Dynamics of Stimulus-Evoked Spike Timing Correlations in the Cat Lateral Geniculate Nucleus

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1Faculty of Computer Science and Engineering, Kyoto Sangyo University, Kyoto, Japan; 2Instituto de Ciencias Biomédicas, Facultad de Medicina, University de Chile, Santiago, Chile; and 3Center for Computational Biology and the Department of Cell Biology and Neuroscience, Montana State University, Bozeman, Montana

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Ito H, Maldonado PE, Gray CM. Dynamics of stimulus-evoked spike timing correlations in the cat lateral geniculate nucleus. J Neurophysiol 104: 3276–3292, 2010. First published September 29, 2010; doi:10.1152/jn.01000.2009. Precisely synchronized neuronal activity has been commonly observed in the mammalian visual pathway. Spike timing correlations in the lateral geniculate nucleus (LGN) often take the form of phase synchronized oscillations in the high gamma frequency range. To study the relations between oscillatory activity, synchrony, and their time-dependent properties, we recorded activity from multiple single units in the cat LGN under stimulation by stationary spots of light. Autocorrelation analysis showed that approximately one third of the cells exhibited oscillatory firing with a mean frequency ~80 Hz. Cross-correlation analysis showed that 30% of unit pairs showed significant synchronization, and 61% of these pairs consisted of synchronous oscillations. Cross-correlation analysis assumes that synchronous firing is stationary and maintained throughout the period of stimulation. We tested this assumption by applying unitary events analysis (UEA). We found that UEA was more sensitive to weak and transient synchrony than cross-correlation analysis and detected a higher incidence (49% of cell pairs) of significant synchrony (unitary events). In many unit pairs, the unitary events were optimally characterized at a bin width of 1 ms, indicating that neural synchrony has a high degree of temporal precision. We also found that approximately one half of the unit pairs showed nonstationary changes in synchrony that could not be predicted by the modulation of firing rates. Population statistics showed that the onset of synchrony between LGN cells occurred significantly later than that observed between retinal afferents and LGN cells. The synchrony detected among unit pairs recorded on separate tetrodes tended to be more transient and have a later onset than that observed between adjacent units. These findings show that stimulus-evoked synchronous activity within the LGN is often rhythmic, highly nonstationary, and modulated by endogenous processes that are not tightly correlated with firing rate.

Introduction

Synchronous activity is a widespread and robust property of neuronal networks in a wide variety of species (Buzsáki 2006; Laurent et al. 2001; Singer and Gray 1995; Usrey and Reid 1999). Its functional significance has long been recognized within the context of synaptic physiology, where it plays a critical role in synaptic integration and plasticity (Alonso et al. 1996; Azouz and Gray 2003; Toyama et al. 1981; Usrey and Reid 1999). In recent years, synchronous activity on a millisecond time scale has been postulated to contribute to higher-level functions including perceptual grouping (Gray 1999; Singer and Gray 1995), attentional selection (Fries et al. 2001; Steinmetz et al. 2000; Womelsdorf et al. 2006), expectancy (Riehle et al. 1997), working memory (Buschman and Miller 2007; Sakurai 1993; Vaadia et al. 1995), motor control (Hatsopoulos et al. 2003), and behavioral arousal (Herculano-Houzel et al. 1999). In each of these contexts, it has been argued that temporally correlated activity should be highly dynamic to provide representational flexibility (Engel et al. 2001; Fries 2005; Fuji et al. 1996; Gerstein et al. 1989; Gray 1999; Gruen et al. 2003; Samonds et al. 2006; Singer 1994; Von der Malsburg 1981).

Such dynamic changes in correlated activity could result from stimulus or task driven influences or be controlled by the dynamic properties of the neuronal networks themselves. In the latter case, spike timing correlations among neuronal groups would be expected to vary even when no temporal structure is provided by the stimulus. A few reports have shown such nonstationary changes of spike synchrony in cortex, even without corresponding changes in the neuronal firing rates (Aertsen and Gerstein 1991; Grammont and Riehle 2003; Gruen et al. 2003; Hatsopoulos et al. 1998; Maldonado et al. 2008; Riehle et al. 1997, 2000; Vaadia et al. 1995), suggesting that time-varying spike timing correlations may be an important feature of cortical networks. These findings raise the question of whether the correlation dynamics observed in cortex can be explained by related dynamics in the thalamic input.

To address this issue with respect to the early visual pathway, this study was motivated by two sets of questions. First, how often does correlated firing occur in the lateral geniculate nucleus (LGN) and what are its properties (i.e., temporal precision, temporal structure, and spatial distribution)? Second, when correlated firing does occur, does it exhibit time-dependent variations under stationary stimulus conditions, and how do the correlations vary with changes in neuronal firing rates? Because synchrony in the LGN often appears in the form of phase-coupled oscillations in the high gamma frequency range (60–100 Hz) (Arnett 1975; Ghose and Freeman 1992; Lauffer and Verzeano 1967; Neuenschwander and Singer 1996), we also characterized the incidence and properties of oscillatory activity in our data. Moreover, previous studies have shown robust and stimulus-dependent spike timing correlations in the LGN (Alonso et al. 1996, 2009; Arnett 1975; Doty and Kimura 1963; Ghose and Freeman 1992; Lauffer and Verzeano 1967; Neuenschwander and Singer 1996), but these studies have largely relied on conventional methods of analysis that limit the evaluation of the time dependence of correlated firing.

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To address these questions, we performed multitetrode recordings in the LGN of anesthetized cats and measured the occurrence and properties of correlated firing using both autocorrelation analysis, as well as unitary events analysis (UEA) (Gruen 1996, 2009; Gruen et al. 2001a,b), the latter method providing an effective means to characterize the time dependence of spike timing correlations. By applying a bootstrap procedure, we extended the method to permit a nonparametric significance test of synchrony in non-Poisson spike trains (Ito 2007). The correlation analysis shows that about one third of the cells display oscillatory firing with a mean frequency near 80 Hz. We find that the UEA is more sensitive to weak and transient synchrony than the cross-correlation analysis. The UEA shows that one half of the single unit pairs display significant synchronous activity, and 65% of them are optimally characterized at a precision of 1–3 ms. We also find that, during the responses to stationary light spots, many unit pairs show nonstationary modulations in their synchrony at time scales that cannot be predicted by the modulation of firing rates. The synchrony between LGN cells also has distinctly different characteristics from that observed between retinal afferents and LGN relay cells. It has a slower onset and a more transient appearance. These findings further show that synchronous activity in the LGN is stimulus dependent, very precise, and often oscillatory in its temporal structure. Moreover, spike-timing correlations in the LGN are highly nonstationary and seem to be regulated by endogenous mechanisms not directly linked to the visual stimulus. Preliminary reports of these findings have appeared in abstract form (Hirata et al. 2002, 2003; Ito et al. 2005).

METHODS

Physiological preparation

Seven adult male cats, weighing 3–5 kg, were studied using extracellular recording methods. On the day of the experiment, each animal was anesthetized with an intramuscular injection of ketamine (12 mg/kg) and xylazine (1 mg/kg), and given atropine (0.05 mg/kg, sc) to reduce salivation. The cephalic vein was cannulated, and a continuous infusion of Ringers containing 2.5% dextrose was given throughout the experiment (4 ml/kg/h). Anesthesia was maintained using halothane (0.6–1.5%) or isoflurane (1.0–2.0%) in a mixture of nitrous oxide and oxygen (2:1) while the animals were actively ventilated using a Harvard respirator pump. The EKG, heart rate, rectal body temperature, and expiratory CO2 were continuously monitored, the latter three being maintained within the ranges of 140–180 bps, 37.5–39.0°C, and 3.5–4.5%, respectively. The animal’s head was mounted in a Kopf stereotaxic frame, and a small craniotomy was made above the LGN (A8, L10) in one hemisphere. Following surgery, the animals were paralyzed with pancuronium bromide (Pavulon) at a dose of 3 mg/kg as an initial bolus followed by a continuous infusion of 3 mg/kg/h by intravenous injection. Antibiotics (Keflin) were given intravenously every 8 h. The eyes were focused on the screen of a computer monitor using the tapetal reflection technique and an appropriate set of gas-permeable contact lenses. Following these procedures, two independently controlled tetrodes (Gray et al. 1995), housed within 25-gauge stainless guide tubes (500-μm separation), were advanced into the brain so that the distal tips of the guide tubes were positioned 1–2 mm above the LGN. A 4% mixture of agar in Ringers solution was applied to the cortical surface to reduce pulsations. The assembly was covered with molten bone wax to prevent drying of the surface. All experimental procedures were in accordance with institutional and National Institutes of Health guidelines.

Recording procedures

The signals from each tetrode were amplified (gain = 10k), band-pass filtered (0.6–6 kHz, 3-dB falloff), and digitized (27 kHz/channel) using a personal computer. Spike waveforms of 1.2-ms duration, centered on the time of occurrence of a user-defined threshold crossing, were stored in a file along with their associated time stamps at a temporal resolution of 37 μs.

Receptive field mapping and visual stimulation

Once stable recordings were obtained, we mapped the receptive field properties (location, size, on or off center) of the multitetrode activity recorded by each tetrode using a mouse-controlled flashing light spot presented on a 19-in color monitor (1,024 × 768 resolution) at a distance of 57 cm from the eyes. Units were stimulated using stationary light spots, presented for durations ranging from 0.5 to 3.0 s, on a dark background over the corresponding receptive fields. When unit activity was recorded from both tetrodes, stationary light spots were presented over both receptive fields simultaneously. On average, the receptive field separation between units recorded on different tetrodes was 10.1 ± 4.8°. When a well-isolated single unit was identified during the recording, the receptive field type of the cell (X or Y type) was further examined by measuring the responses to a counterphase sine wave grating (Hochstein and Shapley 1976a,b). The ocular dominance of the unit was tested at each recording site, and the transition of ocularity along each penetration track was used to identify the lamina (A, A1, and C). We sampled units from all the layers without bias. We typically ran 10–30 trials for each stimulus and advanced the tetrode to a new location and repeated the process.

Data analysis

SPIKE SORTING. Multitetrode activities recorded by each tetrode were sorted off-line to recover the activity of individual single units using custom spike sorting software (Gray et al. 1995). The spike trains were downsampled to a resolution of 1 ms before analysis. Therefore, although the raw data were collected at a higher temporal resolution, the spike trains available for analysis had a temporal resolution of only 1 ms. The tetrode recordings allowed us to isolate as many as seven single units at a given site. Because spike sorting was conducted off-line, we were unable to map the receptive fields of each cell isolated from the same tetrode.

SPIKE TRAIN ANALYSIS. For each recording and stimulus, we computed the peristimulus time histogram (PSTH; bin width, 50 ms). To study the properties of rhythmic neuronal firing during the visual response to the stimuli, we defined a sampling window for stimulus-evoked activity and computed the autocorrelation histogram (ACH) averaged over trials with time lags of ±128 ms. Neuronal firing time-locked to the stimulus was estimated by computing the shift-predictor control correlogram. We calculated the power spectrum of each ACH and extracted the frequency and amplitude of the peak value in the frequency range from 30 to 160 Hz. The statistical significance of the spectral peak was evaluated by comparing the experimental data to the same calculations performed on a surrogate distribution derived from a set of trial-shuffled spike trains (P < 0.002) (Friedman-Hill et al. 2000). The peak strength was defined by the ratio of the experimental peak to the largest value in the surrogate control (cut-off).

The vertical refresh of the video monitor (80 Hz) was synchronized with the data acquisition at a resolution of 300 μs. When a cell followed the monitor refresh, oscillatory structure was not eliminated.

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in the trial-shifted ACH. This effect tended to occur mainly in the responses to large spots (Neuenschwander and Singer 1996). When such an effect occurred, we used the data from the responses to a smaller spot size for the cumulative statistics, provided these data did not show the same stimulus locking.

We evaluated the spike-timing correlations between pairs of cells by computing the trial averaged cross-correlation histogram (CCH) for all possible combinations of cell pairs recorded simultaneously. We used a measure for the magnitude of correlated firing that occurred within ±10 ms of zero time-lag that we refer to as the significance ratio (see Maldonado et al. 2000 for details). This measure is a ratio of two integral values: a peak value, computed by taking the sum of the bins in the central 20 ms of the CCH that exceed the 95% confidence limits, and a variance value, representing the expected occurrence of coincident spikes, computed from the sum of the central 20 ms in each histogram lying between the 95% confidence limits. Because this measure yielded values over a wide range, we computed a separate confidence limit for statistical significance ($P < 0.002$) using the same shuffling procedure described above.

Because of nonstationary changes of firing rates in the sampling window, some CCHs may be judged as significant even when spike timing correlations are not occurring (Brodsky 1999a,b). To address this issue, we computed the significance ratio for the shift predicted CCH. Based on the scatter plot of the experimental significance ratio versus the value of the shifted CCHs over all the unit pairs, we found that most of the false positives could be screened from the data by imposing an additional criterion: the experimental significance ratio had to be greater than twice the value of the shifted CCH. Finally, to test the oscillatory component of the CCHs, power spectra and peak frequencies were computed and tested for significance as done for the ACHs.

SLIDING WINDOW CORRELATION HISTOGRAM. We also computed the ACH and CCH for each unit and unit-pair using a sliding window analysis (Castelo-Branco et al. 1998; Neuenschwander and Singer 1996). This method enabled us to visualize nonstationary changes in spike timing correlations during the course of the trial duration. We used a sampling window of 200 ms, with time lags of ±32 ms, advanced in steps of 10 ms. The resulting histograms were normalized by the corresponding spike count (for ACH) in the sampling window or the geometric mean of the spike counts of the two units (for CCH), and the amplitude at each delay value was represented using a color scale.

UEA. To examine the nonstationary characteristics of spike timing correlations during the stimulus presentation, all the unit pairs were further evaluated by the method of UEA (Gruen 1996; Gruen et al. 2001a,b). The original formulations of the method are given by the following procedures, which are depicted in Fig. 1.

1) The data are binned over a small interval $b$ ranging from 1 to 6 ms. Each bin has a value of either 0 or 1, determined by the occurrence of a spike in that bin (Fig. 1A). Even when the bin contains more than one spike, a value 1 is assigned (clipping).

2) Select a specific time delay $d$ having a precision equal to the bin width $b$.

3) For each pair of spike trains on each trial, detect the occurrence of coincident spike events (CE, indicated in bold in Fig. 1) with the time delay $d$ throughout the trial duration. For the example shown in Fig. 1B, the CE has a constellation of $d = b$: unit B fires $b$ ms after the firing of unit A. By definition, the states of other bins indicated by asterisks can be either 0 or 1.

4) At each bin position $t$, apply a small sampling window of duration $T_w$ (100 ms, extending ±50 ms around the central bin). Count the number of CEs within the sampling window to get a local variable $n_i(t)$, the number of coincident events in the $i$th sampling window of the $n$th trial. Sum up $n_i(t)$ over $M$ trials to obtain the raw CE number, $N_{raw}(t)$.

5) Advance the sampling window in steps of $b$ to obtain the temporal modulation of the raw CE number as a function of time.

SIGNIFICANCE TEST OF COINCIDENT EVENTS. The raw CE number must be tested for statistical significance within each sampling window. For this, the null hypothesis of independent firing of the two units is given by the product of the firing rates of the two units. In the original formulation (Gruen 1996; Gruen et al. 2001a,b), successive CEs in the sampling window are assumed to occur independently and their total count over $M$ trials follows a Poisson distribution. The raw CE number is judged as significant [unitary event (UE)] when it exceeds the significance limit of the Poisson distribution ($P < 0.05$ or 0.01). Recently, we showed that highly periodic firing leads to a strong correlation between the successive CEs, even when the two spike trains are independent (Ito 2007). The distribution of the number of chance CEs has greater variance than the Poisson distribution. Therefore the significance test based on the Poisson distribution leads to more false positives than the $P$ value of the significance limit. We showed that the significance test based on bootstrap sampling works adequately for such non-Poisson spike trains. In this nonparametric test, the distribution of the null hypothesis is predicted by the statistics of the bootstrap samples generated by the following steps (Gruen 2009; Ito 2007; Pipa and Gruen 2003; Ventura et al. 2005):

1) Generate a bootstrap sample by randomly shuffling the combination of $M$ trial pairs of the spike trains, so that no spike train of unit A makes a pair with that of unit B in the same trial.

2) For each trial shuffled pair, count the CEs within the sampling window and sum over $M$ trial pairs to get one sample of the CE number.

3) Repeat steps 1–2 $N$ times to get $N$ samples of the CE number within each sampling window. Because the number of possible shuffled combinations becomes very large, we apply uniform Monte Carlo samplings ($n = 1,000$).

4) For each sampling window, the significance limit is given by the 99th percentile of the $N$ values. When the raw CE number exceeds the significance limit, all the CEs in the sampling window are judged as significant ($P < 0.01$) and regarded as unitary events.
The expectation of predicted CEs occurring by chance, \( \hat{N}_{\text{raw}}(t) \), represents the null hypothesis and is estimated by the average of all bootstrap samples. Because every bootstrap sample is generated by shuffling the original spike trains, the PSTHs remain unchanged. Trial shuffling destroys any precise spike timing correlation existing only in the simultaneously recorded trials. When the CE number in the sampling window is very small because of low firing rates, the significance test may be unstable (Roy et al. 2000). Therefore we imposed an additional condition for significance: the raw CE number should exceed the significance limit and also be greater than the absolute limit \( M/2 \). An example of the unitary event analysis is shown in Fig. 2.

**SELECTION OF OPTIMAL PARAMETERS FOR UES.** We are interested in any transient synchrony that may not be detected as significant in the temporally averaged CCH. For each unit pair, the significance of the CE is tested for every possible combination of the two parameters, bin width \( b \) for 1 to 6 ms and the delay \( d \) for 0 to \( \pm 10 \) ms in steps of \( b \). For each parameter combination, we compute the strength of synchrony, which we refer to as the significance index (SI). For this, we first compute the amount of significant synchrony by calculating the area of any contiguous portion of the raw CE number that exceeds the significance limit. If there is more than one noncontiguous epoch within the trial duration, we take the sum of all the areas. For example, the strength of synchrony represented by the red hatched area in Fig. 2 is the sum of three disjoint portions. We also compute the area below the predicted CE number (green hatched area). The SI is given by the ratio of these two sums. The normalization by the predicted CE number (green line) below the predicted CE number (green hatched area) below the predicted CE number (green line) \( b = 2 \) ms, \( T_w = 100 \) ms, \( d = -2 \) ms, SI = 0.17).

**TIME COURSE AND ONSET OF SYNCHRONY.** To characterize the time course of synchronous activity, we implemented a simple metric. For each cell pair having UEs, we define the strength of synchrony at each time step by the \( z \)-score, \( z(t) = \frac{[N_{\text{raw}}(t) - \hat{N}_{\text{raw}}(t)]/\sigma_{\text{raw}}(t)]}{\text{SD}} \), where \( N_{\text{raw}}(t) \) and \( \sigma_{\text{raw}}(t) \) are, respectively, the mean and the SD of the distribution of the chance CE number generated by the bootstrap samplings. The \( z \)-score is an appropriate measure, because the distribution of the bootstrap samples is Gaussian (Ito 2007). Because this measure of synchrony is independent of firing rates, we can isolate the time course of synchrony from that of the firing rates. For each unit pair, \( z(t) \) is normalized by its maximum value over the trial duration, and its temporal variation is represented using a color scale. For unit pairs having UEs during the \( on \) response, the time course of synchrony \( \{z(t)\} \) is aligned at the stimulus onset. In each case, the onset of significant synchrony is detected when \( z(t) \) first exceeds \( P = 0.01 \) after stimulus onset. The unit pairs are sorted in ascending order of synchrony onset. For unit pairs having UEs during the \( off \) response, \( z(t) \) is aligned to the stimulus offset.

**SIGNIFICANCE TEST OF NONSTATIONARY MODULATION.** For all the cell pairs having UEs, we test whether \( z(t) \) shows significant modulation within the response duration. The modulation in \( z(t) \) (red line in the middle plot in Fig. 6A) is tested for its nonstationarity in a specified test interval (from \( t = T_s \) to \( T_e \), between the 2 dashed lines). This interval is chosen to exclude rapid changes in spike density that occurs at the onset and offset of the stimuli. This selection is necessary because sharp changes in spike density can lead to inaccurate estimates of \( z(t) \) (see Supplementary Material for details). For the null hypothesis, we assume a statistical model in which the fluctuations of synchrony strength throughout the test interval follow a stationary distribution with the mean defined by \( \bar{z} = \frac{1}{T_e - T_s} \sum_{t=T_s}^{T_e} z(t) \) (see green line in Fig. 6A). As shown in Appendix A, \( \bar{z} \) is given by the average of the local variable \( \tilde{z}_i(t) = \frac{N_{\text{raw}}(t) - \hat{N}_{\text{raw}}(t)}{M} \) over all the sampling windows \( t \in [T_s,T_e] \) and all the trials \( i = 1, \ldots, M \). To estimate the breadth of this distribution, we introduce another bootstrap sampling.

1. Choose \( M \) different bins uniformly and randomly in the test interval. Let the \( i \)th sampled bin locate at \( t_i \in [T_s,T_e] \) and consider the sampling windows around those bins.
2. Sum up the local variables \( \tilde{z}_i(t) \) at the sampling windows to get one bootstrap sample, \( z_{\text{BS}} = \sum_{i=1}^{M} \tilde{z}_i(t) \).
3. Repeat steps 1 and 2 \( N \) times to get \( N \) samples of \( z_{\text{BS}} \). Perform uniform Monte Carlo samplings \( (N = 10,000) \) and apply the distribution of those samples to the stationary distribution as defined by the null hypothesis.
4. We ignore the temporal structure of \( \tilde{z}_i(t) \) in the test data and regard its variation \( \{ \tilde{z}_i(t), t \in [T_s,T_e] \} \) as a statistical fluctuation of a single quantity \( \tilde{z}_i \). A single sample is randomly selected from the distribution \( \{ \tilde{z}_i(t) \} \) for each trial \( i \) and, the sum of \( M \) samples is used to estimate the chance departure from the mean \( \bar{z} \).

If large or small values of \( \tilde{z}_i(t) \) consistently appear at a unique sampling window over many trials, either \( \tilde{z}_{\text{max}} \) or \( \tilde{z}_{\text{min}} \) is considered significantly different from the distribution specified by the null

\* The online version of this article contains supplemental data.

**FIG. 2.** Definition of the significance index (SI) that serves as a measure of the strength of synchrony between 2 spike trains. Top plots: the spike rasters for 2 units recorded simultaneously over 20 trials (unit 0, unit 1). The result of the UEA is plotted below. For the points in time where the raw CE number (red line) exceeds the bootstrap significance limit of \( P = 0.01 \) (black line), all the corresponding CEs are regarded as significant (unitary events) and highlighted by red circles in the raster plots. SI is given by the ratio of the area of significant synchrony (red hatched area) above the significance limit to the area of chance synchrony (green hatched area) below the predicted CE number (green line) \( b = 2 \) ms, \( T_w = 100 \) ms, \( d = -2 \) ms, SI = 0.17.)
TABLE 1. Incidence and properties of oscillatory firing among single units recorded in the LGN

<table>
<thead>
<tr>
<th>Animal</th>
<th>Single Units</th>
<th>Incidence, n (%)</th>
<th>Frequency, Hz</th>
<th>Peak Strength, med</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>7 (21%)</td>
<td>83.9 ± 30.2</td>
<td>1.41</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>34 (64%)</td>
<td>68.9 ± 12.8</td>
<td>2.19</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>25 (47%)</td>
<td>84.0 ± 28.0</td>
<td>1.83</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>8 (24%)</td>
<td>80.7 ± 10.8</td>
<td>2.95</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>30 (45%)</td>
<td>85.7 ± 19.0</td>
<td>2.2</td>
</tr>
<tr>
<td>6</td>
<td>59</td>
<td>12 (20%)</td>
<td>87.6 ± 23.2</td>
<td>3.3</td>
</tr>
<tr>
<td>7</td>
<td>80</td>
<td>6 (8%)</td>
<td>74.6 ± 20.8</td>
<td>1.05</td>
</tr>
<tr>
<td>Total</td>
<td>378</td>
<td>122 (32%)</td>
<td>79.9 ± 21.4</td>
<td>2.02</td>
</tr>
</tbody>
</table>

Values are mean ± SD. med, median.

hypothesis. We test for the presence of such a temporal inhomogeneity within the test interval.

5) The test data are judged to have significant nonstationary modulation if either \( z_{\text{max}} \) exceeds the 0.9975 quantity of the bootstrap samples or \( z_{\text{min}} \) is smaller than the 0.0025 quantity (\( P < 0.005, \) limits shown by black horizontal lines in Fig. 6A).

Because the number of trials (\( M \)) ranges from 10 to 30 and the test interval contains 500–1,000 bins, the sampled bins distribute sparsely. For this reason, we apply the above nonparametric significance test. The mean of the bootstrap samples estimates the mean \( \bar{z} \) fairly well.

RESULTS

Stimulus-evoked oscillatory activity

We sampled single unit activity (\( n = 378 \)) from the LGN of seven anesthetized cats (Table 1). In this analysis, we focus our attention on the activity evoked by stationary light spots. Because the stimulus itself does not introduce any temporal structure other than its duration, we are able to isolate the nonstationarity in the spike timing correlations that is intrinsic to the network. For our initial analysis, we examined the spiking pattern of each individual unit using the ACH and its power spectrum. The results of this analysis showed that 123 of 378 units (33%) showed significant oscillatory structure in their firing patterns. An example of this finding is shown in Fig. 3. The oscillatory activity was stimulus induced but not stimulus locked, as shown by the reduction of the periodic structure in the trial-shifted ACH (Fig. 3, E and F). Two cells showing rhythmic firing locked to the stimulus presentation were removed from the analysis. Of the 123 rhythmically firing cells, only 1 showed a significant oscillation during the period of spontaneous activity preceding the stimulus. Therefore 122 of 378 samples (32%) showed significant stimulus-induced oscillatory activity.

We also evaluated the properties of oscillatory firing in the LGN with respect to their receptive field properties (ON- or OFF-center, X or Y) and laminar position (lamina A, A1, or C). The results are summarized in Table 2. ON units showed a greater incidence of significant oscillation than OFF units (\( P < 0.0001, \) Mann Whitney \( U \) test), but the distributions of peak frequencies showed no significant difference between the two groups (\( P > 0.83, \) \( U \) test). ON units tended to show oscillatory activity with a larger peak strength than OFF units (\( P < 0.016, \) \( U \) test). We found no significant laminar dependence on the incidence or magnitude of significant oscillation. Finally, we...
found that the incidence of oscillation was greater in Y cells than X cells ($P < 0.0018$, $U$ test), but neither the distributions of peak frequency nor peak strength showed a significant difference between the two cell types.

To compare these findings from the LGN with our previously published calculations on unit activity in the visual cortex of anesthetized cats (Gray and Viana Di Prisco 1997), we re-evaluated the cortical data ($n = 148$) based on our current criteria (see METHODS). ACHs of oscillatory activity are shown in Fig. 4 for an LGN relay cell (A) and a cortical neuron (B). The distributions of oscillatory frequency and peak strength for all significant cells are shown in Fig. 4, C and E for the LGN and Fig. 4, D and F, for area 17. The incidence of oscillatory activity shows no significant difference between the LGN and area 17 (122/378, 32% for the LGN and 46/148, 31% for the cortex, $P > 0.79$, $U$ test; Table 2). However, as shown in the histograms, the frequency of the oscillatory activity in the LGN ($79.9 \pm 21.4$ Hz) is significantly greater than that found in the cortex ($43.1 \pm 11.9$ Hz; $P < 0.0001$, $U$ test) (Castelo-Branco et al. 1998; Ito et al. 1994). The distributions of peak strength in the two structures also indicates that the oscillatory activity in the LGN tends to be greater in magnitude (median, 2.02) than that occurring in the cortex (median, 1.49; $P < 0.0066$, $U$ test). Note that the oscillatory activities were evoked by different optimal stimuli in the two areas, that is, a stationary light spot for the LGN and a drifting light bar for the cortex. We found similar results, however, for the oscillatory activity in the LGN evoked by drifting bars (82.6 ± 21.2 Hz).

The mean frequency of oscillatory firing to spot stimuli (79.9 ± 21.4 Hz) was very close to the refresh rate of the video monitor (80 Hz). Several lines of evidence indicate that the oscillatory responses are not the result of phase locking to the monitor refresh. First, in all but two cases, the trial shuffled control reduced or eliminated the periodic structure of the ACH. Second, we ran a separate control by stimulating some oscillatory units using a DC light source (data not shown). In these cases, oscillatory activity occurred in the same frequency range. Third, the frequency of oscillation showed considerable variation across cells and animals (Fig. 4C; Table 1). Finally, Castelo-Branco et al. (1998) reported a mean oscillation frequency of 79 Hz using stationary spot stimuli with a video

### TABLE 2. Summary statistics of oscillatory activity

<table>
<thead>
<tr>
<th>Category</th>
<th>Single Units</th>
<th>Incidence, n (%)</th>
<th>Frequency, Hz</th>
<th>Peak Strength, med</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON</td>
<td>197</td>
<td>92 (47%)*</td>
<td>79.2 ± 17.5</td>
<td>2.32†</td>
</tr>
<tr>
<td>OFF</td>
<td>155</td>
<td>28 (18%)*</td>
<td>82.7 ± 31.7</td>
<td>1.61†</td>
</tr>
<tr>
<td>A</td>
<td>173</td>
<td>51 (29%)</td>
<td>81.3 ± 20.5</td>
<td>1.79</td>
</tr>
<tr>
<td>A1</td>
<td>109</td>
<td>37 (34%)</td>
<td>75.0 ± 23.1</td>
<td>1.85</td>
</tr>
<tr>
<td>C</td>
<td>62</td>
<td>22 (35%)</td>
<td>85.1 ± 21.9</td>
<td>2.09</td>
</tr>
<tr>
<td>X</td>
<td>79</td>
<td>22 (28%)†</td>
<td>83.0 ± 26.2</td>
<td>2.00</td>
</tr>
<tr>
<td>Y</td>
<td>72</td>
<td>38 (53%)‡</td>
<td>82.3 ± 20.8</td>
<td>2.26</td>
</tr>
<tr>
<td>LGN</td>
<td>378</td>
<td>122 (32%)‡</td>
<td>79.9 ± 21.4*</td>
<td>2.02†</td>
</tr>
<tr>
<td>Area 17</td>
<td>148</td>
<td>46 (31%)</td>
<td>43.1 ± 11.9*</td>
<td>1.49†</td>
</tr>
</tbody>
</table>

Values are means ± SD. *$P < 0.0001$; †$P < 0.02$; ‡$P < 0.002$.

![Image](https://example.com/image.png)

**FIG. 4.** Example autocorrelation histograms of significantly oscillatory units in the LGN (A) and striate cortex (B). In these examples, which are representative of the population sample, oscillatory activity in the LGN has a higher frequency (82 Hz) and a larger peak strength (13.8) than comparable oscillatory activity in the cortex (35 Hz and 6.6, respectively). Distributions of peak frequencies (C: LGN; D: cortex) and peak strengths (E: LGN; F: cortex) computed from the power spectra of the autocorrelation histograms over all significantly oscillatory units in the 2 structures. The oscillatory activity in the LGN occurs in a significantly higher frequency range (mean frequency: 79.9 ± 21.4 Hz) than that in striate cortex (mean frequency: 43.1 ± 11.9 Hz). The peak strength is normalized by the significance limit estimated by the Monte Carlo analysis.
monitor having a 100-Hz refresh rate. Agreement among these measurements further supports the conclusion that evoked high gamma oscillations in the LGN are induced rather than phase locked to the stimulus.

**Correlated spike timing**

Using the significance test for the CCH, we examined the incidence and properties of correlated spike timing for all single unit pairs \( (n = 405) \). A substantial fraction of the sample consisted of cell pairs in which one cell responded to the onset (on-cell) and the other to the offset (off-cell) of the stimulus. This resulted in little or no temporal overlap of their activity. Consequently, none of these data \( (n = 148) \) satisfied the criteria for significant correlation and were excluded from further analysis. Furthermore, pairs of cells that were driven by input from different eyes \( (n = 31) \), and those including cells at inter-lamina regions (lamina undefined, \( n = 31 \)) were excluded from the remaining sample. As a result, our sample for correlation analysis \( (n = 195) \) was limited to cell pairs driven by the same eye and having the same sign of response (on or off). We also divided our analysis into pairs of cells recorded on the same tetrode (intratetrode pairs, \( n = 121 \)) or from different tetrodes separated by 0.5 mm (intertetrode pairs, \( n = 74 \)).

From this sample, 59 of the 195 cell pairs (30%) showed significant spike timing correlations. Among these, cell pairs recorded on the same tetrode showed a higher incidence of correlated firing \( (47/121, 39\%) \) than those recorded on different tetrodes \( (12/74, 16\%; P < 0.0009, U \) test). However, for cell pairs showing significant synchrony, the strength of the correlations, as measured by the significance ratio, showed no difference between intra- (median, 2.7) and intertetrode (median, 3.4) pairs \( (P > 0.69, U \) test). We tested for the occurrence of oscillatory synchronization in the CCH and found that 36 of the 59 cell pairs \( (61\%) \) showed a significant spectral peak in the high gamma frequency band \( (26 \text{ intratetrode and 10 intertetrode pairs}) \). Other significant CCHs were categorized as having a single peak near the center \( (12 \text{ intratetrode and 1 intertetrode pair}) \) and weak spike correlation without any distinguished peak \( (9 \text{ intratetrode and 1 intertetrode pairs}) \). We also compared the properties of spike timing correlations over the different layers in the LGN. For both intratetrode and intertetrode unit pairs, neither the incidence nor the strength of synchronous firing had significant layer dependencies (see Appendix B and Table 5 for more in detail).

We also found that spike timing correlations were closely associated with the occurrence of oscillatory activity. When both cells of a pair showed significant oscillations, the incidence of significant correlation \( (30/41, 73\%) \) was far greater than that occurring for all other combinations of oscillatory and nonoscillatory activity \( (29/154, 19\%; P < 0.0001, U \) test). Interestingly, for those pairs in which both cells showed oscillatory responses, we found no difference in the incidence of significant correlations between intertetrode pairs \( (9/14, 64\%) \) and intratetrode pairs \( (21/27, 78\%; P > 0.48, U \) test).

An example of the time dependence of oscillatory synchronization is shown in Fig. 5. A pair of cells, recorded on different tetrodes, were stimulated together by two separate light spots that spanned the center and surround regions of the receptive field of each cell (Fig. 5A). One cell was recorded in the A lamina (unit 0) and the second in the C lamina of the LGN \( (unit 2) \). The stimulus evoked vigorous on and off responses in both units, as shown by the PSTHs in Fig. 5B. Conventional auto- and cross-correlation analysis showed pronounced oscillatory firing at a frequency of 96 Hz during the on response \( (C: \text{unit 0}, E: \text{unit 2}) \) that was tightly synchronized with an average phase difference of 1 ms, indicating that \( unit 2 \) tended to fire 1 ms before that of \( unit 0 \) (Fig. 5G). We applied a sliding window correlation analysis (Castelo-Branco et al. 1998; Neuenschwander and Singer 1996) to examine the time course of the oscillatory firing and its synchronization between the two cells. These results, shown in Fig. 5, \( D, F, \) and \( H \), show that the rhythmic synchronization is nonstationary during the response to the stimulus. The synchronized oscillatory firing...
appeared at short latency following the onset of the stimulus, but lasted only for \(~400\) ms and then suddenly decreased in magnitude. Interestingly, this change in synchronization was not associated with an abrupt change in the firing rates of the two units (Fig. 5B).

**UEA**

Although the sliding window correlation analysis enables the visualization of the time course of synchrony, this method has limitations for the quantification of correlation strength (Aertsen et al. 1989; Ito and Tsuji 2000). For example, in the plots of Fig. 5, D, F, and H, there is some elevated correlation structure before the stimulus onset and in response to the stimulus offset. Such correlations at low firing rates are overestimated because of the normalization by spike count. Therefore to more accurately characterize the time-dependent changes in spike timing correlations with respect to changes in firing rate, we applied UEA to our data. An example of the results, for the same pair of cells as shown in Fig. 5, is shown in Fig. 6A. The UEA confirmed that the synchronous firing was nonstationary, occurred primarily during the central portion of the ON response, and was entirely nonsignificant during the off response. The red line in the bottom traces of Fig. 6A shows that the raw count of the CE \((b = 1\) ms and \(d = -1\) ms) exceeds the significance limit \((P < 0.01, \text{ black line})\). Moreover, the predicted CE number (green line), obtained by the average of 1,000 bootstrap samples, is in good agreement with the expected number of chance CEs estimated by the firing rates (Ito 2007). This confirms that the occurrence of the CEs across trials was only loosely time locked to the stimulus onset. We also confirmed that nonstationary synchrony was not a statistical fluctuation, because it tended to occur over a consistent interval across repeated trials. Figure 6B shows the UEA applied to the same data after random shuffling of the trials. The spike synchrony is reduced to the chance level by the shuffling procedure and no UEs are detected.

We applied the same analysis to the entire data set and found that 96 of 195 cell pairs (49%) showed statistically significant UEs. In contrast to the conventional cross-correlation analysis, the incidence of UEs did not differ between cells recorded on the same (57/121, 47%) or different tetrodes (39/74, 53%; \(P > 0.45, U\) test). However, the strength of the synchrony, as measured by the SI, was greater for the intratetrode pairs (median, 0.086) compared with the intertetrode pairs (median, 0.038; \(P < 0.0003, U\) test). A summary of the results from the two significance tests is given in Table 3. Note that a number of nonsignificant samples in the CCH test become significant in the UEA test, particularly for the intertetrode cell pairs. Although the CCH and the UEA provide largely consistent results, the UEA is more sensitive to weak synchrony. The results also show that the synchronous firing detected by the UEA tends to occur transiently, as shown by the example in

![FIG. 6. UEA of the same intertetrode unit pair as shown in Fig. 5. A: UEA based on the bootstrap significance test. Top plots: spike rasters of the 2 units (20 trials). Bottom plots: the raw CE number (red), predicted CE number (green), and the 99% bootstrap significance limit (black) (bin width \(b = 1\) ms, delay \(d = -1\) ms). The horizontal bar below the plots represents the period of stimulus presentation. The horizontal bar on the right shows the size of the sampling window (100 ms). The scale bar along the ordinate represents 50 counts. When the number of CEs exceeds the significance limit, all the CEs within the sampling window are assigned as unitary events and highlighted by red circles in the raster plots. Middle plots: the significance test of nonstationary modulation of spike synchrony. A red line represents the normalized excess synchrony \(z(t)\). The significance of nonstationary modulation is tested in the interval between 2 vertical dashed lines. A green horizontal line and 2 black circles on the red line represent, respectively, the mean, the maximum \((z_{\text{max}})\) and the minimum \((z_{\text{min}})\) of \(z(t)\) in the test interval. The modulation in \(z(t)\) is judged as significantly nonstationary when \(z_{\text{max}}\) exceeds the upper significance limit (upper black line) or \(z_{\text{min}}\) goes below the lower significance limit (lower black line). The scale bar along the ordinate represents 5 (unit 0: A-lamina, Y-type; unit 2: C-lamina, Y-type, \(M = 20\) trials, SI = 0.17, \(z_{\text{max}} = 7.2, z_{\text{min}} = -1.5\), upper significance limit = 6.5, lower significance limit = -0.02). B: UEA applied to the same data after a random shuffling of the trials. Shuffling eliminates the fine temporal synchrony and reduces the raw CE number close to the predicted CE number. The normalized excess synchrony \(z(t)\) shows a small fluctuation around 0 and does not exceed the significance limits (SI = 0, \(z_{\text{max}} = 1.3, z_{\text{min}} = -1.5\), lower significance limit = -2.5).](J Neurophysiol • VOL 104 • DECEMBER 2010 • www.jn.org)
This transient synchrony can be detected during intervals as brief as 200 ms but tends to be averaged out in the CCH.

On the other hand, we found counter examples in which significant correlations detected in the CCH could not be confirmed by the UEA. Unit pairs showing this effect tend to have low firing rates and weak sustained spike correlations as shown in Fig. 7, C and D. Although the cumulative correlated spike events in the CCH showed a statistically significant peak, the number of CEs in a short sampling window was small, showing evident discreteness. Although the UEA was sensitive enough to detect the raw CEs exceeding the significance limit in Fig. 7D, the significance limit itself was unreliable because of the discreteness (Roy et al. 2000). For a conservative significance judgment, we introduced the absolute limit $M/2$ (see METHODS). Significance was not confirmed in this sample because the raw CE number did not exceed $M/2$. Different characteristics of the two methods are summarized in DISCUSSION and the estimation of the minimum firing rate needed for the UEA.

Finally, the incidence and properties of synchronous activity across animals, as measured by the CCH and the UEA, are summarized in Table 4. Although the incidence of the UEs had no significant layer dependencies for intratetrode unit pairs, the synchrony strength, as measure by the SI, was significantly larger in the C layer than the A layer (see Appendix B and Table 5).

**TABLE 3.** Comparison of synchronous activities revealed by CCH and UEA

<table>
<thead>
<tr>
<th></th>
<th>Significant CCH</th>
<th>Non-Significant CCH</th>
<th>Total UEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intratetrode pairs (121)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant UEA</td>
<td>37</td>
<td>20</td>
<td>57</td>
</tr>
<tr>
<td>Non-Significant UEA</td>
<td>10</td>
<td>54</td>
<td>64</td>
</tr>
<tr>
<td>Total CCH</td>
<td>47</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Inter-tetrode pairs (74)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant UEA</td>
<td>8</td>
<td>31</td>
<td>39</td>
</tr>
<tr>
<td>Non-Significant UEA</td>
<td>4</td>
<td>31</td>
<td>35</td>
</tr>
<tr>
<td>Total CCH</td>
<td>12</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>

CCH, cross-correlation histogram; UEA, unitary events analysis.

**TABLE 4.** Summary statistics of synchronous activity: Animal dependencies

<table>
<thead>
<tr>
<th>Animal</th>
<th>Unit Pairs</th>
<th>Sig. CCH</th>
<th>SR, med</th>
<th>Sig. UEA</th>
<th>SI, med</th>
<th>Sig. modulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>3 (15%)</td>
<td>1.25</td>
<td>9 (45%)</td>
<td>0.022</td>
<td>3/9 (33%)</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>27 (49%)</td>
<td>2.71</td>
<td>26 (47%)</td>
<td>0.082</td>
<td>14/26 (54%)</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>1 (20%)</td>
<td>6.59</td>
<td>1 (20%)</td>
<td>0.122</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>8 (42%)</td>
<td>3.78</td>
<td>10 (53%)</td>
<td>0.327</td>
<td>2/10 (20%)</td>
</tr>
<tr>
<td>6</td>
<td>34</td>
<td>10 (29%)</td>
<td>1.85</td>
<td>21 (62%)</td>
<td>0.037</td>
<td>12/21 (57%)</td>
</tr>
<tr>
<td>7</td>
<td>62</td>
<td>10 (16%)</td>
<td>2.47</td>
<td>29 (47%)</td>
<td>0.062</td>
<td>15/29 (52%)</td>
</tr>
<tr>
<td>Total</td>
<td>195</td>
<td>59 (30%)</td>
<td>2.66</td>
<td>96 (49%)</td>
<td>0.064</td>
<td>46/96 (48%)</td>
</tr>
</tbody>
</table>

No unit pair sample in the recordings from animal 4. SR, significance ratio; SI, significance index.

---

**FIG. 7.** Two examples of unit pairs that show different results between the cross-correlation histogram (CCH) and UEA analyses. In both examples (A and B and C and D), the plotting conventions are similar to those in Fig. 6. In A, the peak in the cross-correlation histogram is not significant as judged by the significance ratio. In B, however, the UEA shows significant synchronizaion for a brief period near the center of the response (bin width, $b = 2$ ms; delay, $d = -b$ ms) (unit 0: A-lamina, unit 4: A-lamina, intertetrode pair, $M = 20$ trials, $SI = 0.045$, $z_{\text{max}} = 5.2$, $z_{\text{min}} = -1.9$, upper significance limit = 4.0, lower significance limit = -2.4). C and D show another example cell pair in which the CCH analysis (C) shows a significant peak, but the UEA (D) cannot confirm significant synchronous firing, attributed to the low spike counts in these data (bin width, $b = 3$ ms; delay, $d = 3b$). Because the 2nd unit pair does not have any UE, the plots of modulation [$z(t)$] are not shown (unit 2: A1-lamina, unit 5: A1-lamina, intertetrode pair, $M = 20$ trials, $SI = 0.0$).
DYNAMICS OF SYNCHRONY IN LGN

TABLE 5. Summary statistics of synchronous activity: Layer dependences

<table>
<thead>
<tr>
<th>Layers</th>
<th>Unit Pairs</th>
<th>Sig, CCH</th>
<th>SR, med</th>
<th>Sig, UEA</th>
<th>SI, med</th>
<th>Sig, modulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intratetrode pairs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-A</td>
<td>63</td>
<td>20 (32%)</td>
<td>2.48</td>
<td>28 (44%)</td>
<td>0.068*</td>
<td>9/28 (32%)</td>
</tr>
<tr>
<td>A1-A1</td>
<td>31</td>
<td>14 (45%)</td>
<td>2.78</td>
<td>15 (48%)</td>
<td>0.086</td>
<td>6/15 (40%)</td>
</tr>
<tr>
<td>C-C</td>
<td>24</td>
<td>12 (50%)</td>
<td>3.72</td>
<td>13 (54%)</td>
<td>0.178*</td>
<td>8/13 (62%)</td>
</tr>
<tr>
<td>Total</td>
<td>118</td>
<td>46 (39%)</td>
<td>2.57</td>
<td>56 (47%)</td>
<td>0.086</td>
<td>23/56 (41%)</td>
</tr>
<tr>
<td>Intertetrode pairs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-A</td>
<td>41</td>
<td>3 (7%)</td>
<td>10.74</td>
<td>27 (66%)</td>
<td>0.038</td>
<td>16/27 (59%)</td>
</tr>
<tr>
<td>A-C</td>
<td>12</td>
<td>2 (17%)</td>
<td>10.10</td>
<td>4 (33%)</td>
<td>0.085</td>
<td>3/4 (75%)</td>
</tr>
<tr>
<td>A1-A1</td>
<td>9</td>
<td>5 (56%)</td>
<td>2.63</td>
<td>4 (44%)</td>
<td>0.085</td>
<td>2/4 (50%)</td>
</tr>
<tr>
<td>C-C</td>
<td>11</td>
<td>2 (18%)</td>
<td>1.80</td>
<td>4 (36%)</td>
<td>0.069</td>
<td>2/4 (50%)</td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>12 (16%)</td>
<td>3.44</td>
<td>39 (53%)</td>
<td>0.038</td>
<td>23/39 (59%)</td>
</tr>
</tbody>
</table>

Cell pairs in which the laminar positions are not identified are excluded (3 intratetrode and 1 intertetrode pairs). *P < 0.02. SR, significance ratio; SI, significance index.

Precision of synchrony

To evaluate the temporal precision of the synchronous firing between cell pairs, we repeated the UEA for all possible combinations of bin width (b, 1–6 ms), and delay (d, ±10 ms) in steps of b. The distribution of the optimal bin width over all the significant samples (n = 96) indicates that 65% of the synchrony was optimally characterized by a bin width of 1–3 ms. Fifty-four of 96 cell pairs had relatively low firing rates in which the raw CE count did not exceed the absolute limit M/2 when using the smallest bin width of 1 ms. For those samples, the optimal bin width could not be set at 1 ms simply because of low firing rates. Thus to further examine the precision of synchrony, we excluded those samples from further analysis. For the remaining sample (n = 42), we computed the normalized SI (NSI) as a function of bin width (Fig. 8A: all samples; Fig. 8B: mean). The histogram of the optimal bin sizes (inset in Fig. 8B) shows that the spike timing correlations are very precise, with 69% of the 42 cell pairs being optimally characterized by the smallest bin size of 1 ms. The decay in synchrony strength at larger bin widths validates limiting the bin width ≤6 ms in the current UEA. Comparison of the distributions of optimal bin width showed no significant difference between intratetrode and intertetrode pairs (P > 0.39, U test; data not shown). These findings indicate that spike timing correlations over distances of 0.5 mm are not necessarily less precise than those occurring between cells recorded on the same tetrode.

Retino-geniculate synchrony

In a number of our recordings, we detected the activity of a retinal afferent in the form of an S-potential (Kaplan and Shapley 1984). In these recordings, spikes from a relay cell were consistently preceded by an S-potential (Fig. 9A). The resulting CCH has a sharp peak at a short delay (Fig. 9B). The UEA of the corresponding CEs (Fig. 9C; b = 1 ms, d = −1 ms) shows that the modulation profile of the raw CE number faithfully followed the change of the firing rates (the predicted CE number). We found 19 such retino-geniculate cell pairs, recorded on the same tetrode, where the S-potential preceded the relay cell spikes by 1–2 ms and the CCH showed a sharp peak of 1-ms width.

Time course and onset of synchrony

Using UEA, we were able to characterize the time-dependent variations of synchronous firing over all the cell pairs having UEs. For each unit pair, the strength of synchrony z(t) was normalized by its maximum value during the trial duration, and variations in correlation strength were represented using a color scale (Fig. 10, A and B). Because the influence of firing rate modulation was isolated by the transformation to a z-score, the plots show the modulation profile of synchrony that is independent of the firing rates of the cells. For samples

FIG. 8. The magnitude of significant spike synchronization varies with the bin size (b) used in the UEA. A: for each significant pair of cells having sufficient spike counts (n = 42), the SI is plotted as a function of bin width (1–6 ms). Each characteristic curve is normalized by the maximum SI [normalized SI (NSI)]. B: the mean and SD of the plots in A indicate that synchronous activity is optimally characterized at the highest level of resolution (1 ms). The same characteristic is confirmed by the histogram of the optimal bin sizes (B, inset).
having UEs during the ON response, the plots of $z(t)$ were aligned to the stimulus onset (red arrow in Fig. 10A). In each trace, the onset of synchrony [i.e., the first epoch when $z(t)$ exceeded $P = 0.01$] is represented by a black vertical line and the stimulus offset by a blue line. The data were sorted in ascending order of the onset time in each of three groups: retino-geniculate pairs, intratetrode pairs, and intertetrode pairs. For samples having UEs during the OFF response, the plots of $z(t)$ were aligned at the stimulus offset (red arrow in Fig. 10B), and the stimulus onset was represented by a blue line. For both ON and OFF responses, retino-geniculate synchrony had a shorter onset and a stable appearance represented by a long band of elevated values (Fig. 9C). On the other hand, intertetrode synchrony tended to have a later onset and a more transient appearance as represented by a short duration of elevated values. Note that, following the stimulus onset, the

![Figure 9](https://www.jn.org/)

**Figure 9.** Stationary synchronization between a retinal afferent and a relay cell. A: raw data trace of the recorded signal shows that the relay cell (large spikes, unit 2) tended to fire $-1$ ms after the retinal afferent potential (S-potentials, indicated by red arrows, unit 4). B: CCH (bin width 1 ms) has a sharp peak at a delay of $-1$ ms. C: UEA of the same spike trains ($b = 1$ ms, $d = -1$ ms) shows a highly significant, but largely stationary, elevation of synchronous firing (bottom plots). The modulation profile of the raw CE number (red line) tends to follow the modulation of the predicted CE number (green line). The normalized excess synchrony $z(t)$ is stationary and does not exceed the significance limits (unit 2: a relay cell, Y-type, Al-lamina; unit 4: S-potential, Al-lamina, $M = 10$ trials, $S_I = 1.58$, $z_{min} = 10.8$, $z_{max} = 5.0$, upper significance limit = 11.0, lower significance limit = 3.9).

![Figure 10](https://www.jn.org/)

**Figure 10.** Population statistics on the modulation profiles of synchrony. For each unit pair having UEs, the strength of synchrony $z(t)$ at each sampling window was normalized by its maximum value during trial duration, and its time course was represented using a color scale. A: for samples having UEs during the ON response, the traces were aligned at the stimulus onset (red arrow). In each trace, the onset time of synchrony is represented by a black vertical line. The stimulus offset is shown by a blue line. The samples were sorted in ascending order of the onset time in each of 3 groups: retino-geniculate pairs ($n = 13$), intratetrode pairs ($n = 39$), and intertetrode pairs ($n = 32$). B: for samples having UEs during the OFF response, the traces were aligned at the stimulus offset (red arrow), and the stimulus onset was represented by a blue line: retino-geniculate pairs ($n = 6$), intratetrode pairs ($n = 18$), and intertetrode pairs ($n = 7$). The example unit pairs shown in Figs. 9, 11, 6A, and 7B are indicated by black, cyan, magenta, and orange arrows, respectively, at the left margin.
increase of the firing rates occurred with a short latency as seen in the raster plots of the examples in Figs. 6A and 7B. Intertetrode pairs showed intermediate characteristics.

For both on and off responses, the distributions of the onset times are significantly different among the three groups (ON response: \( P < 0.0001 \); OFF response: \( P < 0.006 \), Kruskal-Wallis test). For on responses, the mean onset time was 70 ± 60 ms in retino-geniculate pairs \((n = 13)\), 220 ± 170 ms in intratetrode pairs \((n = 39)\), and 300 ± 160 ms in intertetrode pairs \((n = 32)\). For off responses, the mean onset time was 130 ± 40 ms in retino-geniculate pairs \((n = 6)\), 230 ± 100 ms in intratetrode pairs \((n = 18)\), and 350 ± 170 ms in intertetrode pairs \((n = 7)\). Because the temporal modulation of synchronous activity is smoothed, because of the low-pass filtering by a 100-ms sampling window, the onset time was likely to be overestimated. Despite this, 16 unit pairs had onset times exceeding 400 ms. Because those pairs were recorded from 10 sites across four animals, these long onset times are not likely to be caused by a sampling bias. We did not observe significant correlation between the onset time and the strength of synchronous activity is smoothed, because of the low-pass filtering by a 100-ms sampling window, the onset time was likely to be overestimated. Despite this, 16 unit pairs had onset times exceeding 400 ms. Because those pairs were recorded from 10 sites across four animals, these long onset times are not likely to be caused by a sampling bias. We did not observe significant correlation between the onset time and the strength of synchrony \((SI, r^2 = 0.04)\), intratetrode pairs \((r^2 = 0.04)\), or intertetrode pairs \((r^2 = 0.06)\). Moreover, although intertetrode pairs have significantly longer onset times than intratetrode pairs at the population level, none of the measures of synchrony strength \((SI, r^2 = 0.16; modulation amplitude, r^2 = 0.21; onset time, r^2 = 0.0)\) of the intertetrode pairs \((n = 39)\) showed a dependence on the separation of their receptive fields.

Nonstationary modulation of synchrony

As discussed above, a substantial number of LGN unit pairs displayed a transient increase of the CE number, and its modulation profile could not be predicted by the changes in firing rates over time (Figs. 6A and 7B). To evaluate the significance of this temporal modulation, we used another bootstrap procedure (see METHODS). The middle plot in Fig. 6A (red line) shows \(zt\) of the corresponding CEs. In this example, the modulation profile in the CE number was fully predicted by the change of firing rates during the OFF response. Therefore \(zt\) remained stationary around the zero value. Testing for nonstationary synchrony during the ON response, we located a test interval between the two vertical dashed lines and tested for the significant departure of \(zt\) with respect to the null hypothesis of stationarity. As explained in METHODS, the distribution of 10,000 bootstrap samples was well fit by a Gaussian distribution (mean, 3.13; skewness, 0.06) and reproduced the mean of \(zt\) in the test interval (3.15, green horizontal line). The two black horizontal lines represent the nonparametric significance limits \((P < 0.005)\) derived from the bootstrap samples. Because both the maximum \((z_{\text{max}})\) and the minimum \((z_{\text{min}})\) of \(zt\) (black circles) lie outside the significance limit, the temporal modulation of \(zt\) is judged to be significantly nonstationary. In contrast, the modulation profile of \(zt\) in the trial shuffled data (Fig. 6B) is not significant. Note also that the shuffled data yields a narrower range of the significance limits.

Nonstationary modulation of synchrony was not a general property of all cell pairs, however. In another example (Fig. 11), both single units from an intertetrode pair showed oscillatory activity at 96 Hz with strong synchronization at a delay of 0 ms (Fig. 11A). The UEA of the corresponding CEs \((b = 1 \text{ ms}, d = 0 \text{ ms})\) confirmed precise synchronization but showed no evidence of significant temporal modulation of synchrony during the test interval (Fig. 11B). The time course of this example pair is indicated by a cyan arrow at the left margin in Fig. 10A, as well as other example pairs (pair in Fig. 9: black arrow, Fig. 6A: magenta, Fig. 7B: orange).

We applied the same analysis to all the single unit pairs displaying UEs \((n = 96)\) and found that approximately one half \((46/96, 48\%)\) showed a significant modulation of \(zt\) \((P < 0.005)\). Thirty-four of the cell pairs showed a \(z_{\text{max}}\) exceeded the upper significance limit, 26 cell pairs had a \(z_{\text{min}}\) that fell below the lower limit, and 14 cell pairs had \(z\) values that exceeded both limits. Modulation exceeding the upper significance limit occurred more often among intertetrode cell pairs \((19/39, 48\%)\) than intratetrode cell pairs \((15/57, 26\%; P < 0.025, U \text{ test})\). This is consistent with the previous finding that intertetrode cell pairs tend to have longer onset times and more transient episodes of synchrony than intratetrode pairs. There

![FIG. 11. An example case of stationary synchrony. Both single units of an intertetrode pair showed vigorous oscillatory firing at 96 Hz. The oscillatory activities were tightly synchronized with a delay of 0 ms as shown in the CCH (A). B: UEA of the corresponding CE \((b = 1 \text{ ms}, d = 0 \text{ ms})\) showed a large departure of the raw CE number from the predicted CE number (bottom plots). The normalized excess synchrony \(zt\) (middle plots) was less nonstationary and did not exceed the significance limits (unit 0: A-lamina, unit 2: A-lamina, \(M = 30 \text{ trials}, SI = 2.57; z_{\text{max}} = 15.0, z_{\text{min}} = 8.9, \text{ upper significance limit} = 16.5, \text{ lower significance limit} = 8.4)\).](image)
was no significant difference in the incidence of modulation falling below the lower significance limit between intertetrode (12/39, 31%) and intratetrode (14/57, 25%) cell pairs ($P > 0.50, U$ test). After combining the two types of significant modulation, we found no significant difference between the two groups (intertetrode pairs: 23/39, 59%; intratetrode pairs: 23/57, 40%; $P < 0.074, U$ test). The occurrence of nonstationary synchrony across animals is summarized in Table 4.

We also compared the properties of the modulation of synchrony between the retino-geniculate cell pairs and those measured between LGN cells. The results showed that the retino-geniculate cell pairs have a lower occurrence of nonstationary synchrony (5/19, 26%) than that seen in the LGN relay cell pairs (46/96, 48%). Although this difference did not reach statistical significance ($P < 0.08, U$ test), analysis of the modulation amplitudes, $z_{\text{max-norm}}$ (see METHODS), showed that the retino-geniculate synchrony displayed a significantly smaller magnitude of modulation than that seen in the LGN relay cell pairs ($P < 0.007, U$ test) (Supplementary Materials, Fig. S1). This relatively stationary synchrony seen in the retino-geniculate cell pairs is consistent with a strong anatomical connection between retinal afferents and LGN relay cells. Although it is possible that the S-potential activity may have been included in the spike sorting of the LGN cells, the waveforms showing the characteristics of S-potentials were generally much smaller in amplitude and were thus likely to have been categorized as multiunit activities in our initial sorting procedure and therefore not included in the sample of single units.

**Discussion**

We studied the properties of rhythmic and synchronous spike activities in the LGN of anesthetized cats using both conventional auto- and cross-correlation analysis and UEA. The autocorrelation analysis showed that 32% of the single units showed stimulus evoked oscillatory activity at a significantly higher frequency range ($79.9 \pm 21.4$ Hz) than that observed in area 17 of the visual cortex. The cross-correlation analysis showed that 30% of the single unit pairs displayed significant synchronization and 61% of these pairs consisted of phase synchronized oscillatory spike trains. Using a modified version of UEA, we found that 49% of the single unit pairs showed significant synchronous activity (UEs). This analysis detected weak and transient synchronous activity that often failed to become significant in the conventional cross-correlation analysis. In many unit pairs, the UEs were optimally characterized at a bin width of 1 ms, indicating that synchronous activity has a high degree of temporal precision. We introduced a significance test of nonstationary modulation of the synchrony based on a bootstrap method. This analysis showed that, in response to stationary light spots, nearly one half of the unit pairs having UEs showed nonstationary modulations at time scales that cannot be predicted by the modulation of firing rates. Population statistics showed that the onset of synchrony between LGN cells occurred later and showed a greater degree of nonstationarity than that observed among retino-geniculate cell pairs. Furthermore, the synchrony between LGN unit pairs recorded on separate tetrodes had a later onset and more transient appearance than that occurring between adjacent unit pairs. These findings confirm and extend previous studies on synchronous oscillatory activity and show that spike timing correlations in the LGN are stimulus dependent, very precise, and often highly nonstationary.

**Comparison between cross-correlation and UEA**

When two units have sufficient firing rates and stationary spike timing correlations, both the CCH and the UEA work adequately and provide consistent results in the detection of significant synchrony. The results from the two methods diverge, however, when firing rates decrease and the correlations become transient and nonstationary. Cross-correlation analysis is capable of detecting correlations among units with lower firing rates than the UEA because it uses activity over the entire trial duration. However, this strength becomes a weakness when strong nonstationarities exist in either the cellular firing rates or spike timing correlations. This problem is overcome by the brief sampling window of the UEA, which enables the quantification of the temporal modulation of spike timing correlations. In turn, the UEA depends on a sufficient number of spikes in the sampling window for a reasonable statistical estimation (Roy et al. 2000). Therefore in our formulation, we imposed an additional condition for significance: the raw CE number should exceed the significance limit and also be greater than the absolute limit of $M/2$. This led us to exclude certain unit pairs with low firing rates that would otherwise have been included (e.g., Fig. 7, C and D). In such cases of low firing rates and weak synchrony, detection of UEs would require an increase in the bin size at the cost of synchrony precision. We estimated the minimum firing rate necessary for exceeding the absolute limit of $M/2$ for different bin widths $b$, and these results are presented in Appendix C.

**Properties and origins of nonstationary synchrony in the LGN**

The time dependence of oscillatory responses and spike timing correlations in the LGN have been documented previously using sliding window correlation analysis (Castelo-Branco et al. 1998; Neuenschwander et al. 2002). These studies showed that responses in the LGN to stationary stimuli display transient, synchronous oscillations that decay in amplitude and frequency over time. This study differs from this work in several respects. First, as we pointed out, the sliding window correlation analysis has difficulties in isolating the temporal modulation of spike synchrony with respect to changes in firing rates (Ito and Tsuji 2000; Nakahara and Amari 2002). UEA is well suited to this type of problem because it enables the objective selection of parameters (i.e., bin width and delay) and provides a rigorous estimate of statistical significance based on a nonparametric bootstrap test. Second, these studies were focused on the properties of thalamo-cortical synchrony and did not apply a systematic analysis of the temporal modulation of intra-thalamic synchrony. Third, our study was based entirely on the activity of pairs of well-isolated single units, whereas the earlier analysis was based largely on multiunit recordings. Because multiunit recordings pool the activities of small groups of neurons, often having heterogeneous characteristics, the temporal characteristics of each single unit cannot be adequately studied.

Our cumulative statistics based on the cross-correlation analysis showed that 61% of the significant synchrony oc-
curred through phase synchronization of oscillatory firing. This suggests a prominent role for oscillations in the generation and maintenance of synchronization. Interestingly, the nonstationary change in synchrony seen in the unit pair in Fig. 5 occurred when the firing patterns shifted from oscillatory to nonoscillatory. This result suggests an interdependence between oscillatory firing and spike timing correlations, as has been reported previously (Maldonado et al. 2000). A related result has been described in cat visual cortex (Samonds and Bonds 2005). These authors found that synchronous activity evoked by drifting gratings was maintained throughout the stimulus period if oscillatory activity was present, whereas the synchronization decayed significantly in the absence of oscillations. On the other hand, time-frequency coherence analysis has shown that cortical synchronous activity can be triggered instantaneously by the stimulus, whereas the gamma oscillations develop more slowly (Zhou et al. 2008). This indicates that the oscillatory activity is not needed for the generation of synchrony but may serve to maintain it over time.

Spike timing correlations in the early visual pathway have often been studied in relation to the underlying anatomical connections (Alonso et al. 1996, 2009; Toyama et al. 1981; Usrey 2002; Usrey and Reid 1999; Usrey et al. 2000; Yeh et al. 2003, 2009). Both anatomical (Hamos et al. 1987) and physiological evidence (Yeh et al. 2009) has shown that retinal afferents make powerful connections with multiple LGN cells within the same layer and across layers. Cells sharing retinal monosynaptic input have overlapping receptive fields (Yeh et al. 2009), and the CCH of such unit pairs has a sharp isolated peak of <1-ms width around 0-ms delay corresponding to the difference of conduction times of the common retinal input to the two cells (Alonso et al. 1996; Usrey and Reid 1999). We conclude that most of the precise spike timing correlations in our study did not originate from common retinal inputs. There are multiple observations supporting this conclusion. First, in our sample, there were only two intratetrode pairs having an isolated single bin (1 ms width) peak within ±1-ms delay, and the other cell pairs had different forms of synchrony (i.e., peaks >1-ms width or larger delay values). Second, spike timing correlations can change their characteristics. An example of this is given by the unit pair shown in Fig. 6A, which showed significant synchrony during the ON response but spike timing became independent during the OFF response. Moreover, synchrony between the same unit pair changed its delay value when a long moving light bar crossed over their receptive fields simultaneously (Fig. 12A). The two units showed a correlation peak with a delay of opposite sign compared with the activity evoked by the stationary spots (Fig. 5G). Specifically, unit 0 tended to fire 1 ms before the firing of unit 2 (CCH, Fig. 12). Third, our analysis showed that about one half of the unit pairs in our sample showed significant nonstationary modulation of their correlation strength that could not be predicted by corresponding changes in firing rates (see also Fig. 12B). Finally, the LGN cell pairs had significantly larger modulation amplitudes and later onset times than the retino-geniculate pairs.

Although the spike timing correlations in our sample are not likely to be the result of common monosynaptic retinal input, the synchronous oscillatory activity observed in the LGN is nonetheless driven by retinal input. Evidence for this comes from multiple sources. LGN relay cells receive ongoing oscillatory input from retinal ganglion cells, even in the absence of light stimulation (Koepse1 et al. 2009), which drives oscillatory firing that is precisely time locked to the cycle of the rhythmic synaptic input. The activities of retinal ganglion cells are known to be synchronized over large spatial distances (Ishikane et al. 2005; Schnitzer and Meister 2003; Shlens et al. 2009), and this precisely correlated retinal input drives long-range spike timing correlations in the LGN (Castelo-Branco et al. 1998; Neuenschwander and Singer 1996). Because there is relatively little lateral connectivity within the LGN (Sherman and Koch 1998), one possible explanation for the nonstationary synchrony could be that the correlated retinal inputs themselves contain nonstationary variations. This notion could be tested by performing an analysis similar to ours on pairs of simultaneously recorded retinal ganglion cells.

An alternative hypothesis suggests that the nonstationary modulation of LGN synchrony arises from the pattern of feedback input from the visual cortex. Relay cells and cells of the thalamic reticular nucleus receive massive feedback input from layer VI cells in the visual cortex (Granseth and Lindstrom 2003; Montero 1991; Murphy and Sillitio 1996). The inhibitory neurons of the reticular nucleus in turn send inputs diffusively over the relay cells (Sherman and Koch 1998). These cortical feedback projections are known to influence LGN activity in a variety of ways including influences on response properties (Cudeiro and Sillitio 1996; Murphy and

![Diagram](A)  
**A**  
- Time (ms)
- 0 to 16
  - Number of CEs
  - Time (sec)
  - Unit 0
  - Unit 2
  - Z
  - 159
  - max
  - 0.5
  - min
  - 0.3
  - significance limit
  - 0.13
  - CCH (bin width, 1 ms) has a peak at a delay of 1 ms.

![Diagram](B)  
**B**  
- Time (sec)
- 0 to 2
  - 100 ms
  - 50
  - raw CE
  - predicted CE
  - bootstrap limit
  - A: CCH (bin width, 1 ms) has a peak at a delay of 1 ms.
  - B: UEA of the corresponding CE (b = 1 ms, d = 1 ms) showed a transient increase of the raw CE number (unit 0: A-lamina, Y-type, unit 2: C-lamina, Y-type, M = 20 trials, SI = 0.13, t_{max} = 6.1, t_{min} = -0.5, upper significance limit = 5.6, lower significance limit = -0.3).

**Fig. 12.** Stimulus context-dependent change of spike synchrony. Synchrony between the same intertetrode unit pair shown in Fig. 5 changed its temporal delay when the 2 receptive fields were stimulated simultaneously by a moving light bar. A: CCH (bin width, 1 ms) has a peak at a delay of 1 ms. B: UEA of the corresponding CE (b = 1 ms, d = 1 ms) showed a transient increase of the raw CE number (unit 0: A-lamina, Y-type, unit 2: C-lamina, Y-type, M = 20 trials, SI = 0.13, t_{max} = 6.1, t_{min} = -0.5, upper significance limit = 5.6, lower significance limit = -0.3).
Sillito 1987; Rivadulla et al. 2002; Varela and Singer 1987; Wörgötter et al. 1998), response variability (Andolina et al. 2007), and information transmission (McClurkin et al. 1994). Thus it seems likely that some, as yet unknown, pattern of cortico-thalamic feedback could regulate the patterns of synchronized activity within the LGN. The late onset times in some of the intertetrode correlations support a role for a feedback mechanism.

**Functional significance of nonstationary synchrony**

We observed a nonstationary change of spike timing correlation having an intrinsic temporal scale (i.e., not dependent on the time course of the stimulus). In the cases shown in Figs. 6A and 7B, the increase of the raw CE number occurs transiently and without a substantial change in the firing rates. Because the stationary light spots do not introduce temporal modulation, other than the duration of the stimulus, we infer that the transient synchrony reflects an intrinsic dynamical organization of the network in which the LGN is embedded. What function might this nonstationary synchrony confer on the thalamocortical network? One possible explanation comes from recent studies showing that the postsynaptic effects of thalamocortical synapses are strongly context dependent. The influence of weak synaptic inputs is greatly augmented when they occur in tight synchrony with that of other synaptic input on the same postsynaptic neuron (Alonso et al. 1996; Bruno and Sakmann 2006; Roy and Alloway 2001; Usrey 2002; Usrey et al. 2000). These findings are consistent with the concept that cortical neurons can act as finely tuned detectors of coincident synaptic input (Azuoz and Gray 2000, 2003, 2008). In this context, even a small transient change of synchrony among the LGN cells could lead to a significant change in the activity of cortical neurons. If, as we speculate above, the nonstationary changes in LGN synchrony are controlled by feedback from the cortex, corticofugal output could be acting to regulate the patterns of synchronous thalamic activity that influence its own activation. Nonstationary synchrony would thus not be surprising if dynamic processing of stimuli (as opposed to simple feedforward processing of stimuli) had an intrinsic dynamical organization having an intrinsic temporal scale (i.e., not dependent on the context of the external input).

**Appendix A: Derivation of Mean of Normalized Synchrony Strength**

The temporal mean of the normalized excess synchrony, $z(t) = N_{raw}(t) - N_{pre}(t) / \sigma_{pre}(t)$, in the test interval $t \in [T_1, T_2]$ is defined by

$$z = \frac{1}{T_2 - T_1} \sum_{i=1}^{T_2} z(t) = \frac{1}{T_2 - T_1} \sum_{i=1}^{T_2} \frac{N_{raw}(t) - N_{pre}(t)}{\sigma_{pre}(t)}.$$

Substituting the definition $N_{raw}(t) = \sum_{i=1}^{M} n_i(t)$,

$$z = \frac{1}{T_2 - T_1} \sum_{i=1}^{M} \frac{n_i(t) - N_{pre}(t)}{\sigma_{pre}(t)} = \frac{1}{T_2 - T_1} \sum_{i=1}^{M} \frac{n_i(t) - \frac{N_{pre}(t)}{M}}{\sigma_{pre}(t)},$$

where $n_i(t)$ is the number of the coincident events in the $i$th sampling window of the $t$th trial. Therefore the temporal mean $\bar{z}$ is given by the average of the local variable $\xi(t) = \frac{n_i(t) - N_{pre}(t)}{M} \sigma_{pre}(t)$ over all the sampling windows in the test interval and all the trials.

**Appendix B: Layer Dependences of Spike Synchrony**

Four unit pairs (3 intratetrode and 1 intertetrode) in the total sample ($n = 195$) were recorded from unidentified layers and showed responses to the contralateral eye (possibly A or C layers). These samples were excluded from the population analysis of the layer dependence. Table 5 summarizes the comparison of various characteristics of synchrony across the different layer combinations for both intratetrode ($n = 118$) and intertetrode pairs ($n = 73$).

The strength of synchrony in the CCH was measured by the significance ratio normalized by the significance limit obtained by the Monte Carlo samplings (see Methods). For the cell pairs showing significant synchrony, the distributions of the strength are significantly different across layers either for intratetrode ($P > 0.83$, Kruskal-Wallis test) or intertetrode pairs ($P > 0.30$). Because the number of significant samples was small for intertetrode synchrony, the statistical comparison may not be well evaluated. Also as for the incidence of significant synchrony, there was no layer dependence for both intratetrode ($P > 0.33$) and intertetrode pairs ($P > 0.17$).

The incidence of UEs also showed no layer dependence for both intratetrode ($P > 0.78$) and intertetrode cell pairs ($P > 0.22$). Distributions of the SI were compared among different layers only for the unit pairs having UEs. Although multiple comparison among three groups of intratetrode pairs did not reach statistical significance ($P > 0.07$), pairwise comparisons showed weaker synchrony in A-A pairs than C-C pairs ($P < 0.02$, U test). For intertetrode pairs, neither multiple comparisons nor pairwise comparisons showed significant differences over different layers.

When the layer dependence was tested for the significance ratio of the CCH over all the cell pairs, including nonsignificant samples, intertetrode synchrony in the A layer was significantly weaker than those in the other layers ($P > 0.003$, Kruskal-Wallis test). As discussed in the text, the UE is more sensitive to weak synchrony than the CCH. Therefore the incidence of significant intertetrode synchrony in the A layer showed an increase from the CCH test (7%) to the UE test (66%). Because those samples were recorded from five animals, this characteristic was not likely to reflect a sampling bias.

As for the significance of nonstationary modulation, both the incidence and distribution of modulation amplitude showed no significant layer dependence for both intratetrode and intertetrode pairs.

**Appendix C: Estimation of Minimum Firing Rate for UEA**

For simplicity, consider the case when both units fire independently with the same firing probability $P$ in a sampling window of size $T_\text{w}$. Each bin contains $b$ elementary bins of $1$ ms and takes a value of $0$ only when no spike event occurs at any of $b$ elementary bins. Therefore each bin of each unit has a value of $1$ with a probability $1 - (1 - P)^b$. The cumulative number of chance coincidence events in the sampling window over $M$ trials that is predicted CE number is estimated as $[1 - (1 - P)^b]^2MT_\text{w}/b$. Suppose that excess coincident events are added with a probability $\alpha P$, where $\alpha \ll 1$, the raw CE number is given by $[b\alpha P + (1 - (1 - P)^b)^2]MT_\text{w}/b$. In the sampling window, the raw CE number should exceed the significance limit to become a UE. Furthermore, we impose an extra condition for reasonable statistical estimation: the raw CE number must be greater than the absolute limit $M/2$. When $P \ll 1$, the probability of the value $1$ at
each bin is approximated by $bP$, which increases linearly to the bin width. The second condition leads to a quadratic inequality with respect to $P$: $P^2 + \frac{\alpha}{b}P - \frac{1}{2TTb} > 0$. With $T_w = 100$, $P > \left( \frac{\alpha}{b} + \frac{1}{50b} \right)^2$ and the net firing rate in spikes/s (sp/s) is given by $(1 + \alpha)P$ multiplied by 1,000. When the excess synchrony is weak with $\alpha = 0.01$, the minimum firing rates are 67, 48, 40, 34, 31, and 28 sp/s for the bin widths $b$ of 1–6 ms, respectively. Although LGN cells often fire at rates $>80$ sp/s, a firing rate $<40$ sp/s in the cortex would make the detection of weak synchrony at a high temporal precision difficult. For stronger synchrony with $\alpha = 0.1$, those rates are decreased to 40, 34, 30, 28, 25, and 24 sp/s.

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DISCLOSURES

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