Spatial range and laminar structures of neuronal correlations in the cat primary visual cortex

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To address these issues, we recorded spiking activity from anesthetized cat V1 with a two-dimensional array of microelectrodes (Fig. 1A) that allowed us to record from tens of horizontally and vertically separated cells simultaneously. By precise correlation that involves neuronal pairs trending to be coactivated within several milliseconds (Toyama et al. 1981b) and loose correlation that occurs on a timescale of tens of milliseconds (Krüger and Aiple 1988; Michalski et al. 1983; Smith and Kohn 2008).

To fully characterize functional properties of these correlations, understanding their spatial organizations and the participating neuronal populations is critical. In particular, knowing whether the two types of correlation have different vertical extents or show different laminar organization is of interest because different layers consist of neurons having different morphologies and functional roles. Previous studies have revealed that precise correlation and loose correlation differ in their horizontal extents: loose correlation was observed with high incidence (≥40%) over a distance of 1 mm (Krüger and Aiple 1988; Michalski et al. 1983) and as far as 10 mm (Smith and Kohn 2008). In contrast, incidence of precise correlation was <10% at 1 mm (Hata et al. 1991; Krüger and Aiple 1988; Michalski et al. 1983) and 17% at 3 mm (Maldonado et al. 2000) and was limited to be <4 mm (Smith and Kohn 2008). However, whether such differences hold for vertical distances or whether they depend on laminar organization is less clear. Determining the mechanisms that generate correlations is also an important issue. The three major projections in V1, namely, thalamocortical projections (LeVay and Gilbert 1976), intrinsic connections (Fisken et al. 1975; Gilbert and Wiesel 1979; Lorente de Nó 1933), and feedback projections from higher visual cortices (Angelucci et al. 2002; Rockland and Virga 1989), may be the predominant sources for neuronal correlation in V1. These projections have different horizontal extents and connect neurons with different functional specificities. For example, intracortical axons connect neurons with similar receptive field (RF) properties and functional roles.

THE FIRING PATTERNS of nearby cortical neurons are often temporally correlated, which may contribute to numerous aspects of cortical processing, including synaptic transmission, sensory information processing, and selective attention (Salinas and Sejnowski 2001; Singer and Gray 1995; Womelsdorf et al. 2007).

To date, a number of studies have examined neuronal correlation in the primary visual cortex (V1) (Aiple and Kruger 1988; Alonso and Martinez 1998; Das and Gilbert 1999; Friedman-Hill et al. 2000; Ghose et al. 1994; Gray et al. 1989; Gray and Viana Di Prisco 1997; Hata et al. 1988, 1991; Kohn and Smith 2005; Krüger and Aiple 1988; Livingstone and Maldonado 1996; Maldonado et al. 2000; Michalski et al. 1983; Smith and Kohn 2008; Toyama et al. 1981a, 1981b; Ts’o et al. 1986; Ts’o and Gilbert 1988) and have revealed that it occurs on a variety of timescales. Specifically, they showed two types of correlation:
Contact lenses of appropriate power with 4-mm artificial pupils were retracted with phenylephrine hydrochloride (Neosynephrine, 5%). Pupils were dilated with atropine sulfate (1%), and nictitating membranes were separated by a range of horizontal and vertical distances (Perkel et al. 1967; Toyama et al. 1981a), we were able to systematically analyze the spatiotemporal structure and laminar organization of precise and loose correlations. On the basis of these results, we discuss the neuronal mechanisms to generate correlation in V1.

METHODS

All animal care and experimental guidelines conformed to those established by the National Institute of Health (Bethesda, MD) and were approved by the Osaka University Animal Care and Use Committee.

Subjects and surgeries. Five normal adult cats (2.5–4 kg) were used. After initial preanesthetic doses of hydroxyzine (Atarax, 2.5 mg) and atropine (0.05 mg), each cat was anesthetized with isoflurane (2–3.5% in oxygen). Cefotiam hydrochloride (Panspolin, 8.3 mg) and dexamethasone sodium phosphate (Decadron, 0.4 mg) were then administered. Electrocardiogram electrodes and a rectal temperature probe were inserted, and femoral veins were catheterized. A glass tracheal tube was inserted by tracheotomy. The animal was subsequently secured in a stereotaxic apparatus with the use of ear and mouth bars and clamps on the orbital rims. Tips of the ear bars were coated with local anesthetic gel (lidocaine). Anesthesia was then switched to thiopental sodium (Ravonal; given continuously at 1.0 –1.5 mg·kg⁻¹·h⁻¹). After the anesthesia became stable, paralysis was induced by a loading dose of gallamine triethiodide (Flaxedil, 10–20 mg) and the animal was placed under artificial respiration with a gas mixture of nitrous oxide (70%) and oxygen at 20–30 strokes/min. The respiratory rate and stroke volume were adjusted to maintain the end-tidal CO₂ between 3.5% and 4.3%. To maintain paralysis and anesthesia for the rest of the surgery and following recording sessions, we continuously infused gallamine triethiodide (10 mg·kg⁻¹·h⁻¹) and thiopental sodium (1.0–1.5 mg·kg⁻¹·h⁻¹) contained in an infusion fluid (Ringer solution, 1 ml·kg⁻¹·h⁻¹) that also included glucose (40 mg·kg⁻¹·h⁻¹). A rectangular hole (typically 3 mm in width and 4 mm in length) was made over representations of area 17 or 18. The dura was dissected away to allow for microelectrode array penetration. Pupils were dilated with atropine sulfate (1%), and nictitating membranes were retracted with phenylephrine hydrochloride (Neosynephrine, 5%). Contact lenses of appropriate power with 4-mm artificial pupils were placed over the corneas. Electrocardiogram, end-tidal CO₂, intratracheal pressure, heart rate, and rectal temperature were continuously monitored and maintained at normal levels throughout the experiments.

Electrophysiology and visual stimuli. Four-shaft silicon microelectrode arrays with 32 recording probes (a4x8_200_400_177, NeuroNexus, Ann Arbor, MI) were used to record neuronal activity (Fig. 1A). The horizontal distance between neighboring shafts was 0.4 mm, while the vertical distance between neighboring probes was 0.2 mm, resulting in a recording range 1.2 mm in width and 1.4 mm in height. Electrode impedance ranged from 1 to 2 MΩ. Electrodes were mounted on a micromanipulator drive (Narishige, Tokyo, Japan) and slowly penetrated into areas 17 and 18 with cortical coordinates between A3 and P8 and between L1 and L3, respectively. The four shafts were typically set parallel to the sagittal plane. We adjusted the electrode angle such that it was perpendicular to the cortical surface and adjusted the depth so as to record from as many probes as possible (Fig. 1B and Fig. 3A). Electrode signals from all but two probes at the most shallow depth were amplified (PXBX2, gain 1,000, Plexon, Dallas, TX), band-pass filtered (100 Hz to 3 kHz), and fed to a custom-made data acquisition system (sampling rate 20 kHz), where they were A-D converted and saved on the PC. Careful spike sorting and data analysis were conducted off-line with these data. Separately, the filtered signals were also processed with an online custom-built spike sorter to roughly isolate single units and monitor neuronal responses to visual stimuli online. The online system was similar to one previously described (Ohzawa et al. 1996) and was extended to 40 channels.

Visual stimuli were produced by a Windows-based PC controlling a graphics card (Millenium G550, Matrox, Dorval, QC, Canada) and were displayed on a color CRT monitor (76 Hz, 1,600 × 1,024 pixels, mean luminance 47 cd/m², 46.6 cm in width and 29.9 cm in height; GDM-FW900, Sony, Tokyo, Japan). In each recording session, the luminance nonlinearity of the monitor was measured by a photometer (Minolta CS-100, Konica Minolta Photo Imaging, Mahwah, NJ) and linearized with gamma-corrected look-up tables. Cats viewed the monitor screen through a custom-built haploscope, which allowed visual stimuli to be presented to the left and right eyes separately. A black separator was placed between the left and right visual fields to prevent stimulation of the unintended eye. The distance between the screen and the eyes (total length of the light path) was set...
to 57 cm, making the screen 23.3° × 29.9° of visual angle for each eye.

After the electrode-penetrated cortex became stable, we first determined the approximate position, size, and basic tuning properties of the classical receptive field (CRF) for one or two cells from each of the four electrode shafts, using small bars or small circular sinusoidal grating patches under manual mouse control. Next, visual stimuli were presented under computer control to determine the accurate orientation and spatial frequency tuning properties of cells recorded from all sites. We used rapidly flashed sequences of sinusoidal gratings that typically included 13 spatial frequencies, 18 orientations (0°–180° in 10° steps), and 4 phases (0°, 90°, 180°, and 270°). Two online, the range of spatial frequencies was adjusted to cover the entire frequency range that evoked responses from all recorded cells and was sampled regularly in logarithmic scale (typical ranges were 0.1–2.0 cycles/° for area 17 and 0.02–0.7 cycles/° for area 18) (Nishimoto et al. 2005; Ringach et al. 1997). We set the stimulus patch sufficiently large to cover the RFs of the initially tested cells. This patch effectively covered RFs of all recorded cells since cells from the same electrode shaft have RFs at similar locations. Using the online data-analysis system described above, we confirmed that the stimulus patch covered the linear CRFs of most simple cells reconstructed from their spatial frequency and orientation (SF-OR) selectivity maps via inverse Fourier transform (Ringach 2002). If necessary, we adjusted the stimulus patch size and conducted measurements again. Presentation of one stimulus sequence lasted ~37 s. Each grating in the sequence was presented for 26.3 ms or 39.5 ms (2 or 3 frames at 76 Hz). The sequence was randomized for each trial and presented 10–30 times. Evoked responses and the stimulus sequence were cross-correlated to obtain SF-OR maps at optimal correlation delays (see Fig. 3B). By these procedures, 94% (n = 195) of recording sites with neuronal activity (total spikes across all trials ≥ 200, n = 208) showed clear SF-OR maps in which the variance of the map at the optimal delay exceeded the mean + 5 SD of the variance for the noncausal maps (21 maps from ~300 to 0 ms in 15-ms steps).

When responses to the flashed gratings were weak and tuning maps were not clear, we also used sinusoidal drifting gratings. As in the case of the flashed gratings, the stimulus patch was set sufficiently large to cover the RFs of the initially tested cells. We used gratings of 24 orientations spaced equally. The spatial frequency was fixed so as to drive most recorded cells. These gratings were interleaved for 4 s each in a pseudorandom order, and this was repeated five times. For both types of stimuli, the stimulus contrast was 50%. Stimuli were presented monocularly for the two eyes. However, response analysis was only done for data obtained from stimulating the eye that drove more cells. Among eight penetrations made in this experiment, flashed gratings were used for seven penetrations and drifting gratings, in which the spatial frequency of the stimulus was fixed at 0.63 cycles/°, were used for one.

In the drifting-grating condition, responses obtained in the aforementioned run were used to calculate neuronal correlation. When the flashed gratings were used, we subsequently conducted more runs by repeating the same stimulus sequence to calculate shift-corrected cross-correlograms (CCGs) (see Cross-correlation analysis) and to compute genuine neuronal correlation. In these runs, sequences of gratings of different contrasts were often used as follows. Among the seven recording sessions using the flashed-grating stimuli, one contrast (50%) was used in one session, two contrasts were used in two sessions (0% and 50%), and four or five contrasts between 0% and 50% were used in the remaining four sessions. Contrast was held steady in one sequence. Sequences of each contrast were presented 10–20 times. Although the use of various contrasts allowed us to examine the effect of the stimulus contrast on neuronal correlation in detail, here we focused our attention on spatial patterns of neuronal correlation in this study and used spikes elicited by different contrasts to obtain single shift-corrected CCGs, unless otherwise mentioned.

In the four sessions using four or five contrasts, sequences of different contrasts were interleaved. For this condition, one might be concerned that responses in the trials with low-contrast sequences might have spuriously covaried between neurons because of adaptation to the contrast level of the most recent trials. However, we confirmed that the mean firing rate in trials with low contrast sequences (3.125% or 6.25%), averaged across all simultaneously recorded single units, did not depend on the contrast used in the most recent trials (r = −0.18 to 0.23, P > 0.4). In the two sessions using two contrasts, sequences of each contrast were consecutively repeated. Typically, these runs were applied only to the dominant eye.

**Spike sorting**. Spikes were sorted off-line with a commercial software package (Offline Sorter, Plexon). Spike segments that exceeded a threshold were extracted and plotted as data points in the PC1–PC2 with principal component analysis. The threshold was set at −2.5 to −5 SD of the raw signal amplitude (1 SD was between 10 μV and 20 μV). If a proportion of the data points appeared to form an isolated cluster, we manually drew a contour that enclosed these points. Templates of spike waves for the clusters were then calculated by averaging all spike segments inside the contours. We next conducted an automatic matching procedure to sort spike segments into the clusters again. Clusters were taken as activity of single units if their average spike amplitude was greater than 3.5 times the background noise level and the proportion of spikes with interspike intervals (ISIs) < 1 ms was <2% of the total spikes. The remaining clusters were deemed multiunit activity.

The average signal-to-noise ratio (SNR) of single-unit activity (n = 197, see results) was 8.65, with 86% of these units having SNRs ≥ 5.0. The SNR was defined as the mean ratio of the spike amplitude to the background noise level. Most of the single units maintained their amplitude throughout the recording session. That is, for 74% and 90% of these units, the mean amplitude of spikes during the first and last thirds of the recording period differed by <10% and 20%, respectively. The SNR of multiunits was always >2.8, with an average value of 5.6.

For 186 of the 197 single units, the proportion of spikes with ISIs < 1 ms was also <2% of the total spikes for both the first and last thirds of the recording period. For 195 units, the proportion was <3% for the two periods.

**Cross-correlation analysis**. To measure the correlation in spike timing of two units, raw spike train cross-correlograms (CCGs) were computed as

\[ \text{CCGs}(\tau) = \frac{\sum_{i=1}^{n} x_i(t) x_i(t+\tau)}{\sqrt{\sum_{i=1}^{n} x_i(t)^2}} \]

where \( x_i(t) \) and \( x_i(t+\tau) \) are the spike counts of the two units for a 1-ms bin at time \( t \) during trial \( i \). \( s_1 \) and \( s_2 \) are the total spike counts of the two cells. \( \tau \) is the correlation delay, which was varied between −100 ms and 100 ms. Shift-corrected CCGs were then calculated by subtracting the shift predictor (Perkel et al. 1967) from the raw CCGs. Two shift predictors were calculated based on the above equation by replacing the two variables in the numerator with \( x_i(t) \) and \( x_i(t+\tau) \) or \( x_i(t) \) and \( x_i(t+\tau) \), respectively. The average of these two predictions was used for the correction. When several contrasts were included in a stimulus set, we computed separate shift-corrected CCGs for each stimulus contrast. We then took the mean of these CCGs to produce an average shift-corrected CCG across all contrasts. These averaged CCGs were analyzed, except when the effects of stimulus contrasts were examined. In the drifting-grating condition, the average shift-corrected CCGs across orientations were calculated in a similar manner.

Shift-corrected CCGs often contain “narrow peaks” reflecting precise correlation with a timescale of milliseconds superimposed on “broad peaks” reflecting loose correlation with a timescale of tens of milliseconds, as shown in other works (Krüger and Aiple 1988; Michalski et al. 1983) and illustrated in Fig. 2A. We conducted the
The resultant CCGs are referred to as filtered CCGs (Fig. 2). Shift-corrected CCGs, which effectively removes narrow peaks. To extract precise correlation, we subtracted filtered CCGs (Fig. 2B) from the shift-corrected CCGs (Fig. 2A). The resultant CCGs showed waves without slow variations, as seen in Fig. 2C. We referred to these CCGs as residual CCGs. When the highest peak in the residual CCG had a width $>8\text{ ms}$ at $33.3\%$ peak level and an SNR $\geq6$ (defined below), we define the peak as a broad peak. Although the amplitude of residual CCGs depended somewhat on the filter width, we confirmed that essentially the same population results were obtained by varying the filter width between $9\text{ ms}$ and $20\text{ ms}$. The SNR of the broad peaks was the ratio of peak amplitude to noise level, defined as the standard deviation (SD) of the amplitude of the filtered CCGs beyond $\pm50\text{ ms}$.

To extract precise correlation, we subtracted filtered CCGs (Fig. 2B) from the shift-corrected CCGs (Fig. 2A). The resultant CCGs showed waves without slow variations, as seen in Fig. 2C. We referred to these CCGs as residual CCGs. When the highest peak in the residual CCG had a width $\leq8\text{ ms}$ and an SNR $\geq6$, we defined this peak as narrow if an additional condition was fulfilled that the peak width of the original CCGs at $66.6\%-\text{peak level}$ was also $\leq8\text{ ms}$. The additional condition avoided mistaking extremely small peaks in the residual CCGs as narrow peaks and did not significantly change the population results. The SNR of narrow peaks was defined similarly as that for broad peaks, except that the noise level was the SD of the amplitude of the residual CCGs beyond $\pm25\text{ ms}$.

C CGs between single units and multunit activity. To show the representative overall correlation structures (Fig. 3), we compiled CCGs of single-unit activity at a reference site with all spikes from multunit activity at each available site. This allowed us to see the overall spatial structure of the CCGs because multunit activity was available for most recording sites and had an advantage over CCGs based on single-unit pairs, in which available sites were limited in a single session because of difficulties in isolating single units. When we calculated CCGs between activities of a single unit and multunit activity from the same site, we subtracted the single-unit activity from the multunit activity.

C CGs generated by activity from isolated single units may be more useful for the quantitative evaluation of correlations than CCGs using multunit activity. Therefore, quantitative population analyses were always based on CCGs made from single-unit pairs (Figs. 5–10).

RESULT S

Spatial extent of loose and precise correlations. We made eight penetrations with four-shaft silicon microelectrode arrays with a total of 32 recording probes (Fig. 1A) in cat area 17 and 18 (6 were in area 17 and 2 in area 18). All penetrations based on the present experimental protocol were included in the analysis presented here. Histological reconstruction (6 of 8 sessions) showed that the electrode array was penetrated vertically with a typical deviation from the vertical of $<15^\circ$ and average deviation of $7^\circ$ (Fig. 1B). For all penetrations, we
could simultaneously record from >10 single neurons and multiunit activity from most recording probes (208/240 probes in total), in response to flashed-grating (7 sessions) or drifting-grating (1 session) stimuli. We used cross-correlation analysis to analyze correlation of neuronal pairs separated by a range of horizontal and vertical distances.

Consistent with previous studies, we observed that neuronal correlations in area 17 and 18 typically occurred on different timescales, as illustrated in Fig. 3. In this recording session in area 17, neuronal activity in response to the flashed-grating stimuli were recorded from L2/3 to L6 (Fig. 3A). Most sites showed clear orientation and spatial frequency tuning maps (Fig. 3B). CCGs of neural activities corrected with our shift predictor (shift-corrected CCGs, see METHODS) were compiled between isolated single units from given reference sites and multiunits from all available sites (partner sites). As an example, CCGs for the b6 single-unit and multiunit partner sites are shown in Fig. 3C according to the location of the partner sites. Most CCGs had broad peaks, which reflect loose neuronal correlation, with their baseline width extending tens of milliseconds. CCGs with background activity at the reference site (b6) and CCGs with activity from sites just above or below the reference site (b5 and b7) had narrow peaks along with the broad peaks, which reflect precise neuronal correlation.

We investigated how correlation on different timescales extends in the horizontal and vertical directions by extracting broad and narrow peaks separately as follows. We first processed the shift-corrected CCGs with a median filter to filter out narrow peaks. The filtered CCGs normalized by peak amplitude are shown in Fig. 3D with gray lines (also see Fig. 2). If the highest peak in the filtered CCGs had a width at the one-third peak level and an SNR > 8 ms at the one-third peak level and an SNR ≥ 6 (see METHODS for...
definition of the SNR), we defined the peak as broad. We then subtracted the filtered CCGs from the shift-corrected CCGs. The resultant CCGs are referred to as residual CCGs. Normalized residual CCGs are shown in Fig. 3D with black lines. When the highest peak in the residual CCGs had a width ≤ 8 ms at the one-third peak level and an SNR ≥ 6, we defined the peak as narrow (see METHODS for the additional condition). In Fig. 3, C and D, CCGs containing broad peaks are seen for nearly all recorded sites. Whereas previous reports have found broad peaks predominantly in horizontally separated pairs, we found a broad peak at one of the most vertically distant sites (a3) that equaled one of the most horizontally distant sites (d6), indicating that these peaks extend widely in both directions. On the other hand, clear narrow peaks were only seen in CCGs with sites close to the reference site.

Analysis of multiunit activity, which was available for most recorded sites, showed clear spatial patterns of correlation from a single recording session. However, these patterns might differ from those solely based on single-unit pairs. We therefore examined the spatial patterns of correlations made from single-unit pairs, although the number of available sites was lower because of the difficulty in isolating single units with a multielectrode array. Shift-corrected CCGs between the same reference cell in Fig. 3 and other single units (partner cells) are shown according to the location of the partner cells in Fig. 4.

Consistent with the results shown in Fig. 3, most CCGs showed broad peaks. Furthermore, narrow peaks were only seen in CCGs with another single unit at the reference site.

We next examined these results in more detail by analyzing the spatial extent of correlations in a quantitative manner based on the entire data set. Population analysis was conducted based on CCGs made solely from single-unit pairs. We isolated 197 single units from a total of eight recording sessions using different electrode-array implants (6 were in area 17 and 2 in area 18) (see METHODS for single-unit criteria). Because results from the two areas were not systematically different, we lumped these data together. We also did not find systematic differences in spatial patterns of correlation under different stimulus conditions (drifting gratings vs. flashed gratings) or under different stimulus contrast (0–50%, shown below). Therefore, we also lumped these data together, unless otherwise stated.

The isolated single units resulted in a total of 2,426 single-unit pairs. We examined how broad and narrow peaks depend on vertical and horizontal distances across the whole population. Figure 5, A and B, show proportions of neuronal pairs with broad and narrow peaks as a function of horizontal and vertical distances. The percentage of pairs with narrow peaks drops rapidly from 60% toward 0% between 0 mm and 0.4 mm in both directions. For distances ≥ 0.4 mm,
a consistent proportion (<10%) of pairs still have narrow peaks. These data indicate that precise correlation on a timescale of milliseconds (mean width of narrow peak = 2.8 ms, $SD = 1.0, n = 169$) is typically found between neurons within 0.4 mm of each other, although they are also observed outside this range at a lower proportion. In contrast, broad peaks were found in 80% of pairs at 0 mm and remained in ~40% of pairs even when separated by 1 mm in either vertical or horizontal direction. These results show that loose correlation on a timescale of tens of milliseconds (mean width of broad peak = 34 ms, $SD = 18, n = 1130$) is highly prevalent over a range of 1 mm in any direction.

The incidence of pairs with narrow peaks was high (53%) at a distance of 0 mm (recorded at the same site). We therefore examined whether this high incidence might have resulted from failures in isolating the units, as follows. Across units used in this analysis ($n = 197$), the median proportions of spikes with ISIs < 1 ms and 2 ms were 0.29% and 3.2%, respectively. We selected well-isolated units that had <3% of spikes with ISIs < 2 ms ($n = 100$). For these units,
proportions of narrow peaks at vertical distances of 0 mm, 0.2 mm, and 0.4 mm were 44%, 10%, and 6%, respectively, which were not highly different from those shown in Fig. 5B (53%, 16%, and 4%). This indicates that the high proportion at 0 mm was not likely due to failed isolations.

For the analysis above, we used CCGs averaged across all stimulus contrasts. Since this manipulation is based on an assumption that spatial patterns of correlation are not substantially affected by the stimulus contrast, which has been indicated by previous studies (Arieli et al. 1996; Tsodyks et al. 1999), we next verified whether this held true for our data. In Fig. 5C, the proportions of neuronal pairs with broad and narrow peaks are plotted against horizontal distance under high-contrast (12.5–50%, mean 34.1%) and low-contrast (0–6.25%, mean 2.6%) conditions, using data from six sessions in which both contrast conditions were tested. Figure 5D shows those for the vertical direction. Note that the proportion curves for the two conditions generally overlap for all cases, with no statistically significant differences (2-way ANOVA, $P = 0.25–0.59$, df = 1). Therefore, our data also indicate that the stimulus contrast does not affect the spatial extents of correlation.

Since the proportions of broad and narrow peaks depend on the SNR with arbitrary criteria for defining them, using other metrics that do not depend on such criteria to show the same differential spatial extents for loose and precise correlations is important. We therefore computed filtered CCGs and residual CCGs averaged across pairs for each vertical and horizontal distance (Fig. 5E) and examined their peak amplitudes. Population-average residual CCGs (Fig. 5E, bottom) at 0 mm and vertical 0.2 mm show large peaks, those at both the vertical and horizontal 0.4 mm show much lower peaks, and those at horizontal and vertical 0.8 mm and 1.2 mm show quite small peaks or do not show clear peaks. The filtered CCGs (Fig. 5E, top), on the other hand, show clear peaks at all of these distances. Although their amplitudes drop as distance increases, they drop more slowly compared with the residual CCGs. To quantify this, we plotted the peak amplitude of the population-average filtered CCGs and residual CCGs for each horizontal and vertical distance (Fig. 5, F and G). Note that the peak amplitudes of the residual CCGs drop rapidly within 0.4 mm in the two directions and approach the peak amplitudes in large delay ranges ($-100$ ms to $-50$ ms and $50$ ms to $100$ ms) for CCGs of 1.2 mm. They also remain much lower than those of the filtered CCGs at most distances. This pattern is consistent with the proportion of broad and narrow peaks shown in Fig. 5, A and B, further supporting the nature of the differential spatial extents observed for loose and precise correlations.

**Horizontal scale of loose and precise correlations for each layer.** In the previous section, we showed that loose correlation, in general, spread widely in the vertical and horizontal directions whereas precise correlations are limited to a narrower range. We next investigated the horizontal scale of loose and precise correlations for each layer. Data were based on the six sessions for which histological reconstruction of recording sites was conducted.

We analyzed only pairs of cells recorded from the same layer and divided them into different groups based on their layers. For each layer group, we compared how the proportion of pairs with broad or narrow peaks depends on the horizontal distance separating them, as shown in each row of Fig. 6A. The proportions of pairs with broad peaks were generally above 25% for horizontal distances up to 1.2 mm, regardless of the layer (14 of 16 data points), indicating that loose correlations exist between neurons separated by up to this distance for all layers. In contrast, the proportions of pairs with narrow peaks decreased from 40% at 0 mm to 0–10% at 0.4 mm for all layers except L2. The proportions mostly remained at this level for further distances for all layers including L2/3. Therefore, regardless of the layers, precise correlation is typically seen between neurons within 0.4 mm of each other, although it does occur outside this range at rather lower proportions.

In L4 and L5, the proportion curves for broad peaks drop and rebound as distance increases. To examine whether this trend was due to layer misalignment, we repeated the analysis after excluding pairs in which at least one neuron was located by 10.2 mm above or below layer boundaries. In L4,
the proportions for 0 mm, 0.4 mm, and 0.8 mm were 50%, 27%, and 57%, respectively (data for 1.2 mm were not used because the sample size was <5). In L5, the proportions for 0 mm, 0.4 mm, 0.8 mm, and 1.2 mm were 84%, 65%, 61%, and 69%, respectively. These changes in proportions by distance are similar to those shown in Fig. 6A, indicating that the observed trend was not caused by layer misalignments.

The above results show that loose and precise correlations differentially extend in the horizontal direction regardless of layers. We further examined this by analyzing the peak amplitude of the population-average CCGs for each layer, as shown in different rows of Fig. 6B. The peak amplitude of residual CCGs sharply decayed toward 0 within 0.4 mm (88% reduction for L2/3, 90% for L4, 88% for L5, and 87% for L6) and became similar to the peak amplitude in large correlation delay periods (−100 ms to −50 ms and 50 ms to 100 ms) for the CCGs at 1.2 mm. It remained at this level for more distant pairs. This pattern of change is consistent with data regarding narrow peaks shown in Fig. 6A.

In contrast, peak amplitude of population-average filtered CCGs decayed more moderately within 0.4 mm (60% reduction for L2/3, 80% for L4, 50% for L5, and 60% for L6) and remained 2.8–5.9 times higher than that of residual CCGs at 0.4 mm. Generally, it also remained higher than that of residual CCGs for further distances. Therefore, these results also suggest that loose and precise correlations have different horizontal extents regardless of layer.

Laminar difference of proportions of correlated pairs. Figure 6A also suggests that, at least for short distances (≤0.4 mm), the proportion of broad peaks was smaller in L4 than other layers. For example, the percentage of broad peaks at 0.4 mm for L4 was 16%, a value less than half that obtained at the other layers. For example, the percentage of broad peaks at 0.4 mm, and other layers (all P > 0.05, 4 groups). Therefore, the weak correlations in L4 are specific to loose correlation.

We further examined whether the reduced proportion of broad peaks in L4 compared with other layers was genuine and robust for errors in drawing layer boundaries. We analyzed whether consistent results were obtained when only pairs at specific distances from layer boundaries were used. From pairs we used in Fig. 7A, we excluded pairs in which at least one neuron was located within 75 μm of a boundary. In this case, the proportions of broad peaks for L2/3, L4, L5, and L6 were 56%, 38%, 73%, and 50%, respectively (n = 9, 21, 48, and 12), showing that L4 still had the lowest proportion and indicating that this result is not likely due to errors in layer assignment.

Vertical range of loose and precise correlations. We next examined the vertical range of precise and loose correlations. For a given reference layer, we sampled pairs consisting of neurons recorded from the layer (reference neuron) and partner neurons recorded from the same electrode shafts and located in any layer. We then divided the pairs for each reference layer into groups based on the absolute vertical distance between the pairs. In different rows of Fig. 8A, we show proportions of pairs with broad and narrow peaks for these distance groups for different reference layers. Regardless of the reference layer, the percentage of narrow peaks starts from ~40% at 0 mm of separation, decays rapidly toward 0% within 0.4 mm, and remains between 0% and 10% for further distances. Figure 8B shows the peak amplitudes of population-average CCGs computed for each distance group. Consistent with the proportion of narrow peaks, peak amplitudes of residual CCGs decayed sharply by ~90% within 0.4 mm for all reference layers. They remained near 0 for further distances. This narrow vertical range of precise correlation is highly similar to its horizontal range (Fig. 6, A and B).

On the other hand, proportions of broad peaks generally remained above 30% for the entire vertical separation range, regardless of the reference layer (Fig. 8A). Consistently, the peak amplitude of filtered CCGs (Fig. 8B) decayed more slowly within 0.4 mm than that of residual CCGs and generally remained higher than the peak amplitude of residual CCGs for further distances. These results indicate that for each reference layer loose and precise correlations differentially extend vertically in a similar manner as they do horizontally.

It may also be important to examine the vertical range of correlations by analyzing how far neurons in each layer share correlations with remote layers. For each reference layer, we used the same set of pairs as in the previous analysis. However, this time, we divided them into four groups based on the layer of the partner cell. Figure 8C, top, depicts proportions of pairs with broad peaks for each group (each bar) when the reference layer is L2/3. Note that regardless of the partner layer, the proportion of broad peaks was always >30%. This trend was generally true for each reference layer, indicating that loose correlations extend vertically even between neurons that are in widely separated layers.

Although the above results show prevalence of loose correlation across layers, does the incidence of correlated pairs for...
each reference layer depend on the partner layer? To examine this on a statistical basis, we compared data for each reference layer with Tukey’s WSD test. For L2/3, the proportion of broad peaks within the layer was significantly larger than that between L2/3 and L4 or that between L2/3 and L6 (all P < 0.05, 4 groups). For L5, the proportion of broad peaks for pairs within the layer was significantly larger than that between L5 and L4 (P < 0.05, 4 groups). Therefore, L2/3 and L5, two output layers of the cortex, were both less loosely correlated with L4.

Figure 8D shows a similar analysis conducted on the proportions of narrow peaks. Proportion values for pairs of neurons in the same layers ranged from 10.5% to 26.7% for the four reference layers. The proportion decreased to 3.6–12.1% for pairs of neurons from adjacent layers and to 3.5–4.5% for pairs of neurons from more distant layers. The results show that precise correlation is most frequently found for pairs of neurons in the same layers and becomes weaker as layers of the two neurons become more distantly separated.

**Temporal offsets of broad and narrow peaks.** To reveal detailed temporal structures of correlation across layers and columns, we analyzed the temporal displacement of broad and narrow peaks as a function of the horizontal and vertical distances.

Figure 3D shows that broad peaks in CCGs with sites located at the same depth as the reference site (a6–d6) tended to have no or quite small displacement, while those located at several upper sites (a3, b3, c3, d3) had positive displacement. In contrast, broad peaks in some CCGs with sites below the reference site had slightly negative shifts (e.g., d7). The results show that the reference cell tended to fire after cells in deeper sites and before cells in upper sites. In contrast, narrow peaks had relatively small offsets, near 0 (b5, b6, b7).

For the population analysis, we examined temporal offsets of the broad and narrow peaks based on CCGs of single-unit recordings.
pairs (Fig. 9). For horizontally separated pairs (Fig. 9A), the average offsets of broad peaks were around 0 irrespective of distance, with no data being judged significantly different from 0 (P = 0.16–0.95, t-test, total data number = 180). Vertically, the average offsets of broad peaks for large distances were deviated from 0 (Fig. 9B). Average offsets at absolute distance of 0.6 mm, 0.8 mm, and 1.0 mm were significantly larger than 0 (t-test, P < 0.05, total data number = 319). This indicates that cells in upper sites tended to fire after cells in deeper sites. The relationship between distance and peak offset is roughly monotonic, fitted by a line with a slope of −0.171 mm/ms. Additionally, we analyzed offsets of broad peaks between reference cells in L6 and partner cells in each layer (Fig. 9C) and found that the offset progressively increased as the layer of the partner cells approached the cortical surface, with the largest offset being 7.3 ms between L6 and L2/3.

The average offsets for the narrow peaks were around 0 irrespective of the horizontal distance (Fig. 9A), with no data being judged significantly different from 0 (P = 0.73–0.81, t-test, total data number = 49). In contrast to broad peaks, the average offset of narrow peaks did not show positive shifts for negative vertical distances (Fig. 9B; t-test, P = ~0.45–0.85, total data number = 80), with 41% (14/34) having offsets of 0 ms for vertical distances of 0.2 mm and 0.4 mm. Seventy-four percent (25/34) of these peaks were located within 1 ms for these same distances (data not shown). These results show highly different temporal structures between precise correlation and loose correlation in the vertical direction.

**Dependence of precise and loose correlation on tuning similarity.** Previous studies have shown that the probability of two cells having correlated activities depends on the similarity of their RFs (Hata et al. 1991; Krüger and Aiple 1988; Schwarz and Bolz 1991; Smith and Kohn 2008; Ts’o et al. 1986). Here we tested whether this held true in this study. Since RF properties usually change along directions tangential to the cortical surfaces, we analyzed neuronal pairs separated horizontally by 0–1.2 mm and vertically by 0–0.4 mm. Data were based on seven sessions in which flashed-grating stimuli were used. Similarities between RFs of two cells were quantified by the correlation coefficients of their tuning maps (Fig. 3B), which we term the similarity index. As this index approaches 1, the tuning maps become more similar. Pairs were sampled for index bins with a width of 0.2 whose locations were varied in 0.1 steps from −1 to 1. These pairs were further grouped according to their horizontal separation. For these groups, the proportions of pairs with broad and narrow peaks are grayscale coded in Fig. 10A. Data are shown only for groups with more than five pairs. Figure 10B shows the proportions of broad and narrow peaks without grouping based on the horizontal separation. Proportions of both broad and narrow peaks decreased with the similarity index, as consistent with previous studies (narrow peak: r = 0.89, P < 0.01, broad peak: r = 0.96, P < 0.01). Furthermore, the proportions of broad peaks remained around 0.4 even for similarity index values around 0. Therefore, some cells with dissimilar RFs are correlated in a loose manner, although the prevalence was smaller than if pairs had similar RFs.

In contrast, cells correlated in a precise manner typically had similar RF properties. Only when pairs had similarity index ≥ 0.5 were the proportion of cells with narrow peaks at least 20% (Fig. 10B). This indicates that precise and loose correlations differ not only spatially but also in their dependence on RF similarity.

**DISCUSSION**

To elucidate details regarding how correlations in V1 neural activity are spatially organized, we examined the horizontal and vertical structures and the laminar organizations of corre-

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**Fig. 9.** Temporal offsets of broad and narrow peaks. Average temporal displacement of broad and narrow peaks is plotted as a function of distance in the horizontal (A) and vertical (B) directions. In B, negative distances indicate that the partner site is above the reference site. The plot is symmetrical around 0 because each pair was plotted twice by taking each cell from a pair as the reference cell. Only data points with >5 samples are shown. Error bars show SEs. C: peak offsets of CCGs between reference cells in L6 and partner cells in each layer.

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correlation timescales. We recorded simultaneous neuronal activities in cat V1 with multielectrode arrays. Because we observed that correlations occurred in different timescales, consistent with previous studies, we separated them into precise (a timescale of milliseconds) and loose (a timescale of tens of milliseconds). We found that loose correlations were frequently observed at distances > 1 mm both horizontally and vertically, regardless of the layers. Additionally, the incidence of observing loosely correlated pairs was lower in L4 than in other layers. Loose correlations showed a consistent delay in firing that was monotonically related to the vertical, but not horizontal, distance between the pairs.

In contrast, precise correlation was more spatially limited, with its incidence dropping sharply within 0.4 mm in both vertical and horizontal directions for all layers. These results show that neuronal correlations in V1 show markedly different structure for horizontal and vertical dimensions depending on correlation timescales.

Spatial distribution of loose and precise correlations. The horizontal spatial range in which broad peaks were observed exceeded 1 mm, which is consistent with previous studies of L2/3 and L5 (Krüger and Aiple 1988; Michalski et al. 1983). Furthermore, Michalski et al. (1983) showed that ~40% of pairs separated by 1 mm had broad peaks. This is generally consistent with the present study, which estimated that broad peaks occurred in 40–60% of pairs that were 1.2 mm apart (Fig. 5, A and C). This range is larger than the horizontal extent of a hypercolumn (a full cycle of orientation columns). Pairs with dissimilar tuning properties consistently show this type of correlation frequently (Fig. 10), as is also shown in Smith and Kohn (2008).

As a measure of loose correlation, Smith and Kohn (2008) used spike-count correlation that reflects loosely correlated activities with a timescale of up to seconds, while our measure of loose correlation reflects correlations of tens of milliseconds. When comparing our results with their study, knowing how our measure of loose correlation is related to spike-count correlation is useful. When we measured trial-to-trial correlation of spike counts for all neuronal pairs (n = 2,426) and calculated the Pearson’s correlation coefficient between them and the peak amplitudes of the filtered CCGs for these pairs, we found that the coefficient value was 0.27 (P < 0.001), indicating that our measure of loose correlation is moderately but certainly correlated with spike-count correlation, despite the differences in timescales. This relationship may be why their results and ours are similar. Additionally, they showed that spike-count correlation decreased by <20% when the distance between neuronal pairs increased from 0.5 mm to 1.5 mm. This slight change is consistent with the small change in peak amplitude we saw for the filtered CCGs between 0.4 and 1.2 mm (Fig. 5F).

While loose correlation on a timescale of tens of milliseconds has been observed previously between horizontally separate pairs (Krüger and Aiple 1988; Michalski et al. 1983), to our knowledge this is the first time such correlation has been shown to frequently occur between vertically separate pairs, including pairs between L2/3 and L6. On the basis of these results, we suggest that populations of neurons across several hypercolumns often share loose correlation.

We also found that loose correlation in L4 is weaker than in other layers. Similar results were reported by Smith et al. in a recent study (Smith et al. 2013). They showed that in monkey V1 proportions of neuronal pairs with positive spike-count correlation are smaller in the middle layer than in superficial and deep layers. This suggests that the laminar organization of loose correlation is common across different species. Smith et al. (2013) also showed that strength of local field potentials (LFPs) in the low-frequency band (<10 Hz) is correlated with spike-count correlation. This suggests involvement of LFPs in the loose correlations analyzed here. However, analysis of laminar patterns for LFP components < 10 Hz (Smith et al. 2013) and < 25 Hz (Maier et al. 2011) appears to indicate that they are weakest in superficial layers, not in the middle layer in which loose correlation is the weakest. Therefore, the relationship between LFP, spike-count correlation, and loose correlations may be complicated and needs further study.

The incidence of precise correlation generally dropped to <10% when pairs were separated by at least 0.4 mm, either vertically or horizontally. This distribution of precise correlation is consistent with Toyama et al. (1981b), who first described vertical neuronal correlations in cat V1. It is also consistent with Krüger and Aiple (1988), who showed that narrow peaks were distributed similarly for the horizontal direction in L5 and L6 of monkey V1. Hata et al. (1991) observed a similar spatial distribution of horizontal correlations for population data sampled from all layers in cat V1.
Many studies have shown that precise correlation is also observed for neuronal pairs that are further apart (<3 mm) if the pairs show similar tuning properties, although the incidence is low (Gray et al. 1989; Maldonado et al. 2000; Schwarz and Bolz 1991; Smith and Kohn 2008; Ts’o et al. 1986; Ts’o and Gilbert 1988). Consistent with these studies, our results show precise correlation between pairs separated by 0.4 mm to 1.2 mm, with typical incidence values between 0% and 10%.

**Underlying mechanisms of loose neuronal correlation.** Long-range horizontal interactions on a spatial scale of a few millimeters in superficial layers (Michalski et al. 1983; Ts’o et al. 1986) or deep layers (Krüger and Aiple 1988; Schwarz and Bolz 1991) have been proposed to be mediated by intrinsic horizontal connections (Ts’o et al. 1986). Therefore, one might consider that the loose correlations shown here are based on intrinsic horizontal and vertical connectivity within V1. On the other hand, Smith and Kohn (2008) indicated that loose correlation is predominantly mediated by feedback projections from higher-order cortical areas, based on the long spatial extent of the correlation (as much as 10 mm) that is wider than the projection range of intrinsic axons (Smith and Kohn 2008). Our results appear to support the feedback proposal. For example, we found that deeper neurons fire before neurons in more superficial layers. These spatial-temporal patterns of correlation might be explained by feedback projection as follows. These projections are known to ascend from deep layers to superficial layers, spreading horizontally. Additionally, axons from a single cell arborize in multiple layers including both deep and superficial layers (Rockland and Virga 1989). Therefore, feedback inputs from the same cell diverge in all layers, possibly causing the target cells to synchronize with subtle time differences that reflect the arrival time of the feedback inputs. Also, feedback inputs tend to avoid L4 (Felleman and Van Essen 1991), an observation consistent with our finding that loose correlation is less common in L4.

Although above we speak as though loose interactions are totally mediated by common feedback inputs in a monosynaptic manner, we do not dismiss the possibility that polysynaptic pathways using intrinsic connectivity also play roles. For example, cells in L5 or L6 that receive feedback inputs then provide inputs to superficial layers, generating temporal displacement of CCGs between deep and superficial layers.

Another factor that may substantially contribute to loose correlations is slow oscillatory neuronal activity (<1 Hz) generated in L5 and propagated to other layers (Sanchez-Vives and McCormick 2000; Smith et al. 2013). However, since this leads to L5 neurons becoming active before neurons in L6, this mechanism alone cannot explain the overall patterns of loose correlation in which L6 neurons tend to fire before L5 neurons (Fig. 9C).

Feedforward projections from the lateral geniculate nucleus (LGN), on the other hand, may not be critically involved in generating loose correlations. These afferents, which maintain myelin sheaths even in the cortex (Ferster and LeVay 1978), transmit signals fast (8–40 mm/ms) (Hoffmann et al. 1972), indicating that they may provide essentially simultaneous inputs to different layers. Additionally, the proportion of loose correlation in L4 is lower than the other layers (Fig. 7A), even though L4 mainly receives thalamocortical inputs. Thus inputs from the LGN are not likely a source for the loose correlations found in V1.

**Underlying mechanisms of precise neuronal correlation.** We reported that 41% of the narrow peaks for neuronal pairs with separation distances of 0.2 mm and 0.4 mm had no temporal offset and that 74% had offset ≲ 1 ms, with heights at the time origin above the 33.3% peak level. Although these peaks might actually have been delayed peaks, high proportions of narrow peaks at or straddling time origins have often been reported (Maldonado et al. 2000; Michalski et al. 1983; Toyama et al. 1981b) and are thought to be mediated by common inputs (Perkel et al. 1967). Because the spatial range (~0.4 mm) that includes a high incidence of narrow peaks is similar in size to a patch of terminal arbors of afferents from LGN (Ferster and LeVay 1978; Freund et al. 1985; Humphrey et al. 1985), Toyama et al. (1981b) and Hata et al. (1991) suggested that afferents from LGN are a plausible source for these common inputs.

Studies have suggested that intrinsic vertical and horizontal connections may also provide common inputs for these correlations, especially in L2 and L5, which do not receive direct thalamocortical projections (Hata et al. 1991; Smith and Kohn 2008). Possibly, these intrinsic connections are stronger sources for precise correlation than thalamocortical connections because the strong dependence of these correlations on tuning similarity is more consistent with the properties of intrinsic axons that connect cells with similar orientation preferences (Kisvarday et al. 1997; Malach et al. 1993). Also, L2 is known to have abundant horizontal connectivity compared with other layers (Fisken et al. 1975; Gilbert and Wiesel 1979). This may explain why precise correlation was slightly more prevalent in L2, although not significantly so (Fig. 7B).

Finally, let us discuss whether feedback inputs from higher cortical areas are involved in the precise correlation. As mentioned in the last section, feedback projections tend to avoid L4. Therefore, if feedback projections act as sources for this correlation this should be particularly weak in layer 4. However, we did not see any reduction in the proportion of pairs showing precise correlations in L4 (Fig. 7B). Therefore, we cannot endorse feedback projection as the primary source of this type of neural correlation.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: H. Tanaka, H. Tamura, and I.O. conception and design of research; H. Tanaka performed experiments; H. Tanaka analyzed data; H. Tanaka, H. Tamura, and I.O. interpreted results of experiments; H.
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