if the firing rate during the delay-period (500-2000 ms from
cue offset) was significantly larger than that during the fix-period at least for one of the cue stimuli (paired t test, \( P < 0.05 \)). The unit exhibiting a significant response either in the cue- or delay-period was referred to as a responsive unit. Unit-pairs were included in the database for the cross-correlation analysis if both of the constituent units exhibited significant responses to at least one common stimulus during a common task-period (cue- and/or delay-periods) and if the available spikes for each constituent unit exceeded 500 (14-15).

**Data Analysis**

Data were analyzed offline using Matlab. Only the data obtained for correct trials were included in further analyses. All of the statistical tests in the present study were two-sided unless otherwise stated.

**Estimation of cortical layers using CSD**

CSD, either at each trial or averaged across trials, was calculated from the depth profiles of the stimulus-evoked LFPs using a three-point-formula that approximates the second spatial derivative of the voltage recorded at each recording contact (11-12). CSD analysis provides a physiological index of the location, direction and density of transmembrane current flow of the corresponding depths of the cortex (10-12). CSD at the n-th contact, \( D_n \), was calculated as follows:

\[
D_n = - \frac{\varphi (n+1) + \varphi (n-1) - 2 \varphi (n)}{\Delta^2},
\]

where \( \varphi (n) \) is the LFP signal at the n-th contact of the electrode, and \( \Delta \) is the spacing between the neighboring electrode contacts (150 or 100 \( \mu \)m for the electrode with 16 or 24 contacts, respectively). Negative or positive \( D_n \) indicates the current sink or current source at the n-th contact, respectively. In fig. S1, we presented CSDs calculated by the second-nearest technique (11) for display purpose.

To physiologically estimate the position of the granular layer, we determined the earliest current sink as follows. First, in each trial, an instantaneous amplitude of CSD at each contact was calculated using a 10 ms time window that was slid in 1 ms steps. Then, we determined the response latency of a CSD for each contact as the first time point after cue onset when the instantaneous amplitude of the CSD was significantly larger than the corresponding value measured at the cue onset (paired t test, \( P < 0.001 \)) for at least three consecutive bins. We thus determined the earliest significant current
sink, and utilized the earliest-sink contact as a physiological marker of the granular layer (for histological verification, see Table S1). The contacts superficial to the earliest-sink contact were estimated to be located in the supragranular layer (SG), and the contacts in deeper positions than the earliest-sink contact were estimated to be located in the infragranular layer (IG). Note that we did not include unit data recorded at the neighboring (< 0.3 mm) contacts on both sides of the earliest-sink contact in further analyses (35-36) except for the case where we calculated the functional connectivity between units without categorizing the recorded units into G, SG and IG units (Fig.4). Although we did not estimate the border between the cortex and the white matter, a recorded unit was regarded as being located in the cortex if the unit exhibited a significant response during either the cue- or delay-period. In fig. S1, we presented CSD profiles of all the penetrations for which we carried out the cross-correlation analysis.

Cross-correlation analysis
We conducted cross-correlation analysis between spike trains (14-15, 37-39) for a given unit-pair using stimuli to which both the constituent units elicited significant responses during either the cue- or delay-period. We constructed raw cross-correlograms (CCGs) for lag times within 100 ms (1 ms resolution) using spikes recorded during a 500 ms period immediately before cue onset (fix-period), during a 500 ms period beginning 80 ms after cue onset (cue-period) and during a 1500 ms period beginning 500 ms after cue offset (delay-period).

In evaluating the strength of spike correlation, trial-to-trial variability in the firing rate and/or response latency is a potential source of estimation bias (40-41). To correct for these effects, we calculated an instantaneous firing rate (IFR) predictor of spike correlation (41) as follows. First, we convolved raw spike trains with a gaussian kernel ($\sigma = 10$ ms) in each trial, and calculated a CCG between these convolved spike trains to obtain the IFR predictor, which estimated the strength of spike correlation due to co-modulation of firing rates within a given trial. This IFR predictor was then subtracted from the raw CCG for each trial, and this calculation was repeated for all trials to compile the IFR-predictor-subtracted CCG, in which the above effects of response variabilities were corrected. The peak of the IFR-predictor-subtracted CCG was detected within ±10 ms lags (14-15, 35, 42), and the peak height was evaluated by calculating z-score using the number of spikes for each unit, bin width, and the total recording time (39, 42-45). The CCG peak was determined to be statistically significant if the z-score of the peak exceeded a value corresponding to $P = 0.05$ (one-tailed, $Z > 2.82$, corrected for multiple comparisons within ±10 ms lags) (14-15).
To measure the net signal flow across cortical layers in each task period, we constructed population-averaged CCGs by averaging the z-scored CCGs of individual pairs with a significant peak (Fig. 2, A and E, fig. S3) (46-47). For the population-averaged CCG during the fix-period, individual CCGs were included if the CCG peak of a given unit pair was significant during either the cue- or delay-period. To evaluate the statistical significance of the population-averaged CCGs, we tested whether the areas of individual CCGs (48-50) within ±10 ms lags (50) were larger than 0 (paired t test). We also compiled the population-averaged CCGs using correlation coefficient of individual CCGs (39, 51-54) instead of z-score (fig. S4). In this calculation, we have added a criterion for the least number of trials as in previous studies using correlation coefficient (51, 53) to correct for large variance due to the shortage of the number of trials. We have calculated correlation coefficient for unit pairs if the spikes were collected for at least 100 trials (51, 53).

**Calculation of Correlation Strength, Asymmetry Index and Center of Mass**

To quantify the strength of functional connectivity between recorded units, correlation strength (CS) (27, 47, 55-56) was defined for each z-scored CCG as follows.

\[ CS = R + L, \]

where R and L indicate the summed z-scores for the bins on the right and left sides of the CCG within 10 ms lags, respectively. The z-score of the center bin (0 ms lag) were equally divided into R and L, and the bins with a negative z-score were not included in the calculation.

To infer the direction of functional connectivity between recorded units, we calculated asymmetry index (AI), which quantified the asymmetry of the CCG peak area against the zero time lag (15, 57-58). AI value for each CCG was calculated as follows.

\[ AI = \frac{R - L}{R + L} \]

We also calculated the center of mass (CoM) of the CCGs (59-61) to evaluate the directional bias of neuronal interactions. CoMs were calculated according to (59), since we subtracted the stimulus-locked component (IFR-predictor) from a raw CCG as in (59).

**Temporal dynamics of neuronal correlation between SG-unit and IG-unit**

We examined the temporal dynamics of functional connectivity between individual SG-IG unit-pairs with a significant CCG peak either in the cue- or delay-period using a sliding time window (‘time-resolved CCG’) (Fig. 3) (14, 45, 62). The time-resolved
CCG at 0 ms was calculated using spikes during the 500 ms window, which started from 250 ms before cue onset. This 500 ms window was then successively shifted in steps of 50 ms (Fig. 3, A and B). The time-resolved CCGs that did not meet the criteria for the number of spikes (> 100 spikes for each unit in each time window) were not included in calculating the population average dynamics in Fig. 3B.

**Analysis of single-unit responses in SG and IG**

To quantify the dynamics of neuronal activities in SG and IG, we calculated the firing rates of cue-responsive single-units and delay-responsive single-units (fig. S6). In estimating the firing rate, the delay-period was subdivided into three consecutive epochs of 500 ms duration (delay 1, delay 2 and delay 3). The firing rate in each epoch was normalized to the corresponding value during the fix-period (63-64) as follows:

$$\text{Normalized firing rates (task epoch)} = \frac{\text{Response (task epoch)}}{\text{Response (fix)}},$$

where 'Response' represents the average firing rate in each epoch.

**AI- and CS-matrices**

Laminar connectivity matrices (35, 65-66) were calculated in terms of the strength (CS) and direction (AI) of the functional connectivity between recorded units (CS- and AI-matrices; Fig. 4, A and B). For each unit-pair, the constituent units were categorized into the putative source- and target-units according to the sign of AI. Then, the AI and CS values for each unit-pair were allocated to the corresponding voxels (voxel size; 0.15 mm) of the matrices to obtain the average value across unit-pairs for each voxel. Unit-pairs recorded by the electrode with 24 contacts (intercontact spacing, 100 μm) were interpolated into the corresponding voxels.

**Supplementary results**

**Cross-correlation results and pair-types**

In Fig. 2, data from SU-SU, SU-MU and MU-MU pairs were merged to examine the direction of the net signal flow across cortical layers. We here present the results of cross-correlation analysis for MU-MU pairs and SU-SU/SU-MU pairs (SU-SU and SU-MU pairs were merged due to a small number of SU-SU pairs).

For SG-IG pairs, AIs were compared between SU-SU/SU-MU pairs and MU-MU pairs, and no significant differences were observed [Wilcoxon signed-rank test; cue-period, $P > 0.2$, median = 0.18 (n = 24) and 0.22 (n = 17) for SU-SU/SU-MU pairs and MU-MU pairs, respectively; delay-period, $P > 0.9$, median = -0.14 (n = 18) and
-0.13 (n = 23)]. Similarly, CoMs were compared between SU-SU/SU-MU pairs and MU-MU pairs, and no significant differences were observed [Wilcoxon signed-rank test; cue-period, $P > 0.4$, median = 1.6 ms (n = 24) and 1.6 ms (n = 17) for SU-SU/SU-MU pairs and MU-MU pairs, respectively; delay-period, $P > 0.3$, median = -0.3 ms (n = 18) and -1.8 ms (n = 23)]. To test whether the reversal of signal flow between SG and IG could occur with only SU-SU pairs, we added a substantial number of SU-SU pairs (see the legend of fig. S5 for the procedures), and found that these SU-SU pairs by themselves revealed significant directional biases in the spike correlation depending on the task periods: SG to IG during the cue-period, IG to SG during the delay-period (fig. S5).

For G-SG pairs, AIs showed no significant differences between SU-SU/SU-MU pairs and MU-MU pairs in either the cue- or delay-period [Wilcoxon signed-rank test; cue-period, $P > 0.4$, median = 0.17 (n = 10) and 0.22 (n = 7) for SU-SU/SU-MU and MU-MU pairs, respectively; delay-period, $P > 0.6$, median = 0.02 (n = 5) and 0.10 (n = 9)]. Similarly, CoMs of G-SG pairs showed no significant differences between SU-SU/SU-MU pairs and MU-MU pairs in either the cue- or delay-periods [cue-period, $P > 0.1$, median = 3.4 ms (n = 10) and 1.2 ms (n = 7) for SU-SU/SU-MU and MU-MU pairs, respectively; delay-period, $P > 0.3$, median = 1.3 ms (n = 5) and 0.1 ms (n = 9)]. AIs of G-IG pairs showed no significant differences between SU-SU/SU-MU pairs and MU-MU pairs in either the cue- or delay-periods [cue-period, $P > 0.9$, median = 0.15 (n = 8) and -0.01 (n = 6); delay-period, $P > 0.6$, median = -0.01 (n = 8) and -0.07 (n = 7)]. Similarly, CoMs of G-IG pairs showed no significant differences between SU-SU/SU-MU pairs and MU-MU pairs in either the cue- or delay-periods [cue-period, $P > 0.8$, median = 1.3 ms (n = 8) and 2.1 ms (n = 6); delay-period, $P > 0.5$, median = 0.4 ms (n = 8) and -1.0 ms (n = 7)].

Next, we calculated the proportions of SG-IG pairs that changed the signs of AI between the cue- and delay-periods (‘flipped pairs’ in Fig. 3, C and D) separately for SU-SU/SU-MU and MU-MU pairs. No significant differences were observed in the proportions of flipped pairs between SU-SU/SU-MU and MU-MU pairs [$\chi^2$ test, $P > 0.9$, 43% (17/40 pairs) and 53% (16/30 pairs) for SU-SU/SU-MU and MU-MU pairs, respectively]. We also compared the proportions of flipped pairs that showed positive AIs (SG to IG) in the cue-period and negative AIs (IG to SG) in the delay-period between SU-SU/SU-MU and MU-MU pairs. No significant difference was observed in the proportions of pairs with positive AIs between these two groups of unit-pairs [$\chi^2$ test, $P > 0.9$, 71% (12/17 pairs) and 75% (12/16 pairs) for SU-SU/SU-MU pairs and MU-MU pairs, respectively].
Cross-correlation results for each monkey

Correlation analyses shown in Fig. 2 were repeated by separating our database into those from individual monkeys.

AIs of SG-IG pairs with a significant CCG peak showed no significant difference between monkeys in either the cue- or delay-period [Wilcoxon signed-rank test; cue-period, \( P > 0.1 \), median = 0.39 (n = 15) and 0.18 (n = 26) for monkeys E and P, respectively; delay-period, \( P > 0.2 \), median = -0.10 (n = 16) and -0.16 (n = 25)]. CoMs of SG-IG pairs with a significant CCG peak showed no significant difference between monkeys in either the cue- or delay-periods (cue-period, \( P > 0.1 \), median = 2.5 ms and 1.3 ms for monkeys E and P, respectively; delay-period, \( P > 0.8 \), median = -1.5 ms and -1.6 ms).

During the cue-period, AIs of SG-IG pairs revealed the same tendency of directional bias (SG to IG) for each monkey (monkey E, \( P < 0.01 \); monkey P, \( P = 0.08 \)). CoMs also revealed the same tendency (monkey E, \( P < 0.02 \); monkey P, \( P = 0.37 \)). During the delay-period, AIs of SG-IG pairs revealed the same tendency of directional bias (IG to SG) for each monkey (monkey E, \( P < 0.05 \); monkey P, \( P = 0.08 \)). CoMs also revealed the same tendency (monkey E, \( P = 0.10 \); monkey P, \( P = 0.08 \)).

AIs of G-SG pairs were not significantly different between monkeys in any of the task periods [cue-period, \( P > 0.2 \), median = 0.09 (n = 6) and 0.21 (n = 11); delay-period, \( P > 0.2 \), 0.27 (n = 4) and 0.06 (n = 10)]. CoMs of G-SG pairs were not significantly different between monkeys in any of the task periods (cue-period, \( P > 0.3 \), median = 0.3 ms and 3.4 ms; delay-period, \( P > 0.3 \), -0.4 ms and 1.0 ms). AIs of G-SG pairs in the cue-period revealed the same tendency of directional bias (G to SG) for each monkey (monkey E, \( P = 0.39 \); monkey P, \( P < 0.05 \)). CoMs revealed the same tendency (monkey E, \( P = 0.34 \); monkey P, \( P < 0.05 \)).

AIs of G-IG pairs were not significantly different between monkeys [cue-period, \( P > 0.5 \), median = 0.16 (n = 7) and 0.14 (n = 7); delay-period, \( P > 0.2 \), -0.16 (n = 6) and 0.02 (n = 9)]. CoMs of G-IG pairs were not significantly different between monkeys (cue-period, \( P > 0.4 \), median = 3.4 ms and 0.8 ms; delay-period, \( P > 0.5 \), 0.4 ms and -1 ms).

Next, we calculated the proportions of SG-IG pairs that changed their signs of AI between the cue- and delay-periods (‘flipped pairs’ in Fig. 3C) separately for individual monkeys. The proportions of flipped pairs were not significantly different between monkeys [\( \chi^2 \) test, \( P > 0.6 \), 44% (12/27 pairs) and 49% (21/43 pairs) for monkeys E and P, respectively]. We also compared the proportions of flipped pairs that showed positive
AIs (SG to IG) in the cue-period and negative AIs (IG to SG) in the delay-period between the monkeys, and did not find a significant difference [$\chi^2$ test, $P > 0.7$, 75% (9/12 pairs) and 71% (15/21 pairs)].

**Estimation of negative neuronal correlation**
For G-SG pairs in Fig. 2, only two pairs and one pair of units revealed significant negative cross-correlations (the criterion for detecting a significant negative neuronal correlation was the same as that for the positive neuronal correlation, but the opposite in sign) during the cue- and delay-periods, respectively. Similarly, for SG-IG pairs, only one pair and three pairs of units revealed significant negative cross-correlations during the cue-and delay-periods, respectively.

**Single-unit responses in SG and IG**
We examined whether the firing rates of the SG-unit reflected the signal flow from IG during the delay-period. The firing rates of IG single-units ($n = 27$) selectively responded throughout the delay-period (fig. S6C) (paired $t$ test with Bonferroni’s correction, $P < 0.01$). By contrast, the responses of SG single-units ($n = 13$) developed during the latter part of the delay-period (fig. S6D) (two-way ANOVA followed by Tukey’s test; time × stimulus interaction, $P < 0.05$), consistent with the gradual appearance of signal flow from IG to SG (Fig. 3, A and B) (for the relation between the connectivity direction of unit-pairs and the firing rates of the constituent units, see fig. S7).
Fig. S1. CSD profiles of all penetrations. (A) Population-averaged CSDs (n = 20). All penetrations were aligned along the cortical depth at the earliest-sink contact. Individual CSDs were normalized by the maximum value of CSDs within each penetration. (B) CSD profiles of individual penetration. CSD amplitude at cue onset (baseline level) was subtracted from the time course of CSD amplitude in each contact.
**Fig. S2.** Proportion of unit-pairs with significant CCG peak in the G-SG, SG-IG and G-IG pairs. The number on each circle indicates the number of unit-pairs that showed significant CCG peak. Proportion of unit-pairs with significant CCG peak ($Z > 2.82, P < 0.05$; one-tailed, corrected for multiple comparisons within $\pm$10 ms lags) was compared among G-SG, SG-IG and G-IG both in the cue- (blue) and delay- (orange) periods using $\chi^2$ test followed by post hoc pair-wise comparisons with corrections for multiple comparisons by the Bonferroni’s method. Colors of daggers indicate task-periods.

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Colors of daggers indicate task-periods.

- † : $P<0.005$
- † : $P<0.05$
Fig. S3. Population results of the functional connectivity for G-IG pairs. Calculation procedure and display format is the same as in Fig. 2. (A) Population-averaged CCGs showed no significant peak in either of the task period. (B and C) Distributions of AIs (B) and CoMs (C) showed no bias in either of the task period.
Fig. S4. Population-averaged CCGs using correlation coefficient for G-SG (A), SG-IG (B) and G-IG (C) pairs in each task period.
Fig. S5. Functional connectivity between SG and IG using only single-units. In addition to the original SG-IG database (Fig. 2, E to G), we constructed a new SG-IG database consisting exclusively of single-unit pairs, and calculated asymmetry index (A) and center of mass (B) of unit-pairs with significant CCG peak during the cue- and delay-periods (cue-period, 31 pairs; delay-period, 24 pairs). To construct the new single unit database, we re-sorted single-units by applying a new criterion (maximum proportion of spikes with the ISI < 2 ms increased from 0.7% to 1.0%), and applied a lower threshold for detecting the CCG peak, that is, $P < 0.05$ without correction for multiple comparisons among different bins. CCGs were calculated only when the available spikes exceeded 700 for each unit to obtain a reliability of the resultant CCGs. Asterisk, significant bias to either side of the histogram. Filled histogram, the task period for which significant bias in the directionality was observed. The same biases of functional connectivity as those in the original SG-IG database were observed in each task period [AI: cue-period, median = 0.16, $P < 0.05$, Wilcoxon signed-rank test; delay-period, median = -0.22, $P < 0.05$; CoM: cue-period, median = 0.8 ms, $P = 0.15$; delay-period, median = -1.8 ms, $P < 0.05$]. During the fix-period, no directional bias was observed (AI: median = -0.03, $P > 0.3$, n= 13; CoM: median = -0.8 ms, $P > 0.5$).
**Fig. S6.** Neuronal responses in IG and SG. (A and B) Firing rates of the cue-responsive single-units in IG (A) and SG (B) during the cue-period. (C and D) Firing rates of the delay-responsive single-units in IG (C) and SG (D) during the delay-period. For each unit, firing rates were calculated using the best (black) and worst (gray) stimuli (the stimuli that elicited the largest and smallest responses during the cue/delay-period, respectively), and were normalized by the firing rate in the fix-period. In (B), a neuron was excluded from the dataset as an outlier (Smirnov-Grubbs test, \( P < 0.01 \), \( n = 13 \)). Firing rate of a delay-responsive single-unit (C and D) was calculated for three consecutive 500 ms time windows (delay 1, delay 2 and delay 3) during the delay-period (from 500 ms to 2000 ms following cue offset). For delay-responsive IG
units (C, n = 27), significant responses were observed throughout the delay-period (delay 1-3) (paired t test, corrected for multiple comparisons across different time windows, $P < 0.001$). For delay-responsive SG units (D, n = 13), the two-way ANOVA on stimulus condition (best stimulus vs. worst stimulus) and time (delay 1-3) revealed a significant interaction between stimulus and time window [$F(2,60) = 3.27, P < 0.05$]. Post-hoc pair-wise comparisons revealed that SG-units showed significantly larger responses in delay 3 than in delay 1 and 2 (Tukey’s test). Error bars represent SEM. Daggers indicate statistical significance assessed by post-hoc Tukey’s test. Asterisks indicate statistical significance assessed by paired t test, corrected for multiple comparisons across different time windows.
Fig. S7. Relation between the direction of functional connectivity of SG-IG pairs and the firing rates of constituent units. AIs (left column) and CoMs (right column) were plotted against the difference of the firing rates (FR) between IG and SG units in each task period.
Fig. S8. Interlaminar connectivity matrices [AI- (A) and CS-matrices (B)] during each task period. Calculation procedure and display format are the same as in Fig. 4 except that the size of a circle in each matrix indicates the number of unit-pairs with significant CCG peak. Blank voxels (three voxels in upper-left and three voxels in lower-right) in each matrix correspond to laminar positions that were not recorded in the present study.
Fig. S9. Functional connectivity within IG during the cue- and delay-periods. Both of the constituent units of a pair were recorded in IG. The putative source- and target-units that showed significant CCG peak during the cue-period showed no difference in their recorded positions. In contrast, during the delay-period, putative target-units were located at positions significantly deeper than the putative source-units (paired $t$ test, $P < 0.02$, $n = 19$ pairs). Arrows represent median values. Overlapped lines were shifted for presentation purpose. Unit-pairs with AI values within the range of -0.2 and 0.2 were excluded in this analysis (56). Results did not change when we repeated the same analysis using all unit-pairs (cue-period, $P > 0.5$, $n = 37$ pairs; delay-period, $P < 0.05$, $n = 39$ pairs).
Table S1. Summary for the histological analysis.

| Track | Subject | $A_{E-CTX}$ (deg) | $D_{ESC-GLC}$ (μm) | $|D_{ESC-GLC}|$ (μm) | $W_{GL}$ (μm) | $|D_{ESC-GLC}|/W_{GL}$ (%) |
|-------|---------|-------------------|--------------------|----------------------|--------------|------------------------|
| #1    | E       | 5                 | +65                | 65                   | 185          | 35                     |
| #2    | E       | 11                | +17                | 17                   | 200          | 9                      |
| #3    | E       | 15                | +13                | 13                   | 220          | 6                      |
| #4    | P (R)   | 12                | -99                | 99                   | 210          | 47                     |
| #5    | P (L)   | 4                 | -100               | 100                  | 220          | 45                     |
| #6    | P (L)   | 3                 | +92                | 92                   | 195          | 47                     |
| Mean  |         | 8.3               | -2                 | 64                   | 205          | 32                     |
| Median|         | 8.0               | +15                | 79                   | 205          | 40                     |
| S.D.  |         | 5.0               | 81                 | 40                   | 14           | 19                     |

R, right hemisphere; L, left hemisphere; $A_{E-CTX}$, electrode penetration angle to cortex; $D_{ESC-GLC}$, signed distance between the earliest-sink contact and granular layer center; +, deviation to supragranular; -, deviation to infragranular; $|D_{ESC-GLC}|$, absolute value of $D_{ESC-GLC}$; $W_{GL}$, width of granular layer; $|D_{ESC-GLC}|/W_{GL}$, proportion of $|D_{ESC-GLC}|$ to $W_{GL}$.

Table S1. Summary for the histological analysis. Penetration angle to the cortex ($A_{E-CTX}$) was estimated by measuring the angle between the vertical axis of the cortex (alignments of cells in radial direction) and the electrode path which was reconstructed from within-penetration lesion marks. Laminar position of the earliest-sink contact was estimated using lesion marks, and overlain on the histological section. Then, the signed distance between the positions of the earliest-sink contact and the granular layer center ($D_{ESC-GLC}$) was estimated. The thickness of the granular layer ($W_{GL}$) was also measured for each penetration, and then proportion of $|D_{ESC-GLC}|$ to $W_{GL}$ was calculated ($|D_{ESC-GLC}|/W_{GL}$) (for details, see ‘Histological analyses’ section in SOM).
**Table S2.** Database of unit-pairs for cross-correlation analysis.

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**Table S2.** Database of unit-pairs for cross-correlation analysis. G, granular layer; SG, supragranular layer; IG, infragranular layer; SU, single-unit; MU, multi-unit. Numbers of G-SG, G-IG and SG-IG pairs analyzed in each task period were presented. A CCG was calculated for each unit-pair for each task-period, and was included into the database if the available spikes for each constituent unit during a given period exceeded 500.
Supporting References


**Supporting Online Material**
www.sciencemag.org
Materials and Methods
Supporting online text
Figs. S1 to S9
Tables S1 and S2