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Visual classification of X and Y perigeniculate neurons of the cat

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Abstract The spike activity of perigeniculate cells evoked by small light spots flashing along the axes of their receptive fields was recorded and presented in response planes. This method allowed the investigated neurons to be grouped into two classes characterized by (1) large receptive fields and phasic-like responses and (2) small fields and tonic responses. The latency measurements for stimulation of the optic chiasma and visual cortex revealed that the cells from the first group are excited by fast, Y fibers and the second by slow, X axons. The spatial tuning curves of the second harmonic component, as measured from the responses of the cells from the two groups for slowly moving square gratings, are also different. We conclude that the X and Y systems of the visual pathway are segregated at the level of the perigeniculate nucleus.

Key words Perigeniculate nucleus X and Y cells Cat

Introduction

There is no doubt that the concept of parallel processing in the visual system via X and Y pathways has been one of the most fruitful hypotheses investigated, from the time when it was first presented by Enroth-Cugel and Robson (1966). Electrical stimulation (Hoffmann et al. 1972), visual stimulation (Shapley and Hochstein 1975) and anatomical (Friedlander et al. 1981) methods have shown that the relay cells in the A layers of the lateral geniculate nucleus (LGN) of the cat can be divided into two distinguishable groups, X and Y. It has been shown that both types of principal cell receive feed-forward inhibition, via intrageniculate interneurons placed with in the main laminae, and recurrent inhibition via pengeniculate interneurons – the thin layer of cells called perigeniculate nucleus (PGN) and located just dorsal to he LGN (Dubin and Cleland 1977; Lindström 1982). Whether both types of interneurons intermingle in the X and Y streams of information was a matter of controversy (Singer and Bedworth 1973).

It is now generally accepted that feed-forward inhibition is subserved by separate X and Y intrageniculate interneurons (Lindström and Wróbel 1990). The remaining possibility of convergence of X and Y pathways at the thalamic level involves the penigeniculate recurrent interneurons. Dubin and Cleland, in their original experiment (1977), postulated from latency measurements that all perigeniculate interneurons would receive information from the Y pathway, but they did not exclude the possibility that some PGN cells could have an additional input from the X system. The possible convergence of X and Y pathways has also not been ruled out by So and Shapley (1981), who used sine wave grating stimuli for response-type differentiation. They found that the responses of the cells in the pengeniculate nucleus were "variable". On the other hand Ahlsen et al. (1983) claimed that in pretrigeminal cats they could readily differentiate two groups of PGN, neurons by slowly moved square-wave gratings.

With the use of intracellular recording from principal cells, we have recently shown (Lindström and Wróbel 1990) that recurrent inhibitory potentials originate from the same (X or Y) pathway as their retinal excitatory inputs, indicating that recurrent interneurons could be separated into different types.

In our previous experiment (Wróbel and Tarnecki 1984) we used the sensitive method of response planes to analyze receptive fields of geniculate interneurons. We could differentiate the X and Y fields of intrageniculate interneurons and also observe the tonic- and phasic-like responses of different perigeniculate cells. In this experiment we investigated the PGN cells in more detail to find if they could be grouped into separate physiological classes.

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Materials and methods

For these experiments we used ten adult cats weighing 2.1—3.3 kg. The brainstem of each animal was transected at the pretrigeminal level under ether anesthesia, which was subsequently discontinued (ernicki 1974). The femoral vein and trachea were cannulated. The animals were then paralyzed with gallamine triethiodide (Flaxedil; initial dose 100 mg, maintenance dose 20mg/h) and artificially ventilated with room air. End-expiratory CO_2 was maintained at 3.5-4% by adjusting the tidal volume delivered by the respirator. The animal's temperature was kept at 38° C with an automatic heating pad, and fluid balance was maintained by subcutaneous injection of 5% glucose in saline solution. The eyelids and nictitating membranes were retracted with neosynephrine, and the pupils were dilated with atropine. Contact lenses were used to protect the corneas and correct the refractive state of the eyes (at the average of + 1 D). The recording started not earlier than 2 h after the surgery.

Hubel-type tungsten microelectrodes were used for single cell recording. The perigeniculate cells were initially recognized by conventional criteria, using electrical and visual stimuli (Ahlsen et d. 1982). An array of six electrodes was inserted into the visual cortex for antidromic activation. Two other macroelectrodes were placed at the level of the optic chiasma for orthodromic stimulation of the pathway. Single (or occasionally paired) rectangular pulses of 0.1 ms duration and strength 100 µA (and 15 µA respectively), were usually enough to evoke the responses of the PGN region. Stimuli stronger then 1 mA were not used. The lateral part of the visual field was beyond the reach of our cortical stimulation, and in these cases we relied on the visual testing and histological verification.

Visual stimuli were displayed on a white perimeter-like screen located 70cm in front of the cat's eye. The screen was illuminated with the background light in the mesopic range 0.5—3 cd/rn^2. With such a background, even non-treated animals have their pupils almost fully dilated. A handheld projector was used for initial characterization of the receptive fields and a bar of light subtending 0.6°/1° for more detailed analysis, by response planes (Stevens and Gerstein 1976). This method is, in our experience, a powerful tool for studying even subtle changes of receptive field organization. The response plane is a stereoscopic view of 30 PSTHs obtained "simultaneously" for cyclic stimulation of 30 separate points spread over the receptive field axis with the typical step of 0.6° (Fig. 1). In our experiments the stimulus is usually switched on for 640 to 1000 ms and then off for consecutive 2 s. The slices cut off from the response plane at different levels of probability of firing result in contour plane pictures (Fig. 1). On the "spontaneous contour plane" dark and white domains correspond to the spatiotemporal areas of the cell activity that are higher or lower than the spontaneous activity. We shall refer to them as "excitatory" and "inhibitory" domains (Stevens and Gerstein 1976).

For the spatial frequency tuning test, a set of slides had been prepared with stripe patterns of rectangular luminosity changes and spatial frequencies from 0.1 to 2 cycles/deg. They had contrast of 0.25 with the luminance of white stripe 5 cd/rn^2. The slides were moved with the speed of 1 deg/s, left and right along the receptive field axis, encompassing always its whole area. The obtained PSTHs were analyzed into their Fourier components: the fundamental component at the temporal frequency of the stimulus modulation and second harmonic component at a frequency that was twice as much. The relation between these Fourier component amplitudes and spatial frequency of the stimulus is called spatial frequency response function (Shapley and Lennie 1985).

Results

General

We positively identified and recorded 45 cells from the perigeniculate nucleus. They were placed at distances from 1300 to 80 µm above the first principal cell encountered, and in general their receptive field positions overlapped the columns described by Sanderson (1971) for LGN. The visual characteristics of PGN cells were reminiscent of those observed elsewhere (Dubin and Cleland 1977; So and Shapley 1981; Ahlsen et at. 1982, 1983; Wróbel and Tarnecki 1984). In general, their receptive fields were roughly circular with on-off responses driven through both eyes. Of these cells, *52%* had a dominant response from the contralateral and 37% from the ipsilateral eye. On average the receptive fields dominated by the input from the ipsilateral eye were located significantly (Mann-Whitney U-test, P <0.02) further from the surface of the LGN (743 i.tm) than the fields with larger contralateral input (384 !.tm). This observation suggests that the perigeniculate nucleus might possess a laminated structure similar to that of the LGN. All cells in the PGN responded to flashes diffusely illuminating the screen, although smaller center spots tended to evoke more tonic responses. This feature differentiated our population from that observed by So and Shapley (1981) and Ahlsen et at. (1983), who found that large flashing stimuli did not excite the cells.

The spontaneous activity was often of a bursting type and did not exceed the frequency of 40 impulses/s (with most cells active in the range 15—20 impulses/s). The average frequencies were not different for the cells from the two groups defined below. Some of the cells showed tonic firing characteristics reminiscent of the firing of lagged LGN cells that have been recently described (Mastronarde 1987). We did not classify these cells separately.

Classification of the spatiotemporal receptive fields

The response plane of the typical PGN cell is dominated by two domains of elevated firing probability, corresponding to the appearance and cessation of the light stimulus within the receptive field (Fig. 1). Most of the response planes had comparable on and off domains; only six cells responded markedly less for one stimulus phase (e.g. Fig. 2C). It appeared easy to classify these excitatory domains in the same way as the primary excitatory domains of reèeptive fields of principal cells within the lateral geniculate nucleus (Stevens and Gerstein 1976). Thus one group of cells was characterized by tonic-like on and off responses in any position within the field. On the spontaneous contour plane, the on domain lasted for such cells during the whole on period (Figs. 1A, B, 2A, B). The observation that on this plane the off domains lasted shorter might result from the lack of the stimulus in the off time. The responses of the cells characterized above suggested that they were excited by X-type geniculate neurons and consequently we called them X PGN cells. Further evidence for such a conclusion will be given in the next section.

The excitatory domains of the other group of cells (Figs. 1C, D, 2C, D) could be described as phasic-like,

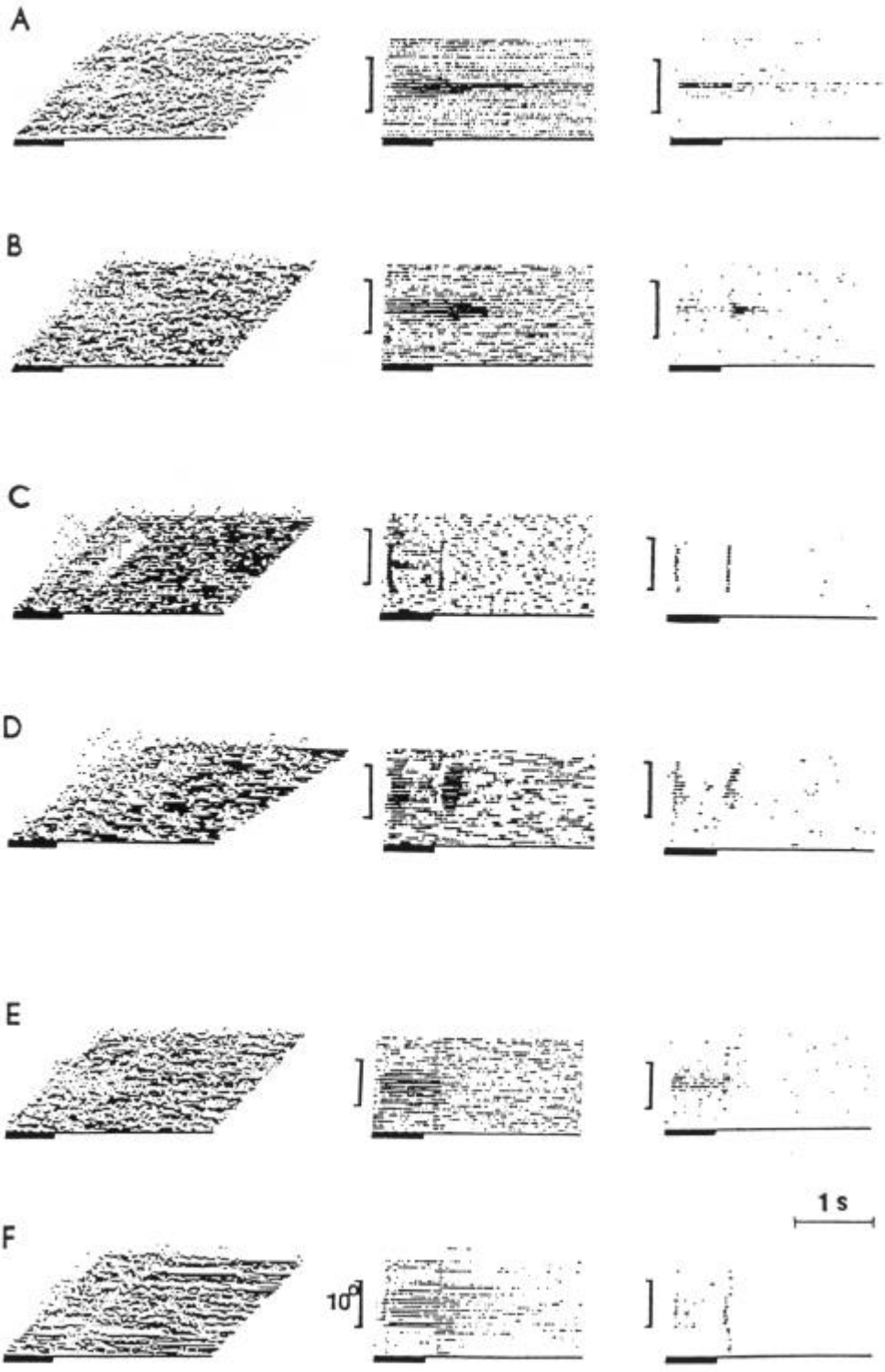


Fig. 1 The spatiotemporal pictures of receptive fields for two X-type (A, B), two Y-type (C, 0) and two mixed type (E, F) perigeniculate neurons. In *the first column* the three-dimensional response planes are shown. The *second column* contains spontaneous contour planes, and the *third* shows the high-probability cut contour planes. The *lowest histogram* on the response planes (or the *lowest line* on the contour planes) shows the activity without the stimulus. The *filled horizontal bar* represents the on-time of the stimulus (light spot 0.60/1°)

i.e., ceasing during the on time of the shortest stimulus (640 ms), with the duration variable along the axis of the field. Cells with response planes characterized by phasic excitatory domains were called Y.

We found that *85%* of the PGN cells could be classified according to these rules, 16 of them having X-type receptive fields and 22 the Y-type. All of these cells had the same type of fields when measured from both eyes (Fig. 2). Thus, we have shown that the majority of the PGN cells can be divided into two separate groups according to the spatiotemporal organization of their receptive fields.

Eight receptive fields dropped out from the above classification with mixed characteristics. Seven of them *(15%)* had mixed tonic/phasic inputs from the same eye (Fig. 1E, F) and one had an X-type field from the

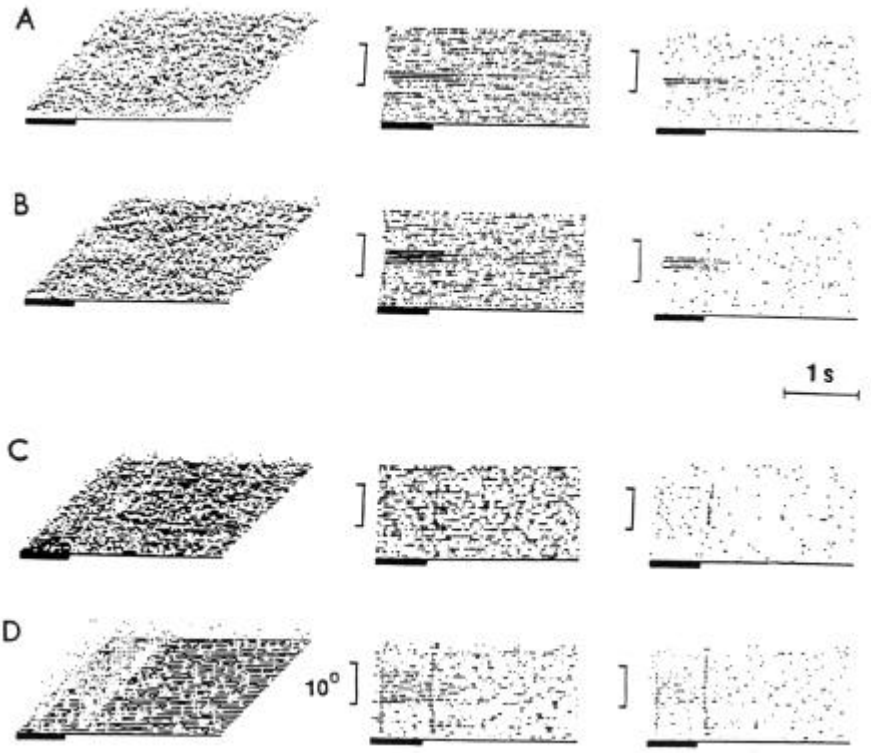
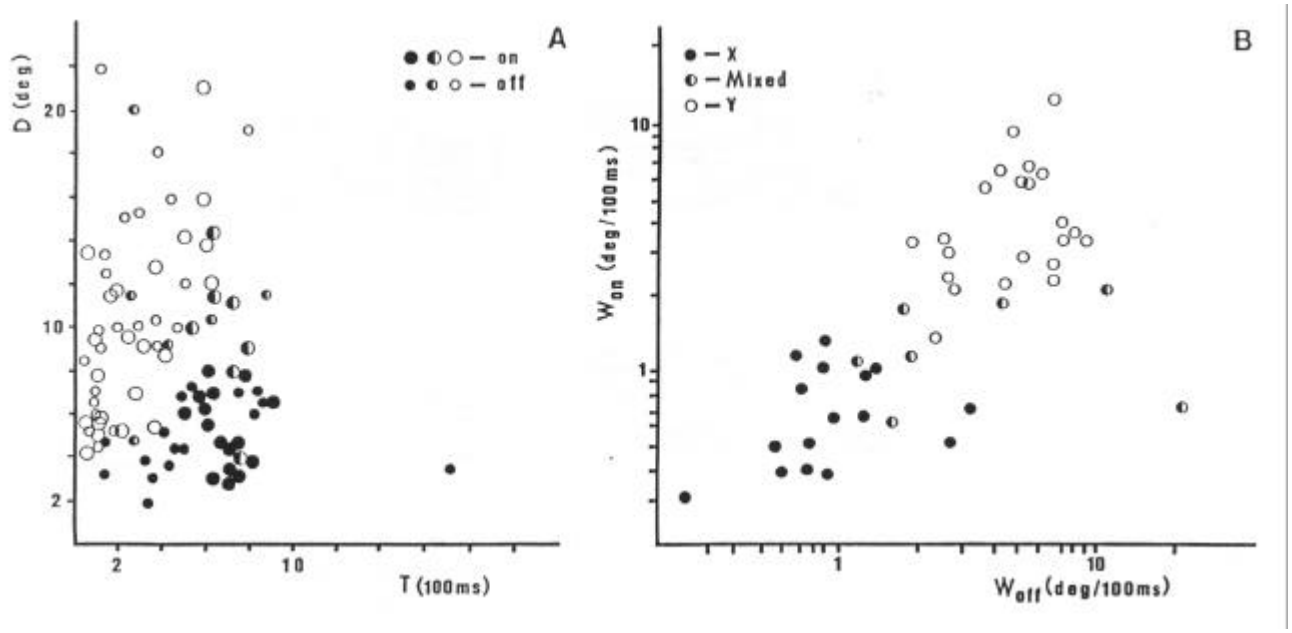


Fig. 2A—D Examples of the receptive fields of two pen-geniculate neurons taken separately from ipsi and contra eyes. **A, B** X cell; **C, D** Y cell. **A, C** Response and contour planes for ipsilateral eyes. **B, D** Corresponding planes taken for the receptive field of the contra eyes. Other explanation as in Fig. 1

contra eye and Y-type field from the ipsi eye (not illustrated).

The spatial (D) and temporal (T) extends of the excitatory domains that have been described were measured and plotted in Fig. 3A. Here the X and Y domains are distinguished by black and white symbols. The mean angular sizes for on and off domains were not different and were calculated as 50 (SE = 0.5°) for X fields and 10.1° (SE= 1.3°) for Y fields. These values were significantly different (t-test, P <0.05). The mean eccentricity was 100 and did not differ between the classes. Field diameters measured in our experiment are about twice as large as those given by Ahlsen et al. (1983), even considering the correction for eccentricity. Such a difference comes from the sensitive averaging method of response plane. The estimation of the receptive field sizes with handheld stimuli and by listening to the response from the loudspeaker gave results smaller by a factor of 0.6.

Fig. 3 A Relation between temporal *(T)* and spatial *(D)* extents of the excitatory domains of the receptive fields of the *45* PGN neurons. B The graph shows relation between W ratios for on and off domains. *W* is defined as the ratio of the spatial extent of the receptive field to the duration of it, as measured on the contour planes cut at the probability of firing doubling the spontaneous rate



Such results can be obtained also from the contour planes but on the firing probability level twice that of the spontaneous level. From such contour planes we also estimated the temporal extent of the domains, since at that cut they were often shorter than the stimulus duration (compare Figs. lB and 2B, right-side columns). The temporal extent was measured as the longest duration of the domain, and for some X-type domains it lasted throughout the whole on period. Four cells had such stable tonic activity that for estimating this parameter we had to elongate the stimulus duration from 640 ms up to 1 s. With this reservation, we calculated the ratios W = D/T for both on and off excitatory domains for each cell, and they are presented in Fig. 3B. It is apparent from this figure that on and off domains for particular cells are similar, and that cells called X and Y form two clearly different groups.

The maxima of firing probability of on and off domains for a given cell were often displaced from each other up to two degrees (Figs. 1 and 2, high contour planes). Sometimes we observed also local minima of activity forming "dimples" within excitatory domains. Both features seem to confirm the hypothesis of the compound nature of PGN receptive fields.

The excitatory domains of X-type PGN receptive fields are spatially flanked by slightly marked inhibitory domains, and excitatory domains in Y-type fields are followed in time by short domains of reduced activity (Figs. 1, 2). The placement of these inhibitory domains resembles the positions of inhibitory domains observed in X and Y receptive fields of the LGN. Whether this decrease of activity results from the intrinsic inhibition within the perigeniculate nucleus or simply reflects the inhibitory domains of LGN cells is difficult to judge, although, in some cases (e.g., Fig. 1A, B) its extent is larger than usually observed in LGN. Thus, it might be due to the reciprocal inhibition between the PGN cells as observed by Ahlsen et al. *(1985)*.

The differentiation of X/Y pathways by electrical stimulation

It has been widely agreed that X and Y pathways from the retina to the visual cortex are conducted by fibers of different caliber and conduction velocity. If there is a segregation between X and Y pathways at the level of PGN cells, there should also be a difference in latencies for orthodromic and antidromic activation of these cells. Using electrical stimulation, Dubin and Cleland (1977), in their original experiment, have confirmed only the Y (fast) pathway input to the perigeniculate interneurons. With the double stimulation technique, Ahlsen et al. (1983) measured latencies long enough to be mediated also by X fibers. As in the last experiment, our data support the notion that different PGN cells can be excited from the optic chiasma and the visual cortex via either X or Y-type fibers. The results are presented in Fig. 4, where the corresponding pairs of latencies for

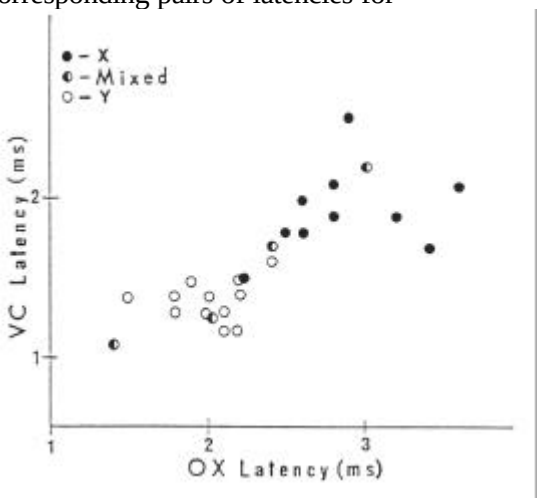


Fig. 4 Latency data for optic chiasma *(OX)* and visual cortex *(VC)* stimulation for 26 cells of the perigeniculate nucleus

activation of neurons classified visually as X and Y are marked correspondingly by black and white circles. The cells classified as X were, as a rule, activated with longer latencies than Y neurons, indicating that the transmitting fibers are slower, as should be expected from the X pathway (Hoffmann et al. 1972; Lindström and Wróbel 1990). Both groups appear to be clearly separated, which gives independent proof that the basis of our classification had been correctly established.

The characteristic latencies within both groups of perigeniculate cells are correspondingly longer by about 0.9 ms than those measured in the same experiment for X and Y principal LGN cells. This is an expected delay for monosynaptic activation of PGN neurons by axon collaterals of principal cells (Lindström 1982). We found the same mean difference (0.8 ins) between corresponding values of orthodromic and antidromic latencies. This difference agrees well with the position of perigeniculate interneurons, which were shown to be driven disynaptically from the optic chiasma and inonosynaptically from the visual cortex (Dubin and Cleland 1977; Lindström 1982). The observed jitter of the first activated spikes and low maximal limit for efficient frequencies (15—30 Hz) give further support for transsynaptic conductance from both stimulation sites.

Spatial frequency tuning of X and Y perigeniculate cells

Spatial frequency analysis of the receptive fields of ganglion, geniculate and cortical cells are commonly used to differentiate between X and Y-type neurons in the visual pathway (Stone 1983). Ahlsen et al. (1983) have used a handheld projector with grating stimuli to differentiate between X and Y perigeniculate cells. In our hands the application of the spatial frequency test for such a purpose was not straightforward. We obtained the full spatial frequency response curves for six X and

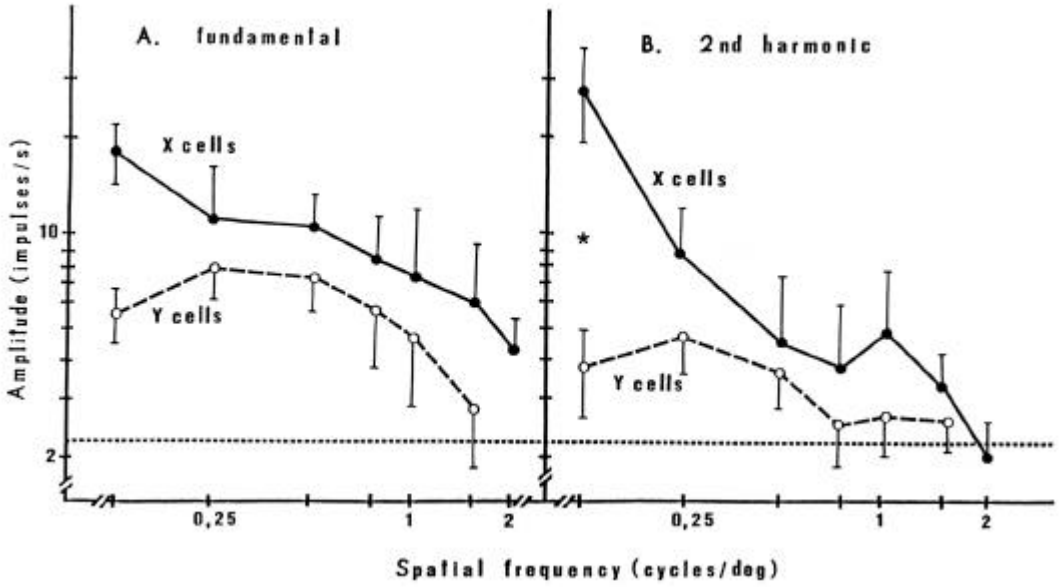


Fig. 5A, B The mean values for the seven Y cells and five X cells of the harmonic amplitudes taken from the Fourier spectra of corresponding periods of the histograms of responses for moving stimuli. The *continuous lines* represent the X cells, the *broken lines* represent the Y cells. Only the curves in B, for the second harmonic responses are significantly different. Star points to significantly different values at given spatial frequency. The *uertical bars* show standard errors. The *dotted horizontal line* above the abscissa represent the mean level of noise in the Fourier spectra as calculated from all cells

seven Y cells classified previously by response plane and electrical stimulation methods. In contrast to the previous report (Ahlsen et al. 1983) all X cells were modulated most strongly for the lowest spatial frequency stimulus (0.125 cycle/degree), whereas Y cells were best modulated for stimuli of 0.25 to *0.75* cycles/degree (Fig. *5).*

In contrast to So and Shapley (1981), we were able to calculate the spatial frequency response functions for most of the cells on which we attempted a test. The data obtained were, however, clearly different from those measured for LGN cells (Troy 1983 and our own unpublished results). In all investigated cells, the activity was modulated with both first and second harmonic components of the visual stimuli in correspondence with on-off type receptive fields of PGN neurons.

Two-way analysis of variance with X/Y cells and spatial frequency as factors (Fig. 5) showed a significant difference between the X and Y groups in second harmonic components (X/Y cells, $F_{1,11}=4.9$, $P<0.05$; spatial frequency, $F_{1,11}=$ 10.1, P<0.001; interaction between factors, $F_{1,11}=8.7$, $P<0.001$). The mean values of the second harmonic amplitudes of the X and Y groups were found to be significantly different only for the lowest spatial frequency (marked by star in Fig. 7B; $F_{111}=8.3$, $P<0.01$, one-way analysis of variance).

The presence of a second harmonic component to a drifting grating has never been reported for X or Y retinal and geniculate cells. It is obvious that further experiments are required to study its origin in the perigenicu-late nucleus circuitry. We did not attempt to solve it in this study. For the sake of the problem undertaken in this experiment, it was enough to conclude that X and Y groups of cells, classified by response plane method, differed also in response to moving gratings, thus supporting the notion that the two groups are functionally different.

Discussion

In this paper we showed that the receptive field description by response plane is a sufficient tool for differentiating two types of cells in the perigeniculate nucleus. The rationale of this classification was further confirmed by a spatial tuning test. The latencies of excitation of the perigeniculate cells from the optic chiasma and the visual cortex allowed us to classify the observed groups as belonging to the X and Y systems of the visual pathway.

Whether the two systems were separated on the level of perigeniculate interneurons was a subject of controversy. The literature concerning the classification of the cells in the perigeniculate nucleus is very limited. Dubin and Cleland (1977) were first to show that PGN cells form a disynaptic recurrent inhibitory loop to principal cells of the LGN. However, they failed to find cells with excitation latencies appropriate to X fibers, and so suggested that the recurrent loop might serve only for the Y pathway. The same difficulties with the excitation of the PGN cells were met by So and Shapley (1981). All the latencies measured by them were too short to fit the X pathway. They reported also that visually evoked responses of PGN neurons were feeble and therefore of no use for systematic measurement of the tuning curves. On the basis of the finding that some of the neurons had a consistent first harmonic response to drifting gratings of high spatial frequency, a response which is lacking in Y LGN cells, they postulated a probable input from X-type principal cells.

Instead of anesthetized cats, Ahlsen et al. (1983) used pretrigeminal cats, which radically improved the responsiveness of the perigeniculate interneurons. With an elegant electrical stimulation technique, they were able to find cells activated with timing appropriate to the X pathway. They noticed, as we did in the earlier study (Wróbel and Tarnecki 1984), that such cells do respond more tonically than those activated by faster fibers. Although we also used a pretrigeminal preparation, we found that classification of the PGN cells by moving gratings, as postulated by Ahlsen et al. (1983), is an uncertain test. In contrast to their findings, the X-type cells found in this study modulated the response for moving gratings of *lower* spatial frequency than did the Y cells. Moreover the unaveraged response is feeble and cannot be the only support for the classification. The ratio of the spatial extent of the receptive field to the duration of the center response that we used has proved to be a very good parameter for differentiating the two classes of the PGN cells.

The mean diameters of X and Y perigeniculate receptive fields were roughly twice as large as their principal cell counterparts in the LGN that we measured previously (Wróbel 1981) at the same eccentricity. Taking into account that a single PGN cell receives convergence from on and off types of LGN neurons from both contralateral and ipsilateral layers, we arrive at about sixteen principal cells connected to one perigeniculate interneuron. All these inputs could be a possible source of functional heterogeneity for PGN cells. In this respect, the similar ratio of mixed cells found in populations of LGN (12%, Wróbel and Lindström 1984) and PGN (15%, this study) supports the notion of physiological segregation of X and Y pathways also on the perigeniculate level. We have recently reached a similar conclusion after obtaining a bimodal distribution of threshold stimuli evoking the inhibitory postsynaptic potentials via a recurrent pathway in principal cells of the LGN (Lindström and Wróbel 1990).

We used the frequency tuning curves as an indication of valid grouping, basically achieved with the response plane method. Nevertheless our results are consistent and promise further possibilities in the study of the processing of visual information within the perigeniculate nucleus.

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