Interhemispheric Synchronization of Oscillatory Neuronal Responses in Cat Visual Cortex

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Neurons in area 17 of cat visual cortex display oscillatory responses that can synchronize across spatially separate columns in a stimulus-specific way. Response synchronization has now been shown to occur also between neurons in area 17 of the right and left cerebral hemispheres. This synchronization was abolished by section of the corpus callosum. Thus, the response synchronization is mediated by corticocortical connections. These data are compatible with the hypothesis that temporal synchrony of neuronal discharges serves to bind features within and between the visual hemifields.

NEURONS IN AREA 17 OF THE CAT visual cortex exhibit oscillatory firing patterns in the frequency range of 40 to 60 Hz (*1*). Such oscillatory responses can synchronize across orientation columns within area 17 (*2, 3*). This synchronization occurs between cells with a spatial separation of up to 7 mm and is sensitive to features of the visual stimulus, such as spatial continuity of contours, similarity of orientation and coherence of stimulus motion (*2*). It was proposed that the synchronization of feature-detecting neurons could serve as a mechanism for the binding of different features of an object and may thus contribute to scene segmentation and figure-ground segregation (*2–4*). We tested whether synchronization also occurs between cells in different hemispheres, which would be expected if such cells were coactivated by stimuli extending across the midline of the visual field.

In three adult cats, we made simultaneous recordings of multiunit responses from area 17 of the left and right hemisphere close to the representation of the vertical meridian (*5*). We computed both auto- and crosscorrelation functions of the recorded spike trains. The oscillatory nature of the responses and the degree to which they were synchronized was inferred from the periodic modulation of the auto- and cross-correlograms, respectively (*1–3*). To obtain a quantitative estimate of this modulation, we fitted damped sine wave functions to the correlograms and defined appropriate significance criteria (*3*).

Interhemispheric interactions were analyzed in 128 response pairs, which were derived from 109 different recording sites. At 90 sites the responses were oscillatory, and, in 89 of 128 response pairs (70%), the responses were synchronized between hemispheres. In 51 of these cases, the high amplitude of the correlogram modulation indicated a strong synchronization (that is, the relative modulation amplitude exceeded a value of 0.2). These results demonstrate that interhemispheric synchronization of oscillatory responses occurs and is of comparable strength to that of synchronization within area 17 (*2, 3*).

In a typical example of two oscillatory responses that exhibited interhemispheric synchronization (Fig. 1), the cells in the two hemispheres had the same orientation preference and their receptive fields were located close to the vertical meridian in the respective contralateral hemifield. The cross-correlogram showed a periodic modulation centered around 0, indicating that the two oscillatory responses synchronized without a phase-lag. This result was confirmed by analysis of the overall data sample. Response synchronization did not crucially depend on the degree of receptive field overlap or angular difference of preferred orientation (Fig. 2). The phase-locking of oscillatory responses between hemispheres occurred, on the average, with a 0-ms phase difference (SEM = ±0.3 ms). Thus, interhemispheric interactions closely resemble those found for spatially segregated neurons within area 17 of the same hemisphere (*2, 3*).

The synchronization of responses between hemispheres suggests that temporal synchrony between neuronal responses is mediated by corticocortical connections rather than by common subcortical input (*2, 3*). Because the visual projections to the two hemispheres remain entirely segregated beyond the optic chiasm, the corpus callosum is the most likely substrate for interhemispheric synchronization (*6, 7*). To test this assumption, we severed the corpus callosum of two additional adult cats and tested whether synchronization of oscillatory responses occurred between hemispheres (*8*). In these experiments, cross-correlation analysis was performed for 82 interhemispheric response pairs. This data sample comprised 71 individual recordings, 65 of which showed an oscillatory modulation, a proportion similar to that obtained in the unlesioned cats. A case where we recorded with two arrays of three closely spaced electrodes from left and right area 17 is illustrated in Fig. 3. The cells recorded in the left and right hemisphere had overlapping receptive fields and all responded to a vertically oriented stimulus. Computation of the cross-correlograms showed that the responses were strongly synchronized within left and right area 17. However, there was no indication of any synchronization between the hemispheres. These results suggest that interhemispheric synchronization is mediated by the corpus callosum.

Analysis of the whole data sample corroborates this conclusion. The vast majority of the interhemispheric response pairs were uncorrelated after lesion of the corpus callosum (Fig. 4). In most of our measurements the conditions were such that, without the callosal lesion, interhemispheric correlations should have occurred (*8*) (Fig. 3). These conditions are (i) strong oscillatory modulation of the responses, (ii) overlapping receptive fields, (iii) similar orientation preference of the recorded cells, and (iv) use of a single coherent stimulus, which facilitates response synchronization (*2, 3*). In seven cases, our algorithm for quantification of the correlogram modulation detected significant interactions even in the absence of callosal connections. However, in all of these cases the correlograms showed only a weak and noisy modulation. We consider these correlations spurious because our quantification

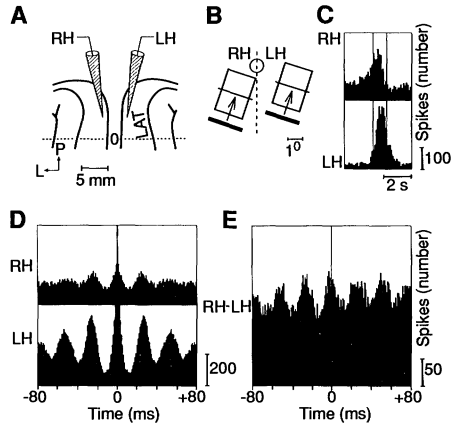

Fig. 1. Interhemispheric synchronization of oscillatory responses. (**A**) The electrodes were located in area 17 of the right hemisphere (RH) and left hemisphere (LH) close to the representation of the vertical meridian. (**B**) The receptive fields of the two multiunit recordings. The cells had the same orientation preferences, and the fields were located in the respective contralateral hemifields within 4° of the vertical meridian (dashed line). Circle, center of the visual field. (**C**) Histograms of the responses evoked simultaneously with two light bars of optimal orientation. (**D**) Autocorrelograms computed for the two responses in a 1-s window centered on the peak of the response. (**E**) The cross-correlogram of the two responses computed within the same time window.

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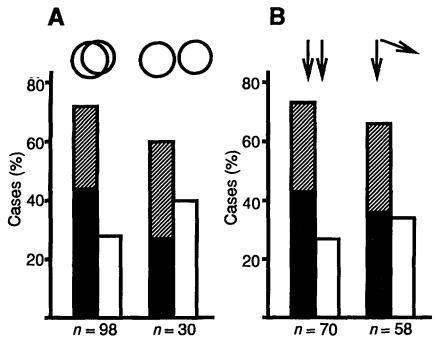

Fig. 2. Quantitative analysis of interhemispheric cross-correlograms. To assess the strength of synchronization, we fitted a damped sine wave function to each correlogram and computed the ratio of the amplitude of this function over its offset (*3*). This relative modulation amplitude (RMA) was used for compilation of the statistics. The histogram columns indicate the percentage of cases that show strong (black, RMA > 0.2), weak (hatched, 0.1 < RMA < 0.2), or no (open) synchronization. (**A**) Comparison of pairs of recordings with overlapping (left) and nonoverlapping (right) receptive fields. In both groups, the majority of pairs show a significant degree of synchronization. In our data sample, pairs with overlapping fields correlated more frequently and more strongly. However, this trend is not significant ($P > 0.05$; U test). (**B**) Comparison of response pairs with small (0° to 22°, left) and large (45° to 90°, right) differences in preferred orientation. In both groups, the great majority of response pairs exhibit a significant degree of synchronization. The two groups do not differ significantly ($P > 0.05$; U test). Further subdivision of the sample did not yield significant differences. However, if both criteria were combined and response pairs with overlapping fields plus differences in preferred orientation below 22° ($n = 59$) were tested against pairs with nonoverlapping fields plus differences in preferred orientation above 67° ($n = 13$), there was a significant difference ($P < 0.025$; U test).

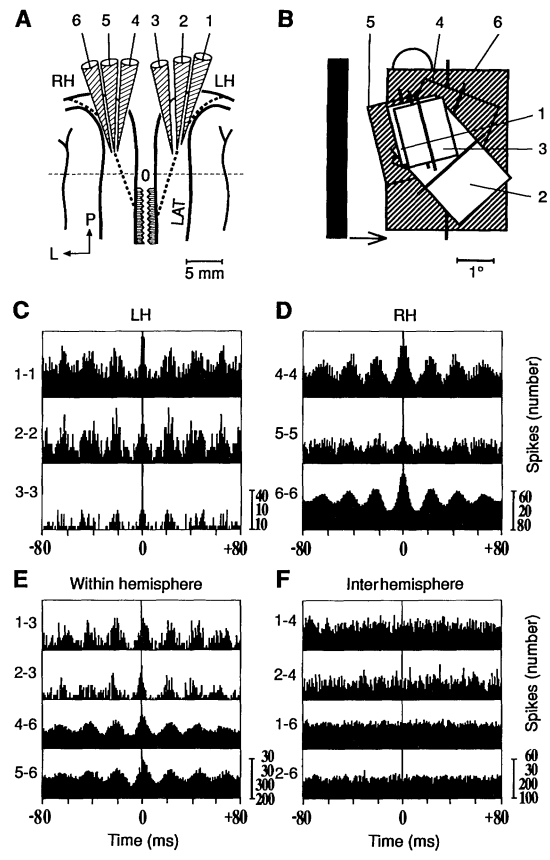

Fig. 3. Interhemispheric synchronization is absent in animals in which the corpus callosum had been sectioned. (**A**) Location of the recording electrodes in the vicinity of the border of areas 17 and 18 (thick dashed line) of the RH and LH. The interelectrode spacing was 0.5 mm for the RH and 1 mm for the LH. (**B**) The receptive fields of the RH (hatched) and LH (open) recordings. Thick lines, orientation preference. Circle, visual field center. All receptive fields were overlapping because the RH receptive fields extended into the ipsilateral visual hemifield. Thus, all recording sites could be costimulated with a single light bar. (**C** and **D**) Autocorrelograms of the responses at the LH and RH recording sites. All correlograms were computed in a 1.5-s window centered on the peak of the responses in the histograms (not shown). All responses show a strong oscillatory modulation in the same frequency range. (**E**) Cross-correlograms between responses from different recording sites within the same hemisphere. The oscillatory responses are strongly synchronized within either hemisphere. Strong temporal correlation occurs even between electrodes separated by as much as 2 mm (1–3). (**F**) Cross-correlograms between responses from different hemispheres. Note the absence of any temporal correlation. Cross-correlations are displayed only for those responses that showed the strongest oscillatory modulation (that is, 1 and 2 with 4 and 6). However, correlograms were also flat for all other interhemispheric combinations.

method operates on a 5% level of statistical significance (*3*) and, therefore, is expected to detect a fraction of false-positive cases in this range.

The results of this study demonstrate that neuronal discharges can be temporally correlated between homologous cortical areas of the two hemispheres representing the two visual hemifields. Furthermore, our results show that this interhemispheric synchronization is mediated by callosal connections. In previous studies on cortical response synchronization it was not possible to decide whether synchrony was achieved by corticocortical connections or by common subcortical input (*2, 3, 9*). Our results demonstrate directly that corticocortical connections can establish synchrony. Because the organization of the callosal pathway is similar to that of the tangential intra-areal connections and the reciprocal projections between different cortical areas of the same hemisphere (*7, 10*), it is likely that the previously described synchronization within and between cortical areas is also mediated by corticocortical connections (*2, 3, 9, 11*).

When interpreting results from correlation studies it is usually assumed that temporal correlation with 0 phase-lag reflects common input and cannot be accounted for by reciprocal connections with finite conduction delays (*12*). Our results prove the contrary by demonstrating interhemispheric synchronization with 0 phase-lag despite transcallosal conduction delays in the range of 4 to 6 ms (*6*). In addition, simulation studies indicate that 0 phase-lag can be established by reciprocal coupling of oscillators if the conduction delays do not exceed one-third of the cycle time (*13*). Given an oscillation frequency of 40 to 50 Hz, this prerequisite is fulfilled in case of the interhemispheric synchronization (*6*).

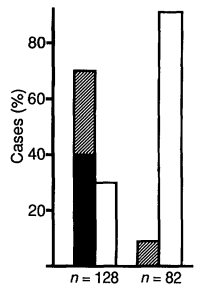

Fig. 4. Comparison of interhemispheric synchronization in normal (left) and lesioned (right) animals. The histogram columns indicate cases that show strong (black), weak (hatched), or no (open) synchronization.

Finally, our results suggest that it is unlikely that long-range connections such as the corpus callosum and other reciprocal corticocortical connections contribute to the feature-specific responses of visual cortical neurons evoked within the classical receptive field (*14*). Rather, callosal and other reciprocal long-range connections may create temporal relations between responses of spatially distributed neurons (*2–4, 11*). Thus, by synchronizing oscillatory responses in visual areas, these connections could contribute to the binding of distributed features of objects that are present in a visual scene. Transcallosal synchronization could then mediate the binding of features across the midline of the visual field.

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5. Animals were anesthetized with a combination of

70% N_2O plus 30% O_2 and 0.2 to 2% halothane. Multiunit activity was recorded with arrays of 4 to 6 Pt-Ir electrodes with a spacing of 0.4 to 1 mm (*3*) that were placed in area 17 of either hemisphere close to the border of areas 17 and 18 [R. J. Tusa, L. A. Palmer, A. C. Rosenquist, *J. Comp. Neurol.* **177**, 213 (1978)]. In one of the experiments, in which 98 pairs of multiunit responses were recorded, the location of the electrodes in the vicinity of the border of areas 17 and 18 was verified histologically. In all recordings, the cells had receptive fields located within 4° of the vertical meridian and velocity preferences typical of area 17 neurons. For further details of recording and data processing see (*2, 3*).
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8. At 9 months of age, two cats were anesthetized with a mixture of ketamine and xylazine. After craniotomy, the corpus callosum was cut, by aspiration, as far forward as stereotaxic level A15 to eliminate all visual connections (*6*). The cats were tested several months after surgery (*5*). The use of arrays of multiple electrodes for recording from either hemisphere enabled us to monitor, as an internal control, the quality of the interactions within left and right area 17. Thus, we recorded 42 intraareal cell pairs, 33 of which displayed synchronized oscillatory responses (Fig. 3). Neuronal responsiveness was normal in the border zone despite the callosal lesion (7). The receptive fields of all cells recorded were located within 4° of the vertical meridian. In 46 of 82 interhemispheric response pairs, the receptive fields were overlapping, and in 40 pairs the orientation preferences at the two recording sites differed only by 0 to 22°. Electrode penetrations were marked by electrolytic lesions and verified in Nissl-stained sections. The callosal lesion was verified by macroscopic inspection of the perfused brain and by myelin-staining of frontal sections through the corpus callosum by the Heidenhain-Woelcke method. (M. Woelke, *J. Phychol. Neurol.* **51**, 199 (1942)].
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Fragile X Genotype Characterized by an Unstable Region of DNA

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DNA sequences have been located at the fragile X site by in situ hybridization and by the mapping of breakpoints in two somatic cell hybrids that were constructed to break at the fragile site. These hybrids were found to have breakpoints in a common 5-kilobase Eco RI restriction fragment. When this fragment was used as a probe on the chromosomal DNA of normal and fragile X genotype individuals, alterations in the mobility of the sequences detected by the probe were found only in fragile X genotype DNA. These sequences were of an increased size in all fragile X individuals and varied within families, indicating that the region was unstable. This probe provides a means with which to analyze fragile X pedigrees and is a diagnostic reagent for the fragile X genotype.

Fragile X syndrome is the most common form of familial mental retardation (*1*). It is associated with a rare, fragile site at Xq27.3 (FRAXA); this association allows for cytogenetic prenatal diagnosis and carrier detection, although incomplete penetrance of the fragile site renders these procedures inaccurate. The genetics of the syndrome are bizarre. Normal men and women can transmit the fragile X, although they do not manifest any symptom of the fragile X syndrome themselves and do not express the fragile site cytogenetically. Such transmitters or carriers can have intellectually handicapped children or grandchildren with the fragile X syndrome (*1*). The diagnosis of the fragile X genotype relies on polymorphic DNA markers that are closely linked (*2*). Neither the molecular basis for the syndrome nor the mechanism of expression of the fragile site is understood. To obtain a better understanding of the syndrome and the site, we have previously isolated a 275-kb fragment of human DNA in a yeast artificial chromosome (XTY26) that spanned the fragile site at Xq27.3 (*3*). This clone was constructed from the DNA of a fragile X–affected individual and therefore ought to contain the sequences necessary for expression of the fragile site.

To identify sequences that constitute the fragile site and to screen for DNA differences between normal and fragile X individuals in the vicinity of the fragile site, we used sequences from XTY26 as hybridization probes. We localized the fragile site by first establishing a contig of λ subclones between the two closest probes that flanked the fragile site. One of these probes (VK16, Fig. 1) was first used to isolate XTY26; VK16 has been localized proximal to the fragile site by in situ hybridization (*3*). We established the distal end of the contig by screening the λ library of XTY26 with an Alu polymerase chain reaction (PCR) product (*4*) referred to as Alu2 (Fig. 1). Subclone 91 was isolated with this probe, and it was subsequently demonstrated by in situ hybridization that the probe mapped distal to the fragile site.We used RNA probes from each end of 91 to chromosome walk away from this

Table 1. Number of individuals with each band type seen in Southern blots probed with pfxa3 (Pst I digests) in 136 fragile X individuals from 25 families and 130 unrelated controls. Males were classified as affected if they had expression of the fragile site in lymphocyte culture (*1*), mental retardation, and dysmorphic features of the fragile X syndrome (*1*). Males were classified as "transmitting" if they were phenotypically normal (no fragile site expression, no clinical features of the syndrome, and intellectually normal) and if they had either the appropriate position in the pedigree or if they had a high probability, on the basis of flanking DNA polymorphisms, of having the fragile X genotype (*9*). Female carriers were classified as affected or normal on the basis of intellectual status, regardless of fragile site expression.

Classification	Normal band	Single band of increased size	Two to four bands of increased size	Multiple bands (smear)
		Males		
Affected	0	18	10	5
Transmitting	0	11	1	0
Normal	65	0	0	0
		Females		
Normal carriers	82	71	5	6
Affected	9	4	3	2
Normal	65	0	0	0

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