Stimulus-Dependent Neuronal Oscillations in Cat Visual Cortex: Inter-Columnar Interaction as Determined by Cross-Correlation Analysis

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Abstract
We have demonstrated previously that neurons in cat striate cortex, in response to their preferred stimuli, exhibit oscillatory responses in a frequency range of 40–60 Hz. Recently, we obtained evidence that such oscillatory responses can synchronize across columns. We have now performed an extensive analysis of this phenomenon for both unit and field potential responses. In addition, we studied the stimulus conditions leading to intercolumnar synchronization. We recorded both multi-unit activity and local field potentials from area 17 of adult cats with arrays of several electrodes. Inter-electrode distances ranged from 0.4 to 12 mm. For all pairs of unit (n = 200) and field potential (n = 174) recordings, we computed auto- and cross-correlation functions. The modulation of the correlograms was quantified by fitting a damped sine wave (Gabor) function to the data. Cross-correlation analysis of the unit data revealed that in 90 out of 200 cases the recorded cells established a constant phase-relationship of their oscillatory responses. This occurred, on average, with no phase difference. If the receptive fields were nonoverlapping, we observed a synchronization primarily between cells with similar orientation preferences. Cells with overlapping receptive fields also showed a high incidence of synchronization if their orientation preferences were different. In this latter group, synchronization occurred even in cases where the stimulus was optimal for only one of the recording sites. Under conditions of monocular instead of binocular stimulation the oscillatory modulation of the responses was attenuated, but the cross-correlogram still indicated a significant interaction. Similar effects were seen with the application of stationary instead of moving stimuli. A synchronization of oscillatory field potential responses was observed in 136 out of 174 paired recordings. At all distances investigated, the probability of synchronization of field potential responses was independent of the orientation preferences of the cells. However, the strength of interaction decreased with increasing spatial separation. Control experiments showed that the synchronization of field potential responses was not due to volume conduction. The results demonstrate that oscillatory responses at separate cortical sites can transiently synchronize. The probability and strength of synchronization are dependent on the spatial separation of the recorded cells and their orientation preferences. In addition, the cross-columnar synchronization is influenced by features of the visual stimulus. It is suggested that this synchronization provides a mechanism for the formation of neuronal assemblies in the visual cortex.

Introduction
Increasing physiological evidence suggests that neurons of various cortical areas, in response to their preferred stimuli, do not merely show an elevation of their average firing rate. Rather, the neuronal responses exhibit a well defined temporal structure. As has been shown first in the olfactory system, both the neuronal firing probability and the local electroencephalogram show an oscillatory modulation which is stimulus-dependent and appears in a characteristic frequency range of 35–90 Hz (for review, see Freeman, 1975, 1983, 1988). In our laboratory, an analogous phenomenon has been discovered in the visual cortex (Gray and Singer, 1987), which has subsequently been confirmed by Eckhorn et al. (1988). In cat visual cortex, a significant fraction of cells within an orientation column show stimulus-dependent oscillations (Gray and Singer, 1987, 1989; Gray et al., 1989c; Engel et al., 1989). This suggested the possibility that oscillatory responses can also synchronize across orientation columns. We assume that the synchronization could provide a mechanism for the functional coupling

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of neurons. In particular, the temporal coherence of the responses could serve to identify the members of a cell assembly representing an object present in the visual field (Abeles, 1982; Crick, 1984; von der Malsburg and Bienenstock, 1986; von der Malsburg and Schneider, 1986; von der Malsburg and Singer, 1988).

We tested this hypothesis by recording from multiple sites in area 17 of cat visual cortex and subjecting the data to cross-correlation analysis. These experiments have shown that synchronization of oscillatory unit responses does indeed occur in cat striate cortex (Gray et al., 1988, 1989a; Singer et al., 1988), and we therefore set out to investigate in greater detail the conditions leading to intercolumnar synchronization of oscillatory responses: (i) we analysed a larger sample of data and applied a more differentiated protocol for the quantification and classification of the correlograms; (ii) we investigated the variability of response synchronization; (iii) we analysed the influence of variations of several stimulus parameters on the synchronization of neuronal oscillations; to that end, we compared suboptimal with optimal stimulus orientations, monocular with binocular stimulation and stationary flashed with moving light bars; (iv) since oscillatory responses can be observed not only in the unit activity, but also in the local field potential (Gray and Singer, 1989), we also investigated the occurrence of synchronization for the latter; (v) we performed control experiments to assess the contribution of volume conduction to the synchronization of field potential responses.

Materials and methods

Animal preparation

The data analysed in this study were recorded from 18 adult cats aged more than 6 months. The preparation and maintenance of the animals followed the standard protocol for in vivo experiments. In brief, anaesthesia was induced by intramuscular injection of a mixture of ketamine hydrochloride (Ketanest®, Parke-Davis; 10 mg/kg) and xylazine hydrochloride (Rompun®, Bayer; 2.5 mg/kg). After tracheotomy, the animals were artificially respirated and maintained on a combination of 70% N₂O/30% O₂ with 0.2–2% halothane (Hoechst). Muscle relaxation was achieved by continuous i.v. infusion of hexachlorobutadienone (Imbretil®, Hormon-Chemie; 1–2.5 mg/h). During the preparation, all wound edges were infiltrated with a local anaesthetic. To further eliminate potential sources of painful stimuli, the animal’s skull was then cemented to a metal rod and the stereotactic ear and eye bars were removed. During the experiment, the body temperature was kept constant at 38°C. Pulmonary pressure and CO₂ content of the expired air were continuously monitored. Fluid loss was compensated by infusion of a glucose-Ringer solution via a gastric catheter.

Recording

Multiunit activity (MUA) and local field potentials (LFP) were recorded from multiple 25 μm thick Teflon coated platinum-iridium wires with tapered tips (Mioche and Singer, 1988). After amplification, MUA and LFPs were obtained from the same original signal by differential bandpass filtering in a range of 1–3 kHz and 1–100 Hz, respectively. We usually employed an array of four to six closely spaced (400–500 μm) electrodes which was inserted into area 17 within the representation of the area centralis. In addition, one or two electrodes were positioned farther anterior at varying distances and advanced down the medial bank. Thus we were able to examine the interaction of cell populations with a spatial separation ranging from 0.4 mm to 12 mm. Prior to quantitative analysis, the receptive field properties of the MUA were assessed with hand-held stimuli. For subsequent measurements, light bars were projected via electronically controlled mirror galvanometers onto a screen located 1.10 m in front of the cat’s eye plane. For each trial, the light bars were moved forward and backward across the receptive fields perpendicular to the axis of orientation. All stimulus trials lasted 10 s and were repeated at intervals of 15–20 s. Blocks of 10 trials recorded under identical stimulus conditions were used for further analysis.

Stimulation was usually binocular, after the receptive fields for the two eyes had been aligned with a prism placed in front of one of the eyes. For each binocular cell, we mapped the receptive field of one eye as carefully as possible. By means of the prism, the receptive field of the remaining eye was then superimposed on the field mapped on the screen. This was usually possible with a precision of about a quarter of a degree of visual angle. For computation of a quantitative orientation tuning curve, we recorded responses to stimuli whose orientation was changed at angular steps of 22.5°. To optimize responses for correlation measurements, we stimulated the cells whenever possible with light bars of preferred length, velocity and orientation. When cells recorded from two different electrodes had overlapping receptive fields, but differing orientation preferences, we used stimuli with orientations intermediate between the respective optima to elicit responses of sufficient strength. For electrode distances above 2 mm, the receptive fields of the recorded neurons were typically nonoverlapping. In these cases we applied two independent light bars. Thus, the units could be activated optimally even if their orientation preferences differed. In a number of cases, we also studied the effect of suboptimally oriented stimuli, of monocular stimulation and of stationary flashed light bars on the interaction of the respective cells.

On three penetrations in three cats, we performed 20 measurements at different cortical depths to examine the contribution of volume conduction to the LFP responses. In these experiments a square-shaped piece of area 17 was isolated by circumcision with a razor blade down to the white matter. Then an array of 5–7 electrodes with an average spacing of 0.4 mm was placed across the cut to record signals from both sides of the lesion (for further explanation, see results section and Fig. 11). At the end of these control experiments, lesions were made by applying DC currents (20 nA, 30 s) through the electrodes. After administration of a lethal dose of barbiturate, the brains were removed and fixed by immersion in 10% formaldehyde. The positions of the electrode tips relative to the transcortical cuts were then verified in Nissl stained sagittal sections.

Data processing

The MUA was fed through a Schmitt trigger, the threshold of which was set to about twice the noise level. The trigger output and the analogue LFP signal were digitized on separate channels with a time resolution of 1 ms.

Several sets of calculations were performed off-line using a MicroVax GPX with graphics display. On the unit data, we first computed peri-stimulus time histograms (PSTH) with a resolution of 100 ms. From trial blocks recorded for different orientations we constructed tuning curves by integrating over the total number of spikes during the responses. For further processing, we selected pairs of recordings according to the following criteria: (i) only those recording sites were included where the MUA had an orientation tuning (defined as width at half-height of 45° or less; (ii) response pairs were subjected to cross-correlation analysis only if both responses had sufficient temporal overlap; and (iii) as we were interested in stimulus-dependent
phenomena, but could not always activate both recording sites with the preferred stimulus, responses were considered for further analysis if they had at least 1/4 to 1/3 of their maximum strength. For a given pair of cells, responses were usually recorded under a variety of stimulus conditions. In these cases, the best cross-correlogram obtained was utilized for compilation of our statistics (for criteria see below).

On the MUA data we computed both autocorrelation functions (ACF) and cross-correlation functions (CCF) for time shifts of up to ±60 ms at a resolution of 1 ms (Perkel et al., 1967a; Toyama et al., 1981a,b; Michalski et al., 1983; Ts’o et al., 1986). In addition, a shift predictor was calculated by recomputation of the ACF and CCF after shuffling the trial sequence by one stimulus period (Perkel et al., 1967a,b). The calculations were performed separately for the first and second half of each 10 s trial, corresponding to the forward and backward movement of the stimulus, respectively. In some cases, we computed ACFs and CCFs for three separate time windows of 1 s duration containing spontaneous activity and the two light responses, respectively. In the majority of our cross-correlation measurements, the correlograms computed for the 10 individual trials of each block were summed up to yield a single CCF. In a number of selected cases, however, we also analysed the interaction between the two unit responses on a trial by trial basis. This was done in order to investigate the variability of the synchronization of the two signals.

The following calculations were performed on the LFP data: in analogy to the PSTH, a ‘field potential histogram’ (FPH) was compiled for each trial block. The LFP trace was subdivided into segments of 100 ms duration and then, for each segment, a power spectrum was computed using a Fast Fourier Transform algorithm. Subsequently, the power values in the frequency range between 24 and 96 Hz were summed to obtain a single value for each 100 ms segment. These FPHs then reflect, as a time series, the stimulus-dependent increase of LFP oscillations in the frequency range between 24 and 96 Hz (see Fig. 10A). From FPHs of responses to stimuli of different orientations we then computed orientation tuning curves for the LFP using the integral of the FPH response as a measure.

With the help of FPHs, three time epochs of 960 ms duration were selected. One comprised spontaneous activity and the other two were centred on the maxima of the responses to forward and backward movement of the stimulus. In these windows, the ACFs and CCFs of the LFP signals were computed by means of a Fast Fourier Transform algorithm in a frequency band of 24–96 Hz (Press et al., 1986). This calculation was performed with a resolution of 1 ms for time shifts between ±63 ms. The correlation functions of the 10 individual trials comprising one block were subsequently averaged. Power spectra of the respective LFP epochs were stored in separate files. The results obtained with the Fast Fourier Transform algorithm are equivalent to those of conventional cross-correlation methods. In addition, this algorithm performs a digital filtering, because it can be carried out selectively for a certain frequency band. We tested the algorithm with white noise filtered in various frequency bands to control for the introduction of artefacts. In addition, a shift predictor was calculated for the LFP cross-correlation.

Quantification of auto- and cross-correlation

The oscillatory nature of ACFs and CCFs was assessed by fitting Gabor functions (damped sine waves) to the data using a nonlinear iterative procedure (Fig. 1). Thus the correlograms could in approximation be characterized by the frequency, phase shift, amplitude and decay constant of the respective Gabor function. The fit was performed by means of the Levenberg-Marquardt-method (Press et al., 1986). This

FIG. 1. Illustration of the rating paradigm used in this study.

(A) For the rating of MUA cross-correlograms, we utilized as parameters the normalized modulation amplitude (ANORM, abscissa) and the quotient of decay constant over cycle time (3/T, ordinate). All three parameters were taken from the respective Gabor function that was fitted to the data. All cases with 3/T > 0.8 and ANORM > 0.1 were considered to be significantly oscillatory.

(B–D) Typical examples of ‘raw’ MUA correlograms which were rated into the categories 2, 4 and 8, respectively. The thick line depicts the Gabor function that was fitted to the MUA correlogram. Note that we did not subtract the shift predictor. The scale bars in B–D indicate the number of spikes.

(E) Rating criteria for the evaluation of LFP cross-correlograms. As for the MUA, we used as a rating criterion the quotient of the decay constant over the cycle time (3/T; vertical axis). For the rating of the amplitude, we used the ratio of the amplitude of the CCF under light stimulation (ASTIM) over the amplitude of the CCF in an epoch of spontaneous activity (ASPION; horizontal axis). All parameters were taken from the respective Gabor function. If 3 was larger than T and, in addition, ASTIM/ASPION exceeded a value of 1.5, we considered the CCF as oscillatory. Examples are given in

(F–H) for rating categories 2, 4 and 8. The thick continuous line indicates the Gabor function that was fitted to the CCF (thick dashed line). The thin continuous line corresponds to the CCF calculated from spontaneous activity. All three curves within a figure are displayed at the same relative amplitude.
algorithm provides estimates of the errors of all four parameters. As a criterion for the significance of the fit, we used the error of the amplitude. To accept the fit as significant, the amplitude of the Gabor function had to be more than twice its standard error, that is, the hypothesis that the correlogram could equally well be described by a Gabor function with zero amplitude had to be discarded at the 5% level. In cases where the fit was significant, the Gabor function describing the auto- or cross-correlogram was subjected to a rating procedure to quantify the degree of rhythmicity of the response or the strength of the synchronization, respectively. Criteria for this were derived from the amplitude and the decay constant of the Gabor function. Auto- and cross-correlograms were subjected to the same rating criteria.

The Gabor function fit to the ACFs and CCFs of the MUA had a variable offset from the x-axis (Fig. 1B–D). This offset corresponds roughly to the amplitude of the shift predictor (Fig. 6C,D; 9E). For assessing the strength of the correlation, it was obviously not appropriate to consider the ‘absolute’ amplitude of the modulation, but rather its amplitude relative to the size of the offset. We therefore performed a normalization by dividing the amplitude of the Gabor function with its offset. This measure will in the following be termed ‘normalized modulation amplitude’ (Fig. 1A). It reflects the proportion of spikes which actually contribute to the periodic modulation of the correlogram and yields a rating which is independent of the absolute strength of the response. In many cases, the offset was larger than the amplitude of the Gabor function. This was due to several factors. First, many units were spontaneously active, and we had calculated ACFs and CCFs over epochs comprising both the response and preceding spontaneous activity. From separate computation of correlograms for the light response and spontaneous activity, respectively, we inferred that the latter does not contribute to the oscillatory modulation. Instead, it adds a constant offset to the modulation. Additional possibilities are that the MUA contains oscillating as well as nonoscillating cells, or that the oscillatory behaviour of individual cells varies from trial to trial (cf. Fig. 7). In addition to the normalized modulation amplitude, we used the decay constant of the Gabor function as the second rating parameter to determine the oscillatory nature of the correlograms (Fig. 1A). We divided the decay constant (i.e. the time after which the envelope of the Gabor function has decreased to 1/e of its maximum) by the respective cycle time. This was necessary because otherwise, a correlogram with only one broad peak, that is, with a very low Gabor function frequency, would obtain the same rating as a CCF that has the same decay of the envelope of the Gabor function, but contains in addition a superimposed high-frequency oscillation. MUA correlograms were classified as oscillatory only if the amplitude of the respective Gabor function exceeded 10% of its offset and if the quotient of decay constant over cycle time exceeded a value of 0.8 (Fig. 1A). Above these thresholds (i.e. rating category 3 or better), correlograms usually displayed at least three distinct maxima (Fig. 1C,D).

LFP auto- and cross-correlograms were evaluated in the same way. In response to light stimulation, the frequency variance of the LFP decreases and the amplitude increases. Without stimulation, the frequency components in the range of 20–100 Hz show only low amplitudes (cf. Gray and Singer, 1989, Fig. 1C). Accordingly, auto- or cross-correlograms computed from epochs of spontaneous LFP activity display a very low amplitude and a rapid decay (Figs 1F–H; 10C,E). To take into account the response-specific increase of the oscillatory activity, we used as an amplitude criterion the quotient of the Gabor function amplitudes of the CCFs computed for light response and spontaneous activity, respectively (Fig. 1E). This measure seemed to be a reasonable assessment of significance because the amplitude of the CCFs computed for spontaneous activity corresponds fairly well to the Gabor function amplitude of the shift predictor (averaged over 10 trials; see Fig. 10C–F). As for the MUA correlograms, the decay constant of the Gabor function was divided by the cycle time (Fig. 1E). To be classified as oscillatory, the modulation amplitude of the LFP correlograms had to exceed by a factor of 1.5 that of the correlated spontaneous activity. In addition, the quotient of decay constant and cycle time had to be larger than 1.0 (Fig. 1E).

For both MUA and LFP, the oscillatory correlograms were further classified into six categories according to their quality as indicated in Figure 1. The rating categories with odd and even numbers are distinguished by the normalized modulation amplitude of the Gabor function, as shown in Figure 1A and E. As categories 1 and 2 comprise all those correlograms with a decay of the function to 1/e within one cycle time or less, they cover a variety of correlogram types including ones with only a single peak as well as those exhibiting troughs to both sides of the centre peak (Fig. 1B,F).

To further analyse the influence of spatial separation on the degree of synchronization of MUA responses, we used our rating scheme with a modification. According to our standard procedure, ratings with even numbers were attributed to MUA correlograms if their normalized modulation amplitude exceeded 0.2 (Fig. 1A). In the modified version of the rating procedure, the amplitude threshold for even numbered categories was raised to 0.4. Subsequently, the proportion of oscillatory CCFs which passed the new threshold was compared for pairs of recordings with a spatial separation of below or above 2 mm. For the LFP CCFs, we investigated the influence of spatial separation on the degree of synchronization in a different manner. For electrode pairs where both ACFs, as well as the CCF, showed an oscillatory modulation, we computed the ratio of the CCF Gabor function amplitude over the geometric mean of the two respective ACF amplitudes. This normalization accounts for the possible enhancement of the CCF modulation by an increased regularity of the ACFs. This measure was compared for electrode pairs with spatial separations of less than 2 mm, between 2 and 7 mm and of more than 7 mm.

Results

From our data sample, we selected 200 pairs of MUA recordings. Responses were only included if they showed sufficient temporal overlap, were of sufficient strength and had an orientation tuning (defined as width at half-height) of 45° or less. These 200 pairs comprised responses from 176 different recording sites. At 119 sites (68%), the periodic modulation of the ACF indicated an oscillatory behaviour of the neuronal firing probability (rating category 3–8). In 64 of these 119 recordings, the ACF obtained a rating value of 6 or 8 indicating a high amplitude and slow decay of the modulation. Recomputation of the ACF after shuffling the trial sequence by one stimulus period eliminated the periodicity. In some of the experiments we performed a separate computation of the ACF for light responses and epochs of spontaneous activity. These cases demonstrate that the contribution of spontaneous activity to the oscillatory modulation of the ACF is negligible. At 22 of the 176 recording sites, the ACF had a broad peak centred around the zero bin and, occasionally, troughs to both sides (category 1 and 2). The half-width of the centre peak was usually in the range of 3–5 ms. The remaining 35 auto-correlograms were flat, apart from the zero bin.
At 144 of the recording sites selected for this study, LFPs were recorded simultaneously with MUA. For the LFP, we routinely performed separate computation of the autocorrelograms for spontaneous activity and light response epochs, respectively. The ACF of the response epochs displayed an oscillatory modulation in 108 of these 144 cases (75%). At 72 recording sites, the ACFs were classified into rating category 6 or 8. As for the MUA recordings, calculation of a shift predictor eliminated the regular modulation of the average ACF. After shuffling the trial sequence (shift predictor), the correlation function of pairs of single trials still showed some modulation. However, averaging over 10 shuffled correlograms yielded an ACF with a very low amplitude (Fig. 10D). This indicates that the LFP oscillations are not phase-locked to the stimulus (Gray and Singer, 1989). In 33 of 144 cases, the ACF of the response epochs decayed rapidly to zero, but the amplitude of the centre peak exceeded the amplitude of the ACF computed for spontaneous activity by a factor of more than 1.5 (rating categories 1 and 2).

Comparison of the MUA and LFP ACFs at the same recording site revealed that in 54% of the cases both ACFs were rated 3 or better (78 of 144). In 30 cases, the LFP ACF was oscillatory whereas the MUA ACF was not, and in 19 cases the reverse was true. The frequency of the oscillatory responses ranged from 30 to 70 Hz. The average frequencies of the modulation of the ACFs were 54 ± 2 and 47 ± 5 Hz for MUA and LFP, respectively (Fig. 2).

For 116 of the recording sites, we computed orientation tuning curves (at angular steps of 22.5°) for both the MUA and LFP responses. In eight cases the LFP tuning curve was flat or displayed two maxima. In 86 of 116 cases, the orientation preferences of MUA and LFP differed by 22° or less. In 14 cases, the maxima of the tuning curves differed by 45°. In the remaining eight cases, the difference of the preferred orientation of MUA and LFP was 67 or 90°. The tuning was usually broader for LFP than for MUA responses. In 62 cases, the LFP tuning width (defined as width at half height of the response) exceeded that of the MUA by about 20°. In only eight cases was the difference of the tuning widths larger than 20°. In 31 cases, the tuning widths of both signals were comparable. In 7 cases, the tuning of the LFP was narrower than that of the MUA.

**MUA cross-correlation analysis**

In 90 out of 200 pairs selected for correlation analysis, the CCF exhibited a significant oscillatory modulation (rating 3–8), indicating a synchronization of the two signals. A single peak was found in 32 cross-correlograms (rating 1 or 2). The remaining correlograms were flat or could not be described significantly by a Gabor function. In all cases where the CCF showed a periodic modulation or a single centre peak, the shift predictors of the cross-correlation were flat (Figs 6D; 9E, F).

For the 200 pairs of recordings, we analysed the dependence of the synchronization of the responses on the orientation preferences of the respective recording sites and their spatial separation. In addition, in a number of cases we investigated to what extent the rhythmicity of the responses and their synchronization varied from one individual stimulus trial to the next.

Figures 3 and 4 illustrate a case where we recorded from 5 electrodes with a spacing of 400 μm. The cells at sites 1, 3 and 5 had similar orientation preferences which were nearly orthogonal to those of cells at sites 2 and 4 (Fig. 4A). All receptive fields were overlapping and located in the centre of the visual field. Figure 3 shows segments of responses to a light bar of 112° orientation recorded simultaneously...
Fig. 4. Synchronization of oscillatory MUA responses across five orientation columns with different preferred orientations and overlapping receptive fields. (A) Normalized orientation tuning curves. For each recording site, the response amplitude (ordinate) is plotted versus stimulus orientation (abscissa). Arrows indicate the orientation of the stimuli applied in B-D: (B1) PSTHs obtained in response to a light bar of 112° orientation. (B2) Auto-correlograms of responses at electrodes 1 (1–1), 3 (3–3) and 5 (5–5) for the first (unfilled) and second (filled) direction of stimulus movement. (B3) Cross-correlograms computed for the three possible combinations. CCFs obtained for the first stimulus direction are displayed with unfilled bars with the exception of the cross-correlation of 1–5. (C1) With a vertical (0°) light bar, strong responses are recorded at sites 2 and 4. (C2) Auto- (2–2, 4–4) and cross-correlograms (2–4) for the responses at channel 2 and 4. Histograms compiled for the first direction of stimulus movement are displayed with unfilled bars except for 4–4. Note that, for the backward movement of the stimulus, only a very weak but significant cross-correlation can be observed, although the response on channel 4 is even stronger for this stimulus direction. (D1) Stimulation with a 135° light bar simultaneously evokes strong responses at sites 1, 2 and 5. (D2) All three cell groups exhibit an oscillatory modulation of their ACFs. (D3) Although the orientation preference of the cells at site 2 differs from that at 1 and 5, strong oscillatory cross-correlations occur between all sites. The correlogram in D computed for the first stimulus direction are displayed with filled bars, with the exception of 1–1 in D2. Vertical scale bars indicate the number of spikes.

At sites 1 and 3. Both the MUA and LFP responses are oscillatory and synchronized between the two recording sites. The spikes are grouped in bursts, which are phase-locked to the negative phases of the LFP oscillation (Gray and Singer, 1989). The oscillatory nature of the responses and their synchronization are also reflected in the ACFs and CCFs computed from 10 consecutive trials (Fig. 4). If the cells were stimulated with a light bar of 112° orientation, strong oscillatory responses were found at sites 1, 3 and 5, but not at sites 2 and 4. As indicated by the CCFs, the three responses synchronized with 0 ms phase difference (Fig. 4B). At this stimulus orientation no interaction occurred with the cells at recording sites 2 and 4. When the stimulus was changed to 0° to activate the units at electrodes 2 and 4, correlated oscillatory responses were observed at these sites (Fig. 4C). At intermediate orientations, synchronization also occurred between columns of different orientation preference. For example, a stimulus with an orientation of 135° evoked strong oscillatory responses at sites 1, 2 and 5. The responses at sites 1 and 5 were clearly synchronized with site 2, although the stimulus orientation was suboptimal for the latter (Fig. 4D; backward direction of stimulus movement). This experiment demonstrates that synchronization of oscillatory responses does occur between sites of similar orientation preference, but also between sites with different preferences. The synchronization does not necessarily require the optimal stimulus orientation. It should be noted that in cases where the responses from two sites were not sufficiently
overlapping in time the CCFs between evoked activity at one site with spontaneous activity at the other did not show an oscillatory modulation.

The influence of orientation preference and spatial separation of the recording sites on the interaction is summarized in Table 1. Based on these two parameters, we classified the 200 pairs of recordings into nine groups. Neurons with a spatial separation of less than 2 mm had overlapping fields. If the cells at both sites responded to the stimulus, they showed a high incidence of response synchronization irrespective of the angular difference of their preferred orientations. At distances of 2–7 mm, when the receptive fields were nonoverlapping, the interaction of cells in separate columns was clearly dependent on their orientation preferences. For this range of interelectrode distances, oscillatory CCFs were found preferably for neuronal groups with similar orientation preference. Above 7 mm, oscillatory CCFs were rarely encountered.

The synchronization of MUA responses occurred on average with a 0 ms phase difference. The largest phase shifts observed were ±5 ms. As shown in Figure 5A, the distribution of phase shifts is monomodal. There was no obvious correlation between phase difference and difference in preferred orientation or spatial separation of the cell groups (data not shown). The frequency of the modulation of the cross-correlograms was in the same range as that of the ACFs with an average of 53±8 Hz (Fig. 5B).

The cross-correlograms with a rating of 1 or 2, that is, those with essentially one center peak, were also differentially distributed among the nine groups of Table 1. In our sample, this type of interaction occurred mainly between cell groups that were separated by less than 2 mm. The difference in preferred orientations was of no obvious

<table>
<thead>
<tr>
<th>Δ OR</th>
<th>Spatial separation</th>
<th>0.4–2.0 mm (overlapping fields)</th>
<th>2.1–7.0 mm</th>
<th>7.1–12.0 mm (nonoverlapping fields)</th>
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<tr>
<td>0–22° a.</td>
<td>32/59 (54%)</td>
<td>a. 10/20 (50%)</td>
<td>a. 1/12 (8%)</td>
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<tr>
<td>b.</td>
<td>15/59 (25%)</td>
<td>b. 0/20 (0%)</td>
<td>b. 1/12 (8%)</td>
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<tr>
<td>45° a.</td>
<td>15/27 (56%)</td>
<td>a. 5/17 (29%)</td>
<td>a. 2/8 (25%)</td>
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<td>b.</td>
<td>6/27 (22%)</td>
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<td>67–90° a.</td>
<td>22/32 (69%)</td>
<td>a. 1/12 (8%)</td>
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<tr>
<td>b.</td>
<td>7/32 (22%)</td>
<td>b. 0/12 (0%)</td>
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Within each group, the ratios 'a' and 'b' refer to the fraction of CCFs with ratings of 3–8 and 1–2, respectively, over the total number of observations.

Fig. 5. Distribution of phaseshifts and frequencies of the Gabor functions of MUA (A,B) and LFP (C,D) cross-correlograms which were rated as oscillatory. Scale bars indicate the number of cases.
importance. In most of these correlograms, the peak was symmetric about zero, indicating that on average correlated firing occurred without time lag. Only occasionally, we observed delays of up to ±3 ms.

Not only the likelihood of synchronization of oscillatory responses, but also the strength of correlation depends on the spatial separation (Table 2). The strength of synchronization is reflected in the amplitude and the decay of the modulation of the CCF. For interelectrode distances below 2 mm, 69 of the CCFs were oscillatory. Out of these, 44 fell into the two highest rating categories 6 and 8, that is, they showed a slow decay and their modulation amplitude exceeded 20% of the shift predictor amplitude (Fig. 1A). If neuronal groups had nonoverlapping fields and a spatial separation of 2–7 mm, such high ratings occurred less frequently (6 of 16 CCFs with an oscillatory modulation). However, even above 7 mm spatial separation CCFs with a rating of 6 or 8 were obtained (4 out of 5 oscillatory CCFs). We then re-examined the quality of the oscillatory CCFs with a more stringent amplitude criterion in order to obtain a better differentiation of cases below and above 2 mm spatial separation. CCFs were only rated into an even numbered category if the ratio between modulation amplitude and shift predictor amplitude exceeded 0.4. Applying this criterion, 27 of the cases with an interelectrode distance of less than 2 mm still obtained a rating of 6 or 8. However, all cases with a spatial separation of more than 2 mm failed to pass the new threshold (Table 2).

As could be expected, oscillatory CCFs should only be obtained if the ACFs at both sites show at least some degree of oscillatory modulation. However, applying our rating algorithm we found that in only 50 of the 90 cases where a MUA CCF exhibited a periodic modulation both ACFs were classified as oscillatory. In 40 out of 90 cases, only one ACF was oscillatory. In about half of these 40 cases, the other ACF was classified into rating category 2 (see Fig. 1B). In the remaining cases, the other ACF was, according to our rating algorithm, not significantly modulated. A closer inspection of these cases resolved this apparent difficulty which turned out to be essentially due to the methodological problems inherent to a rating procedure: (i) in most of the 40 cases the periodic modulation of the CCF was not very pronounced; (ii) on the other hand, in many of those cases where one ACF was rated into category 2, the correlogram exhibited two small satellite peaks at both sides of the centre peak (Fig. 1B); therefore, these correlograms bear at least some degree of rhythmicity which may be sufficient to contribute to a modulated cross-correlogram; (iii) in those cases where one ACF could not be fitted by a Gabor function, a periodic modulation was frequently visible, but due to noise, the fit had just failed to pass the significance level. We conclude from these observations that the majority of the oscillatory MUA CCFs can be considered to reflect the synchronization of two oscillatory responses. This conclusion is further supported by the observation that results similar to that of Table 1 are obtained if only those response pairs are considered where both auto-correlograms are oscillatory (Table 3). In contrast, Table 1 contains all pairs selected for cross-correlation analysis, irrespective of the rating of the ACFs obtained for the two recording sites. In both samples, the distribution of oscillatory CCFs is similar.

For 13 cases out of our data sample, we investigated the variability of the response synchronization on a trial by trial basis. In these cases, the ACFs and CCFs were recomputed for individual trials of one measurement. These correlograms were subjected to our rating procedure. Thus we could analyse variations of the modulation amplitude, frequency and phaseshift which occur from one 10 s trial to the next. In addition, the spike trains were inspected to examine the phase-relationships of individual bursts. The results of this variability analysis are exemplified in Figures 6 and 7. The cells recorded were separated by 2 mm and differed in their preferred orientation by 45°. Both recordings displayed clear oscillatory firing patterns (Fig. 6B). The cells were stimulated with a vertical light bar which was optimal for recording site two. Using this stimulus, the response at site 1 reached about half its maximum and was still strong enough to contribute to a well modulated cross-correlogram (Fig. 6C). As can be inferred from the single trial ACFs and CCFs (Fig. 7A), all parameters which characterize the correlogram modulation may vary considerably from one trial to the next, most notably the response amplitude and the frequency of the modulation (cf. e.g. sweeps three and four in Fig. 7A3). Occasionally we also observed a complete cessation of the oscillatory modulation of the spike train despite an unaltered response strength (e.g. sweep eight in Fig. 7A3). In addition, the average phase of the cross-correlation varied between individual trials (cf. e.g. sweep four and six in Fig. 7A1). The variability of the synchronization between the two recorded cell groups is illustrated further in Figure 7B which displays epochs of spike trains taken from the peak of the response. The phase-relationship of the bursts at the two recording sites clearly shows some jitter, as indicated by the distribution of filled and open symbols in Figure 7B. Typically, the

<table>
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<tr>
<th>Table 2. The proportion of cases with a rating of 6 or 8, expressed as a function of the spatial separation of the recording sites and the angular difference of preferred stimulus orientation (Δ OR)</th>
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<td>Δ OR</td>
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In each group, the ratio 'a' refers to the fraction of CCFs with a rating of 6 or 8, over the total number of observations. The ratio 'b' indicates the number of cases left in these two rating categories after the amplitude criterion had been changed so that the normalized modulation amplitude had to exceed 0.4 (cf. methods).

<table>
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<th>Table 3. The influence of differences in orientation preference (Δ OR) and of spatial separation of the recording sites on the inter-columnar interaction. Only those cases are included where both auto-correlograms of the response pair display an oscillatory modulation</th>
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<tr>
<td>Δ OR</td>
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Conventions as in Table 1.
two responses kept their average phase-lag for periods of 50–100 ms, interrupted by short epochs in which they adopted a different temporal relationship (see e.g., sweeps four and five in Fig. 7B). In addition, the strength of synchronization varied markedly from trial to trial (cf. Fig. 7A1 and B). Similar observations were made in all other cases investigated.

Feature dependence of the synchronization of MUA responses

To further investigate the extent to which the synchronization of oscillatory responses depends on the visual stimulus, we varied three parameters: we tested suboptimal stimulus orientations, applied monocular instead of binocular stimulation, and compared the effect of stationary flashed light bars with that of moving stimuli.

The results of these experiments show that stimulation with the cells preferred orientation is not necessarily required for the synchronization of the responses. As already demonstrated in Figure 4, oscillatory CCFs with very similar normalized modulation amplitudes and decay constants were found for optimally as well as suboptimally oriented stimuli. This effect was tested in 28 pairs of MUA recordings which exhibited oscillatory CCFs. In 19 of these cases the cells had overlapping receptive fields. In the remaining cases the receptive fields were nonoverlapping and thus the cells could be stimulated simultaneously with their optimal orientations even if their orientation preferences differed. In addition, suboptimal stimuli in varying combinations could be tested. Other parameters like bar length as well as amplitude and velocity of movement were kept constant. The results of these measurements show that response synchronization is facilitated by simultaneous optimal stimulation of the cells at both recording sites, but does not necessarily depend on it. For pairs of recordings with overlapping receptive fields, oscillatory CCFs could be obtained for all stimuli which evoked oscillatory responses at both recording sites with a sufficient degree of overlap in time (19 out of 19 cases). In 3 of 9 pairs of recordings with nonoverlapping fields, the orientation preferences of both cell groups differed by less than 22°. In these cases, the strongest synchronization was obtained if both fields were stimulated with the optimal orientation. In the remaining six cases, the angular difference of the tuning maxima amounted to more than 22°. In 4 out of these 6 cases, the strongest CCF modulation was obtained if stimuli with identical orientations were presented to both fields, that is, stimuli which were optimal for one cell group but suboptimal for the other. The synchronization was less pronounced if both fields were stimulated with their respective optimal orientation.

In the recordings collected for this study we routinely applied binocular stimulation. In 15 of those cases where both ACFs as well as the CCFs showed a strong periodic modulation, we tested the effect of monocular stimulation. The cell groups at all sites were binocular. In only 1 out of 15 cases did the responses of the participating neurons show a strong binocular summation. The results of these measurements
Fig. 7. Variability analysis (continued from Fig. 6). (A) Cross-correlograms (A1) and auto-correlograms (A2, A3) recomputed for individual trials (1 – 10) which were recorded under identical stimulus conditions. Those trials which could not be fitted by a Gabor function are labelled by asterisks. Note that in some of the correlograms a modulation is clearly visible, but due to the low number of spikes, the fit did not pass the significance level (e.g. sweep 10 in A1 and A2). Scale bars indicate numbers of spikes. (B) Selected epochs of unit data taken from the peak of the response (cf. Fig. 6A). The phase-relationship of the bursts at the two recording sites is indicated by open and filled symbols. Filled circles mark those instances where the burst at site 2 occurs 1 – 5 ms prior to that at site 1 (corresponding to the average phase-lag, see Fig. 6C). Open circles indicate pairs of bursts with the reverse temporal relationship. Half-filled symbols indicate ambiguous cases. Note that pairs of bursts are labelled only if their temporal association is closer than 5 ms.

demonstrate that binocular interactions influence the oscillatory modulation of the signals. A typical example is shown in Figure 8. The cells at the two recording sites had a clear preference for stimulation through the left eye, but did not exhibit binocular summation (Fig. 8A1 – C1). Binocular stimulation evoked strong oscillatory responses at both sites (Fig. 8C2) which were well synchronized for both directions of stimulus movement (Fig. 8C3). When the cells were stimulated through one eye only, the oscillatory modulation of the ACFs was reduced or disappeared completely (Fig. 8A2, B2). The CCFs, however, still exhibited a clear centre peak which was only slightly reduced in amplitude (Fig. 8A3, B3). These effects were observed in 9 out of 15 cases. Except for one case, where the correlation was completely eliminated, a single peak remained in the CCFs under monocular stimulation. In these cases, the amplitude of the centre peak was moderately reduced, if compared to the offset of the respective Gabor function. This indicates that the strength of correlation is only slightly diminished, although the oscillatory modulation of the spike trains dissolves into a more random temporal structure. In one additional case, the CCF retained a periodic, but weakened modulation under monocular stimulation. In the remaining 5 out of 15 cases, the temporal structure of the responses and their synchronization were not influenced by binocular interactions.

In another series of measurements, we compared moving contours with stationary stimuli of the same length and orientation. The effects observed are similar to those of monocular stimulation as described above. In all of the eight cases investigated, the cells responded well to light bars flashed ON and OFF within their receptive field (Fig. 9B). In seven cases, the oscillatory modulation of the ACFs and CCFs disappeared when stationary stimuli were used (cf. Figs 9C, E and 9D, F). In three cases, the cross-correlograms exhibited a single peak
under this condition (Fig. 9F). In 4 of the 7 cases, the correlation was completely eliminated. In only one of the cases tested, was the CCF still classified as oscillatory under stationary stimulation.

LFP cross-correlation analysis
From 174 electrode pairs, LFPs were simultaneously recorded with MUA. In the great majority of these cases, we obtained significant
Fig. 9. Comparison of responses to moving and stationary stimuli. The recorded cells had overlapping receptive fields, matched in their orientation preferences and were separated by 400 μm. (A,B) when stimulated with their preferred orientation, the cells responded strongly to a moving light bar (A) as well as to a stationary stimulus which was turned ON (B, black arrow) and OFF (B, light arrow) within the receptive field. Auto- and cross-correlograms were computed within a 1 s window centred on the peak of the responses. In case of the ON and OFF responses, the first 100 ms of the response were omitted in order to remove some of the stimulus co-ordination artefact. (C,D) Auto-correlograms for both responses (1–1, 2–2) computed within the second window, that is, for the backward movement of the stimulus (C) and the OFF response (D), respectively. (E) Cross-correlogram for the responses to the moving light bar. The lower histogram corresponds to the shift-predictor for this cross-correlogram. (F) Cross-correlogram and shift-predictor (lower histogram) for the correlation of both OFF-responses. Note that the responses evoked by the stationary stimulus are non-oscillatory, but nevertheless they show a significant correlation as indicated by the centre peak in the CCF. Scale bars refer to the number of spikes.

<table>
<thead>
<tr>
<th>Δ OR</th>
<th>0.4–2.0 mm (overlapping fields)</th>
<th>Spatial separation</th>
<th>2.1–7.0 mm (nonoverlapping fields)</th>
<th>7.1–12.0 mm (nonoverlapping fields)</th>
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<tr>
<td>0–22°</td>
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<td>a. 11/15 (73%)</td>
<td>a. 7/12 (58%)</td>
<td></td>
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<tr>
<td></td>
<td>b. 10/46 (22%)</td>
<td>b. 0/15 (0%)</td>
<td>b. 0/12 (0%)</td>
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<td>45°</td>
<td>a. 22/26 (85%)</td>
<td>a. 11/12 (92%)</td>
<td>a. 6/8 (75%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b. 3/26 (20%)</td>
<td>b. 1/12 (8%)</td>
<td>b. 0/8 (0%)</td>
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<td>a. 24/30 (80%)</td>
<td>a. 10/12 (83%)</td>
<td>a. 9/13 (69%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b. 6/30 (20%)</td>
<td>b. 0/12 (0%)</td>
<td>b. 0/13 (0%)</td>
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Conventions as in Table 1.

Table 5. The proportion of LFP CCFs with a rating of 6 or 8, expressed as a function of both the spatial separation of the recording sites and the angular difference of preferred stimulus orientation (Δ OR)

<table>
<thead>
<tr>
<th>Δ OR</th>
<th>0.4–2.0 mm (overlapping fields)</th>
<th>Spatial separation</th>
<th>2.1–7.0 mm (nonoverlapping fields)</th>
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<tr>
<td>67–90°</td>
<td>22/30</td>
<td>7/12</td>
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of the spike activity was usually narrower than that of the LFP. The results indicate that oscillatory LFP responses synchronize even over long distances irrespective of differences in orientation preference (Table 4). Even for electrode separations as far as 12 mm, we found a high incidence of synchronization of the two LFP responses. At all distances investigated, a high proportion of the oscillatory CCFs obtained a rating of 6 or 8 (Table 5).

A typical example is illustrated by Figure 10. The data were collected from the same recording sites as the MUA in Figures 3 and 4. As shown in the field potential histograms, the power in the frequency band of 24–96 Hz increases at all recording sites when the receptive fields are stimulated with a light bar of 112° orientation (Fig. 10A). This increase in FPH amplitude is very similar at all sites, although the cells at site 2 and 4 respond only weakly (cf. Fig. 4B1). Compared to spontaneous activity, the frequency range shifts to higher values, and the variance decreases under light stimulation (data not shown). The high amplitude and slow decay of the ACFs reflect the increased regularity of the signals (Fig. 10C). In these and related cases with overlapping receptive fields the LFP responses exhibited a tight synchronization across all recording sites (Fig. 10B,E). Only the amplitudes of the LFP oscillations varied across neighbouring cell populations (Fig. 10B). For epochs of spontaneous LFP activity, the CCFs do not show a significant synchronization between different sites (displayed with thin continuous lines in Fig. 10E). Thus the occurrence of oscillatory responses as well as their synchronization are clearly stimulus-dependent phenomena. As a control for the significance of these cross-correlations, we calculated a shift predictor (Fig. 10D,F). The CCF, averaged over 10 shifted trials, exhibits only a low modulation amplitude. This indicates that the strong modulation of the CCF for the nonshifted trials is not merely reflecting the similar frequency contents of the two oscillatory responses. Another control

oscillatory CCFs (136 out of 174). In 116 of these cases with an oscillatory CCF both ACFs exhibited an oscillatory modulation. Very similar to the results of MUA cross-correlation, the synchronization of LFP responses occurred in most cases with zero phase difference (Fig. 5C). The average Gabor function frequency of the LFP CCFs was 47±5 Hz (Fig. 5D).

As for the unit activity, we investigated to what extent the probability of LFP correlation depended on the spatial separation of the recording sites and on differences of orientation preference. Orientation preference and tuning were determined from the respective MUA, because tuning
Fig. 10. Synchronization of oscillatory LFP responses across different recording sites. The data are taken from the same experiment as in Figure 3. (A) FPIs from the five recording sites obtained during stimulation with a 112° light bar. The traces demonstrate a stimulus-dependent increase of power in the frequency band between 24 and 96 Hz. (B) Selected data epoch from a single stimulus trial. The LFPs were bandpass filtered between 1 and 100 Hz. Arrows indicate the onset of the response to the first direction of stimulus movement. The amplitude of field potential oscillations was usually in the range of 10–100 µV. (C) ACFs of the responses of the five recording sites. (E) Cross-correlation functions computed for each pair of neighboring channels. The cross-correlation of, for example, 1–4 or 1–5 is of similar strength (not shown). The thick continuous and thick dashed line represent the correlograms from forward and backward response epochs, respectively. The thin continuous line depicts the correlogram calculated for spontaneous activity. (D and F) show the results of computation of ACFs and CCFs, respectively, after shuffling the trial sequence by one. The shift predictors are displayed at the same scale as the non-shifted CCFs. The graphs show the results of averaging over 10 shifted trial combinations. If averaging is performed over all possible shift combinations (90 for CCF, 45 for ACF), the amplitude of the shift predictors approaches zero. The scale bars in E and F indicate the amplitude of the curves relative to that of C and D to permit comparison of the ACFs and CCFs.

For artefacts was the application of our correlation algorithm to bandpass filtered white noise. The respective auto- and cross-correlograms obtained decrease very rapidly to zero (not shown). The fact that spontaneous LFP activity has a very similar ACF suggests that its frequency contents in the range of 24–96 Hz are randomly distributed.

To further quantify the influence of spatial separation on the degree of synchronization, we computed the ratio of the CCF Gabor function amplitude over the geometric mean of the two respective ACF amplitudes (see methods). This was done for all cases where the CCF as well as both ACFs showed an oscillatory modulation. If the electrodes
Fig. 11. LFP oscillations are volume-conducted only to a very weak extent from active to neighboring inactive sites. (A) Schematic display of the experimental paradigm. Electrodes one and two were inserted into a nonresponisive piece of cortex obtained by circumcision with a razor blade. The interelectrode interval corresponds to roughly 400 μm. GL, lateral gyrus; P, posterior; L, lateral. (B) PSTHs from the five recording sites obtained at a depth of 500 μm. Only at electrode four and five can spike activity be recorded. (C) At the same sites, a stimulus-dependent increase in amplitude of the spectral components between 24 and 96 Hz can be observed. Note that only a negligible FPH increase occurs at site 3 which is unresponsive in terms of unit discharge. (D) Selected epochs of LFP data. The arrow indicates the end of the second light response. Note the very low amplitude of high-frequency oscillations on channel three. The low-frequency components of the EEG, however, spread into the lesioned area. (E) ACFs of the five signals. Their oscillatory modulation reflects the stimulus-induced decrease in frequency variance. (F) CCFs of neighboring cell groups. Note that mainly the signals at site four and five are correlated. However, a weak cross-correlation appears also between channels three and four. The scale in F indicates the amplitude of the curves relative to that of the graphs in E. Correlation functions displayed with thick continuous and thick dashed lines are calculated from the first and second light response epochs, respectively. The thin continuous line corresponds to the ACF and CCF computed from spontaneous activity within the first 960 ms of the trials.

were separated by less than 2 mm, this ratio was on average 0.87 (with a standard deviation of 0.11) indicating a high degree of synchronization (n = 69; see also Fig. 10B and E). Between 2 and 7 mm distance, the quotient was markedly reduced to 0.39 ± 0.20 (n = 26) and above 7 mm it was 0.20 ± 0.14 (n = 21). Irrespective of recording distance, these values did not show any obvious dependence on differences in
orientation preference. If the CCF was oscillatory, but one or both
ACFs showed only weak periodic modulation (rating categories 1 or
2), this quotient was also reduced (below 2 mm interelectrode distance:
$0.67 \pm 0.09, n = 11$; above 2 mm: $0.14 \pm 0.10, n = 7$). These results
demonstrate that the degree of synchronization decreases with increasing
spatial separation of the recording sites.

As for the MUA, the degree of synchronization of LFP responses
was dependent on binocular interaction. In seven experiments we
compared the synchronization of LFP responses under binocular and
monocular stimulation. In all of these cases, the CCFs had a lower
amplitude and decayed more rapidly for monocular than for binocular
responses.

As described above, LFP responses from nearby recording sites
almost always showed a strong synchronization suggesting the
possibility of volume conduction. To test this, we isolated a piece of
cortex by circumcision that extended down to the white matter. Then
we recorded with an electrode array placed across the lesion (Fig. 11A).
Spike responses were abolished within the lesioned area and the first
electrode outside the isolated patch usually failed to record unit activity.
However, electrodes more than $400\text{–}500 \mu m$ away from the cut
recorded MUA responses with normal orientation tuning and associated
oscillatory LFPs (Fig. 11B–D). Cross-correlation of LFPs revealed
a good synchronization between the respective sites. At the sites within
the lesioned area there was neither a stimulus-dependent increase in
LFP power in the high frequency range (Fig. 11C,D) nor was there
any evidence for a significant cross-correlation with the LFPs from
neighbouring responsive sites (Fig. 11F). The first recording site
outside the isolated piece of cortex, where unit activity was also absent,
still showed a minute stimulus dependent increase of power in the FPH
(Fig. 11C). This is possibly due to subthreshold synaptic currents at
this site which are still activated by afferents from neighbouring intact
regions of tissue, but no longer sufficient to produce postsynaptic spike
responses. Similar results were obtained in all 20 measurements of
this kind. Since the electrode spacing in our experiments was typically
close to $400 \mu m$, the half-decay distance for the passive spread of the
LFP can be estimated to be maximally in the range of $100\text{–}200 \mu m$.

As a second measure of neuronal group activity, we utilized the high-
frequency oscillations that appear in the local field potential. It is
assumed that this signal reflects the sum of the membrane currents in
a certain volume of cortical tissue (e.g. Nicholson and Freeman, 1975;
Mitzdorf, 1985, 1988). The activity reflected by the LFP seems to be
recruited essentially from a single orientation column, because the
LFP usually exhibits an orientation preference which is similar to that
of the MUA activity (present results; Gray and Singer, 1989). The
neighbouring columns with different orientation preferences appear
to contribute only little to the LFP oscillations. Supportive evidence
for this comes from our lesion experiments. Our results indicate that
the LFP oscillation evoked within an orientation column can hardly
be recorded across a distance of $400 \mu m$ at a neighbouring site of
inactive tissue. Assuming a half-decay distance of $100\text{–}200 \mu m$ for
the LFP amplitude and an average spacing of iso-orientation columns
of $800\text{–}1000 \mu m$ (Löwel et al., 1987), it seems reasonable to assume
that the LFP response is volume-conducted into neighbouring columns
only to a minor extent.

Cross-correlation analysis of these two types of signals yielded three
major CCF types. The first group comprises CCFs with a regular
periodic modulation which reflects the establishment of a constant
phase-relationship between two oscillatory signals. In our rating
scheme, these CCFs were classified as categories 3–8 (Fig 1C,D,G,H).
The second type of CCF exhibits mainly one peak centred around zero which is occasionally flanked by troughs and minute satellite peaks (Fig. 1B,F; rating categories 1 and 2). These
correlograms reflect a correlation between two essentially nonoscillatory
signals. The third group comprises flat CCFs indicating the absence
of correlation between the two signals.

These CCF types were distinguished by means of a rating procedure
based on the assumption that the correlograms can be described by
damped sine wave (Gabor) functions. In the case of CCFs with an
oscillatory modulation, it seems obvious that damped sine waves are
an appropriate model (Fig. 1D,H). In addition, correlograms with only
one centre peak can be described by a Gabor function with a rapid
decay. The degree to which two oscillatory signals are synchronized
is reflected in the amplitude and the decay of the modulation of the
CCF. As outlined in the method section, we used modifications of these
two parameters to classify the correlograms. The significance of the
fit of the Gabor function to the correlogram was assessed by means
of the amplitude error provided by the algorithm. To further increase
the reliability of our rating procedure, we excluded all correlograms
with a very low modulation amplitude from further classification
(Fig. 1A,E). In addition, the CCFs were considered to be oscillatory
only if they displayed at least three distinct maxima, that is, the
Gabor function had to exceed a certain decay value (Fig. 1). Altogether,
we assume that these criteria permit a reliable distinction between the
various correlogram types.

Besides assessing whether a CCF exhibits a significant modulation
it is necessary to establish that this periodicity is actually due to the
phase-locking of two signals rather than to accidental coincidences.
This can be tested by recomputation of the MUA and LFP correlograms
after shuffling of the trial sequences (Perkel et al., 1967a,b). These
shift-predictors control for the possibility that correlations result from
changes of statistical properties, such as an increase of the spike rates
or a decrease in frequency variance which lead to an increased similarity
of the temporal structure of the signals. In the case of the MUA, the
shift predictors were consistently flat (Figs. 6D, 9E,F). This has three
implications: first, the oscillatory modulation of the CCF is not simply

Discussion

Methodological considerations

This study focuses on the analysis of high-frequency oscillations in
multi-unit activity and local field potentials recorded from cat area 17.
The reason for choosing these two types of signals rather than single
unit activity is that we intended to measure the activity of functional
cell assemblies. Our approach is justified by the well-known fact that
neighbouring neurons in the visual cortex have similar feature
specificity. Moreover, the results of a previous investigation and of
the present study show that oscillatory firing patterns can indeed be
observed in a large fraction of multi-unit recordings. This indicates
that nearby cells, when exhibiting oscillatory responses, tend to
discharge in synchrony (Gray and Singer, 1987, 1989). It was proposed
therefore that assemblies of synchronously oscillating cells within an
orientation column could be envisaged as a basic functional unit.
Another argument for using multi-unit activity is merely a pragmatic
one. The detection of significant interactions in a unit-unit cross-
correlogram is considerably facilitated if the statistics are improved
by a large number of spikes. For the same reason, spontaneous activity
can also be investigated more reliably.
due to similarities in the temporal structure of the signals, second, the oscillatory responses are not phase-locked to the stimulus, and third, the oscillatory responses exhibit a considerable degree of variance (Fig. 7). This holds true because CCFs of two very regular periodic signals, e.g. two sine waves with a similar frequency, will always show a periodic modulation even if the two signals are independent. For the LFP shift-predictor, individual pairings yield a CCF with a weak modulation due to the stimulus-dependent increase in the regularity of the signals. However, the modulation disappears after averaging over a sufficient number of shifted correlograms because there is no consistent phase-relationship (Fig. 10). An additional control was performed by applying our algorithm for LFP cross-correlation to two random noise signals. This was done to exclude the possibility that the digital filtering performed by the Fast Fourier Transform introduced artefacts. These tests showed that the resulting auto- and cross-
correlograms decay to zero very rapidly. Therefore the bandpass filtering, as part of the algorithm, does not produce oscillatory ACFs or CCFs. From these controls, we infer that the algorithms for computation and classification of CCFs which we applied in this study are appropriate to detect significant correlation between oscillatory responses.

Cross-correlation of MUA oscillations: comparison to previous studies

The results of this study extend our previous findings and demonstrate that spatially separated neuronal groups can synchronize their stimulus-induced oscillatory responses. Both the probability and the strength of synchronization depend on the spatial separation of the neurons recorded. If the cells have nonoverlapping receptive fields, the synchronization of oscillatory responses is influenced by their orientation preference. In addition, the results show that the occurrence of oscillatory responses and their synchronization also depend on stimulus features, that is, both are enhanced with binocular as compared to monocular stimulation and require the movement of contours.

Although several studies are available of unit cross-correlation in cat and monkey striate cortex, none of them have provided evidence for synchronization of oscillatory activity (Toyama et al., 1981a, b; Michalski et al., 1983; Ts’o et al., 1986; Hata et al., 1988; Ts’o and Gilbert, 1988; Aiple and Krüger, 1988; Krüger and Aiple, 1988, 1989). This may have several explanations. First, cross-correlograms of rhythmic responses were interpreted to be misleading or artefactual and therefore were excluded from further analysis and documentation (Toyama et al., 1981a; Ts’o et al., 1986; Hata et al., 1988). Second, the probability of finding oscillatory responses depends on the type and the depth of anaesthesia and is reduced under treatment with barbiturates (unpublished observations). Third, averaging procedures such as those applied by Aiple and Krüger (1988) can be expected to mask the oscillations because they are not phase locked to the stimulus. Fourth, the systematic use of certain stimulation conditions may preclude the occurrence of oscillatory behaviour. For example, Toyama et al. (1981a) used only monocular stimulation which according to our results significantly weakens the rhythmicity of responses of binocular cells in anaesthetized preparations. Ts’o et al. (1986) regularly stimulated cells with two light bars even if the receptive fields were overlapping. We have evidence that the oscillations can be disturbed if light bars of different orientations are applied to the same field simultaneously, even in cases where the absolute response strength is not modified (Gray et al., 1989b). Finally, rhythmic firing patterns have in fact been observed, but were not investigated further (cf. the example of an area 18 complex cell in Fig. 5 of Hubel and Wiesel, 1965; see also Krüger, 1983).

In previous studies of cat visual cortex, cross-correlation analysis of unit activity has been performed for recording distances of up to 2–3 mm (Toyama et al., 1981a, b; Michalski et al., 1983; Ts’o et al., 1986). Analysis of interactions showed that in the majority of the cases co-ordinated firing occurred with almost zero time lag. In a minority of recordings, the correlograms indicated that one cell fires or is inhibited with a short delay relative to the other. These three types of correlograms have been interpreted as indicative of common input (from the lateral geniculate body or from intracortical cells), of monosynaptic intracortical excitation or monosynaptic inhibition, respectively (see e.g. Toyama, 1988; Ts’o et al., 1986). In our data sample, only a minority of the MUA cross-correlograms resemble these classical correlogram types with a single centre peak. In our rating scheme, these types are pooled in the categories 1 and 2, depending on their peak amplitude. The overall morphology of these CCFs agrees well with that described by Ts’o et al. (1986), who frequently observed correlograms with relatively broad peaks (with a half width in the range of several milliseconds), which often are symmetrically flanked by slight troughs. In accordance with their results we found in most of these correlograms the centre peak was symmetric about zero. In a minority of the cases, the peak was shifted to one side by 1–3 ms. Our results also demonstrate that CCFs with a single symmetrical peak occur across a spatial separation of up to 2 mm. Toyama et al. (1981b) found this type of CCF to be largely independent of the receptive field type and orientation preference of the respective cells, whereas Ts’o et al. (1986) claimed that this type of interaction occurs primarily between cells with similar orientation preference. In our sample, the distribution of single-peak CCFs is independent of the orientation preference of the participating cell groups (Table 1).

As far as correlograms with a single peak are concerned our results may be interpreted within the conventional framework of correlation theory. However, we wish to emphasize that our results do not merely extend previous studies to the observation of long-range interactions. Our results demonstrate a hitherto unnoticed type of interaction which is reflected in the periodic modulations of the cross-correlograms (Gray et al., 1989a). As shown in this study, this correlogram type was encountered much more frequently than the conventional CCF types and was obtained for much larger interelectrode distances. For reasons discussed below, we believe that this finding introduces new aspects to the interpretation of cross-correlograms and the investigation of functional connectivity.

Traditionally, cross-correlation analysis serves as a quasi-anatomical method (Perkel et al., 1967b; Ts’o et al., 1986; Toyama, 1988). The basic assumption of this paradigm is that correlated firing of spatially distributed neurons directly reflects their mutual axonal connection or their monosynaptic input from a third cell. In contrast, our results suggest that no direct inferences about anatomical wiring can be made from cross-correlograms with a periodic modulation. We propose that the visual cortex can be envisioned as a system of distributed oscillators which can rapidly synchronize or desynchronize in a stimulus-dependent manner. Thus, peak latencies in cross-correlograms are not interpreted in terms of transmission delay, but are rather considered to reflect a constant phase-relationship between two oscillatory signals.

The results of this study support the notion that the synchronization of oscillatory responses represents a functional coupling which is not only determined by the underlying anatomical substrate, but also dependent on properties of the visual stimulus. As indicated by the
effects of monocular stimulation and stationary flashed contours, the
oscillations do not represent a stereotyped response property of a given
group of cells. Rather, these stimulus features exert a strong influence
upon the coherency of the firing of a local group of cells within an
orientation column, as shown by our multunit auto-correlograms. In
addition, modification of the stimulus can qualitatively change the
interaction between spatially separate columns, for example, in those
cases where the replacement of moving by stationary stimuli leads to
a disappearance of the correlation between the recorded cells. Further
evidence for this comes from previous experiments where we recorded
from two cell groups with separate fields which were aligned co-linearly
(Gray et al., 1989a). Thus, we could stimulate the cells independently
or with a single long light bar. In these experiments, the strongest
synchronization was obtained with a single contour stimulating both
fields simultaneously. The synchronization was weaker if two
independent light bars of identical orientation and movement direction
were applied. It disappeared completely if the light bars moved in
opposite direction over the receptive fields. We conclude from these
results and the present findings that the synchronization of oscillatory
responses is not activation-gated in a nonspecific manner, but rather
sensitive to features of the visual stimulus. As demonstrated by our
results, changes of stimulus features can strongly influence the
functional connectivity between cortical neurons.

The anatomical substrate responsible for the synchronization is
unknown. The intra-areal horizontal connections as well as reciprocal
projections from other visual areas are likely candidates (Rockland and
Lund, 1982; Gilbert and Wiesel, 1983; Martin and Whitteridge, 1984;
Luhmann et al., 1986; Sain et al., 1989). It should be noted that the
synchronization described in this study does not have to depend on
monosynaptic connections. In a network of spatially distributed
oscillators, mutual coupling could well involve polysynaptic pathways.

Cross-correlation of oscillatory field potential responses
Our results on synchronization of LFP responses confirm the unit data.
Both indicate that, upon appropriate stimulation of a given area of visual
field, the corresponding part of visual cortex responds with a coherent
burst of oscillatory LFP activity in a frequency range of 30–60 Hz.
However, we observed one clear difference between the LFP and unit
activity: for sites with nonoverlapping receptive fields, significant
correlation of the LFP responses can be observed even if the cells differ
in their orientation preferences (cf. Tables 1 and 4).

This result implies that the two types of signals do not reflect the
same neural processes. This is further indicated by the fact that, under
a variety of conditions, field potential oscillations can be observed in
the absence of unit activity. This is the case, for example, when the
cells are driven with a stimulus whose orientation is orthogonal to the
optimal (Gray and Singer, 1989). The same observation is made with
direction selective cells. Usually, the LFP oscillation amplitude is not
reduced to the same extent as the unit response if the suboptimal
stimulus direction is applied (see e.g. Gray and Singer, 1989; Fig.
1B,C). We saw similar effects when recording from cells which
exhibit a strong ocular dominance. In these cases, an oscillatory LFP
response can be obtained without unit response when stimulating the
nondominant eye (unpublished observation).

One possibility to explain these differences is that activity of
neighbouring orientation columns contributes via volume conduction
to the LFP oscillations at the actual recording site. However, a large
contribution of volume conduction can be ruled out on the basis of
our lesion experiments. Another possibility is that the LFP does not
merely reflect the sum of the action potentials of a given functional
column, but also subthreshold membrane events. Indeed, there is
evidence that dendritic currents substantially contribute to the
extracellular field (Nunez, 1981; Freeman, 1983; Mitzdorf, 1985,
1988). Thus, synaptic activity from afferents originating in
neighbouring columns with different feature selectivity is likely to
contribute to the LFP. Altogether, the greater uniformity of LFP cross-
correlation as compared to the MUA is likely to result from: (i) some
volume conduction; and (ii) differences between dendritic inputs and
axonal output of the cells.

Cross-correlation analysis of LFP oscillations in the β range has been
performed in various regions of cat, rabbit and monkey brain (Freeman,
1978; Rougeul et al., 1979; Freeman and Schneider, 1982; Freeman
and van Dijk, 1987; Bouyer et al., 1981; Basar, 1988). These
studies demonstrate the widespread occurrence of synchronized
oscillatory LFP responses. A cross-correlation analysis of LFP
oscillations in cat visual cortex has recently been reported by Eckhorn
and coworkers (1988). By calculating spike-triggered averages the
authors demonstrate that LFP oscillations at one electrode are phase-
locked to the unit activity at a second, spatially remote recording site.
Their results indicate synchronization of oscillatory activity over several
millimeters within area 17 as well as between adjacent sites of area
17 and 18. One obvious discrepancy between this study and our results
relates to the issue of specificity of intercolumnar LFP synchronization.
Eckhorn et al. (1988) assume that synchronization of LFP responses
occurs primarily between columns of similar orientation preference.
However, they give only a few examples to support this hypothesis
and do not provide any statistical confirmation. Our results indicate
that only the synchronization of MUA responses, but not of the LFP
depends on the orientation preferences of the cells, if these have
nonoverlapping receptive fields.

Functional significance of phase-synchronization of oscillatory
responses
It is well established that in sensory cortices, maps representing various
feature domains coexist with a topographic map of the sensory surface.
In the primary visual cortex, parameters like orientation and direction
of stimuli are evaluated for any location in the visual field. Thus the
local features of an object will activate spatially distributed neurons
with appropriate specificity. However, to permit identification of the
object and its segregation from background, the global coherency of
features constituting this object must be evaluated (Treisman and
Gelade, 1980; Treisman, 1986). Cues which can be used to achieve
this figure-ground segregation include, for instance, the continuity or
c-linearity of contours, the common direction of movement, similarities
of spatial frequency content, interocular disparity, contrast
and colour (Marr and Poggio, 1976; Julesz, 1981; Ballard et al., 1983;
von der Malsburg and Singer, 1988). On the cortical level, this implies
that relations are established between neurons coding for these coherent
features. Thus these cells become distinguished as a functional assembly
representing the object. In the model originally suggested by Hebb
(1949), a functional assembly is defined by the common elevation of
firing frequency of the participating cells. However, it has been
recognized that such a concomitant elevation of the average firing
frequency remains ambiguous, because in natural scenes several objects
are usually present on a complex background. This stimulus
configuration would lead to simultaneous enhancement of the activity
in several spatially interleaved cell assemblies which would thus become
indistinguishable (von der Malsburg and Bienenstock, 1986;

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Abbreviations
ACF auto-correlation function
CCF cross-correlation function
FPH field potential histogram
LFP local field potential
MUA multiunit activity
PSTH peri-stimulus time histogram

References
Basar, E. (1988) EEG-dynamics and evoked potentials in sensory and cognitive

von der Malsburg and Schneider, 1986). Therefore, it was suggested that assemblies are not defined by enhanced responses of the participating neurons but by the temporal coherence of their firing patterns (Abeles, 1982; Crick, 1984; von der Malsburg and Bienenstock, 1986; von der Malsburg and Schneider, 1986).

The results of the present study suggest that synchronization of oscillatory responses of cortical neurons could be a suitable mechanism for the formation of assemblies. The respective members of an assembly would oscillate in synchrony, whereas no constant phase-relationship would exist among different assemblies. Thus the phase information contained in oscillatory responses could be used to solve the superposition problem mentioned above. Recent evidence from computer simulations suggests that two functional assemblies encoding two different objects can indeed coexist in the same network if they are distinguished by the phase-relationships of their members (von der Malsburg and Schneider, 1986). This concept of assembly formation implies that the degree of synchronization between cortical neurons should be determined in a flexible way by features of the object. Thus, synchronization should not occur between similar feature detectors only, as was suggested, for example, by Ts’o et al. (1986).

For instance, to form an assembly representing the outlines of a figure, certainly neurons with different feature selectivity have to interact. Our results on short-range interactions clearly demonstrate the possibility of synchronization of feature detectors with widely differing orientation preference. The hypothesis that synchronization of oscillatory responses fits the requirements of a feature-sensitive mechanism of assembly formation is further substantiated by our experiments with monocular and stationary stimuli. Finally, the proposed synchronization mechanism seems to be advantageous because it would establish assemblies by rapid and subtle changes of frequencies and relative phases. Thus a particular assembly would be formed only transiently for brief periods, after which the participating cells could join a different assembly. In summary, we conclude from the results of this study that the synchronization of oscillatory responses may provide a powerful mechanism to form assemblies of cortical neurons and thus to encode relationships between different features of an object.


