Thesis

Diversity, Heterogeneity and Orientation Dependent Variation of Spike Count Correlation in the Cat Visual Cortex

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Abstract

Cortical neurons are known to be noisy encoders of information, showing large response variabilities with repeated presentations of identical stimuli. These spike count variabilities are correlated over the cell population and their neuronal mechanism and functional significance have not been well understood. Recently there has been much debate over the magnitude of the population mean of the correlation, ranging from 0.1 ~ 0.2 down to nearly zero. We performed multi-neuron recordings on the cat visual cortex and found that the population mean did not necessarily represent the nature of correlated variabilities because the spike count correlation showed significant diversity and heterogeneity. Although the population mean was relatively small (0.06), the correlations of individual unit pairs were distributed over a broad range, extending to both positive and negative values. In most of the recording sessions of local cell populations (83%), significantly positive correlations coexisted with significantly negative ones in different unit pairs. Furthermore, nearly 20% of the unit pairs showed significant variation in the spike count correlation for different stimulus orientations. Correlation analysis between the spike count correlation and the firing activity of the unit pair suggested that the orientation tuning properties of the two quantities were unlikely to have originated from a common neuronal mechanism. Diversity, heterogeneity and context dependent variation suggests that the correlated spike count variabilities originate not from fixed anatomical connections but rather from the dynamic interaction of neuronal networks.

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圓山　由子

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1. Introduction

A pioneering study of the visual cortex was conducted by Hubel and Wiesel in 1960's. They stimulated the cat's retina by presenting various pictures and investigated in what manner individual neurons in the visual cortex responded to each stimulus. Surprisingly, they found that each neuron in the primary visual cortex did not respond to the visual stimulus extending to a full field but responded to the stimulus only in a tiny spatial range, which was called as the receptive field of the neuron. They also found that each neuron in the primary visual cortex responded optimally to a simple object (bar and slit) with a specific orientation, which was called as optimal orientation of the neuron. After systematic experimental examinations of neuronal properties in the visual cortex, Hubel and Wiesel established an architectural model of the visual cortex, named as *hyper column*. The hyper column has a dimension of 1mm × 1mm on the cortical surface and 3~4mm depth. The hyper column has a highly organized structure for parallel computations of various features (location, orientation, movement direction, texture, binocular disparity) of the stimulus presented at its corresponding receptive region, called as hyper field. For example, each hyper column consists of multiple vertical columns named as orientation columns. All the neurons in a single orientation column have the same optimal orientation. Detection of the stimulus orientation is processed parallelly by different orientation columns tuned to different optimal orientations.

Response property of a neuron is generally examined by the mean firing rate averaged over reasonably large number of trials (>20) presenting the same stimulus. However, in our ordinary perception, we can recognize a shape of the object in a single presentation. Therefore, in our brain, there should exist a mechanism that can decode the information of the given stimulus encoded by single trial neuronal activities. However, the modeling of such decoding mechanism turned out to be difficult, because individual neuron shows a large response variability to the repeated presentations of the identical stimulus. Figure 1 shows the response property (mean firing rates) of the recorded neuron to stimuli of different orientations. Although the trial averaged firing rates showed a relatively clear orientation tuning property, there existed significantly large amount of trial-to-trial variabilities. The responses to the optimal orientation stimulus sometimes showed lower firing rates than those to the least optimal orientation stimulus. We can not decode the stimulus orientation with enough confidence by the single trial response of a single neuron. To overcome this difficulty, a scheme of population coding was proposed (Georgopoulos *et al*., 1992; Salinas & Abbott, 1994). We assume a large number of neurons showing either the same or a reasonably

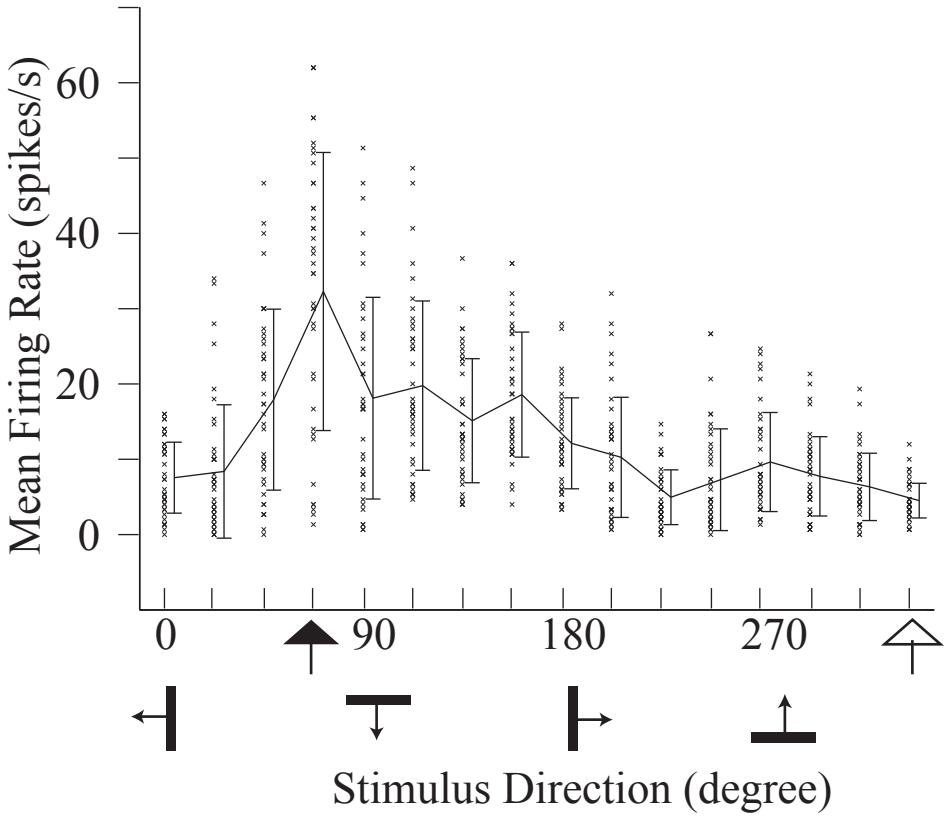

Figure 1. Example of spike count variabilities. Orientation tuning curve of a single unit based on the mean firing rate over 40 trials. Firing rates of individual trials are shown by dots for each stimulus. A large amount of trial-to-trial variabilities lead to rather large error bar (±*SD*). The filled (open) arrow represents the optimal (the least optimal, null) orientation. The distribution of the responses to the optimal stimulus was broader and overlapped with that to the null stimulus.

redundant statistical response characteristic and that the fidelity of the neuronal response emerges as a result of ensemble averaging over these populations. However, when the response variabilities are correlated within the population, the standard deviation of the population average over N neurons no longer decreases at a rate of $1/\sqrt{N}$ but, saturates to a finite quantity (Zohary *et al*., 1994). Correlated response variabilities, which is also called spike count correlation, have actually been observed in various cortical areas (IT: Gawne *et al.*, 1993, MT: Zohary *et al*., 1994; Bair *et al*., 2001, V1: Reich *et al*., 2001; Kohn & Smith, 2005; Smith & Kohn, 2008; Gutnisky & Dragoi, 2008; Ecker *et al*., 2010, V4: Cohen & Maunsell, 2009; Mitchell *et al*., 2009, M1: Lee *et al*., 1998; Maynard *et al*., 1999, A1 and S1: Renart *et al*., 2010). Most studies have previously reported that the population mean of the correlation coefficients were weakly positive, lying within a range of 0.1 ~ 0.2. However, recent reports of values one order of magnitude smaller (0.01 Ecker *et al*., 2010; 0.005 Renart *et al*., 2010) have opened the debate regarding the degree of this correlation as well as the efficacy of the population coding (Cohen & Kohn, 2011).

In previous spike count correlation studies, the magnitude of the population mean was the main interest. However, the mean value can only represent the nature of the correlated spike count variabilities when the correlations of individual samples distribute about the mean with a reasonably low variance. Fine spatial structure and stimulus dependent variation of the individual correlations, even when they may exist, are averaged out in the population mean. Furthermore, many studies restricted their analysis only to unit pairs that displayed similar orientation tuning characteristics. Since both the neuronal mechanism and functional significance are still open questions, the analysis of limited samples could provide unnecessary bias in the property of spike count correlation. Therefore we characterized the physical properties of the correlated spike count variabilities extensively without any restriction of the tuning properties of the sample units.

We found that the correlations of individual unit pairs were distributed over a broad range, extending to both positive and negative values. Our small population mean of 0.06 was only the result of averaging out those diversities. The spike count correlation was found to have only a weak relationship with the similarity in orientation tunings of the two units. Significant correlation was also observed in the unit pairs having dissimilar orientation tuning properties and those in which one or both units did not show significant orientation preference. The spike count correlations were spatially heterogeneous in most of the recording sessions of local cell populations (83%), that is,

significantly positive correlations coexisted with significantly negative correlations in different unit pairs. Furthermore the spike count correlation was not necessarily context invariant. Nearly 20% of the samples showed significant variations of the spike count correlation for different stimulus orientations. The spike count correlation and the firing activity of the unit pairs were not likely to originate from a common neuronal mechanism because the orientation dependent variations of two quantities were mostly independent. Diversity, heterogeneity and context dependent variation observed in the spike count correlation may suggest that the correlated spike count variabilities originate not from fixed anatomical connections, but rather from the dynamic interaction of neuronal networks.

The study presented in this thesis will be published in European Journal of Neuroscience, 2013.

2. Materials and Methods

2-1 *Physiological preparation*

Acute experiments were performed on five adult male cats weighing between 3 and 5 kg (American short hair, Liberty Research, *Inc.*, USA). Each animal was premedicated with Atropine (0.03mg/kg, s.c.) to reduce salivation and anesthetized with an intramuscular injection of Medetomidine HCL (0.02mg/kg) and Midazolam (0.3mg/kg). During the experiment, animals were maintained by intravenous infusion of an electrolyte solution (Salita T-3, 2 ml/kg/hr) and ventilated with a mixture of nitrous oxide and oxygen (2:1) via a respirator pump (Shinano, Japan). During the surgical operation, animals were anesthetized with isoflurane (1.5 – 2.0 %). The EKG, heart rate, rectal body temperature, expiratory CO_2 and SpO_2 were continuously monitored, the latter four being maintained within the ranges of 140 - 180 bps, 37.5 -39.0 degrees centigrade, 3.5 - 4.5 % and 98-100%, respectively. The animal's head was mounted in a stereotaxic frame and a small craniotomy was made above the primary visual cortex (A10-P10, L0-5) in one hemisphere. Before an incision was made into the dura matter, glycerin (Glyceol) was given intravenously (1.67g/kg/hr) by mixing it with the maintenance solution to reduce intracranial pressure. The eyes were focused on the tangential screen at a distance of 57 cm using the tapetal reflection technique and an appropriate set of gas permeable contact lenses. The pupils were dilated using phyenylephrine hydrochloride (Neosynesin eye solution). After penetration of electrodes, we switched to the balanced anesthesia by intravenous infusion of fentanyl (0.00785mg/kg/hr, Fentanest), droperidol (0.25mg/kg/hr, Droleptan) and pancuronium bromide (0.1mg/kg/hr, Mioblock) mixed with the maintenance solution. The dosage of thirty minutes was injected as an initial bolus. During the balanced anesthesia, the heart rate was maintained within the range of 180~200 bpm. Antibiotics (cefotiam hydrochloride) were given intravenously (0.25g) every eight hours. All experimental procedures were in accordance with institutional and NIH guidelines and approved by institutional Animal Welfare Committee.

2-2 *Recording procedures*

Two types of electrodes arrays were adopted for the recordings (a 4-tetrode array and an array of 8 single microelectrodes, both of which were fabricated in our laboratory). Each array is assembled from different quartz-platinum/tungsten

microelectrodes (Thomas Recordings, Germany). The tetrode array consists of 4 tetrodes (96 μm diameter) arranged in a 2×2 square matrix with a 500 μm inter-electrode distance. The array of single electrodes consists of 8 single microelectrodes (40 μm diameter) arranged in a 3×3 square matrix (excluding the center) of a 310 μm inter-electrode distance. Recordings by both arrays collected multiple single unit activities at a local cortical area of the size of a single hyper column. The penetration depth of each electrode was independently adjustable with a step-size of 1 μm performed by a stepping motor drive (EPS, Alpha Omega, Israel). Generally, in one recording session, all electrodes were positioned roughly at the same depth. After all of the electrodes were embedded in the cortical surface, a 4% mixture of Agar in saline was applied to the surface to reduce pulsations. Except for the tuning similarities of the units isolated from the same electrode (see Appendix), unit pairs recorded by the two types of arrays did not show any significant difference in their spiking properties. We performed global statistics over all unit pairs without making a distinction between the two arrays, with the exception of the analyses explicitly mentioned. The signals from each electrode were amplified (gain=10000, Cheetah, Neuralynx, USA), band pass filtered (0.6 kHz – 6 kHz, 3dB falloff), and digitized (27 kHz/channel Data Translation DT2821). Spike waveforms 1.2 ms in duration and centered at the time of occurrence of a user-defined threshold crossing were stored in a file along with their associated time stamps with a temporal resolution of 37 μs (see Ito *et al.*, 2010).

2-3 *Stimulus presentation*

Once stable recordings were obtained, we mapped the receptive field properties (location) of the multiunit activity recorded by each electrode using a mouse-controlled moving light bar presented on a 21 inch color monitor (1024×768 resolution, vertical refresh rate of 80Hz) at a distance of 57 cm from the eyes. Since the receptive fields of the units recorded by the high-density electrode arrays had significant overlap, we stimulated the units by moving the light bars on a dark background crossing over the region covering all of the receptive fields. The stimuli consist of the light bars of 16 orientations equally spaced (i.e. with an angular separation of 22.5°) that move along the direction of the normal. We ran 40 trial blocks in which each of the 16 stimuli were presented in a pseudo-random order with an intertrial interval of 3 s. The bars traveled an angular distance of $3 \sim 5^{\circ}$ over a period of 1.0~1.7 s (speed 3°/s).

2-4 *Spike sorting*

Multiunit activities recorded by each electrode were sorted to recover the activities of individual single units using custom spike sorting software (Gray *et al*., 1995). The sorting was carried out based on peak-to-peak amplitudes of available channels, spike width, peak time and the principal component analysis (PCA) of the waveforms. To avoid contamination by multiunit activities, we imposed a strict criterion for the number of spike events falling within an absolute refractory time of 1 ms. For the tetrodes, this number should be less than 1.5% of the total isolated spike counts to be judged as a single unit. Since single microelectrodes have a weaker sorting ability, stricter criterion was applied (1.0%). The spike trains were down-sampled to a resolution of 1 ms before analysis.

2-5 *Sample selection*

For the analysis of spike count variabilities and correlation, we selected single units satisfying the following two requirements. 1) *Significant stimulus response*: For each single unit, we selected the optimal stimulus orientation giving the maximum trial-averaged firing rate. Then we tested whether the firing rates changed significantly before (duration of 0.1~0.8 s) and after (duration of 1.5~2.8 s) the stimulus presentation using the Wilcoxon signed rank test (40 trial samples, $P<0.05$). 2) *Stationary response*: Since a single recording session lasted approximately 30~40 minutes, unit activity might have non-stationary modulation over a long temporal scale due to electrode drift or unstable anesthesia. Co-modulation of the activity levels of the unit pairs poses the danger of creating an artificial spike count correlation. We tested how stationary each unit's activity levels were over 40 successive trials to the corresponding optimal stimulus via the bootstrap trial shuffle test. At first, we calculated the mean firing rate over 40 trials and counted the maximum successions of the trials, keeping the firing rate more (less) than the mean firing rate, $MS+_{\mathrm{test}}$ ($MS-_{\mathrm{test}}$). If there was a long-term non-stationary modulation, we expect that the activity levels consistently remained above (below) the mean rate for a significantly longer duration than the cases of stationary response. Significance was tested against the null hypothesis that trial-to-trial variabilities of the firing rate had the same distribution as the test data, but occurred independently in each trial. The distribution of the maximum successions ($MS+$, $MS-$) in the null hypothesis was predicted by bootstrap samples generated using the following

methodology:

1) Generate a bootstrap sample by randomly shuffling the order of the 40 trials. Mean firing rate remains the same as the test data.
2) For each trial shuffled sample, count the maximum successions of trials *MS*+ (*MS*-) keeping the firing rate more (less) than the mean firing rate.
3) Repeat steps 1-2 *N* times to get *N* samples of both *MS*+ and *MS*-. Since the number of possible shuffled combinations becomes very large, we apply uniform Monte Carlo samplings (*N*=1000*)*.
4) For both *MS*+ and *MS*-, the significance limit is given by the 99th percentile of the *N* values. When either the $MS+_{test}$ or $MS-_{test}$ exceeds the significance limit, the activity levels during the recording session are judged as significantly non-stationary ($P<0.01$) and the sample is excluded from further analysis.

2-6 *Stimulus tuning properties of single units*

For all the units showing significant stimulus-evoked and stationary responses, we computed the orientation tuning curves over 16 stimuli based on the trial averaged mean firing rate. We then calculated two kinds of tuning amplitudes: a direction selectivity amplitude A_d and an orientation selectivity amplitude A_o. The direction selectivity amplitude was defined by the vector sum of the 16 orientations θ_s weighted by the mean firing rate of the corresponding stimuli r_s standardized by the firing rate averaged over all stimuli (Ringach *et al*., 2002).

$$A_d = \sqrt{A^2 + B^2},$$

$$A = \sum_{s=0}^{15} r_s \times \frac{\cos\left(2\pi \times \frac{\theta_s}{360^\circ}\right)}{\sum_{s=0}^{15} r_s}, \quad B = \sum_{s=0}^{15} r_s \times \frac{\sin\left(2\pi \times \frac{\theta_s}{360^\circ}\right)}{\sum_{s=0}^{15} r_s}, \quad \theta_s = 22.5^\circ \times s,\ s = 0, \ldots, 15.$$

The orientation selectivity amplitude was calculated in a similar manner except that θ_s was replaced by $2\theta_s$ and r_s was replaced by the sum of the firing rates to the stimuli of the opposite moving directions $r_s' = r_s + r_{s+8}$.

$$A_o = \sqrt{C^2 + D^2},$$

$$C = \sum_{s=0}^{7} r_s' \times \frac{\cos\left(2\pi \times \frac{2\theta_s}{360^\circ}\right)}{\sum_{s=0}^{7} r_s'}, \quad D = \sum_{s=0}^{7} r_s' \times \frac{\sin\left(2\pi \times \frac{2\theta_s}{360^\circ}\right)}{\sum_{s=0}^{7} r_s'}, \quad \theta_s = 22.5^\circ \times s,\ s = 0, \ldots, 7.$$

If either A_d or A_o exceeded 0.1, the unit was regarded as showing a significant stimulus tuning property. When $A_o > A_d$, we ascertained that the unit was orientation selective. Conversely, when $A_d > A_o$, the unit was deemed to be direction selective. The optimal orientation spanning 0~180° was determined differently depending on whether the unit was orientation selective or direction selective. For the direction selective unit,

$$\text{optimal orientation} = 180^\circ \times \frac{\tan^{-1}\left(\frac{B}{A}\right)}{\pi},$$

where the argument of the arctangent was taken within the range of [0, π] irrespective of the sign of *B*. For the orientation selective unit,

$$\text{optimal orientation} = 90^\circ \times \frac{\tan^{-1}\left(\frac{D}{C}\right)}{\pi},$$

where the argument of the arctangent was taken to fall within the range [0, π] for $D>0$, and [π, 2π] for $D<0$.

2-7 *Correlation of spike count variabilities*

Firing rates of the unit *i* in the stimulus duration of the *k*- th trial ($k = 1,\ldots, 40$) to the stimulus *s* ($s = 0,\ldots, 15$), x_{ik}^s were normalized to the *z*-score,

$$z_{ik}^s = \frac{x_{ik}^s - r_s}{\sigma_s},$$

where r_s and σ_s are the mean and the standard deviation of the firing rates to the stimulus *s* over the trials, respectively. We at first computed *z*-scores taking all trials into consideration, and then recomputed the *z*-scores using only the samples which had not been excluded as outliers having a *z*-score of more than 3 or less than -3 (Zohary *et al.*, 1994). Covariation of spike count variabilities (spike count correlation) between the two units i and j for the stimulus s was evaluated by the correlation coefficient,

$$R_{ij}^S = \mathrm{E}[z_i^S \times z_j^S],$$

where E[ ] represents the expectation value over trials. Significance of the spike count

correlation should be tested against the null hypothesis of independent variabilities. Because of the limited number of trials (N=40) and low firing rates (especially to non-optimal stimuli evoking a firing rate less than 5 Hz), the distribution of the firing rates could not necessarily satisfy the assumption of the normal (Gaussian) distribution. Therefore, instead of Fisher's z-transformation and the t-test, we adopted a non-parametric bootstrap test based on trial shuffling. Trial shuffling destroys correlated spike count variabilities between the two units existing in the same trial, but keeps the distribution of variabilities of each unit. The distribution of the spike count correlation in the null hypothesis was predicted by the statistics of the bootstrap samples generated by the following steps:

1) Generate a bootstrap sample by randomly shuffling the combination of all trial pairs of the spike trains, so that no spike train of one unit makes a pair with that of other unit in the same trial. Note that we have already excluded the outlier data at either unit.
2) For each trial-shuffled sample, calculate correlation coefficient R_{BS}.
3) Repeat steps 1-2 N times to get N samples of the R_{BS}. Since the number of possible shuffled combinations becomes very large, we apply uniform Monte Carlo samplings (N=1000).
4) For each unit pair, the significance limits are given by the 97.5 percentile (positive limit) and the 2.5 percentile (negative limit) of the 1000 values. When the spike count correlation of the test data is either more than the positive limit or less than the negative limit, that value is judged as significantly departing from zero (P<0.05).

When either unit presented with a very low firing rate, the distribution of the bootstrap samples became discrete due to many tied values and the significance test was insufficient. When the bootstrap samples consisted of less than 500 different values, we assigned the spike count correlation as non-significant to avoid false positives.

For each unit pair, the mean spike count correlation $\overline{R_{ij}}$ was computed by averaging over all stimulus orientations. We examined its dependences on tuning similarity and physical distance between the two units. The tuning similarity was quantified by the signal correlation SC_m, which is a correlation coefficient between the two orientation tuning curves over the 16 stimuli. The tuning curves used in this computation were calculated by the responses in even trials for one unit and by the responses in odd trials for another unit to exclude a contribution of the spike count correlation to the signal correlation. Physical distance between the two units was estimated using the inter-electrode spacing. For pairs recorded by the same electrode,

their distance was zero. For pairs isolated from different tetrodes, the distance was either 500 or 710 μm depending on layout of the two tetrodes. For pairs isolated from different microelectrodes, the distance was either 310, 430, 610, 680, or 860 μm .

2-8 *Orientation dependence of spike count correlation*

When the unit pair showed that the spike count correlation significantly departed from zero for at least two stimulus orientations, we tested significant variation of the spike count correlation over the 16 stimuli. Distributions of the product of *z*-scores over the trials were tested for their significant differences by the analysis of variance (Kruskal-Wallis test, P=0.05). Corrections for multiple comparisons were applied in all statistical analyses in the current study by Kruskal-Wallis test. When the unit pair showed significant orientation dependent variation in spike count correlation and had similar stimulus tuning properties of the mean firing rates (SC_m>0.3), we examined the relationship between the spike count correlation and the geometric mean of the firing rates of the two units. We calculated the correlation coefficient between the two tuning curves and tested its significance using a non-parametric shuffle test. We generated 1000 samples of stimulus index shuffled combination of the two variables to obtain bootstrap samples of the correlation coefficients. The positive and negative significance limits were, respectively, given by the 97.5 and 2.5 percentile of the 1000 values. When the test correlation coefficient was greater (smaller) than the positive (negative) limit, the spike count correlation and the unit pair's firing rates were judged to be positively (negatively) correlated (P<0.05).

3. Results

We recorded multiple single units simultaneously in the visual cortex (area 17) of 5 anesthetized, paralyzed male cats. The stimulus set consisted of 16 moving light bars at different orientations and we ran 40 blocks in which each of the stimuli was presented in a pseudo-random order (640 trials in total). As explained previously, there have been contradictions among previous studies, especially with respect to the magnitude of spike count correlation. This diversity was thought to be derived from different experimental and extrinsic factors in those studies (Cohen & Kohn, 2011). In order to investigate the intrinsic spike count correlation of stimulus evoked activities, we imposed three basic criteria in selecting sample units for our analysis (see Methods). Firstly, we selected well isolated single unit activities. The portion of spike events falling within the absolute refractory time of 1 ms should be less than 1.0% and 1.5% of the total number of spikes for each unit isolated from single microelectrodes and tetrodes, respectively. In total, 515 units were isolated as a single unit from 48 recording sessions (25 sessions with the 4-tetrodes array and 23 sessions with the 8-single microelectrodes array). Secondly, we selected units (N=464) which increased their firing rate significantly to the optimal stimulus compared with the ongoing activity before the stimulation (Wilcoxon signed rank test P<0.05). Thirdly, by the bootstrap test, we extracted 310 units that kept stationary responses throughout the recording session (204 units recorded by the tetrode arrays and 106 units by the single microelectrodes arrays). The distribution of the firing rates of the sample units to their optimal stimulus (peak firing rates) had a median of 6.0 Hz and 69.4% (215/310) of the sample had a rate less than 10Hz. For the mean firing rate averaged over the 16 stimuli, a large part of the sample units (86.8%, 269/310) had a rather small rate less than 10 Hz (median 2.7 Hz). Spike count variabilities were computed for those 310 units and their correlations were investigated between all the simultaneously recorded unit pairs (N=1090, 857 pairs recorded by the tetrode array and the 233 pairs by the single microelectrode array).

Spike count correlations were often studied for the unit pairs that had similar orientation preferences (Zohary *et al.*, 1994; Kohn & Smith, 2005). In our study, we performed unbiased samplings for orientation tuning properties. Our single unit samples consisted of both significantly tuned units (N=256) and units showing no significant tuning (N=54). Therefore the unit pairs (N=1090) were classified into three groups based on the combination of their tuning characteristics. Both units of the pairs in Group 1 showed significant and similar stimulus tuning characteristics (signal correlation, SC_m >0.3, N=265). Although both units of the pairs in Group 2 also showed significant

stimulus tuning characteristics, they were dissimilar (SC_m<0.3, N=517). Finally, one or both of the units of the pairs in Group 3 showed non-significant stimulus tuning characteristics (N=308).

3-1 *Correlation of spike count variabilities*

Firing rates showed a considerable amount of variability over repeated trials involving identical stimuli. For the stimuli of all the units (N=310), standard deviations (SD) of firing rates over trials were well fitted by a power function of the mean firing rates (M), $SD=2.53M^{0.63}$ for three orders of magnitude (regression line in Figure 2, r^2=0.89). The power 0.63 of our data is consistent with the previous reports on the cortical activities (V1: 0.6 Vogels *et al*., 1989, M1: 0.57 Lee *el al*., 1998; 0.50 Maynard *et al*., 1999). For all of the unit pairs (N=1090), we calculated the correlation coefficient of the firing rates over all trials (spike count correlation). Considering a possibility of orientation dependent change of the covariance, we computed the correlation coefficient R for each stimulus orientation. Significant departure from the null hypothesis of independent variabilities was tested for each correlation coefficient by the bootstrap shuffle test (see Methods). Figures 3A-C show three different modes of spike count correlation observed in different unit pairs: (A) strong positive correlation, (B) strong negative correlation and (C) non-significant correlation. One of the units in Figure 3A showed significant stimulus tuning (dashed line) and the stimulus tuning of the other unit was non-significant (solid line). Although the two units were isolated from different tetrodes separated by 500 μm , their spike count variabilities were highly correlated over trials as seen in the scattergram between the firing rates (z-scores) of the two units (R=0.83, significant with P<0.01 by the Bootstrap test). On the other hand, the two units in Figure 3B were isolated from the same tetrode and showed no significant stimulus tuning. Their spike count variabilities were found to be negatively correlated over the course of the trials (R= -0.82, P<0.01). Finally, the unit pairs in Figure 3C were isolated from the same tetrode and had a similar stimulus-tuning characteristic (SC_m=0.62). However, their spike count variabilities had no significant correlation over the trials (R=0.06, P>0.3). If the spike count correlation originated from a long-term co-modulation of the activity levels due to unstable anesthesia or electrode drift, the firing rates should show a monotonic increase/decrease over successive trials. Since we excluded those samples initially by the stationary response test (see Methods), the above samples did not show such non-stationary modulations (middle plots in Fig. 3A-C).

Figure 4A shows a cumulative histogram of the correlation coefficients over all the

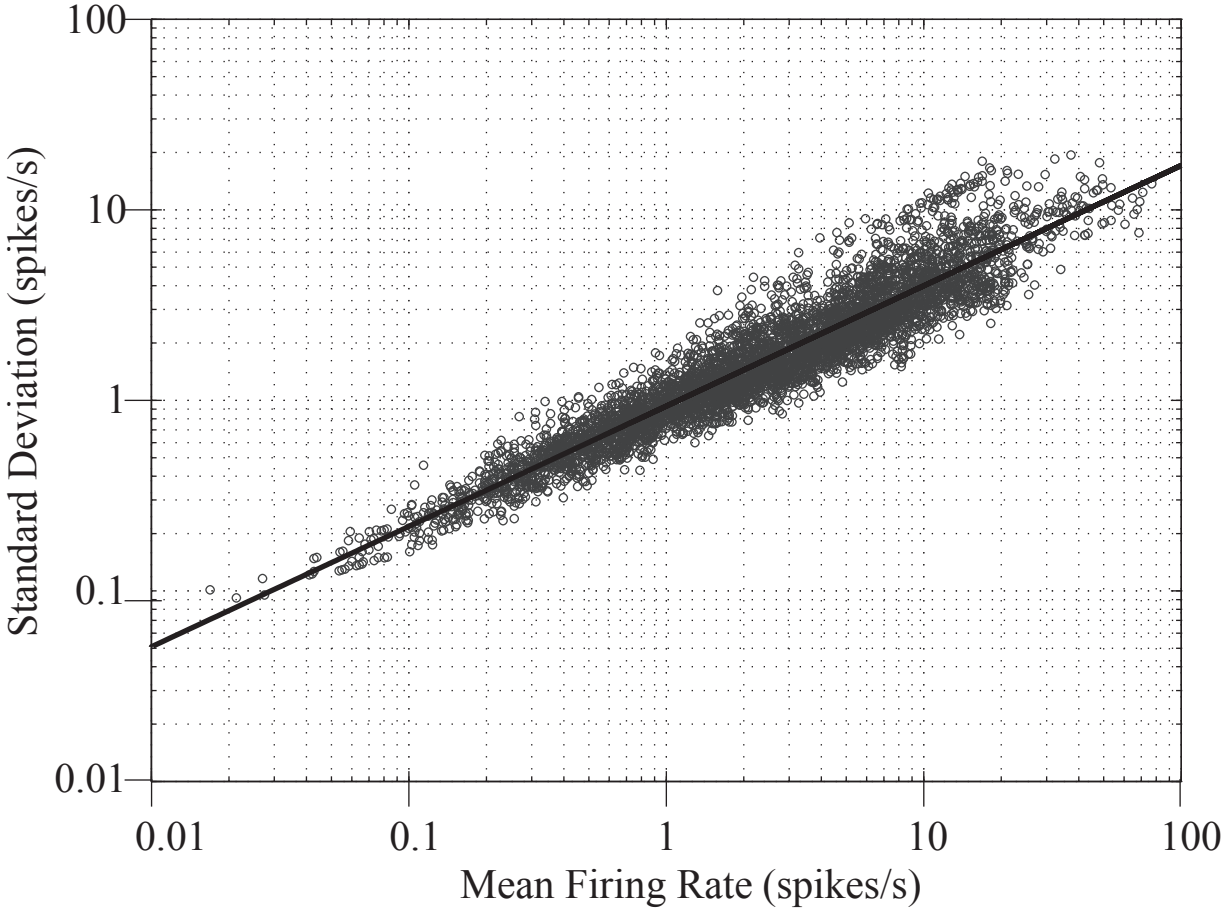

Figure 2. For all the stimuli of all the units (N=310), the relationship between the mean firing rate (M) and the standard deviation (SD) over trials are plotted. Both the abscissa and the ordinate are in logarithmic scale and in units of spikes per second.
Their relationship was well fitted by a power function (regression line, r^2=0.89)
$SD = 2.53M^{0.63}$ in three orders of magnitude.

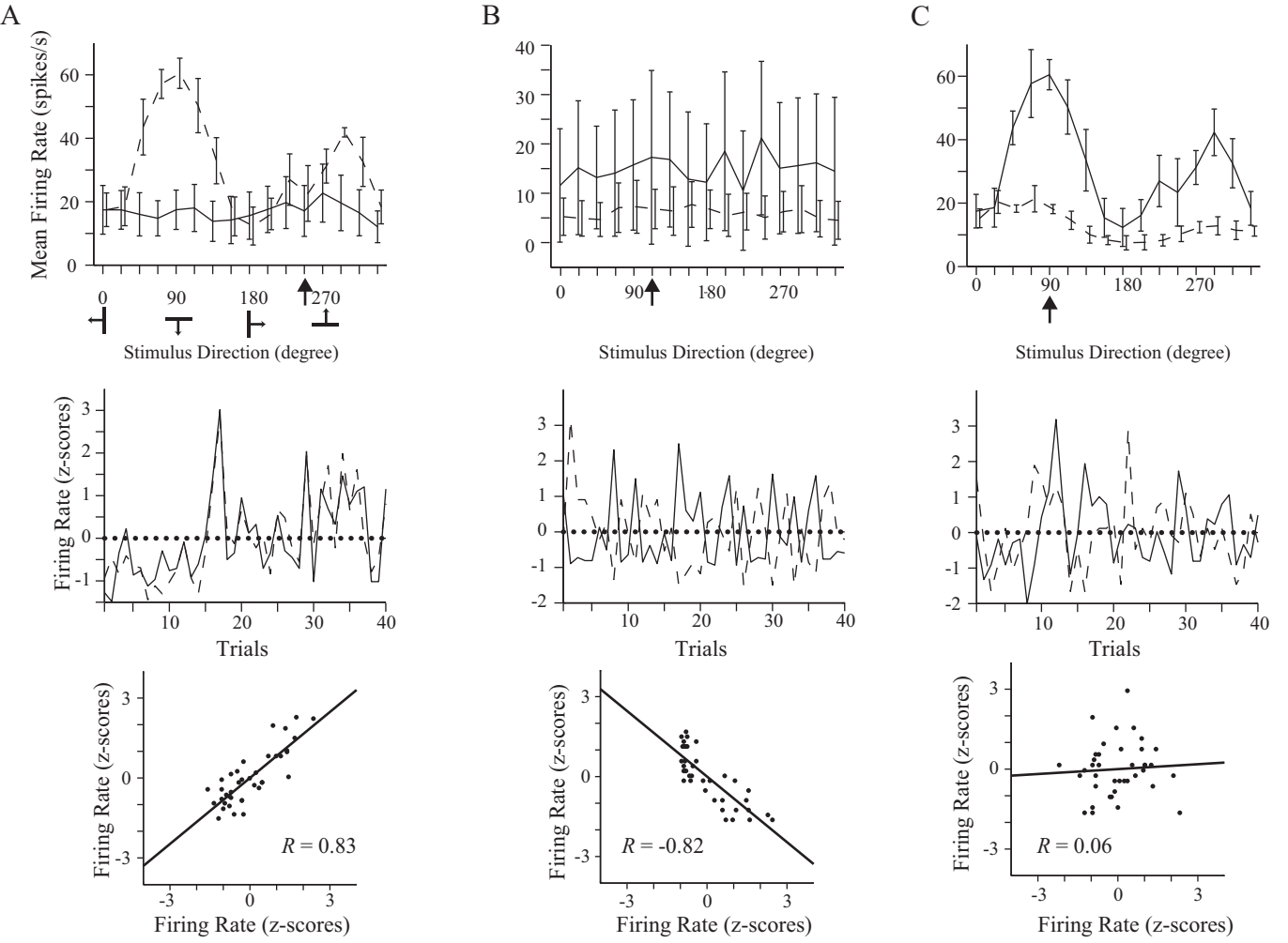

Figure 3. Three examples of unit pairs showing different modes of spike count correlations. In each panel of A-C, the tuning curves of the mean firing rates of the two units (solid line and dashed line) are shown on the top. In the middle, the variations of the firing rates (*z*-scores) of the two units are plotted (solid line and dashed line) over 40 successive trials to the stimulus indicated by an arrow in the corresponding tuning curve. Dotted lines represent zero of *z*-score. The bottom plots are the scattergrams between the firing rates (*z*-scores) of the two units over 40 trials. Each data point represents a pair of *z*-scores in the same trial. The regression lines were obtained by the least square fitting. (A) Unit pair showing a positive spike count correlation. The two units were isolated from different tetrodes separated by 500 μm. Stimulus tuning of one unit (solid line) was not significant. Modulation profiles of the firing rates were correlated in phase (middle) and the scattergram shows a positive correlation significantly departing from zero (bottom, R=0.83, P<0.01, Bootstrap test). The relation between the *z*-scores are well fitted by the regression line (r^2=0.68). The absence of monotonic drifts of the firing rates along successive trials (middle) suggests that significant correlation was not due to long term non-stationary modulations of the activity levels. (B) Unit pairs showing a negative spike count correlation. Both units, which were isolated from the same tetrode, have non-significant stimulus tuning (top). The firing rates were correlated out of phase over trials (middle) and the scattergram shows a negative correlation significantly departing from zero (bottom, R= -0.82, P<0.01, linear regression r^2=0.67). (C) Unit pair without significant spike count correlation. The units were isolated from the same tetrode and had similar stimulus tuning characteristics (*SC*m=0.62, top). The correlation coefficient between the firing rates over trials does not significantly depart from zero (R=0.06, P>0.3, middle and bottom). The linear regression fit was very poor (r^2=0.004).

stimuli of all the unit pairs (N=16×1090=17440). Although a population mean of 0.06 was weakly positive, the distribution was rather broad with a large standard deviation (0.22) and extended to both positive and negative values. The correlation between the unit pair was often characterized by a single correlation coefficient obtained by the covariance of the *z*-scores over all the different stimulus presentations as well as all the trials. When the correlation coefficients were averaged over the 16 stimuli for each pair, the distribution has the same mean (0.06 ± 0.14) but with less variance (Fig. 4B, N=1090). Although a slight bias toward positive correlation is qualitatively consistent with the previous reports, our data provides smaller mean value than those results (V1: 0.25 Reich *et al*., 2001; 0.20 Kohn & Smith, 2005; 0.18 Smith & Kohn, 2008, MT: 0.12 Zohary *et al*., 1994; 0.20 Bair *et al*., 2001, IT: 0.22 Gawne *et al*., 1993, M1: 0.12 Lee *et al*., 1998; 0.21 Maynard *et al*., 1999, however V4: 0.04 Cohen & Maunsell, 2009; 0.05 Mitchell *et al*., 2009]. However, our value is greater than the recent result (mean value 0.01) in V1 of awake macaques (Ecker *et al*., 2010). Figure 4C shows the histogram of correlation coefficients significantly departing from zero (P<0.05, 2682 out of 17440, 15%). Since values around zero were judged to be non-significant, the distribution becomes bimodal. Although the cases of positive correlation (N=1937) were more plentiful, there existed many cases of negative correlation (N=745). There were unit pairs showing highly correlated firing rate variabilities with $|R|$>0.8. Nearly half of the entire samples (575 out of 1090, 53%) had correlation coefficients significantly departing from zero for at least two stimulus orientations. The histogram of correlation coefficients averaged over the 16 stimuli for those pairs (N=575, Fig. 4D) had a larger mean value 0.08±0.18 than that of the total samples shown in Figure 4B. If spike count correlation of each unit pair would be reasonably invariant over different stimulus orientations, the distribution of the averaged correlations (Fig. 4D) should have a similar shape as the distribution of individual samples (Fig. 4C). Differences between the two distributions suggest that spike count correlations were not necessarily constant over different stimuli. Figure 4E shows a distribution of the number of stimuli showing the correlation coefficient significantly departing from zero (all the unit pairs, N=1090). A large portion of the unit pairs had a significant correlation coefficient for only 2 or 3 stimuli (mean 2.5). Only thirty percent of the entire samples (331 out of 1090, 30.4%) had a significant correlation coefficient at more than two stimuli. However, this distribution is significantly distinct from what we could get when the significant cases appeared by chance, that is, the binomial distribution with P=0.05 (mean 16×0.05=0.8). Therefore we conclude that significant spike count correlations to multiple stimulus orientations are an intrinsic property of the local cell population in the visual cortex.

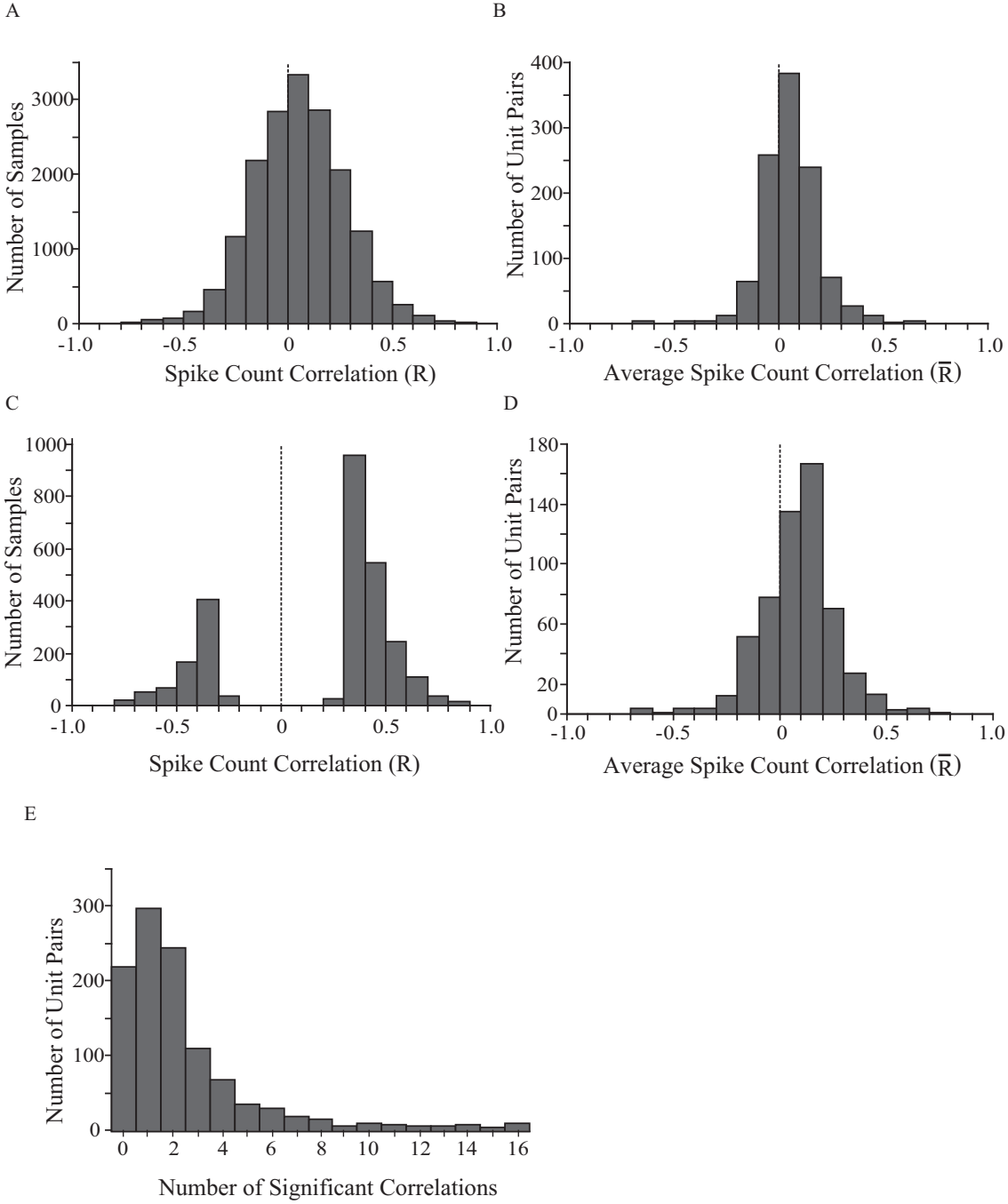

Figure 4. (A) Cumulative histogram of correlation coefficients of spike count variabilities over all of the stimuli for all unit pairs (N=16 × 1090=17440, mean: 0.06 ± 0.22, maximum: 0.88, minimum: -0.82). (B) Histogram of the correlation coefficient averaged over 16 stimuli (N=1090, mean: 0.06 ± 0.14, maximum: 0.80, minimum: -0.70). The distribution has the same mean as A but with less variance. (C) Histogram of the correlation coefficients significantly departing from zero (P<0.05) judged by the bootstrap shuffle test (2682 out of 17440 samples in A). The bimodal distribution is a result of the removal of non-significant samples around zero. Samples of positive correlation (N=1937) are more plentiful than those of negative correlation (N=745). (D) Histogram of the correlation coefficients averaged over 16 stimulus orientations for the pairs having correlation coefficients significantly departing from zero for at least two stimuli (N=575, Mean: 0.08 ± 0.18). Note that scales of the ordinate differ in the histograms A-D. (E) Distribution of the number of stimuli showing significant correlation (over all unit pairs, N=1090, mean: 2.5).

3-2 *Dependences of spike count correlation on receptive field properties*

Physical distance of units

In our experiment, multiple single units were recorded by closely spaced multiple electrodes. The unit pairs recorded simultaneously had spatially overlapped receptive fields and were likely to receive shared feed-forward inputs from lateral geniculate nucleus (LGN). Since we assume that units that are close together share a larger amount of common input, if the spike count correlation originates from those common inputs then the magnitude of the correlation coefficient should decrease as their physical distance increases. For all of the unit pairs (N=1090), average correlation coefficients over the 16 stimuli were plotted as a function of the distance between the two electrodes that recorded the corresponding units (spatial extent up to 1mm, Figure 5). For units isolated from the same electrode (either the tetrode or the single microelectrode), we assigned zero distance. For the unit pairs recorded by the tetrode array, the model of the linear relationship between the correlation coefficient and the distance was rejected (χ^2 =5.26 for 1 degree of freedom, P<0.02). Also the analysis of variance suggested a non-significant difference among the mean values at different distances (P>0.16 Kruskal-Wallis test). On the other hand, for the unit pairs recorded by the single microelectrode array, the model of the linear relationship was not rejected (χ^2 =4.39 for 4 degree of freedom, P>0.36). The linear decay had an intercept of 0.138 ± 0.018 and a slope of 0.119 $mm^{-1} \pm 0.031$. We found that units isolated from the same microelectrode (zero distance, N=16) had a significantly larger degree of correlation than the unit pairs located at other distances (distance 0 μm : 0.15 ± 0.09, 310 μm : 0.08 ± 0.14, 430 μm : 0.12 $\pm$ 0.16, 610 μm : 0.07 ± 0.12, 680 μm : 0.05 ± 0.13, 860 μm : 0.04 ± 0.10, P<0.013 Kruskal-Wallis test). When the data of zero distance was excluded, the data became a bit more consistent with the linear relationship (χ^2 =3.01 for 3 degree of freedom, P>0.39) with a more gradual slope of 0.078 $mm^{-1} \pm 0.047$ and an intercept of $0.112 \pm$ 0.028. The difference among the mean correlation values of the different distances became non-significant (P>0.17, Kruskal-Wallis test). Since the spatial extent of our recordings was limited to 1 mm, if the correlation decreased very gradually over a longer distance, we were not able to confirm a significant decrease. However our result of the linear slope seems to be comparable to the previous result 0.048 mm^{-1}, which was estimated by the data over the larger spatial extent of up to 10 mm (Smith & Kohn 2008). Due to weak distance dependence and the broad distribution at any distance (Figure 5), there existed a large amount of diversity. While adjacent units may have a

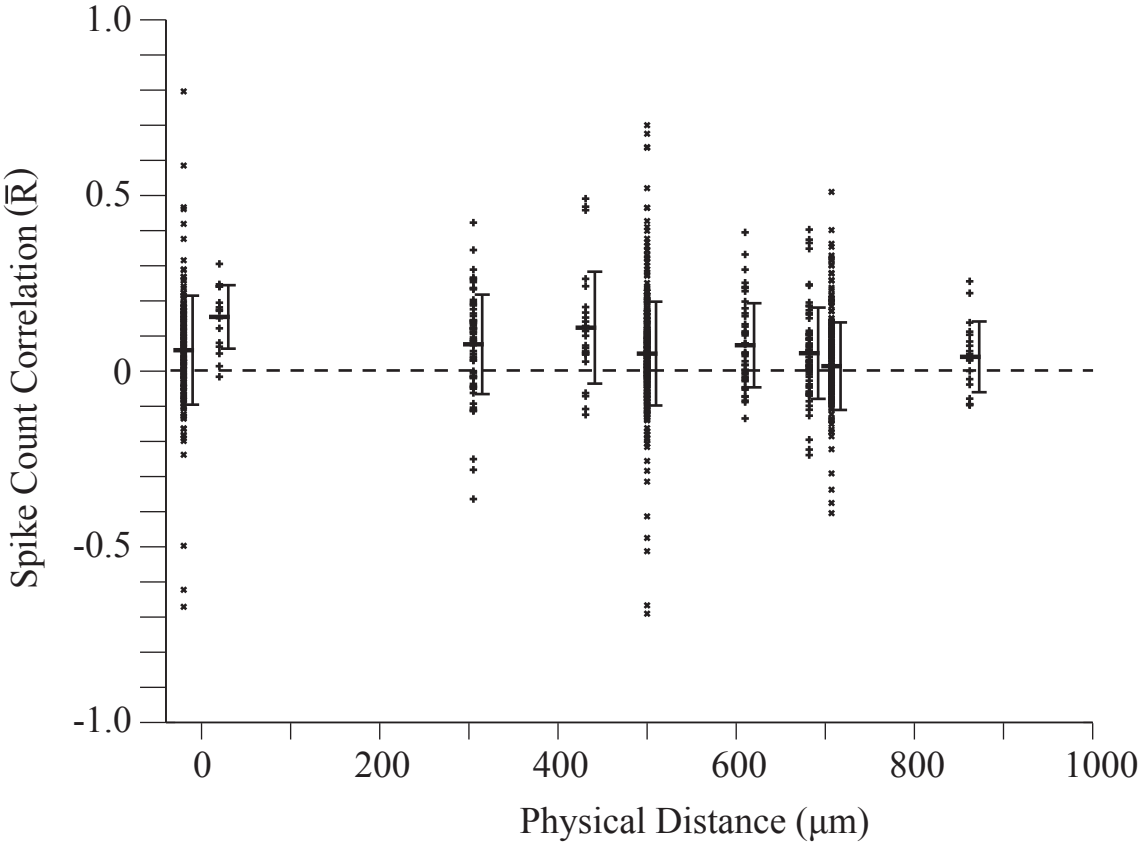

Figure 5. Dependence of the spike count correlation on the physical distance between the unit pairs. For all the unit pairs (*N*=1090), correlation coefficients were averaged over the 16 stimulus orientations and plotted as a function of physical distance between the electrodes that recorded the corresponding units. For units isolated from the same electrode (either the tetrode or the single microelectrode), we assigned a zero distance. Distances of the pairs recorded by the tetrodes (represented by ×) were either 0, 500, or 710 μm) and those recorded by the microelectrodes (+) were either 0, 310, 430, 610, 680, or 860 μm. At each distance, the mean and the standard deviation of the distribution are indicated (tetrode array: 0 μm: 0.06 ± 0.16, 206 samples; 500 μm: 0.05 ± 0.15, 418 samples; 710 μm: 0.04 ± 0.13, 233 samples, single electrode array: 0 μm: 0.15 ± 0.09, 16 samples; 310 μm: 0.08 ± 0.14, 66 samples; 430 μm: 0.12 ± 0.16, 25 samples; 610 μm: 0.07 ± 0.12, 44 samples; 680 μm: 0.05 ± 0.13, 64 samples; 860 μm: 0.04 ± 0.10, 18 samples). A dashed line represents zero spike count correlation. Since the sample sizes were different over different distances, we performed a χ^2 fit of the data to a straight line. Uncertainty of the mean value at each distance was estimated by the standard deviation over the samples divided by the square root of the sample size. For the unit pairs recorded by the tetrode array, the model of linear relationship between the correlation coefficient and the distance was rejected (χ^2 =5.26 for 1 degree of freedom, P<0.02). Also the analysis of variance suggested a non-significant difference among the mean values at different distances (P>0.16 Kruskal-Wallis test). On the other hand, for the unit pairs recorded by the single microelectrode array, the model of a linear relationship was not rejected (χ^2 =4.39 for 4 degrees of freedom, P>0.36). The linear decay had an intercept of 0.138 ± 0.018 and a slope of 0.119 mm^{-1} ± 0.031. We found that units isolated from the same microelectrode (zero distance) had a significantly larger correlation than the unit pairs of other distances (P<0.013 Kruskal-Wallis test). When the data of zero distance was excluded, the data became a bit more consistent with the linear relationship (χ^2 =3.01 for 3 degrees of freedom, P>0.39) with a more gradual slope of 0.078 mm^{-1} ± 0.047 and an intercept of 0.112 ± 0.028, and the difference among the mean correlation values of different distances became non-significant (P>0.17, Kruskal-Wallis test).

very small correlation coefficient, distant units (~860 μm) could show significantly correlated spike count variabilities.

By further comparison between the unit pairs isolated from the two types of electrodes (see Appendix), we found that the units isolated from a single microelectrode located very closely (<120 μm) and had more similar stimulus tuning characteristics than the units isolated from a single tetrode. Our observation of significantly larger correlations of those unit pairs suggests that the effect of common thalamic inputs to increasing spike count correlation could be very short range. On the other hand, since the tetrode records units over a wider spatial range, the unit pairs were distributed over a larger spatial extent of at most ~260 μm. We suppose that such inhomogeneous spatial samplings by the tetrode could smear a weak distance dependence which was observed in the data recorded by the single microelectrode array.

Similarity of stimulus tunings

Orientation selectivity of the cortical unit is considered to be organized by coordinated samplings of afferent inputs from the LGN. We expect that, within a small spatial extent (such as a single hyper column), the units having similar stimulus tuning characteristics should share more common inputs from the LGN. Therefore positive correlation would be expected between the signal correlation and the spike count correlation of the unit pairs. The average correlation coefficients over the 16 stimuli were plotted as a function of the signal correlation for all the samples (N=1090, Figure 6). There exists a significant but very weak positive linear correlation between the two variables (slope 0.04, P<0.001, r^2=0.01). Our result did not support the previous reports that unit pairs of non-similar receptive field properties had a considerably smaller spike count correlations than those with similar properties (Zohary *et al*., 1994; Bair *et al*., 2001; Kohn & Simth, 2005). As we have explained previously, the unit pairs isolated from the same microelectrode (N=16) tended to have a larger signal correlation and a larger spike count correlation (Figure 6).

In order to further examine the relationship between the spike count correlation and the similarity in tuning properties, we compared the distributions of the spike count correlation among the three groups, Groups 1~3, defined previously based on the combination of their tuning characteristics. We found that the distributions were significantly different among the three groups (P<0.05 Kruskal-Wallis test, see Table I). Unit pairs in Group 2 tended to have a significantly smaller spike count correlation than those in Group 1 (P<0.01 Mann-Whitney test). Although this property agrees with the previous reports (Zohary *et al*., 1994; Bair *et al*., 2001), unit pairs in Group 2 tended to

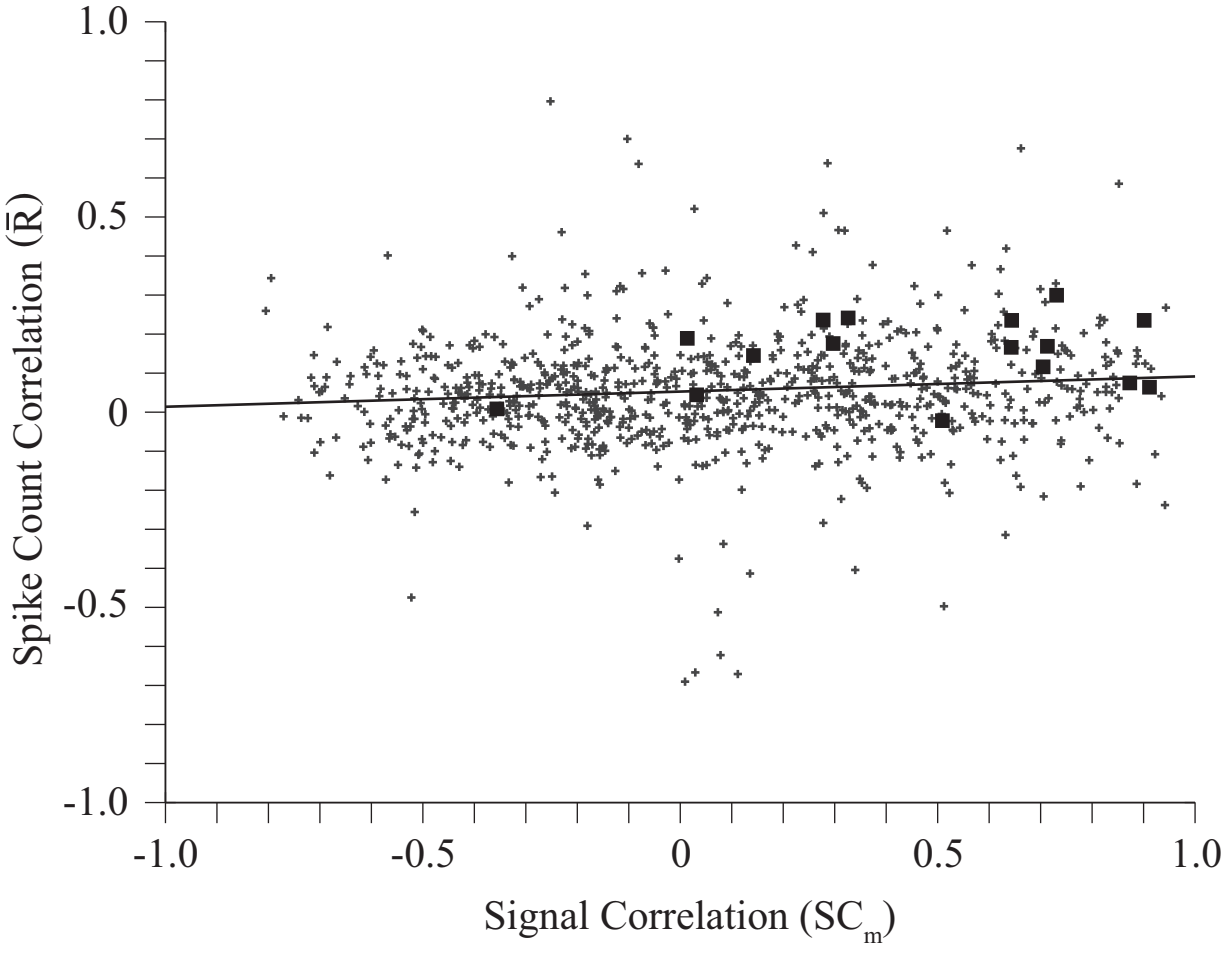

Figure 6. The correlation between the spike count correlation and the similarity in stimulus tuning characteristics. For all unit pairs (N=1090), the correlation coefficients were averaged over the 16 stimulus orientations and plotted as a function of the similarity between the stimulus tuning curves of the two units (signal correlation, SC_m). Two variables show a significant but very weak positive linear correlation (regression by the least square fitting, slope 0.04, P<0.001, r^2=0.01). The unit pairs isolated from the same microelectrode (N=16, indicated by filled squares) tend to have a larger signal correlation and a larger spike count correlation.

Table Ⅰ. Spike count correlation and its orientation dependent variation

Group	Number of pairs	Spike count correlation $\overline{R}$ (Mean)	Number of pairs with significant correlation (%)	Degree of orientation dependent variation $-\log_{10}P$ (median)	Number of pairs with significant orientation dependent variation (%)
1	265	0.07±0.14 *	150/265 (56.6) #	0.51	28/150 (18.7)
2	517	0.05±0.12 *, #	247/517 (47.8) #, *	0.64 *	54/247 (21.9) #
3	308	0.06±0.18 #	178/308 (57.8) *	0.44 *	24/178 (13.5) #
total	1090	0.06±0.14	575/1090 (52.3)	0.53	106/575 (18.4)

Two entries with the same symbol (* or #) in each column are significantly different (Mann-Whitney U-test, * $P<0.01$, # $P<0.05$).

have a significantly smaller spike count correlation even in comparison to those in Group 3 (P<0.05). The distributions of the spike count correlation were not significantly different between Groups 1 and 3 (P> 0.6). We found the same statistical results for the incidences of significant spike count correlations (see Table I). In summary, due to the large diversity of our samples, the spike count correlation showed very weak dependence on both physical distance and similarity in stimulus tuning characteristics. Therefore, our data do not necessarily support the hypothesis that the spike count correlation between unit pairs originates from shared afferent inputs from the thalamus. The shared thalamic inputs affect only the unit pairs located very close together and were isolated from the single microelectrode. When the unit pairs were separated further, even for the units isolated from the same tetrode, the effect of common thalamic inputs to increasing spike count correlation was very weak.

3-3 *Dependence of spike count correlation on firing rates*

We have examined the relationship between the spike count correlation and the firing rates of the two units. Figure 7 shows the population statistics results over 17440 samples, that is, the responses of all the unit pairs (N=1090) to the 16 stimulus orientations. Most samples (85.9%, 14989/17440) had the firing rates in a range of 1 to 10 spikes/s and their average spike count correlations were below 0.1, showing little rate dependence. The unit pairs of firing rates higher than 10 spikes/s tended to have stronger spike count correlations. We also examined the dependence of spike count correlation on the geometric mean of the firing rates of the two units (N=17431, Figure 8). Although the increase of the correlation can be observed, the model of the linear relationship between the two variables was rejected (χ^2 =33.5 for 4 degrees of freedom, P<0.001). The geometric mean may not adequately represent the response strength of the unit pair when their tuning characteristics are not similar. However, even when we limited the analysis to the unit pairs with similar orientation tunings (SCm>0.3, Group 1, N=4234), the linear relationship was still rejected (χ^2 =19.5 for 4 degrees of freedom, P<0.001). Interpretation of the rate dependence is not straightforward, since the dependence of the correlation strength on the firing rates can originate from multiple independent factors. We continue to discuss the rate dependence in the discussion section.

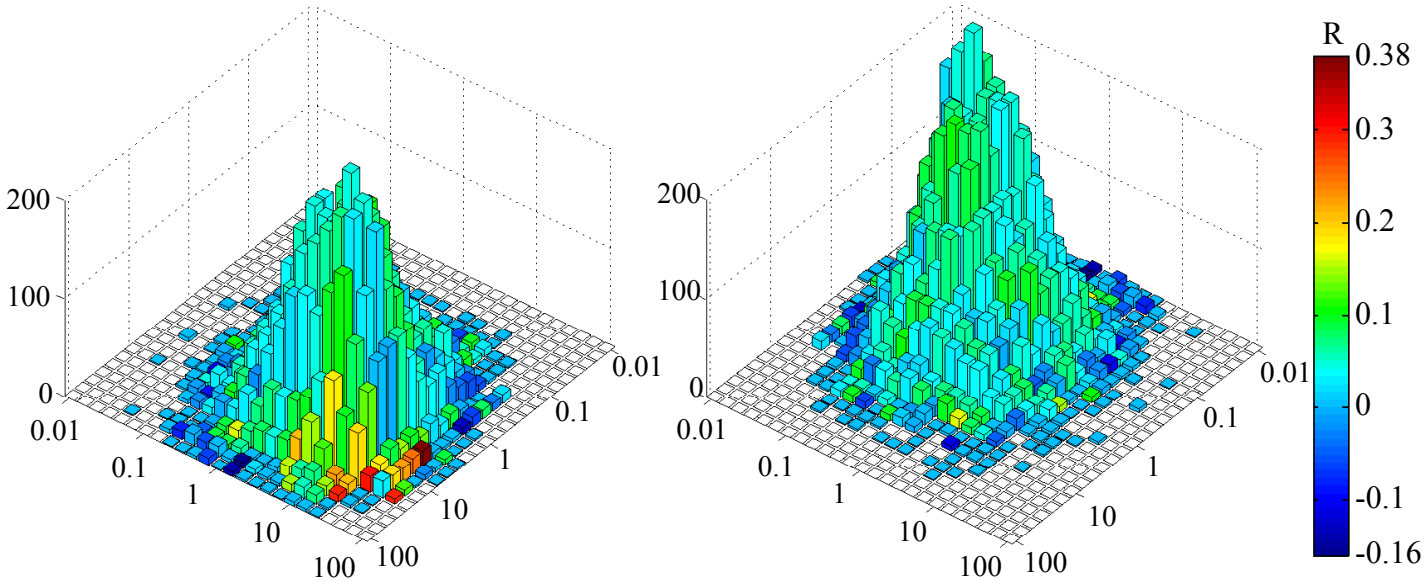

Figure 7. Population statistics of the relationship between the spike count correlation and the firing rates of the two units. Two 3-D histograms show different views from the opposite corners. Each axis of the firing rate is in a logarithmic scale and the range of 4 orders of magnitude (from 0.01 to 100 spikes per second) was subdivided into 25 bins. For all the unit pairs (N=1090), samples of spike count correlation to each of the 16 stimulus orientations (totally 17440 samples) were assigned at the compartment corresponding to the trial averaged firing rates of the two units. In the three-dimensional plot, the height and color of the bar represent, respectively, the sample count and the average correlation value over the samples in the corresponding compartment. To avoid a statistical fluctuation due to the small sample count, we do not show the values of the compartments containing less than 5 samples. Most samples (85.9%, 14989/17440) had firing rates in a range of 1 to 10 spikes/s and their average spike count correlations were below 0.1, showing little rate dependence. However, when both units had firing rates higher than 10 spikes/s, the unit pairs tended to have a larger spike count correlation.

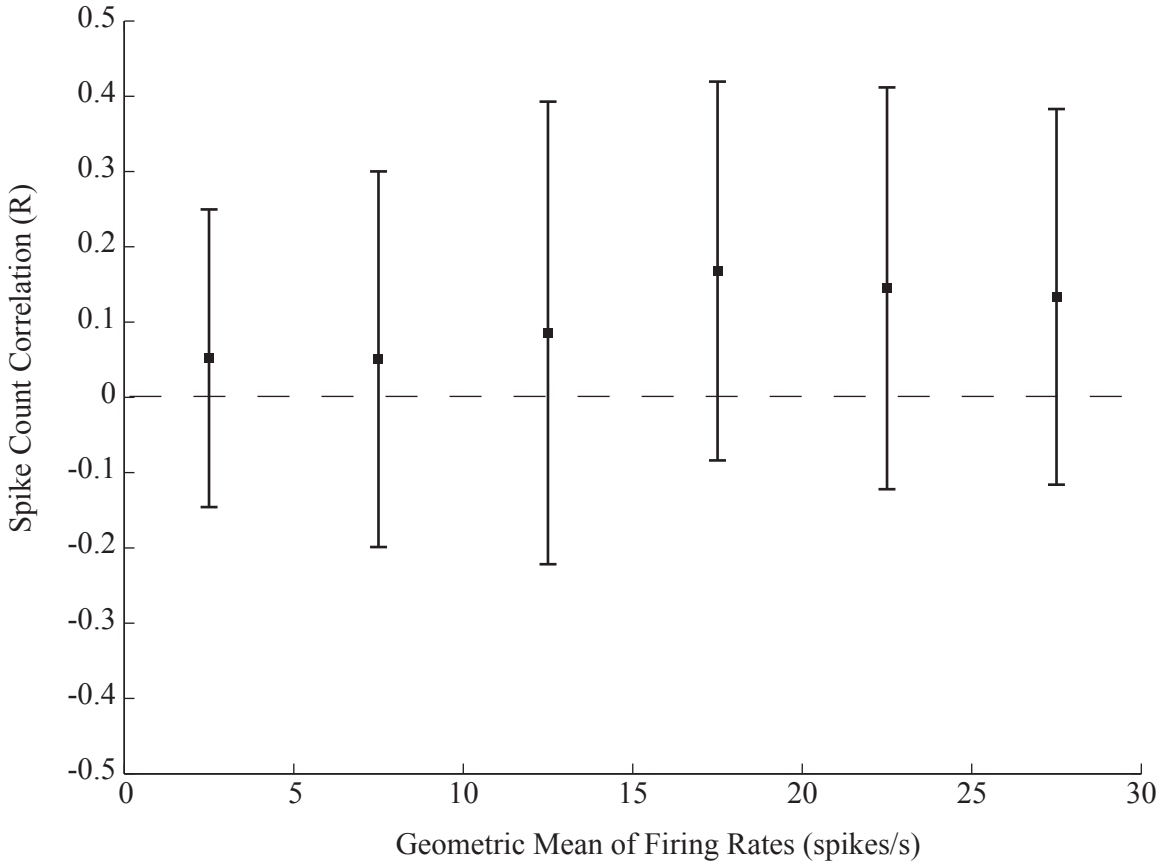

Figure 8. The relationship between the spike count correlation and the geometric mean of the firing rates of the two units. We at first drew a scatter plot between the spike count correlation and the geometric mean over all stimuli for all unit pairs (N=17440), and then the data were averaged within bins 5 spikes/s in size. The plot shows the mean and the standard deviation of each bin (0~5 spikes/s: 0.052 ± 0.198, 12612 samples; 5~10: 0.051 ± 0.249, 3574 samples; 10~15: 0.086 ± 0.307, 914 samples; 15~20: 0.168 ± 0.252, 232 samples; 20~25: 0.145 ± 0.267, 60 samples; 25~30: 0.134 ± 0.250, 39 samples). A dashed line represents a zero spike count correlation. We have excluded 9 samples corresponding to larger geometric mean rates due to a sample size that was too small (30-35 spikes/s: 7 samples; 35-40 spikes/s: 2 samples). We performed a χ^2 fit of the data to a straight line. The uncertainty of the mean value at each bin was estimated by the standard deviation over the samples divided by the square root of the sample size. Although an increase in the correlation can be observed, the model of the linear relationship between the two variables was rejected (χ^2 =33.5 for 4 degrees of freedom, P<0.001).

3-4 *Spatial heterogeneity of spike count correlation*

Recent studies suggested that the correlation of spike count variabilities could originate from non-stationary transitions of the activity state (UP state and DOWN state) in the local cell population induced by the top-down projection from higher cortical areas (Renart *et al.*, 2010; Kelly *et al.*, 2010). If the spike count correlation arises from the state transition of the local cell population, we could expect spatial homogeneity. We have tested homogeneity of the spike count correlations over unit pairs recorded in the same session. Figure 9 illustrates an example session showing spatially heterogeneous correlations. Two unit pairs shared one common unit. Each of the three units was isolated from a different tetrode and had distinct stimulus tuning characteristics (Fig. 9A and D). To the same stimulus, both unit pairs had spike count correlations significantly departing from zero but with opposite signs. The variation of the firing rates over successive trials (Fig. 9B) and their scattergram (Fig. 9C) show that the first unit pair had positively correlated spike count variabilities (R=0.71, P<0.01 Bootstrap Test). On the other hand, the spike count variabilities were negatively correlated in the second unit pair (R= -0.67, P<0.01, Fig. 9E and F). The global statistics over all recording sessions (N=48) concluded that 83% of the total sessions (40 out of 48) showed heterogeneity in spike count correlations, that is, significantly positive correlations coexisted with significantly negative correlations over different unit pairs recorded in the same session. Those results suggest that spike count correlations were spatially heterogeneous even in the local cell population within an extent less than 1 mm. The spike count correlations in our data are unlikely to have originated from non-stationary transitions of the activity state in the local cell population.

3-5 *Orientation dependence of spike count correlation*

In the foregoing analysis, we examined the properties of spike count correlation based on the averaged correlation coefficient over the 16 stimulus orientations. The averaged value only validly represents a property of the unit pair under the assumption that correlation coefficients are reasonably invariant over different stimuli. We tested the significance of orientation dependent variation of the spike count correction over the 16 stimulus orientations. For all the unit pairs that have spike count correlations significantly departing from zero for at least two stimulus orientations (N=575), we computed the distribution of the products of two units' firing rate z-scores over the trials. We then performed a multiple comparison test (Kruskal-Wallis test) to elucidate

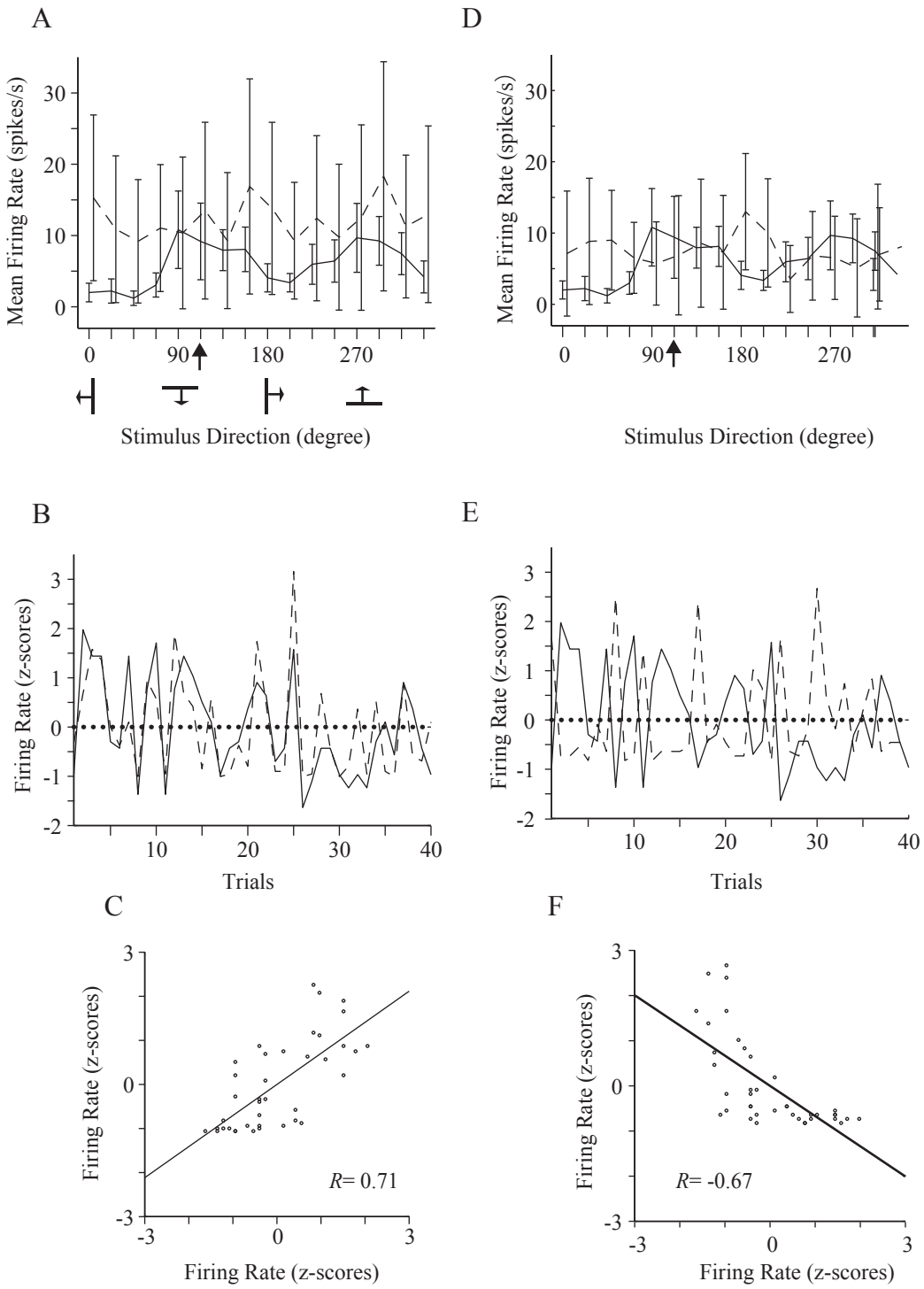

Figure 9. Example of spatially inhomogeneous spike count correlations among the units recorded in the same session. The ways of the plots are the same as in Figure 3, that is, (A) and (D) tuning curves of the mean firing rates of the two units (solid line and dashed line), (B) and (E) variations of the firing rates (*z*-scores) (solid line and dashes line) along 40 successive trials to the stimulus indicated by an arrow in the tuning curve (dotted line represents zero of *z*-score), (C) and (F) scattergrams between the *z*-scores of the two units. Each of the three units was isolated from a different tetrode and had a distinct stimulus-tuning characteristic. The unit with the tuning curve of solid lines is common in the two unit pairs. The spike count variabilities of the first unit pair, which was isolated from different tetrodes separated by 500 μm, were correlated in phase (B) and lead to a significantly positive correlation (C, R=0.71, P<0.01 Bootstrap test, linear regression r^2=0.50). On the other hand, the second unit pair, which was isolated from different tetrodes separated by 710 μm, showed a significantly negative spike count correlation to the same stimulus (E, F, R= -0.67, P<0.01, linear regression r^2=0.45).

whether or not there was a significant difference among the mean values in the distributions of different stimulus orientations (see Methods). Correction for multiple comparisons was applied in the computation of the degree of significant variation, the *P*-value. Figure 10 shows an example of orientation dependent variation of the spike count correlation. The two units were isolated from the same tetrode and showed similar stimulus tuning curves (Fig. 10A, SC_m=0.80). As shown in the orientation tuning of the spike count correlation (Fig. 10B), the spike counts of the two units showed significantly positive correlation to three stimulus orientations and significantly negative correlation to two stimulus orientations (indicated by asterisks). A horizontal bar moving upward (filled arrow in Fig. 10B) evoked the most positive correlation (R=0.51, P<0.01), which is confirmed by the scattergram of the *z*-scores in Figure 10C (linear regression r^2=0.26, P<0.001). The most negative correlation (R= -0.36, P<0.05) was evoked by a vertical bar moving leftward (open arrow in Fig. 10B) and confirmed by the scattergram (Fig. 10D, linear regression r^2=0.13, P<0.05). In this example, the spike count correlation had significant variation among different stimuli (P<0.02 Kruskal-Wallis test). When we compute the averaged correlation coefficient over the 16 stimuli, those variations are averaged out to get a very small amount 0.01. Therefore, when the spike count correlation shows significant orientation dependent variation, the mean correlation value does not represent the nature of correlated spike count variabilities of the unit pair. Figure 11 shows a distribution of the degree of orientation dependent variation, $-\log_{10}P$, over all of the samples (median of $-\log_{10}P$ 0.53), where P is computed by Kruskal-Wallis test and is the probability with which the null hypothesis of no orientation dependence realizes the test data by chance. We found that 18.4% of the sample (106 of 575) showed significant orientation dependent variation satisfying the condition P<0.05 ($-\log_{10}P$>1.3). When we limited the sample to the unit pairs having significant spike count correlations for at least three stimulus orientations (N=331), the incidence of significant orientation dependent variation increased to 24.5% (81 of 331).

For further examination of orientation dependence, we classified our pool of unit pairs (N=575) into three groups based on how significant their correlation coefficients were. Since those samples have correlation coefficients significantly departing from zero for at least two stimuli, there are three possible groups. Group A: the significant cases consist of only positive correlations (N=349, 60.7%, average spike count correlation over 16 stimuli $\overline{R}=0.19\pm0.12$), Group B: the significant cases consist of only negative correlations (N=118, 20.5%, $\overline{R}=-0.15\pm0.14$) and Group C: the significant cases consist of both positive correlations and negative correlations (N=108,

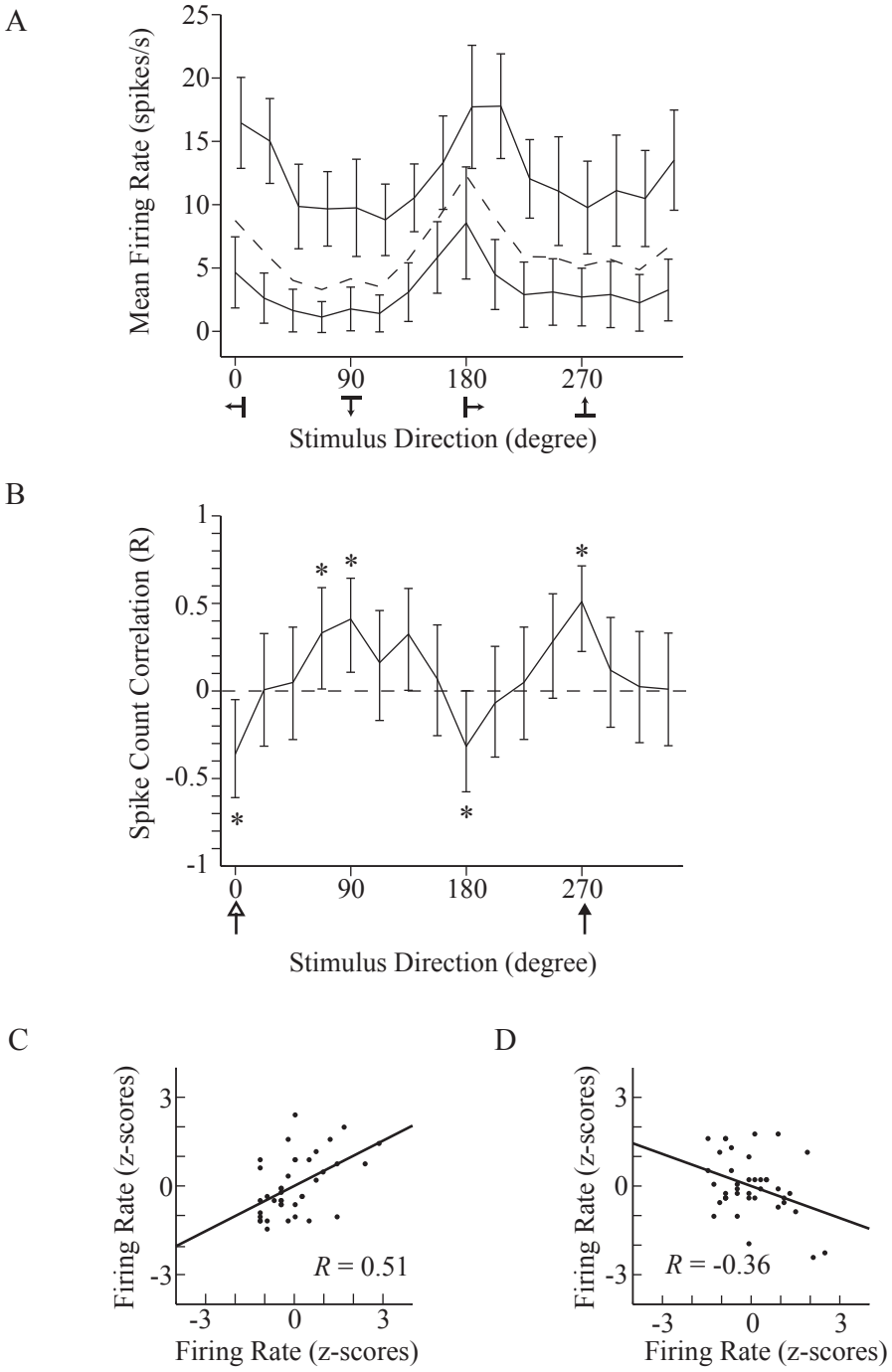

Figure 10. Example unit pair showing a significant variation in the spike count correlation among different stimulus orientations. (A) Tuning curves of the mean firing rates (two solid lines) over 16 stimulus orientations. The two units were isolated from the same tetrode and showed similar tuning chracteristics (SC_m=0.80). The dashed line represents the geometric mean of the two firing rates. (B) The tuning curve of the spike count correlations over different stimulus orientations. Spike counts of the two units showed a significantly positive correlation to the three stimuli and a significantly negative correlation to the two stimuli (indicated by asterisks). A dashed line represents zero correlation. (C) Scattergram of the firing rate *z*-scores of the two units over 40 trials to the stimulus (filled arrow in B) that evoked the maximum correlation (R=0.51, P<0.01; linear regression r^2=0.26, P<0.001). (D) Scattergram of the *z*-scores to the stimulus (open arrow in B) that evoked the most negative correlation (R= -0.36, P<0.05, linear regression r^2=0.13, P<0.05). The spike count correlation has significant variation among different stimulus orientations (P<0.02 Kruskal-Wallis test).

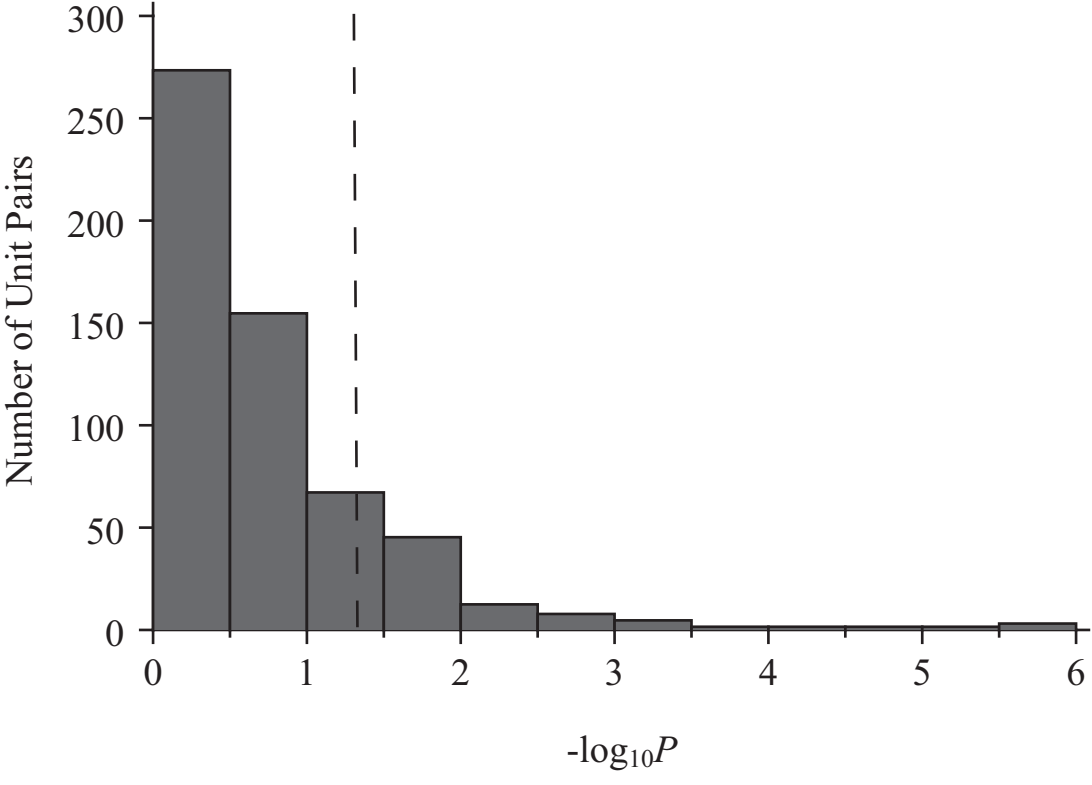

Figure 11. The degree of orientation dependent variation in the spike count correlation was quantified by *P*-value of Kruskal-Wallis test. The histogram plots the distribution of $-\log_{10}P$ (N=575, median of $-\log_{10}P$ 0.53). Orientation dependent variation was judged to be significant for 18.4% of the unit pairs (106 of 575) satisfying the condition $-\log_{10}P>1.3$ ($P<0.05$, dashed line).

18.8%, $\overline{R} = 0.01 \pm 0.07$). Example unit pair shown in Figure 10 belongs to Group C. The distribution of the degree of orientation dependent variation in Figure 11 was split into three groups (Figure 12). The distributions were significantly different among the three groups ($P<0.0001$, Kruskal-Wallis test). Pair-wise comparison concluded that the unit pairs in Group C tended to have a larger degree of variation (larger $-\log_{10}P$, median 0.89) than the unit pairs in Group A (median 0.45) and B (median 0.53, $P<0.0001$ Mann-Whitney test). There was no significant difference between Group A and B ($P>0.1$). Incidences of significant variation were also different among the three groups ($P<0.0001$, Kruskal-Wallis test). Group C had significantly larger incidences (35.2%, 38 out of 108) than Group A (13.4%, 47 out of 349) with $P<0.0001$ (Mann-Whitney test) and Group B (17.8%, 21 out of 118) with $P<0.01$. When the unit pair showed significantly negative correlations as well as significantly positive correlations to different stimuli, such a large variation was likely to be judged as significant.

We investigated whether orientation dependent variations of the spike count correlation had any relation with the physical distance and the signal correlation between the two units. Significant differences were not found in the degree of variation among different distances for both types of the electrode arrays (the single electrode array $P>0.6$, the tetrode array $P>0.3$, Kruskal-Wallis test). We also compared the degree of variation among the three groups, Group 1, 2 and 3 (see Table I). While Group 3 had a significantly larger incidence of significant spike count correlation (58%) than Group 2 (48%), Group 3 had a significantly smaller degree of variation (smaller $-\log_{10}P$, median 0.44) as well as significantly smaller incidences of significant variation (13.5%) than Group 2 with $P<0.01$ (median of $-\log_{10}P$, 0.64) and $P<0.05$ (21.9%), respectively (Mann-Whitney test). This result might suggest that the pairs of stimulus tuned units (Group 2) were more likely to show orientation dependent variation of spike count correlation than the pairs including non-tuned units (Group 3). In fact, Group 3 had a marginally smaller degree of variation than Group 1 (median of $-\log_{10}P$, 0.51, $P<0.072$). However, similarity between the tuning characteristics of the two units seemed not to be essential to orientation dependent variation, because there was no significant difference between Group 1 and 2. Smaller incidence of orientation dependent variation in Group 3 is considered to be derived from the property that this group contained a smaller percentage of Group C (13.5%) than the other groups (Group 1: 21.3%, Group 2: 21.1%). We arrived at the same conclusion when the unit pairs were classified with respect to the two types of recording electrodes.

Finally, we examined the mechanism leading to orientation dependent variation of the spike count correlation. In particular, we were interested in whether this dependence

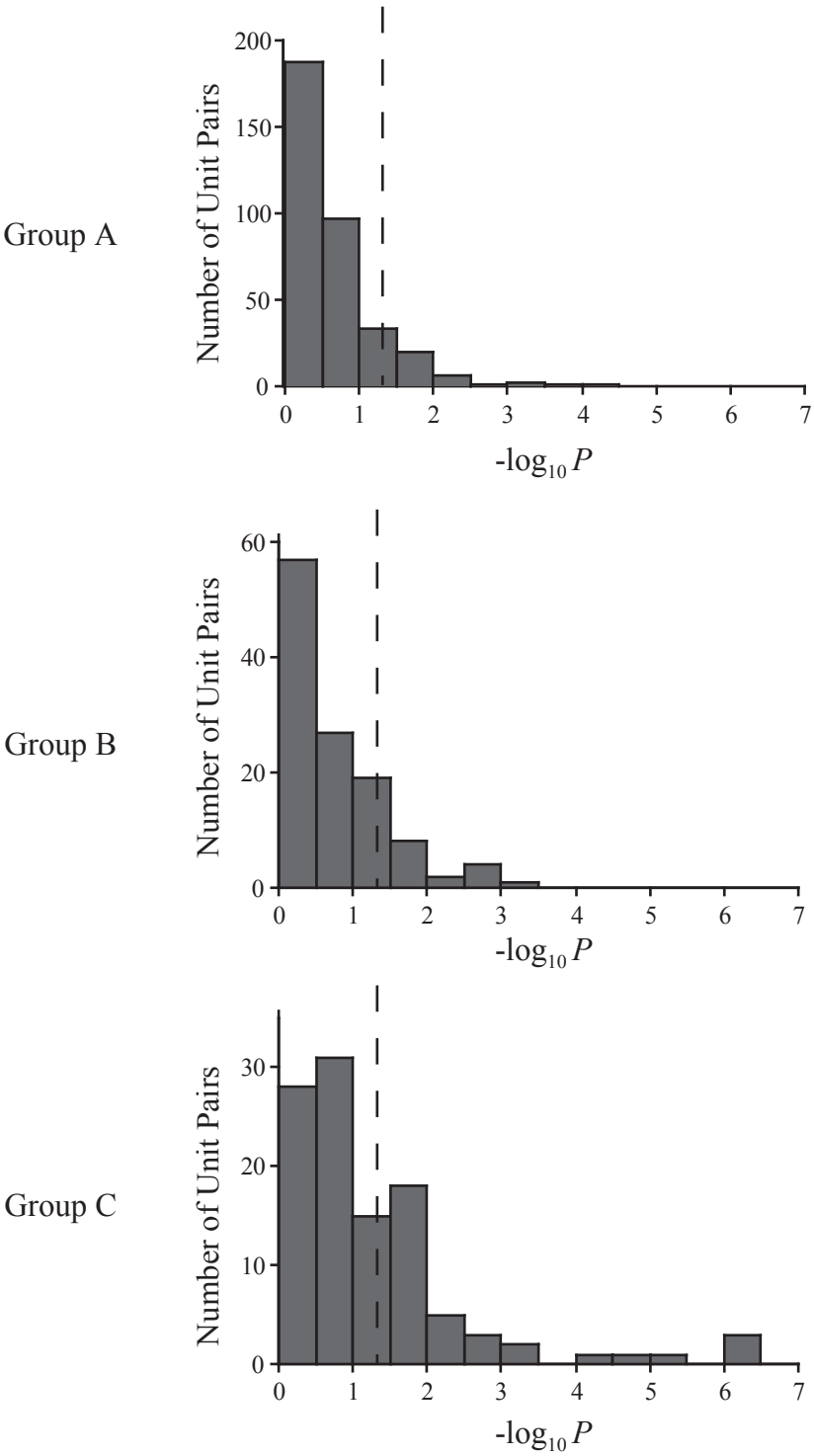

Figure 12. The degree of orientation dependent variation in the spike count correlation was quantified by the *P*-value of the Kruskal-Wallis test. The histogram of $-\log_{10}P$ over all the samples (N=575) shown in Figure 11 was split into three groups, Group A: significant cases that consist of only positive correlations (N=349, 60.7%), Group B: significant cases that consist of only negative correlations (N=118, 20.5%) and Group C: significant cases that consist of both positive correlations and negative correlations (N=108, 18.8%). The variation is judged to be significant when $-\log_{10}P>1.3$ ($P<0.05$, dashed lines). The distributions are significantly different among the three groups ($P<0.0001$ Kruskal-Wallis test). Group C (median of $-\log_{10}P$ 0.89) has significantly larger degree of variation than Group A (median 0.45) and B (median 0.53).

originated from the same neuronal mechanism leading to orientation tuning characteristics of the firing rates. For the unit pairs showing significant variation of the spike count correlations (N=106), we performed global statistics on the relationship between the tuning characteristics of the two variables. The geometric mean of the firing rates of the two units appropriately characterizes the orientation tuning properties of the unit pair's activity only when the two units have similar tuning characteristics, that is, Group 1 with SC_m >0.3. Only 10 out of the 28 pairs in Group 1 (35.7%) showed a significant correlation between the spike count correlation and the geometric mean, and 4 of 10 showed a negative correlation. For example, the spike count correlation of the unit pair shown in Figure 10 was negatively correlated with the geometric mean (correlation coefficient -0.72, P<0.01). On the other hand, both units of the pairs in Group 2 showed significant orientation tuning of their mean firing rates but they were not similar (SC_m<0.3). We can no longer define an adequate single variable characterizing the orientation tuning of the unit pair's activity. However, not a small portion of the unit pairs in Group 2 showed significant orientation dependent variation of the spike count correlations (54 out of 247, 21.9%, see Table I). Finally, one or both units of the pairs in Group 3 did not show significant orientation tuning of their firing rates. Nevertheless, 13.5% (24 out of 178) of the unit pairs in this group showed significant variation. For example, neither unit of the pair shown in Figure 13 showed significant orientation tuning of its mean firing rate (Fig. 13A). However, the spike count correlation still showed significant orientation dependent variation (P<0.01 Kruskal-Wallis test, Fig. 13B). In summary, correlation analysis between the spike count correlation and the activity level of the unit pairs suggested that orientation dependent variations of the two quantities were mostly independent and were not likely to originate from a common neuronal mechanism.

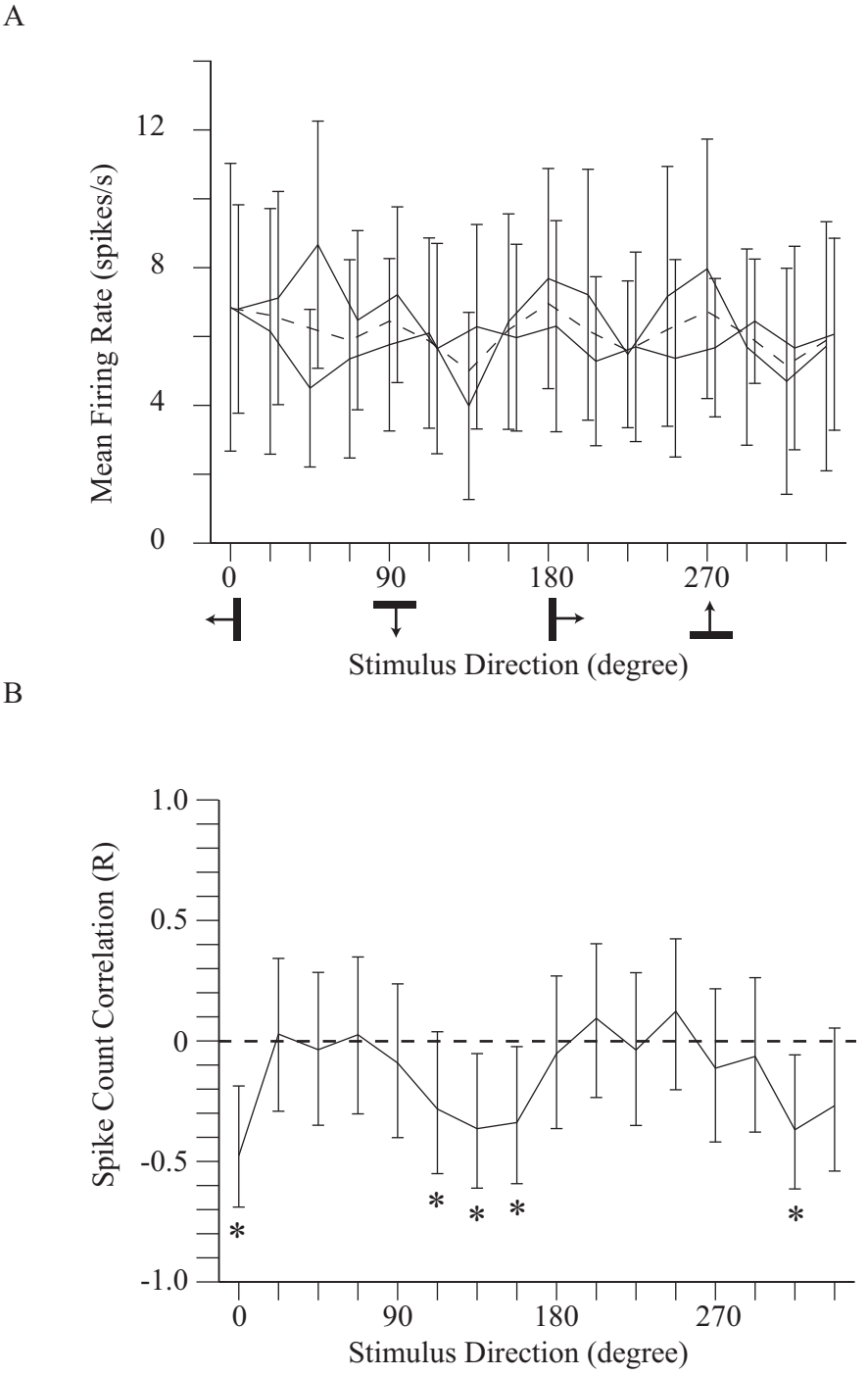

Figure 13. Example unit pair showing orientation dependent variations of the spike count correlations that appeared independently from variations of the mean firing rates. (A) Orientation tuning curves of the mean firing rates of the two units showed no significant variation over different stimulus orientations. Both units were isolated from different tetrodes separated by 710 μm. The dashed line represents the geometric mean of the two firing rates. (B) Orientation tuning curves of the spike count correlation. A dashed line represents zero correlation. The spike count correlation significantly departed from zero (negative) for five stimulus orientations (P<0.05 Bootstrap test, marked by asterisks) and its orientation dependent variation was significant (P<0.02 Kruskal-Wallis test).

4. Discussion

The present findings reveal that the spike count correlation in the visual cortex has a larger diversity and heterogeneity than those reported previously. Although the population average of the spike count correlation is relatively small (0.06), the correlations of individual unit pairs are distributed rather broadly, extending to both positive and negative values. More than half of the population (53%) shows correlations significantly departing from zero to at least two stimulus orientations and nearly 20% of those samples show significant variations of the spike count correlation for different stimulus orientations. Correlation analysis between the spike count correlation and the firing activity of the unit pair suggests that orientation tunings of the two characteristics are mostly independent and are not likely to originate from a common neuronal mechanism. Context dependent variation suggests that the correlation does not necessarily originate from fixed anatomical connections. Furthermore, in most of the recording sessions of local cell populations (83%), significantly positive correlations coexist with significantly negative correlations in different unit pairs. We suppose that both orientation dependent variation and spatial heterogeneity lead to a large diversity in the spike count correlation.

4-1 *Relationship to previous studies*

Magnitude of correlation

Spike count correlation has been investigated extensively in previous studies (Zohary *et al*., 1994; Lee *et al*., 1998; Maynard *et al*., 1999; Bair *et al*., 2001; Kohn & Smith, 2005; Smith & Kohn, 2008; Gutnisky & Dragoi, 2008; Ecker *et al*., 2010; Renart *et al*., 2010). However, there have been contradictions among their conclusions, especially with regard to the magnitude of spike count correlation (see extensive review by Cohen & Kohn, 2011). Most of the previous studies reported that the population mean of the spike count correlation was within the range of 0.1 ~ 0.2 (V1: 0.25 Reich *et al*., 2001; 0.2 Kohn & Smith, 2005; 0.18 Smith & Kohn, 2008, MT: 0.12 Zohary *et al*., 1994; 0.20 Bair *et al*., 2001, IT: 0.22 Gawne *et al*., 1993, M1: 0.21 Maynard *et al*., 1999, however V4: 0.04 Cohen & Maunsell, 2009; 0.05 Mitchell et al., 2009). However correlated spike count variability was questioned by the recent studies reporting values one order of magnitude smaller than the previous results (0.01 Ecker *et al*., 2010; 0.005 Renart *et al*., 2010). We have confirmed weakly positive value of the mean correlation 0.06, which is a bit smaller but comparable to previous results. However, due to the

large diversity in the correlations between individual unit pairs, we do not think that the population mean is a unique statistic that characterizes the nature of correlated spike count variabilities.

Dependences of receptive filed properties

Some of previous studies limited the sample to the units showing significant orientation preference and the unit pairs having similar orientation tuning characteristics (Zohary *et al*., 1994; Kohn & Smith, 2005). In the current study, we sampled unit pairs without any restriction on their tuning properties. We analyzed the spike count correlation of the samples having dissimilar tuning preferences and the samples in which one or both units did not show significant orientation preference. As a result, we found that those unit pairs showed significant spike count correlation as with the samples having similar tuning properties. Although most studies supported positive correlation between the spike count correlation and the signal correlation, the degree of correlation varied from weak (Maynard *et al*. 1998; Ecker *et al*., 2010) to strong (Zohary *et al*., 1994; Bair *et al*., 2001; Kohn & Smith, 2005; Smith & Kohn, 2008; Cohen & Maunsell, 2009). Very weak correlation was found in our analysis.

We found that the correlations of the unit pairs isolated from the single microelectrode array decreased gradually as the distance was increased. Our result of the linear slope 0.078 mm^{-1} seems to be comparable to the previous result 0.048 mm^{-1}, which was estimated by the data over the larger spatial extent of up to 10 mm (Smith & Kohn, 2008). Since the spatial extent of our recordings was limited to 1 mm, we were not able to fully confirm a very gradual decrease over longer distance. Significant distance dependence was not concluded in other reports (Maynard *et al*., 1998; Cohen & Maunsell, 2009; Ecker *et al*., 2010). For both the signal correlation and the unit distance, their influences to the spike count correlation are very weak and there exists a large amount of diversity over individual samples.

Finally we found that the unit pairs in close proximity isolated from a single microelectrode ($<120\,\mu m$) have significantly larger signal correlation and spike count correlation than the pairs isolated from distant single electrodes and isolated from the single tetrode (see Appendix). The same property was reported in the study performed on monkey motor cortex (Lee *et al*., 1998).

Significance test of spike count correlation

Significant departure of the correlation from statistical independence was tested in some of previous studies (Maynard *et al*., 1998; Ecker *et al*., 2010). Due to weak

correlation and a limited number of trials, we needed to adopt an adequate statistical test to judge whether the unit pair has a significantly larger amount of correlation than the value expected by chance. We calculated spike count correlation for each orientation stimuli and examined its significance using the bootstrap test. Orientation dependent variation of the spike count correlation was investigated only for the unit pairs showing a significant correlation.

Heterogeneity due to different cell types

Heterogeneity of the spike count correlation over individual unit pairs might originate from distinct characteristics for different combinations of two cell types: the regular spiking excitatory neuron and the fast spiking inhibitory neuron (Middleton *et al*., 2012). However, this possibility could not be tested since we did not succeed in isolating two cell types with sufficient confidence based on their spike waveforms.

4-2 *Influence of extrinsic factors*

Although our main interest is to characterize intrinsic neuronal properties of the spike count correlation, estimation of the correlation is influenced by several extrinsic factors (Ecker et al., 2010; Cohen & Kohn, 2011).

Spike sorting

Contamination of spike events of another unit in the pair leads to an artificial increase (overestimation) in both the signal correlation and the spike count correlation (Ecker *et al.*, 2010). We imposed a strict criterion for the portion of spike events falling within the absolute refractory time of 1 ms. Since microelectrodes provide less information for the spike isolation, we imposed more strict criterion, that is, less than 1.0% of the total spikes within 1 ms, compared with 1.5% for tetrode. On the other hand, Cohen and Kohn (2011) showed that excessively restrictive criterion in the spike sorting can lead to an underestimation of the spike count correlation. They discussed that this factor could lead to a very small correlation reported recently (0.01 Ecker *et al*., 2010; 0.005 Renart *et al*., 2010). However, this possibility is not supported by our result. We adopted more restrictive criterion than those studies (3% Ecker *et al.*, 2010; 10% within 2 ms Renart *et al*., 2010), and obtained a finite level of correlation (mean 0.06) which is comparable to that of previous studies.

Non-stationarity

Previous studies discussed a danger of artificial spike count correlation due to non-stationary modulation of the firing activity in a long temporal scale (van Kan *et al*., 1985; Zohary *et al*., 1994; Bair *et al*., 2001; Ecker *et al*., 2010; Renart *et al*., 2010). Such modulation may originate from global state transitions by unstable anesthesia or by different behavioral/cognitive states. Also selective attention was known to increase the spike count correlation (Roelfsema *et al*., 2004; Cohen & Newsome, 2008; Cohen & Maunsell, 2009; Mitchell et al., 2009). In our anesthetized preparations, non-stationary modulation by top-down projections from higher cortical areas was not likely to be a dominant factor. Physical drift of recording electrodes also leads to non-stationary modulation. Our single recording session, which consisted of 40 trials of the 16 different stimuli, took 40 to 60 minutes. We screened out the units showing a long-term drift of their activity levels within the session using the bootstrap test of stationary responses.

Low firing rate

When the stimulus evoked very weak activity, the distribution of spike counts became non-Gaussian and discrete. Correlation coefficients could no longer be used as a basis for determining the extent to which the spike counts of the unit pairs were related (Hoel, 1984; Cohen & Maunsell, 2009; Cohen & Kohn, 2011). Simulation studies showed that the correlation was systematically underestimated when one unit of the pair had a very low firing rate of less than 0.1 spikes/s (Cohen & Kohn, 2011). As known from the distribution of the firing rates in Figure 7, most of our sample unit pairs had firing rates in the range of 1~10 spikes/s (85.9%, 14989/17400). According to the above simulation study, the correlation can be adequately estimated in this range. In fact, the median of the firing rates in our samples, 2.7 spikes/s was comparable to the mean firing rate of the previous report of V1 (3.4 spikes/s, Smith & Kohn, 2008). For a low firing data, cares were taken also in the test of significant departure of the correlation from zero. We adopted a non-parametric test based on the bootstrap samplings of trial shuffled data. When either unit had a very low firing rate, the distribution of the bootstrap samples became discrete due to many identical values and the significance test did not work adequately. When the bootstrap samples (N=1000) consisted of less than 500 different values, we assigned the correlation as non-significant to avoid false positives.

4-3 *Rate dependence of spike count correlation*

The spike count correlation of our sample showed little rate dependence in the range of 1~10 spikes/s (Fig. 7). For the unit pairs exceeding 10 spikes/s, an increase in the correlation was observed. Although this trend was observed in the plot against the geometric mean of the two firing rates (Figure 8), the model of linear increase reported previously (Ecker et al., 2010) was rejected.

Dependence of the correlation strength on the firing rates can originate from three independent factors. First, as discussed just before, the correlation coefficient gives an inadequately small value when one unit of the pair has a very low firing rate. This is not a physiological issue but a mathematical one. When the assumption of a continuous Gaussian distribution is strongly violated, we may no longer rely on the correlation coefficient. Second, correlated common input to the two units fails to transmit to correlated spike outputs when the amplitude is below a finite threshold of a non-linear transfer function (de la Rocha *et al*., 2007; Cohen & Kohn, 2011; Middleton *et al*., 2012). They found that, even when the strength of the input correlation remained fixed, the correlation of the output spike counts increased nonlinearly with the firing rates. Note that this mechanism of rate dependence applies only in the case when the spike count correlation originates solely from shared common inputs to the two units. Our result differed from this type of rate dependence, since the samples showed little rate dependence in the range of 1~10 spikes/s (Fig. 7) where significant increase was observed in *in vitro* experiments (de la Rocha *et al*., 2007). Third, quantitative comparison of correlation strength between the data of different firing rates is inevitably model (measure) dependent (Ito & Tsuji, 2000; Kass *et al*., 2005). Simulation studies (Mitchell *et al*., 2009 Supplementary Material) showed that even when the unit pairs have sufficient spike counts, the correlation still increases as a function of the firing rates. They also computed the spike count correlation of the two neurons receiving partially correlated fluctuating inputs. Even for a fixed strength of the input correlation, the correlation of the output spike counts increased with the firing rates and was smaller than the input correlation even at very high firing rates (~200 spikes/s). Similar rate dependence was observed experimentally in spike synchrony among spinal motoneurons (Binder & Powers, 2001). We suppose that this *rate artifact* (Mitchell *et al*., 2009 Supplementary Material) was due to the confusion of two different correlation measures, one being a model parameter controlling input correlation, and the other being a correlation coefficient of the output spike counts. It was known that, even from the same spike train data, different correlation measures normalize the covariance by the

firing rates in different ways and lead to quantitatively different rate dependences (Aertsen *et al.*, 1989; Ito & Tsuji, 2000; Binder & Powers, 2001; Dorn & Ringach, 2003; Kass *et al.*, 2005; Kohn & Smith, 2005 Appendix).

4-4 *Orientation dependence of the spike count correlation*

Orientation dependent variation of the correlation was mainly investigated with respect to its relationship with the orientation dependence of the activity levels of unit pairs (Kohn & Smith, 2005). By aligning the tuning curves of the correlation on the peak of the geometric mean of the firing rates, they found little relationship between the magnitude of the spike count correlation and the activity level. In addition to the population analysis, they also tested the correlation between the tuning curve of the spike count correlation and the tuning curve of the geometric mean for each individual unit pair. They found a significant correlation in only 7 of 100 unit pairs. However, their results did not necessarily rule out an orientation dependent correlation, which varies independently from the rates. In fact, we have shown that, although both variables had significant orientation dependent variations, they were not significantly correlated in many unit pairs having similar orientation preferences (Group 1 in our classification). We can no longer investigate the relationship between the correlation and the geometric mean of the rates when the two units have dissimilar tuning preferences (Group 2) or one or both units do not have significant orientation tuning (Group 3). Therefore significant variation of the correlation should be tested independently from the variation of the rates. We found that a comparable portion of the samples (~20%) in each group showed significant variation of the correlation.

Maynard *et al*. (1999) reported that 78% of the unit pairs in monkey M1 associated with arm movements showed significant variation in spike count correlations over a range of directions. Currently we are unable to explain the smaller incidence (~20%) of orientation dependent correlation given by our results. Orientation dependent variation of the correlation has been examined and it was determined to be a non-robust property in previous studies (Zohary *et al*., 1994; Bair *et al*., 2001; Kohn & Smith, 2005). In those studies, significance of the variation was tested for all recorded unit pairs. On the other hand, we tested significance only for the samples showing spike count correlations that departed from zero significantly. Previous studies might underestimate the incidence of orientation dependent correlation because the samples with non-significant correlations were not likely to show significant variation over different orientations.

As we discussed above, the correlation strength depends on the firing rates in a

multitude of ways. However, as far as any of these factors are concerned, variations of the two variables are strongly correlated. We have shown that orientation dependent variation of the spike count correlation did not correlate with the variation of the geometric mean of the firing rates (Group 1). Furthermore, significant variation of the spike count correlation was observed even for the unit pairs of dissimilar rate tunings (Group 2) and non-significant rate tuning (Group 3). Therefore we conclude that the orientation dependent variation of the spike count correlation we have reported did not originate simply from the rate dependence.

Orientation dependent variation suggests that the spike count correlation does not necessarily originate from a fixed anatomical structure of shared inputs. Context dependent change of the spike count correlation was reported in cortical areas of monkeys performing attention tasks (MT: Cohen & Newsome, 2008, V4: Cohen & Maunsell, 2009; Mitchell et al., 2009). Cohen and Newsome concluded that the changes in correlation were due to changes in top-down or recurrent functional inputs to MT whose strength was context dependent. Gutnisky & Dragoi (2008) found that, in V1 of awake monkey, brief adaptation to a stimulus of fixed structure reorganized the distribution of correlations across the entire network by selectively reducing their mean and variability. They concluded that adaptive decorrelation improved the accuracy of population coding to optimal performance.

4-5 *Neuronal mechanism of spike count correlations*

Common input from feed-forward and horizontal connections

Shared input to the two neurons has been considered as a primary factor leading to the spike count correlation. Three possible sources of input were pointed out (Smith & Kohn, 2008), feed-forward inputs by thalamocortical axons, horizontal intra-cortical connections and feedback connections from the extrastriate cortex. Feed-forward, thalamocortical axons extend tangentially in layer IV within 1 mm (Blasdel & Lund, 1983). Our observations of gradual linear decrease to the increase of unit distances (also Smith & Kohn, 2008) and very weak dependence on the signal correlation do not support the feed-forward origin of the spike count correlation. However, we have shown that, for the unit pairs in close proximity isolated from a single microelectrode, the spike count correlation was likely to be affected by shared thalamocortical inputs (see Appendix). As for the horizontal intra-cortical connections, neurons with a similar orientation preference are interconnected over distances of several millimeters, but connections are locally (<0.5mm) nonspecific (Gilbert & Wiesel, 1983, 1989; Ts'o *et al*.,

1986; Malach *et al*., 1993; Bosking *et al*., 1997). Although the spatial extent we have examined (< 1 mm) was too small to test the contribution of the horizontal connections, we did not find a significant increase in the spike count correlation for the unit pairs of a large signal correlation in the distance range of 0.5 ~ 1.0 mm (data not shown).

Feedback input from the extrastriate cortex

Feedback connection from the extrastriate cortex differs from feed-forward inputs and horizontal connections in respect of their far-reaching and non-specific target projections (Angelucci *et al*., 2002; Shmuel *et al*., 2005). Cell population of the cortex often shows globally synchronized transitions between a high firing rate state (Up state, depolarized state) and a low firing rate state (Down state, hyperpolarized state) in a sub-second time scale (Destexhe & Contreras, 2006). Renart *et al*. (2010) reported that, during the spontaneous neuronal activity in both the rat auditory cortex and the somatosensory cortex, network activity alternated between the activated state and the inactivated state. During the activated period, population activity was tonic and the spike count correlation was small (median 0.0053). On the other hand, during the inactivated period, the Up-state and the Down-state alternated over a short time scale (~500 ms) and the modulations were synchronized globally over the cell population. They found that such a global gain modulation caused a relatively large positive spike count correlation (median 0.095). Since the time scale of the state transitions could be less than the single trial duration (1.0~1.7 s), our bootstrap test of the stationary response over trials might fail to exclude this type of non-stationary data. Therefore we need to perform additional tests to confirm that our results were associated with locally correlated activities of the two units. First, if the spike train data contain discrete transitions between Up and Down-states, the scattergram of the firing rates (*z*-scores) of the two units over trials should show two isolated clusters corresponding to the high firing state and the low firing state. The regression line connecting those two clusters provides an artificially large spike count correlation. The distribution of *z*-scores in the scattergram (Figs. 3, 9, 10) did not show such discrete clusters. Second, we found that spike count variabilities between the two units can be negatively correlated and even orientation dependent. Finally, if the spike count correlation arises from the state transitions of the cell population, we expect some degree of spatial homogeneity. We have shown that 83% of the total sessions (40 out of 48) showed spatial heterogeneity, that is, significantly positive correlations coexisting with significantly negative correlations over different unit pairs recorded in the same session. Those results suggest that the spike count correlation observed in our data did not originate from stochastic

state transitions of the cell population by the feedback interactions.

Network dynamics

In the above discussion, we assumed that shared anatomical connections necessarily lead to spike count correlation. However, recent studies demonstrated that the postsynaptic effects of thalamocortical synapses are strongly context dependent. The influence of weak synaptic input is greatly augmented when they occur in tight synchrony with that of other synaptic input on the same postsynaptic neuron (Alonso *et al*., 1996; Roy & Alloway, 2001; Usrey *et al*., 2000; Usrey, 2002; Bruno & Sakmann, 2006). These findings are consistent with the concept that cortical neurons can act as finely tuned detectors of coincident synaptic input (Azouz & Gray, 2000, 2003, 2008). Non-stationary modulation of spike timing synchrony in a sub-second time scale has been observed in the cortex (Aertsen & Gerstein, 1991; Vaadia *et al*., 1995; Riehle *et al*., 1997, 2000; Hatsopoulos *et al*., 1998; Grammont & Riehle, 2003; Gruen *et al*., 2003; Maldonado *et al*., 2008) and the thalamus (Ito *et al*., 2010). Those results suggest that correlated neuronal activity could be controlled by dynamic properties of the neuronal networks even without any change in anatomical connection (Aertsen *et al*., 1989; Sporns, 2011). Thus, there is a possibility that spike count variabilities and their correlations do not originate from fluctuations of the firing rates at a single shared input, but from temporal fluctuations of the spike timings among shared synchronous inputs. Orientation dependent variation of the spike count correlation also could reflect the mechanism that different stimuli evoke changes in the temporal coordination of synchronous inputs to the two neurons. Firing rates and spike synchrony might independently encode stimulus orientations.

Middleton *et al.* (2012) suggested that a combination of threshold nonlinearity and feed-forward inhibition from the fast spiking (FS) inhibitory neuron to the regular spiking (RS) excitatory neuron can lead to network activity dependent changes of the spike count correlation, that is, stimulus-evoked decorrelation of FS-RS activity. Luczak *et al*. (2009) reported that stimulus evoked activities had similar spatio-temporal structure as on-going activities. They discussed that such intrinsic structure was imposed by spike count correlations not just locally, but also between unit pairs separated by 1 mm. Since examination of cross-correlogams did not indicate a functional monosynaptic connection between correlated pairs, they concluded that the spike count correlation may reflect large-scale network interactions, such as the consistent participation of cells in neuronal assemblies spread over wide cortical areas (Harris, 2005). Renart *et al*. (2010) discussed other possibility of network dynamics

influencing the spike count correlation. They demonstrated theoretically that densely connected recurrent network dynamics can lead to an active decorrelation of the synaptic current, resulting in a state of arbitrarily low mean spike count correlation. They concluded that shared input does not inevitably cause correlated activity.

Appendix

Difference between two recording electrodes

Multiple single units were isolated from multi-unit activities recorded by either the single microelectrode or the single tetrode. Although we have assigned zero physical distance for those unit pairs, the unit pairs recorded by the two types of electrodes had distinct properties with respect to both the signal correlation and the spike count correlation. Unit pairs isolated from the microelectrode tended to have a larger spike count correlation (Fig. 14A, N=16, mean 0.15 ± 0.09) than those isolated from the tetrode (Fig.14C: N=206, mean 0.06 ± 0.16, P<0.01 Mann-Whitney test). This difference can be explained by different spatial ranges for unit sampling of the two types of electrodes. Our microelectrode consists of a single platinum-tungsten core (diam. 12 μm) embedded in a quartz-glass fiber (diam. 40 μm). The range of the microelectrode was estimated to be 60 μm (Lemon, 1984), so that physical distances between the isolated units were at most 120 μm. We could isolate at most a few single units based on the principal components of spike waveforms. Since isolated units were located in close proximity, the distribution of their signal correlations were biased to positive values as shown in Figure 14B (N=16, median 0.57). On the other hand, the tetrode consists of four platinum-tungsten cores (diam. 26 and 14 μm) embedded in a quartz-glass fiber of larger diameter (96 μm). The tetrode samples units over a wider spatial range (130 μm) (Gray *et al*., 1995) and has superior performance in spike isolation. Therefore, even isolated from the same tetrode, unit pairs were distributed over a larger spatial extent of at most 260 μm. Reflecting a heterogeneous distribution of orientation selective units (pin wheel structure) in the visual cortex (Maldonado & Gray, 1996), the signal correlation has a broader distribution as shown in Figure 14D (N=206, median 0.33). However, the difference between the two distributions was only marginally significant (P=0.10, Mann-Whitney) due to the small sample size of the unit pairs isolated from the microelectrode.

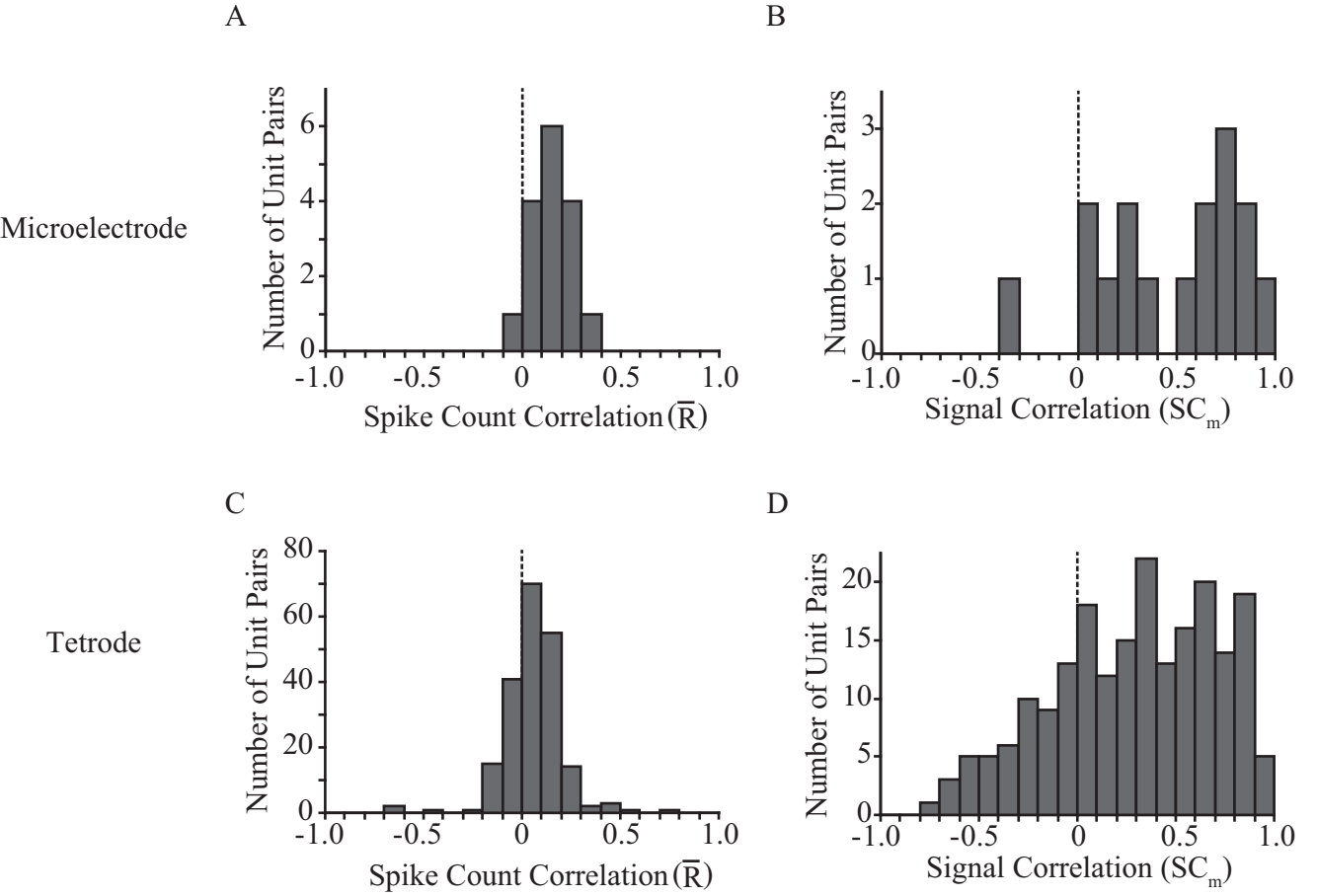

Figure 14. Comparison of characteristics of the unit pairs isolated from single microelectrode and single tetrode. Distributions of mean spike count correlations averaged over 16 stimuli, (A) microelectrode *N*=16, mean 0.15 ± 0.09; (C) tetrode N=206, mean 0.06 ± 0.16. Unit pairs isolated from the microelectrode tended to have a significantly larger spike count correlation than those isolated from tetrode (*P*<0.01 Mann-Whitney test). Distributions of signal correlations, (B) microelectrode *N*=16, median 0.57; (D) tetrode *N*=206, median 0.33. Although both distributions showed bias to positive correlations, tetrode tended to sample unit pairs over a wider range of signal correlation.

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