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Correlated activity of lateral geniculate neurones in binocularly deprived cats

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Abstract. Pairs of single neurones were recorded simultaneously from lateral geniculate nucleus of adult cats binocularly deprived of pattern vision by rearing in masks. Receptive fields of cells in laminae A and A1 were highly abnormal with unusually small proportion of Y-type fields and some neurones characterized by ON/OFF type of responses. In spite of these changes all crosscorrelogram patterns between spontaneous firing of these cells were similar to those previously observed in normal cats.

Key words: cat, binocular deprivation, lateral geniculate nucleus, correlated neuronal activity

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INTRODUCTION

It has been postulated that correlated temporal activity of single neurones in the vertebrate visual system plays a crucial role in establishment of the appropriate pattern of connections during development. This postulate is supported by several lines of experimental evidence. First, appearance of binocular cells in the visual cortex requires binocularly corresponding visual input to both eyes (Hubel and Wiesel 1965). Second, silencing ganglion cells with blocker of sodium channels tetrodotoxin in the neonatal kitten leads to abnormally high degree of convergence of ON/OFF and X/Y retinal inputs to cells at the dorsal lateral geniculate nucleus (dLGN) of the adult cat (Archer et al. 1982, Dubin et al. 1986). Such an atypically high degree of convergence has not been reported after monocular deprivation, lid-suturing or dark rearing in which ganglion cell activity was preserved despite of the fact that all these deprivation methods resulted in abnormal neuronal responses in dLGN (Kratz 1982, Sherman and Spear 1982).

In our previous study concerning cats binocularly deprived of pattern vision throughout the first 6-7 months of postnatal life we have reported abnormal receptive fields of dLGN neurones (Michalski and Wróbel 1986). These abnormalities included seven percent of neurones with receptive fields in which ON/OFF regions spatially overlapped. Unlike in the adults, in the young kittens a substantial proportion of dLGN neurones has spatially overlapping ON and OFF discharge regions within their receptive fields (Daniels et al. 1978). In the present experiment we investigated whether such abnormalities found in the binocularly deprived cats were accompanied by atypical correlations in maintained firing of geniculate neurones.

METHODS

Six cats were binocularly deprived of pattern vision by rearing in masks from the time of eye opening. The masks prevented pattern vision but allowed access of scattered light to the retinae. Average reduction of eye illumination (about 2-6 log units) was comparable to that produced by eyelid suture (for details see Żernicki 1991). While three cats wore masks for 5 to 7 months up to the time of acute experiments, three others were reared in masks to the same age and thereafter trained in visual pattern discrimination tasks for another two months before the experiment. Since the observation in both groups were similar all data presented in this paper are pooled together.

The details of the acute experiments are described in detail in our earlier publication (Michalski and Wróbel 1986). In brief, under ether anaesthesia the brainstem transections were performed at the pretrigeminal level (Żernicki 1986). The femoral vein and trachea were cannulated. The animals were paralysed with gallamine triethiodide (Flaxedil: initial dose 100 mg, maintenance dose 20 mg/h) and artificially ventilated with room air. End-expiratory CO_2 was maintained at 3.5% by adjusting the tidal volume delivered by respirator. Temperature was kept at about 38°C with an automatic heating pad. Fluid balance was maintained by subcutaneous injection of 5% glucose in saline solution. The eyelids and nictating membranes were retracted with neosynephrine and pupils dilated with atropine. Contact lenses were used to protect the corneas and correct the refractive state of the eyes. The recording started not earlier than 2 h after the surgery.

Tungsten in lacquer single microelectrodes with the 20 μm free tips were used for multiunit recordings from the dLGN. Two to three cells were sorted out on the basis of the amplitude of their action potentials and shape by means of amplitude separation method. The original signal was stored on magnetic tape (Racal recorder) for off-line analysis. An array of five additional electrodes was fixed in the primary visual cortex (areas 17 and 18) and used for antidromic activation of dLGN relay cells. Geniculate cells with receptive fields outside the zone of stimulation were classified on the basis of position and physiological properties. The skull openings were sealed with agar. Small electrolytic lesions were made at selected microelectrode depths for future track reconstructions. After the ex-

periment the animals were perfused and brains stored in formaline for further verification with standard histological procedures.

Visual stimuli were displayed on a white tangent screen located 57 cm in front of cat's eyes. A hand-held projector was used for initial characterization and plotting of neuronal receptive fields. An electronically controlled light slit subtending 0.4 x 0.75 degrees was used for detailed analysis. The stimulus intensity was 10 cd/m^2 and the background level was routinely 1 cd/m^2.

Data analysis was done on-line with a Cromemco Z-80 computer. The spike train blocks from different neurones encompassing at least 3 min were stored as different time events. The autocorrelation functions were then calculated to characterize individual trains. The crosscorrelation functions between simultaneous firing of two cells at a time, during spontaneous (against the standard background) and evoked (taken during visual stimulation of the receptive field) activities were then build and displayed at two standard time windows: 0.3 and 0.05 s (Fig. 1A and B). All receptive fields were characterized by "response plane" method (Stevens and Gerstein 1976a). Under computer control the slit of light was switched on and off at 30 different positions along the vertical diameter of the receptive field. The step size was usually 0.5°. Each particular position of the slit corresponded to one peristimulus time (PST) histogram. One complete traverse of the stimulus added one repetition to each of 30 PST histograms. Fifteen repetitions were routinely used. All PST histograms were displayed together (Figs. 2 and 3, left columns) to form a plot of the averaged time course of cell firing rate (Z axis) as a function of time counted from stimulus onset (X axis) and location of the stimulus in space (Y axis). One PST histogram (the lowest in each response plane) was taken without a stimulus and, therefore, represented spontaneous activity. The slice through the response plane on the spontaneous firing level (Figs. 2 and 3) was called contour plane and consisted of holes and solid regions representing respectively domains of lowered and increased probability of cell firing.

RESULTS

Though three of the six cats used in this experiment had postdeprivational visual experience (see methods), the results obtained in both groups were not different and therefore they are presented together. The activities of 33 pairs of single neurones recorded from main dLGN laminae (A and A1) were analyzed. The selection was based on histological reconstructions of electrode tracks and a sequence of receptive fields (RFs) in the track. Since some neurones had RFs outside the access of antidromic activation via cortical electrodes, it is possible that a recorded sample includes intrageniculate interneurones. The crosscorrelograms and autocorrelograms were computed for all selected pairs. Six units were clearly not action potentials but S potentials as characterized by Bishop and his colleagues (1962). Accordingly, correlograms of these units with simultaneously recorded spiking cells were of typical monosynaptic type (Levick et al. 1972, Wróbel 1981, Mastronarde 1987). The sharp peak in each of these correlograms indicated high probability of spikes occurring at a fixed interval after the S potentials. Figure 1 A and B shows one of these pairs.

Altogether, 39 units were recorded in groups of three (9 triplet cases) or two (12 pairs). This sample contains only those cells which could be classified as X or Y units. The classification was based on the rules established before (Michalski and Wróbel 1986). The X type cells differed from Y type in that they possessed spatially non-overlapping center and surround excitatory domains. Their center responses were also typically more sustained than it was found for Y type cells. The diameter of the receptive field centres in given eccentricity was also taken into account as differentiation factor with the reservation that the deprived cells could have quite large receptive field diameters in the area centralis (Michalski and Wróbel 1986). In more ambiguous cases we also relayed on the antidromic electrical stimulation of the visual cortex provided that the measured latencies were in the non-overlapping zones for the two (X and Y) populations (Lindström

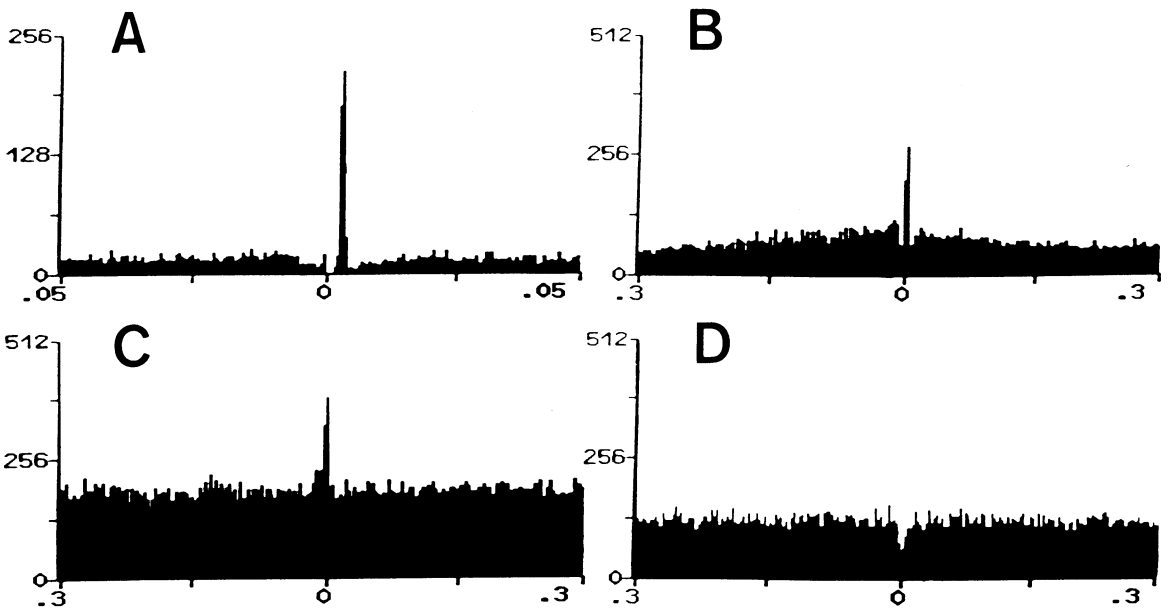

Fig. 1. A and B, the crosscorrelograms of spontaneous activity of simultaneously recorded S potential and action potential. The receptive fields of both of the corresponding cells were characterized as X type / OFF-center. Note different time windows in (A) and (B). The shifted peak in (A) indicates the monosynaptic connection. C, excitatory crosscorrelogram between two spontaneously active, X type / OFF-center geniculate cells with the centrally located peak. D, inhibitory type of crosscorrelogram between spontaneous firing of two Y type / OFF-center geniculate cells.

and Wróbel 1990). For half of the sample the response planes have been also obtained and the detailed analysis confirmed the previous classification based on the analysis with hand-held stimuli. Only five units were found to be of Y type and this proportion (13%) agrees well with that of the larger sample obtained previously in our laboratory (Michalski and Wróbel 1986). This finding confirmed the large decrease of Y cell ratio in dLGN after binocular deprivation since the sample measured in normal cats with the same type of electrodes and method of analysis reached 48% (Stevens and Gerstein 1976b).

All 6 pairs that consisted of S potential and dLGN spike were identified as X input to X cell. Judging from their responses to the visual stimuli, three of these cells could belong to the lagged category as described by Mastronarde (1987, see example in Fig. 1A and B). The average contribution of S events to firing of dLGN cell and the duration of excitatory input peak calculated for our recordings (20%, 1.16ms and 50%, 1.16ms for presumed lagged and nonlagged cells respectively) resembled the values of normal sample given by Mastronarde (17%, 1.17ms and 71% and 1.16ms). This fit is worth noting since the receptive field of most of the target dLGN cells were atypical with the surround weaker as compared to normal fields and one of them characterized by the ON/OFF overlapping responses as exemplified in Fig. 3C. (The strength of the surround was here defined as proportional to the spatiotemporal extent of its domain on the spontaneous contour plane, as described in detail in our previous study, Michalski and Wróbel 1986). It is possible that larger sample could show significant deficit in the calculated S potential contribution to the dLGN cell firing since the disparity between receptive field centres measured for two such pairs could indicate existence of other excitatory inputs.

Among 27 pairs of units identified as dLGN cells, fourteen did not show any deviation from flat correlograms when spontaneous activity was used for computation (Fig. 2C, left correlogram). Since all the involved cells were recorded by single electrodes, they had also partially overlapping receptive fields. Similar proportion of unrelated cells (14 out

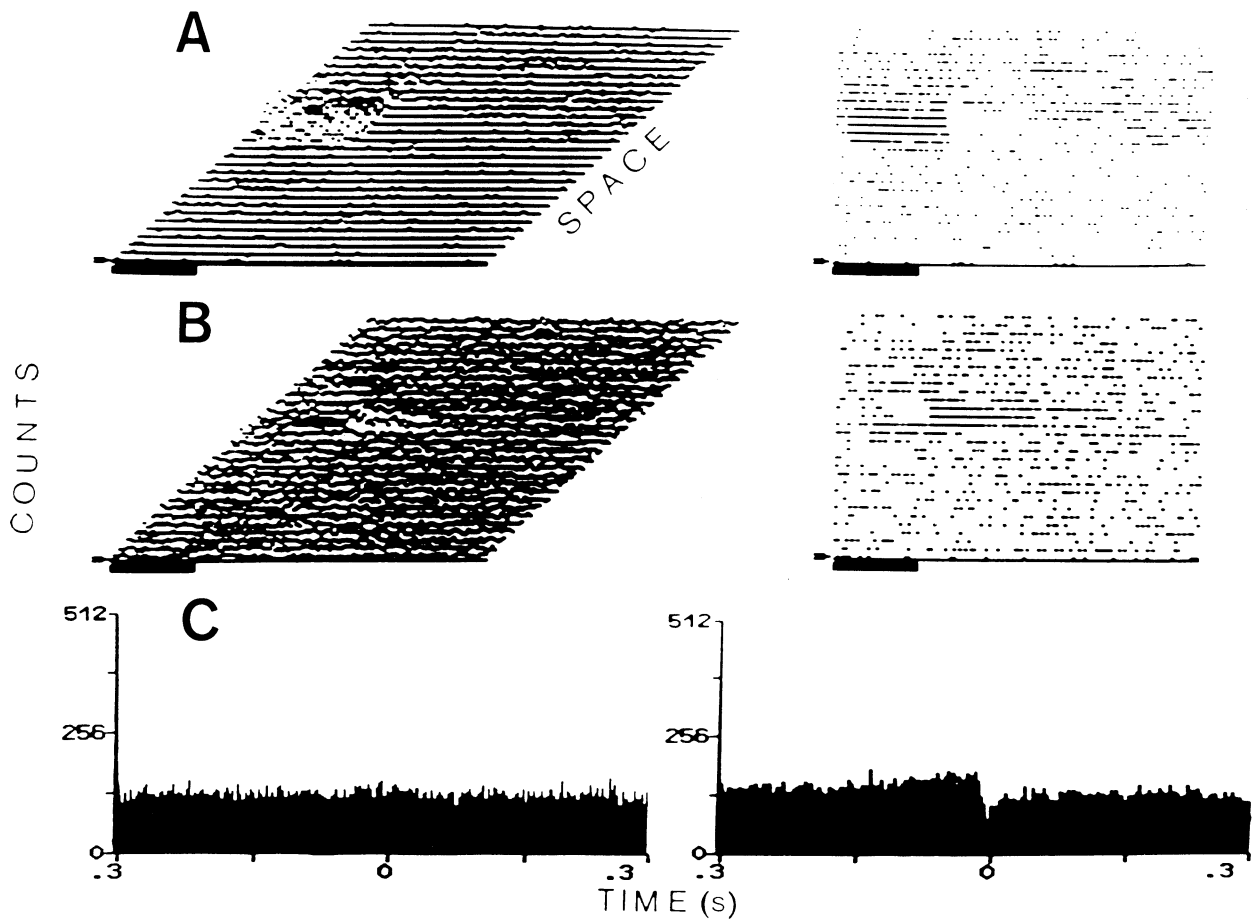

Fig. 2. A and B, response and contour planes of two geniculate X cells: one ON-center (A) and one OFF-center (B). C, the crosscorrelograms of spike trains of these cells during spontaneous firing (left) and during receptive field stimulation (right). The space axes for A and B overlapp.

of 31) has been reported for dLGN cells in normal cats (Stevens and Gerstein 1976b). Some of these cells exhibited non-flat correlograms when their overlapping fields were simultaneously stimulated by the same stimulus. The example of such a coordination of firing evoked by stimulation of both receptive fields by the common stimulus (testing spot) is shown in Fig. 2C (right correlogram). The central well indicates that both cells tended to withhold firing at the same time and that after the cassation of the light stimulus there was an increased firing probability off the OFF-center cell.

Eight pairs showed correlograms between spontaneous firing with a narrow peak around zero (Fig. 1C). Only the peaks that exceeded the noise level by at least 20 % were considered significant. Average duration of these peaks at the background level was 8.5 ms for seven X cell pairs (range 4.5 to 15 ms) and 12 ms for one Y pair. These values are in the same range as those described by Mastronarde (1989) for fast shared inputs to ganglion cells. Four other pairs in our experiments had excitatory peaks which were between 30 and 90 ms wide (average 57 ms). Examples of correlation peaks of similar duration were presented by Stevens and Gerstein (1976b) in dLGN of normal animals. All neurally coordinated pairs of neurones were of similar center types: ON or OFF, though some of the cells had abnormal surrounds of their receptive fields as previously described (Michalski and Wróbel 1986; see also response plane in Fig. 3A). Some of the correlograms also exhibited wider central peaks during

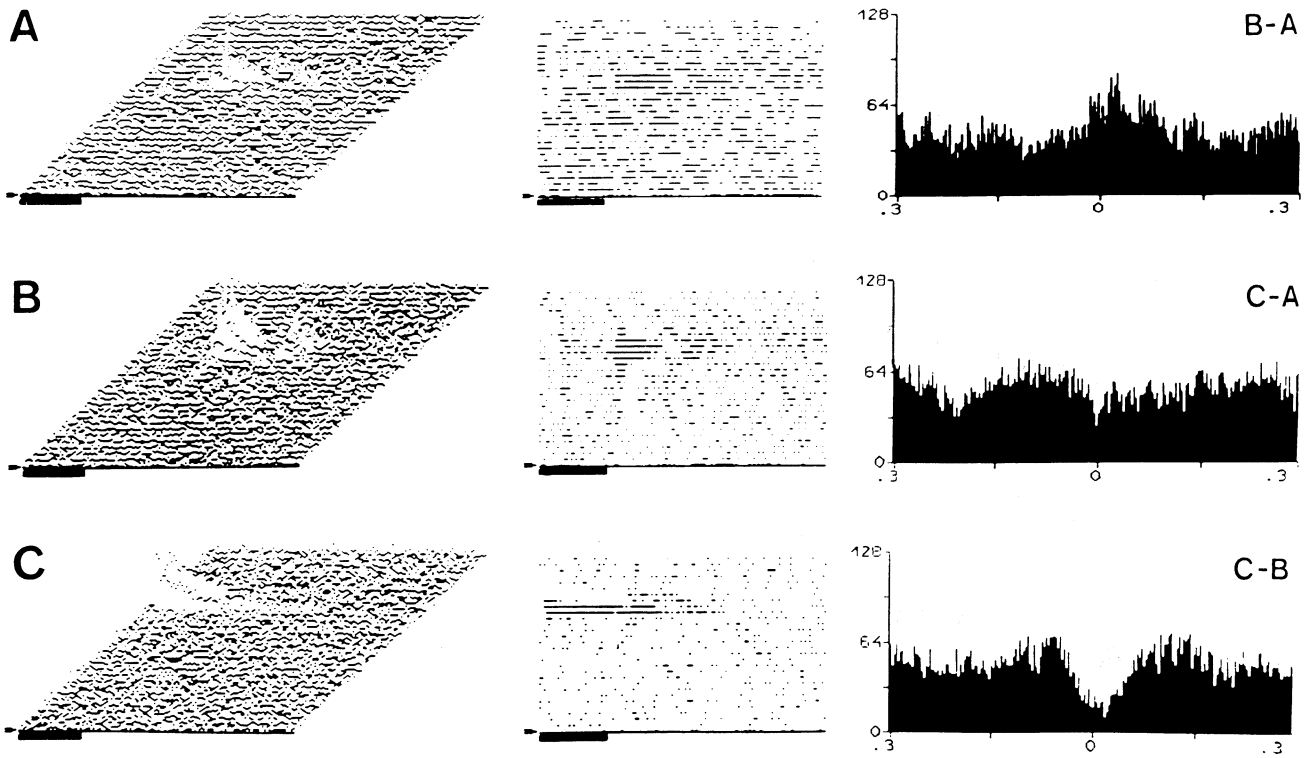

Fig. 3. The response planes (left column) and contour planes (middle column) of three cells (A, B, C) recorded simultaneously from dLGN of deprived cat. The crosscorrelograms in between their spontaneous activities are shown in the right column. The directions of correlations are indicated above the right parts of the crosscorrelograms. Space axes aligned.

epochs of visual stimulation. Since our experiment was not designed to study these effects, we have not analyzed these correlograms. They are in line with recent data indicating that principal cells might be neurally coordinated via the layer 6 pyramidal cells in the visual cortex (Sillito et al. 1993).

We found only three pairs of cells with central well in the correlograms calculated with trains of spontaneous activity. The wells were centred at zero time, deep for at least 30% of the background noise level and wide for 10, 14 and 150 ms. They are shown in Figs. 2D, 3B and 3C. All these pairs consisted of cells with opposite ON- and OFF-center types. The limited number of selected pairs with such correllograms was due to the fact that some of the central wells had to be disqualified since they could be produced by triggering system during elaboration of the data from a single electrode (Wróbel 1982). It is known that such a method can introduce the artificial trough in the center of the correlogram which in our setup could last for at least 4 ms. Thus only the wider inhibitory troughs could be analyzed.

Among the analyzed pairs, those of special interest possessed the abnormal receptive fields. ON center cell with receptive field plot is shown in Fig. 3C had abnormal OFF responses from the receptive field center similar to responses previously found in young kittens (Daniels et al. 1978) and in binocularly deprived animals (Michalski and Wróbel 1986). The OFF response was not a lagged feature since the cell was classified as nonlagged on the base of its short latencies for visual (Fig. 3C, middle column) and antidromic stimulations. Nevertheless, neither of two correlograms with overlapping OFF center receptive fields showed a central peak which could reveal the common or shared input originating in OFF fibers. Instead, both had the central wells indicating that the cells of the opposite type (ON and OFF center cells) tend not to fire simultaneously.

DISCUSSION

Correlation analysis of simultaneously recorded units provides an important method for studying connectivity, yet the interpretation of results can be difficult. In particular, such an interpretation should take into account the neuronal interactions among afferent inputs which could be responsible for correlated activity. For example, the central well in correlograms between the ON- and OFF-center LGN cells has been taken as evidence for reciprocal inhibition (Stevens and Gerstein 1976b, Wróbel 1982). It was shown later, with direct intracellular recordings from principal dLGN cells, that they receive feed-forward inhibition exclusively from intrageniculate interneurones of the same center type (S. Lindström and A. Wróbel, unpublished observations). It appears that the tendency of an ON and OFF dLGN cells not to fire at the same time can be explained simply by the tendency of their respective ON and OFF retinal inputs not to fire simultaneously (Mastronarde 1989).

All central wells in correlograms appearing among the spontaneously active dLGN cells could be therefore thought of as having the retinal origin. Mastronarde (1983 a,b) found two processes causing decremental central correlations between retinal ganglion cells: a fast shared input at all light levels, responsible for wells of 4 to 20 ms and a slow shared input at low light levels which was a source of 80 to 100 ms long wells. It appears that both processes can be traced on the level of geniculate activity. Similar correlograms were found in the dLGN of normal cats by Stevens and Gerstein (1976b). Our experiment indicates that they are present in visually deprived cats as well.

Similar interpretation can be applied for central incremental correlograms. Both short and long duration central peaks were found in correlograms of normal (Stevens and Gerstein 1976b) and binocularly deprived (the present study) cats and could also be predicted by retinal coordination of firing. We started the present study hoping to trace the abnormal convergence of inputs in highly abnormal ON/OFF cells found in our previous experiments (Michalski and Wróbel 1986). The mixed responses of these cells resembled the activity of immature neurones found by Daniels et al. (1978). It was plausible to think that convergence from both ON and OFF type cells could underlie such responses. However, this notion was not supported by the present data.

The source of the OFF excitation in the ON center cells as found in binocularly deprived cats (Michalski and Wróbel 1986) remains obscure. It might be produced by small active inputs which cannot be traced by the correlation technique as used in this study. The larger displacement of the retinal input receptive fields to the principal cells of deprived cats as found in the present experiment might suggest that they indeed receive more convergent inputs than normal. On the other hand, it cannot be excluded that the prolonged central excitation as shown on the response plane in the Fig. 3C could be evoked by other membrane or network rebound-type mechanisms.

The finding that the crosscorrelations of firing between the pairs of neighbouring neurones in the main laminae of dLGN in the binocularly deprived cats are not different from those in the normal animals is surprising in the view of the data indicating the functional role of correlated firing in development (Archer et al. 1982, Dubin et al. 1986, Meister et al. 1991). There are two possible explanations of this finding. Either "the spontaneous activity" in neonatal kittens (Meister et al. 1991, Goodman and Shatz 1993) is more correlated than it was expected or the deprivation method used by us allows for some coordination of neuronal activity due to the non-patterned visual stimulation.

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