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NASAL FIELD LOSS IN KITTENS REARED WITH CONVERGENT SQUINT: NEUROPHYSIOLOGICAL AND MORPHOLOGICAL STUDIES OF THE LATERAL GENICULATE NUCLEUS

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SUMMARY

1. Recordings of single cells were made in layers A and A1 of the lateral geniculate nucleus of kittens raised with convergent squint in one eye, and morphological studies of cells representing the different parts of the visual fields in these layers were also made from histological sections.

2. For the normal eye, cells receiving inputs from the nasal and temporal visual fields were evenly represented up to the periphery, whereas for the squinting eye, no cells which permitted quantitative studies of receptive field properties could be found in the periphery of the nasal field.

3. The loss of nasal field, represented by the loss of functional cells in the LGN layer A1 fed by the squinting eye, depended on the severity of the squint. The greater the angle of convergent squint, the greater the loss of nasal field represented by the loss of functional cells.

4. The cells fed by the squinting eye's temporal visual field retained their brisk function, although minor modifications in the receptive field organisation were apparent.

5. The mean perikaryal size was smaller and the cell-density higher for cells in layers fed by the squinting eye. As found for the functional loss of cells, the shrinkage of perikaryal size and the increase of cell-density was smallest in the zones fed by the temporal visual field, and greatest in the zones fed by the peripheral nasal visual field.

6. The functional and morphological changes in the cells in the LGN, which receive inputs from the nasal field of the squinting eye, are attributed to part of the temporal retina being hidden behind the bridge of the nose. It is proposed that this is a consequence of disuse atrophy, due to lack of stimulation during the sensitive period of development.

INTRODUCTION

In clinical cases of convergent strabismus (esotropia), a major symptom is the reduction in visual acuity of the fovea of the deviating eye, i.e. central scotoma or amblyopia. We have studied this symptom in kittens raised with convergent squint, and found that cells in the dorsal lateral geniculate nucleus (LGN) driven from the area centralis of the squinting eye showed poor spatial resolution (Ikeda & Wright, 1976*a*). Furthermore, behavioural tests showed poor visual acuity for the squinting eyes of these kittens (Franklin, Ikeda, Jacobson & McDonald, 1976).

The present investigation arises from a puzzling observation made in the course of our previous work. When the LGN was examined histologically, the poor spatial resolution of cells fed from the area centralis of the squinting eye could be correlated with morphological changes of cells in the corresponding projection zones (Ikeda, Plant & Tremain, 1976), but, in addition, we were surprised to find extensive changes in regions of the LGN which received inputs from the peripheral retina. These changes, though of a lesser degree, were similar to those previously reported following monocular deprivation (Wiesel & Hubel, 1963; Guillery & Stelzner, 1970; Chow & Stewart, 1972). A closer examination showed that the most marked changes in the peripheral projection regions of the LGN occurred in the zones which received an input from the nasal visual field of the eye with convergent squint.

We then searched the literature for visual perimetry studies in human subjects with squint in early life. In clinical cases of strabismus, there is not only amblyopia and lack of binocular vision, but also a significant loss in the peripheral visual field (Tron, 1925; Travers, 1936; Duke Elder & Wybar, 1973). The literature dealing with peripheral visual field loss appears to be somewhat confusing and disorganised, due to the diversity of conditions of strabismus in clinical situations. However, it may be concluded that the field loss occurs in the direction of the deviation of the eye, that is, a loss of nasal field occurs in convergent squint whereas a loss of temporal field occurs in divergent squint. Thus, there appears to be some similarity between the changes found in squinting kittens and the field losses noted in clinical conditions of convergent squint.

In this paper, we report the results of a neurophysiological and morphological investigation of nasal visual field defects in the LGN of kittens raised with convergent squint. The results of behavioural studies will be reported in the following paper (Ikeda & Jacobson, 1977).

METHODS

1. *Production of convergent squint in kittens*

Six kittens, born in a specific pathogen-free cat colony, were raised with their mother in separate cages for each litter. At the 21st–23rd day after birth, in four of the six kittens, an operation to produce convergent squint in one eye was carried out under anaesthesia, induced by I.M. injection of Ketalar (ketamine hydrochloride; Parke-Davis at 0·5 ml./100 g body wt.). The lateral rectus muscle, the superior oblique muscle, the nictitating membrane and connective tissue on the lateral side of the eyeball were removed. After the surgery, an antibiotic (usually 0·5 ml. of Crystamycine, 25 % in water) was injected intramuscularly and Nivemycin eye-ointment placed around the operated eye.

Since the action of ketamine hydrochloride wears off rapidly, the kittens could be returned to their mother within an hour. Two kittens were kept as controls and all were weaned at 6 weeks. At 10 weeks they were let out from the cage and allowed to explore within the colony room (11′ × 8′) illuminated by two-time controlled fluorescent tubes (60 W each, 6 a.m.–8 p.m.), thus providing an equivalent bright day-light condition of 14 hr/day. An additional high window overlooking the Houses of Parliament provided a favourite spot for these kittens with aeroplanes, birds and politicians to be seen through the window. Thus, they had plenty of exercise in jumping up on the window-sills and reasonable visual experience. At the age of 4–6 months, they were investigated neurophysiologically by single cell recording from the LGN and subsequently the LGN was examined histologically.

2. *Neurophysiological experiments in the LGN*

The kittens with convergent squint in their left eyes were prepared surgically under thiopentone sodium B.P. (60 mg/kg I.P.). The deviation angle of the squint was determined 1 hr after the commencement of the I.V. infusion mixture (5 ml./kg hr), containing gallamine triethiodide (7–10 mg/kg) and Crystamycine (4·7 mg/ml.) in 2·5 % dextrose saline. Artificial respiration with 70–80 % N_2O, 19–28·5 % O_2 and 1–1·5 % CO_2 and additional halothane was given. Since in anaesthetized and paralysed cats the area centralis of the two eyes usually deviates by 3·5–5°, it is possible to determine the angle of deviation of the area centralis of each eye using a beam reflected through an ophthalmoscope. The area centralis of the squinting eyes in three out of four kittens projected 20–30° into the right temporal visual field of the non-squinting right eye, instead of projecting to 3·5–5° into the left nasal field of the right eye. Thus, by this method, the angle of squint in all kittens varied between 23·5–35°. The remaining kitten had a squint of 14°.

After the determination of the angle of squint, a scleral suture was placed on the lateral limbus margin and pulled gently laterally until the area centralis of the squinting eye was pointing at the normal visual axis. Thus, the stimulating conditions during the experiment were equivalent in both eyes. In some cats, the squinting eye appeared to have become almost embedded in the socket of the eyeball at the convergent squint position, and a further dissection to free the eyeball was necessary before the scleral suture was pulled to bring the eye into position. Accurate retinoscopy was carried out at this stage. Throughout the experiment, no distortion of the squinting eye had occurred in any of the squinting eyes, due to the scleral suture.

Two craniotomies were centred at A 6·5 and L 9·0 and R 9·0. Glass pipettes filled with 1 % pontamine blue in 0·5 M sodium acetate (adjusted to pH 7·7) were used to sample cells from layers A and A1 of both the left and right LGN. Therefore, we could sample cells evenly from both layers representing the corresponding points

of the visual field of the normal eye and the squinting eye in a single electrode penetration. The e.e.g. was monitored, enabling us to adjust the gas mixture to maintain stage II–III of anaesthesia (Ikeda & Wright, 1974); the body temperature was maintained at 38·5° C by a thermostatically regulated heating-blanket; the end expired CO_2 level was also monitored throughout, and maintained at 4·5 %–5·0 % by regulating the stroke volume.

The pupils of both eyes were dilated and accommodation paralysed by phenylephrine hydrochloride 10 % (phenylephrine eye drops B.P.C.) and Atropine sulphate (1 % in water). A contact lens with a 3 mm artificial pupil was centred on each eye, but when recordings were made from layers A or A1, the ipsilateral eye or the contralateral eye, respectively, was covered with a black circular disc in front of the correction lens. Thus, all cells were studied under monocular viewing conditions.

Quantitative measurements of the receptive field properties of each cell encountered were made using a grating stimulator (Dench, Ikeda & Wright, 1974) and the number of cells encountered at each layer of the LGN and their precise receptive field positions were analysed. Any intorsion of the eyes during paralysis, or a small torsional rotation (usually between 2 and 3° of downward rotation) due to the pull on the scleral suture, was assessed and corrected for the receptive field position. The determination of the receptive field position was carried out using an optimal-sized spot (luminance 20 cd/m^2) for each cell, back-projected on a vertical translucent screen (luminance 1 cd/m^2) and flashed at 1 Hz to plot the receptive field centre. A clear, thin Perspex screen placed at 45° to the translucent screen allowed transmission, and also reflexion, of the spot onto a horizontal plotting table.

3. *Histological and morphological procedure*

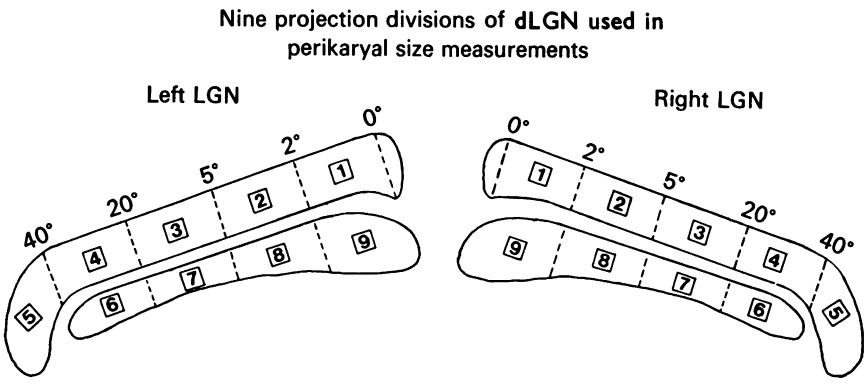
After the neurophysiological experiment, the cat was deeply anaesthetised by intravenous injection of thiopentone sodium and intravitally perfused with heparinised normal saline followed by 10 % formal saline. The brain, still encased in the skull, was then placed in 10 % formal saline for 2–3 days. The fixed brain in the skull was then placed in a stereotaxic frame and blocked to dissect out the thalamus. From the thalamus, 40 μm thick frozen sections of the LGN were prepared in the coronal plane and stained with cresyl fast violet.

The LGNs of two strabismic cats were selected for measurements of cell-density and perikaryal size, since these were the best histological preparations of all brains of the kittens with a convergent squint in the left eye. A total of eighteen sections (ten from cat 1 and eight from cat 2) were selected for the study of the portion of the LGN which represents the central horizontal strip of the visual field (nine sections for the left and nine sections for the right LGN).

Such sections were examined by a microprojection technique (Matthews, Cowan & Powell, 1960) and divided into nine zones according to the visual field projection data of Sanderson (1971). The divisions were made by drawing lines perpendicular to the uppermost outline of layer A of the LGN and across to the end of layer A1, dividing layer A into five approximately equal parts and layer A1, into four equal parts, as shown in Text-fig. 1. The diagonals were drawn on each projection division indicated in Text-fig. 1, and a 100×100 μm square was chosen at the intersection of the lines. If the square included artefacts (histological or electrode-track), the nearest clean neighbouring region was chosen. This sample area was projected at a higher power, and the outline of every cell with a clear nucleolus and clear axonal or dendritic processes visible at their junction with the soma was then traced on paper. Various methods of measuring the area of the traced cells were tried including the use of a planimeter, a light pen tracing on a T.V. screen computed by a general-

purpose computer, and a painstaking counting of squares on transparent graph paper of 1 mm scale. Most (150 out of 162 divisions) of the data included in this series were based on the counting of squares, since this was found to be the most reproducible of the three methods.

In addition, the density of cells was determined by counting the number of traceable cells within an area of 150×150 μm enclosing the 100×100 μm square (see Text-fig. 1) chosen for the measurement of perikaryal size.



Text-fig. 1. Diagrams of coronal sections of layers A and A1 of the left and right LGN representing the central horizontal strip of the visual field with the 9 projection divisions used for perikaryal size measurements. The dashed lines indicate the borders of the divisions with approximate projection line in terms of eccentricity from the area centralis, i.e. 0°. The small squares illustrate areas of 100×100 μm in each projection division chosen for sampling cells (the squares are not to scale).

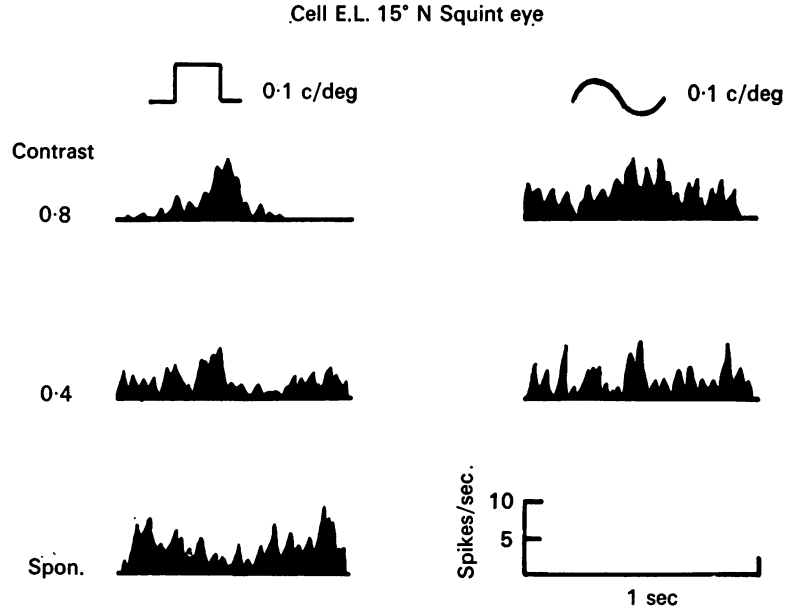
RESULTS

Loss of function in cells receiving projections from the nasal visual field of the squinting eye

During electrode penetrations through the LGN in regions representing the mid-periphery of the horizontal meridian of the visual field, it was noted that in the right LGN, cells were sampled evenly from layer A, fed by the squinting eye, and from layer A1, fed by the normal eye. At the left LGN, however, cells from layer A fed by the normal eye were encountered readily, but no cells which permitted a quantitative measurement of their receptive fields were encountered in layer A1. Instead, the records of the experiments consist of remarks such as 'sluggish', 'large receptive field with ill-defined surround', 'only responds to an ophthalmoscope beam moving in or out', or 'no response to the grating of 0·1 c/deg' and 'no response to a grating of any spatial frequency'. To illustrate the 'sluggishness' of the cells in the nasal field of the squinting eye, Text-fig. 2 shows five post-stimulus histograms obtained from a cell

with a receptive field at approximately 15° in the nasal field of the eye with a squint of 30° deviation.

The cell responded to a large square wave grating (0·1 c/deg, i.e. 5° width bar) of high contrast (0·8) but barely responded to a sinusoidal grating of the same spatial frequency and moderate contrast (0·4), and



Text-fig. 2. Post-stimulus histograms (16 stimulation cycles, bin width = 20·48 msec) obtained from a cell in layer A1 of the left LGN receiving inputs from approximately 15° in the nasal field of the squinting eye, showing sluggish responses to a square-wave modulation of a large, high contrast grating (0·1 c/deg, i.e. 5° width bar, contrast: 0·8, mean luminance of the grating: 10 cd/m²) moving across the receptive field centre at a speed of 1 Hz (1 spatial cycle of the grating travelled across a given spatial position in 1 sec). Note that the cell does not respond well to a sinusoidal grating of 0·1 c/deg, even at the contrast of 0·8, suggesting that the contrast sensitivity of the cell is extremely depressed, and determination of spatial frequency tuning is impossible. *Spatial frequency* is defined as number of grating cycles (bright and dark period pair) per degree of visual angle. *Contrast* is defined as $(L_{max}-L_{min})/(L_{max}+L_{min})$, where L_{max} is the luminance of bright phase of grating and L_{min} is that of dark phase. The contrast of the grating could be raised without altering the space averaged luminance of the grating screen, i.e. mean luminance.

not at all to gratings of higher spatial frequency. (For each cell we routinely checked responses for a possible low spatial cut in frequency). The receptive field of the cell was large ($>10°$) and it did not respond

consistently to stationary flashing spots of sizes ranging from 8′ to 3°. The approximate receptive field position could only be determined by a moving spot of 3°, though again the response was inconsistent. Such characteristics of cells fed by the nasal peripheral field of the squinting eye cannot be attributed to variation in anaesthetic level or to deterioration of the electrode or preparation, since immediately before and after recording from these cells, we could find cells fed from the normal eye which permitted the usual quantitative studies on the receptive field properties.

TABLE 1. Number of 'briskly functioning'* cells from the peripheral visual field (>10°) in the central horizontal meridian (within 5° above and below the visual axis) in two normal kittens and three kittens with a squint of 23·5–35° and one kitten with a squint of 14°. The layer A1 of the left LGN and the layer A of the right LGN correspond to the squinting eye. Each electrode penetration sampled cells from layer A and layer A1 immediately below

	LEFT LGN			RIGHT LGN		
	No. of penetrations	Layer A R eye Temporal	Layer A1 L eye Nasal	No. of penetrations	Layer A L eye Temporal	Layer A1 R eye Nasal
† NK	10	28	26	8	26	24
‡ SK (23·5–35°)	7	17	0	8	17	11
SK (14°)	2	7	5			

* Cells which permitted a quantitative measurement of spatial and temporal resolving power using sinusoidal grating.

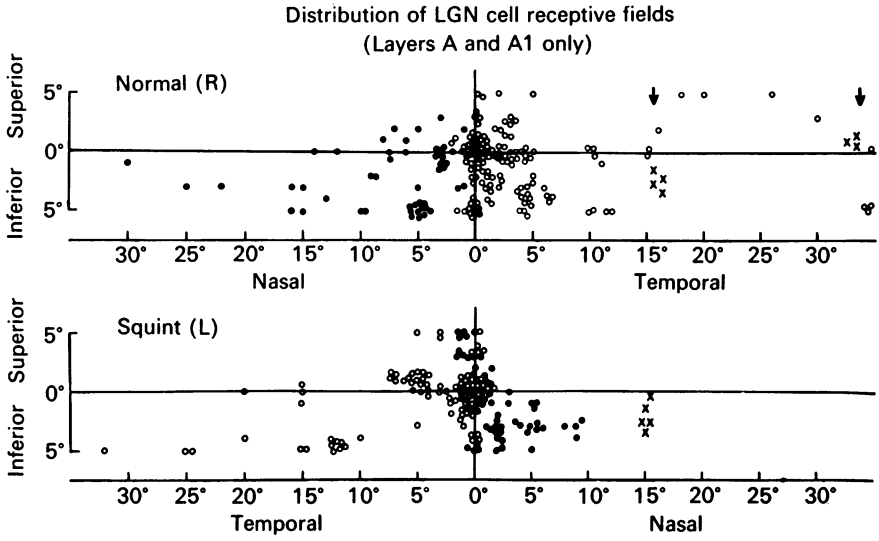
† NK, Normal kittens.

‡ SK, Squint kittens.

Table 1 compares the numbers of cells which could be studied thoroughly, with inputs from the mid-horizontal meridian of greater than 10° from the visual axis in layers A and A1 of the left and right LGN from 2 normal kittens and 3 kittens with convergent squint of 23·5–35° in the left eye, and a kitten with a squint of 14°. For the kitten with 14° squint, electrode penetrations were made only in the left LGN.

Table 1 shows that, in each LGN of the normal kitten, cells with normal receptive fields were equally common in layers A and A1, whereas in those kittens with over 23·5° of squint no 'briskly functional' cells could be recorded from the layer A1 of the left LGN where the squinting eye's nasal field projects. (In fact, our records showed that an attempt had been made to investigate the cell properties quantitatively in a total of 20 cells at layer 1A of the left LGN, expected to have received projections from the mid-horizontal meridian of greater than 10° to nasal from the visual

axis, though no cells were totally visually unresponsive). However, normal cells with receptive fields beyond 10° in the nasal field were found in the kitten with the smaller angle of squint, i.e. 14°. Text-fig. 3 shows a plot of the receptive field centre positions of 'brisk' LGN cells encountered within a central strip of visual field, i.e. within 5° of the line dividing the superior and inferior visual fields. This is because our morphological studies have been done exclusively on that part of the LGN receiving projections from the visual field within 5° of the horizontal meridian.



Text-fig. 3. Receptive field distribution of the 'briskly functioning' cells encountered within a central (below and above 5° of the visual axis) horizontal strip in layers A (open circles) and A1 (filled circles) of the left and right LGNs in four kittens raised with convergent squint in the left eye. Three kittens had a squint of 23·5–35° deviation and one had a squint of 14°. The crosses (marked by arrows) are cells encountered in two electrode penetrations in the left LGN of the kitten with a squint of 14°. Note that no cells were encountered during 7 electrode penetrations in the nasal field of the squinting eye beyond 10° in the kittens with a squint of 23·5–35° whereas cells at 15–16° but not at 34–35° were found in the kitten with a squint of 14°.

The upper map of Text-fig. 3 is from the normal eye and the lower map is from the squinting eye. For both eyes, a disproportionately large number of cells with receptive fields near the area centralis are seen. This is due to the magnification of the area centralis projection zone at the LGN. For the normal eye, cells having nasal and temporal receptive fields are evenly represented up to 30–35° in the periphery. (We did not study the cells with inputs from the extreme peripheral visual fields, and all

cells are well within the so-called 'binocular segment' of the visual field of the cat.) It is striking to note that there is an abrupt stop at about 10° of the nasal field for cells fed by the squinting eye, showing the lack of briskly-functioning cells at the periphery of the nasal field in the kittens with a convergent squint of 23·5–35°.

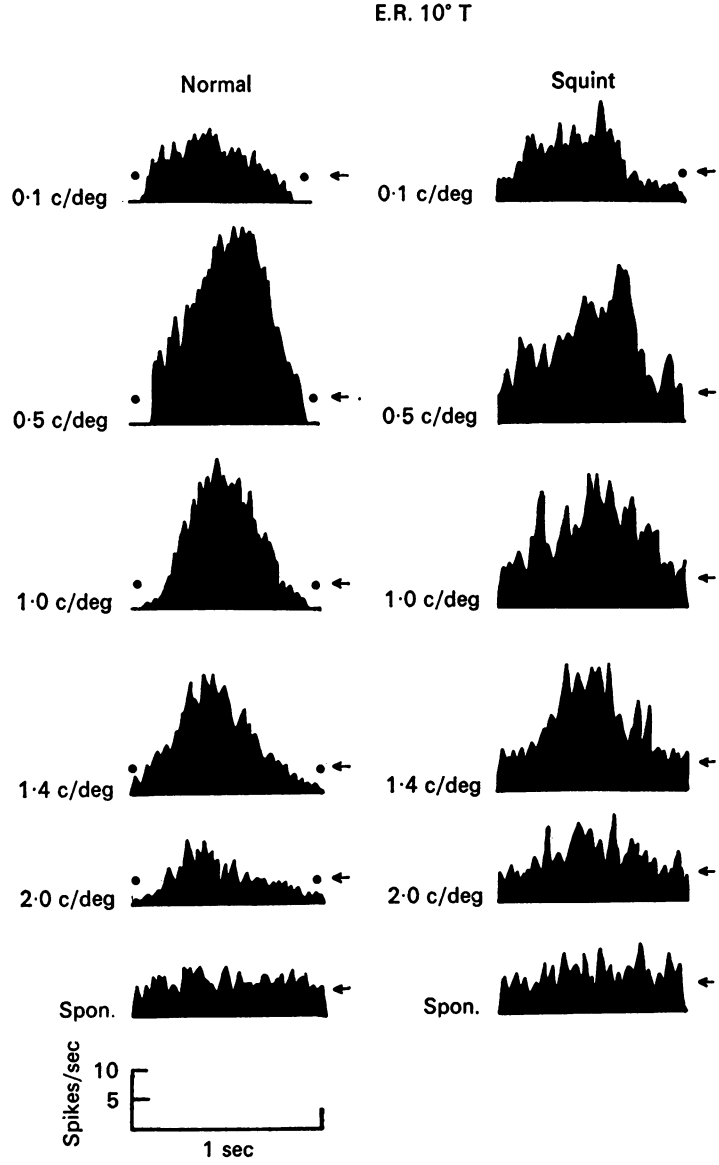
It can also be noted in Text-fig. 3 that in the kitten which had only 14° of squint, we were able to study four cells with receptive fields of 15–16° in the temporal field of the normal eye (shown as × symbols below an arrow) at layer A and five cells representing 15–16° in the nasal field of the squinting eye (also shown as × symbols) at layer A1. But, in the same kitten, another electrode penetration which encountered three cells with receptive fields approximately 34–35° in the temporal visual field of the normal eye, failed to sample cells from the squint eye in layer A1, although this penetration was well within the 'binocular segment' of the LGN. Cells found in A1 only responded to an intense ophthalmoscope beam shone directly into the region of the receptive field, and even this response was inconsistent. In this kitten, also, the spatial resolution of cells fed by the area centralis of the squint eye was poor, to the same degree as that found in the other kittens with a greater degree of squint. Thus, the loss of nasal field represented by the loss of functional cells in the LGN depends on the severity of squint, whereas amblyopia (loss of spatial resolution of the cells fed by the area centralis zone) does not do so.

Minor modifications in the receptive field organization of cells with projections from the temporal visual field of the squint eye

All the cells plotted in Text-fig. 3, regardless of whether they received an input from the normal eye or from the squint eye, showed clear cut 'on' or 'off' centre receptive fields. They could give either 'sustained' or 'transient' firing to a stationary spot of optimal size for the cell, located at the receptive field centre. All responded with clearly modulated firing to a sinusoidally modulated grating drifting across the receptive field centre, thus permitting us to determine spatial and temporal frequency tuning properties. As in the previous work of Ikeda & Wright (1976*a*), the spatial resolution of the cells fed from the area centralis zone of the squinting eye was considerably reduced, but cells which received inputs from the peripheral temporal field of the squinting eye showed a similar spatial resolution to those cells which received inputs from the equivalent region in the temporal visual field of the normal eye.

However, there was a minor difference in the receptive field properties of cells fed by the periphery of the temporal visual field of the squinting eye compared with those from the normal eye, as illustrated in Text-fig. 4.

In Text-fig. 4 post-stimulus histograms were obtained from two cells,



Text-fig. 4. Post-stimulus histograms showing the response of an on-centre sustained cell receiving projection from 10° in the temporal visual field of the normal eye (left) and that of an on-centre sustained cell receiving projection from 10° in the temporal visual field of the squinting eye (right), to moving sinusoidal gratings of different spatial frequencies (number of grating cycles per degree of visual angle). *Contrast of gratings*: 0·4, mean luminance of gratings: 10 cd/m^2, *drift speed*: 1 Hz, 16 stimulation cycles. *Bin width*: 20·48 msec. Note that both the normal and the squinting eye cells respond up to 2·0 c/deg but the squinting eye cell does not show an inhibitory response that is apparent in the response of the normal eye cell (marked as dots) except for a grating of 0·1 c/deg. The arrows shown at the right-hand side of each post stimulus histogram indicate the level of spontaneous firing of the cells.

one fed by the normal eye and the other by the squinting eye, in response to grating stimuli of different spatial frequencies. Both cells had receptive fields approximately 10° from the area centralis in the temporal visual field, and they were both 'sustained' on-centre cells. Both cells responded with firing, which followed approximately the sinusoidally modulated contrast of the grating. Spontaneous firing (the lowest post-stimulus histogram in Text-fig. 3) is the firing of the cell to a uniform field of the same mean luminance as the grating stimuli (10 cd/m^2), without a grating.

Although the spatial frequency threshold (the highest spatial frequency to which the cell responded with modulated firing) was 2·2 c/deg for both the normal eye cell and the squinting eye cell, there is a difference in the mode of firing of the two cells. The cell fed by the normal eye showed a clear inhibition when bright phases of the grating drifted across the region surrounding the receptive field centre even at 2·0 c/deg, whereas the cell fed by the squinting eye showed no such inhibition, except for the grating of 0·1 c/deg. Observations similar to this were often (ten pairs out of fifteen pairs of cells which had equivalent receptive field in the periphery of the temporal visual field) made throughout the experiments, and thus the finer receptive field organization involving excitatory and inhibitory mechanisms (Singer & Creutzfeldt, 1970) is indeed altered in cells fed by the squinting eye.

It appears, therefore, that there is a graded deterioration in the function of cells fed by the squinting eye, ranging from minor alterations in the receptive field properties in the temporal visual field projection zones, and the loss of spatial resolution at the central retinal projection zone leading to a complete loss of function at the extreme nasal field projection zone.

Morphological changes in the different visual field projection zones of the LGN

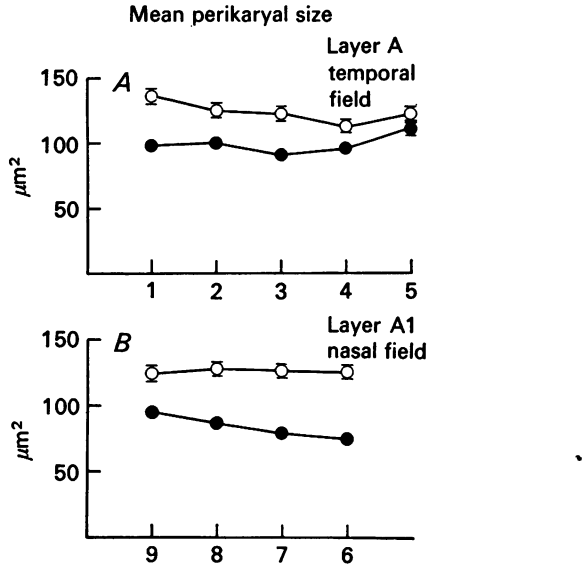
Pl. 1*A* shows sections of the left and right LGN, from zones receiving inputs from the central horizontal strip of the visual field. Layer A (upper layer) and layer A1 (lower layer) are separated by an interlaminar zone (IL). Layer A of the left LGN in Pl. 1*A* has inputs from the normal eye's temporal visual field and layer A1 from the squinting eye's nasal field. Further to the left is the region which receives a peripheral projection. Layer A of the right LGN was fed by the squinting eye's temporal visual field, and layer A1 by the normal eye's nasal field. At a glance, the layers fed by the normal eye appear to be thicker, more darkly stained by cresyl fast violet, and the cells somewhat larger than in the layers fed by the squinting eye. Furthermore, the difference between layer A, representing the temporal fields of the left LGN (normal eye layer) and that of the

right LGN (squint eye layer) appears to be relatively small, whereas the difference between the layers A1, representing the nasal fields of the left and the right LGN, is more apparent.

These observations could be made more readily in the sections with higher magnification as shown in Pl. 1 *B*.

Mean perikaryal size

The mean perikaryal size of cells in the nine zones representing different parts of the visual field (see Text-fig. 1), measured from eighteen histological sections, are shown in Text-fig. 5 *A* and *B*.

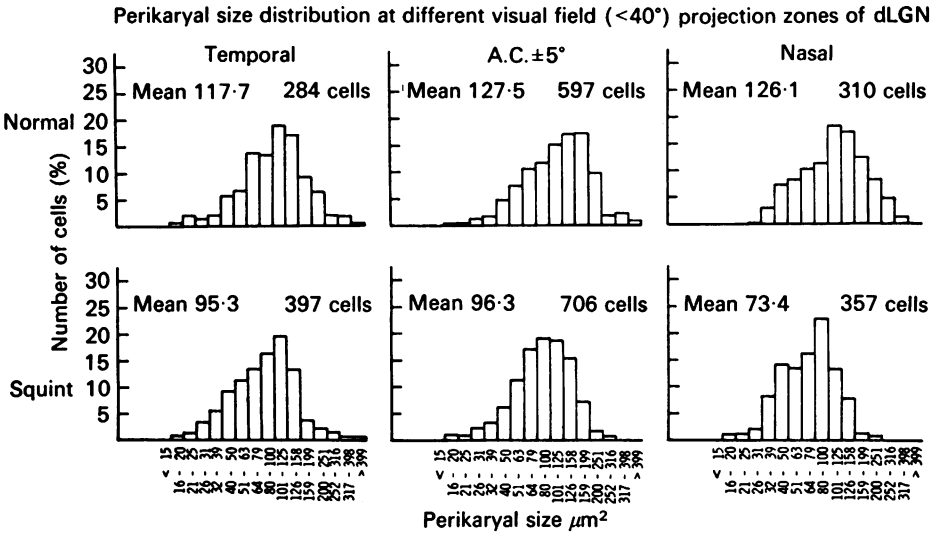


Text-fig. 5. *A*, mean perikaryal size of cells sampled at the five different visual field projection zones in layer A representing the area centralis (zone 1) to progressively peripheral temporal visual fields (zone 2–5). *B*, those sampled at the four zones in layer A1 representing the area centralis (zone 9) to progressively peripheral nasal visual field (zones 8, 7 and 6) of the normal eye (open circles) and the squinting eye (filled circles). The nine projection zones are as shown in Text-fig. 1. The vertical bars are ± 1 S.E. of mean. Where no S.E. of mean values are shown, symbols are larger than S.E. of mean.

The size of cells from zone 1, representing the area centralis, to progressively more peripheral parts of the temporal visual field (zones 2–5) of the normal eye and the squinting eye are shown in Text-fig. 5 *A*, whereas Text-fig. 5 *B* compares cells from the zone 9, representing the area centralis, through to the peripheral nasal visual field (zones 8–6)

of the normal eye and the squinting eye. It can be seen that the perikaryal sizes of the cells fed by the normal eye are greater than those of the squinting eye throughout. This difference is much greater for the cells representing the nasal field ('shrinkage' = 39·3 %, $t = 11{\cdot}54$, $P < 0{\cdot}0001$) than for those representing the temporal visual field ('shrinkage' = 18·9 %, $t = 5{\cdot}04$, $P < 0{\cdot}0001$) as shown in Text-fig. 5.

Furthermore, the difference between the perikaryal sizes of the cells in layer A fed by the normal eye and the squinting eye decreases with increasing eccentricity from the area centralis zone for the temporal visual field, whereas it increases with increase in eccentricity from the central



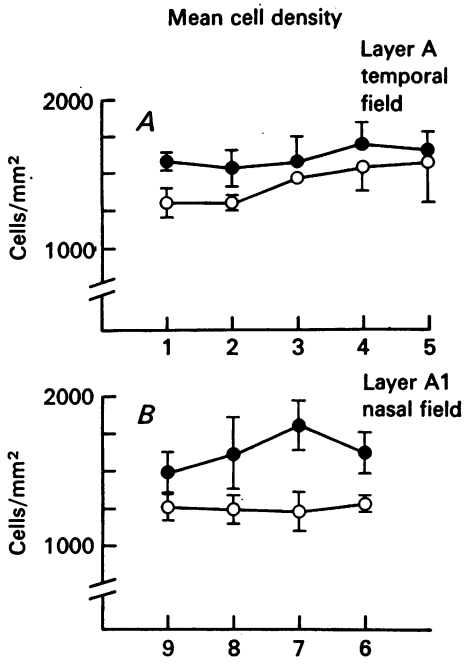
Text-fig. 6. Comparisons of perikaryal size distribution of cells receiving inputs from the normal eye (upper histograms) and those receiving inputs from the squinting eye (lower histograms). The cells representing 6–40° of the temporal visual field are shown in the left-hand column, whereas those receiving inputs from the central 5° of visual field in the middle, and those from 6–40° of the nasal field, in the right-hand column. Each bin shift is equivalent to a change in the perikaryal size of approximately 20–25 %. Note that the greatest shift of the histogram of the squinting eye cells can be seen for the nasal field.

retinal zone for the nasal visual field (layer A1). If the differences are expressed in percentage shrinkage of the cells from the squinting eye, the shrinkage for the projection zones 1, 2, 3, 4 and 5 in layer A were 27, 19, 25, 15 and 10 %, respectively. The percentage shrinkage for projection zones 9, 8, 7 and 6 in layer A1 were, on the other hand, 30, 33, 37 and 39 %, respectively.

In fact, for the normal eye, the mean perikaryal size for all the cells in layer A1, representing the nasal visual field, is slightly larger ($t = 1{\cdot}35$, $P < 0{\cdot}1$) than that for the cells in layer A, representing the temporal visual field. For the squinting eye, the reverse occurs; cells in layer A1 are significantly smaller than those in layer A ($t = 5{\cdot}21$, $P < 0{\cdot}0001$).

Perikaryal size distribution

Perikaryal size distribution for cells representing the central 5° of the visual field, 6–40° in the nasal field and 6–40° in the temporal field, were compared between the layers fed by the normal eye and those fed

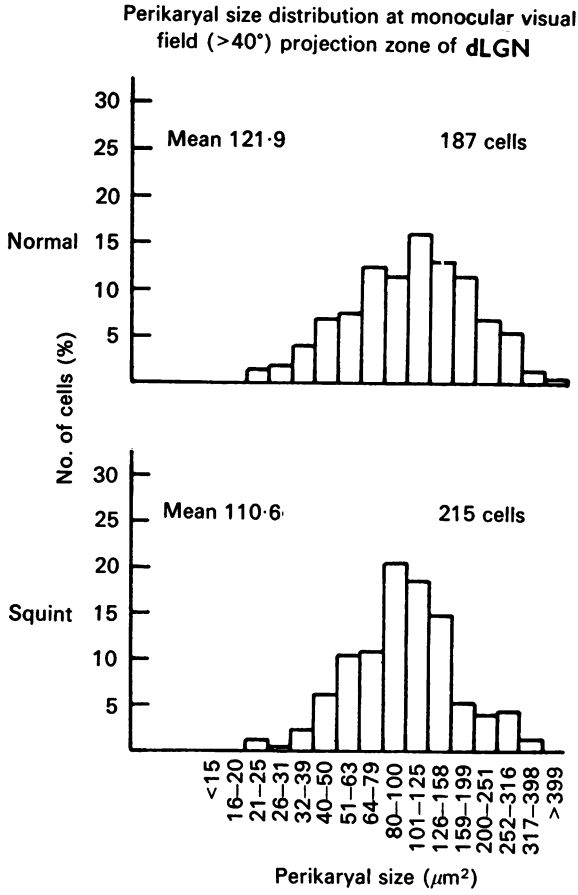


Text-fig. 7. *A*, mean cell-density of cells counted at the five different visual field projection zones in layer A representing the area centralis (zone 1) to progressively peripheral temporal visual field (zones 2–5). *B*, those counted at the four zones in layer A1 representing the area centralis (zone 9) to progressively peripheral nasal visual field (zones 8, 7 and 6) of the normal eye (open circles) and the squinting eye (filled circles). The nine projection zones are as shown in Text-fig. 1. The vertical bars are ± 1 S.E. of mean. Where no S.E. of mean is indicated, the symbol is larger than S.E. of mean.

by the squinting eye in Text-fig. 6. Here, the projection zones (see Text-fig. 1) 1,2,9 and 8 were combined to represent the central visual field while the zones 3 and 4 represented the peripheral temporal visual field, and 6 and 7, the peripheral nasal visual field. Projection zone 5, which

represents the extreme periphery of the temporal visual field, i.e. the so-called 'monocular segment' of the LGN, was excluded from the comparisons.

The histograms in Text fig. 6 are all of unimodal shape, similar to the distribution in the LGN of the monkey reported by Von Noorden &



Text-fig. 8. Comparisons of perikaryal size distribution of cells receiving inputs from the normal eye (upper histogram) and those receiving inputs from the squinting eye (lower histogram) in the monocular segment of the LGN (zone 5 in our projection division shown in Text-fig. 1). Note that a slight shift of the histogram for the squinting eye to the left can be seen ($\chi^2 = 14{\cdot}17$ d.f. $= 7$, $P < 0{\cdot}05$).

Middleditch (1975). The size bands are log scale, to equate the percentage shrinkage of size; thus one bin shift to the left represents approximately 20–25% shrinkage. In this way a change from 40 to 30 μm^2 may be

compared with that from 400 to 300 μm^2. The histograms for the squinting eye are shifted towards a smaller size, compared with those for the normal eye, and this shift is most prominent for the nasal visual field and less apparent for the temporal visual field cells. Thus, the reduction in the mean perikaryal size of cells from the squinting eye layers may be due to an over-all shrinkage of cells in all perikaryal size groups, but it is impossible to be certain without being able to identify neurones by criteria other than size alone.

Cell density

The observations that there is an over-all shrinkage of all types of cells, rather than a loss of large cells, in the layers fed by the squinting eye, is supported by the observation that the cell-density was higher in the layers representing the squinting eye than those representing the normal eye ($t = 4{\cdot}09$, $P < 0{\cdot}0001$). Text-fig. 7*A* and *B* show the mean cell-density in terms of no. of cells/mm^2 in each of the nine visual field projection zones.

The difference between the cell-density for the squint eye cells is much greater in layer A1, representing the nasal field ($t = 3{\cdot}61$, $P < 0{\cdot}005$), than in layer A, representing the temporal visual field ($t = 0{\cdot}96$, $P > 0{\cdot}3$).

Monocular segment perikaryal size

It can be seen in Text-fig. 5*A* that the difference between the normal eye and the squint eye is smallest at projection zone 5, which is the so-called 'monocular segment' of the LGN. Here, the difference in mean perikaryal size for the cells of the normal eye and the cells of the squinting eye was found to be barely significant ($t = 1{\cdot}76$, $P < 0{\cdot}08$).

Thus, the changes in perikaryal size and cell-density found for cells fed by the squint eye are graded effects, being least at the zone of the extreme periphery of the temporal field and greatest at the zone of the extreme periphery of the nasal field projection.

DISCUSSION

There are three main symptoms in clinical cases of strabismus: (i) lack of binocular vision and stereopsis, (ii) reduction in visual acuity of the fovea for the deviating eye, i.e. amblyopia or central scotoma, and (iii) peripheral field loss in the direction of the deviation of the eye. Since the first demonstration by Hubel & Wiesel (1965), many neurophysiological studies in experimental strabismus have put their emphasis on the first symptom, lack of binocular vision, revealing the importance of synchronous inputs from both eyes for preserving normal binocularly driven cells in the visual

cortex (Baker, Grigg & van Noorden, 1974; Blakemore & van Sluyters, 1975; Yinon, Auerbach, Blank & Friesenhausen, 1975).

Neurophysiological demonstration of the second symptom, amblyopia of the deviating eye, has recently been made in kittens raised with convergent squint by Ikeda & Wright (1976), from a study of the responses of LGN cells to gratings. The present study is the first neurophysiological demonstration of the third symptom, the peripheral field loss in the deviating eye in experimental strabismus.

We found that the LGN cells, driven from the squint eye's peripheral nasal field, cannot respond adequately to stimuli which would enable a quantitative study of their receptive field properties to be made. These cells only respond sluggishly to a large, high contrast stimulus moving in or out of their ill defined receptive fields. Thus, it appears that the LGN cells which receive projections from the squinting eye's nasal field are functionally moribund.

An important observation which emerges from this study is that the extent of the visual field from which functioning cells can be driven appears to depend upon the degree of squint angle of the eye, i.e. a greater functional loss of cells which receive inputs from the peripheral nasal field is found in the kittens with a larger angle of convergent squint (Text-fig. 2). This result is different from that found for the symptom of the loss of spatial resolution in the cells receiving an input from the area centralis of the squinting eye, since this was independent of the degree of squint. This suggests that the two symptoms may stem from different causes.

The single cell recordings in the LGN of the kittens raised with convergent squint also suggested that, even though the apparent spatial resolution of cells with an input from the peripheral temporal visual field appeared to be the same for both the normal and the squinting eye, the cells fed from the squinting eye showed no clear inhibition following excitation to a sinusoidal modulation of contrast (Text-fig. 3). Thus, a finer modification of the receptive field organization and the synaptic interaction which produces centre and surround antagonism (Singer & Creutzfeldt, 1970; Hammond, 1973) had occurred in these cells.

Thus, a significant observation in the present neurophysiological study is that the change we found is a graded effect. The loss of function of the cells ranged from a minor change in the receptive field organization in cells receiving an input from the temporal visual field, and a loss of normal spatial resolving power in the cells fed by the area centralis, to the total loss of function in those with an input from the periphery of the nasal visual field.

These functional deficits in the LGN cells could be correlated with morphological changes in the layers fed by the squinting eye. In these

layers, the perikaryal size was smaller and the cell-density higher, in agreement with the findings of Von Noorden (1973) and Von Noorden and Middleditch (1975) for the monkey with convergent squint in one eye.

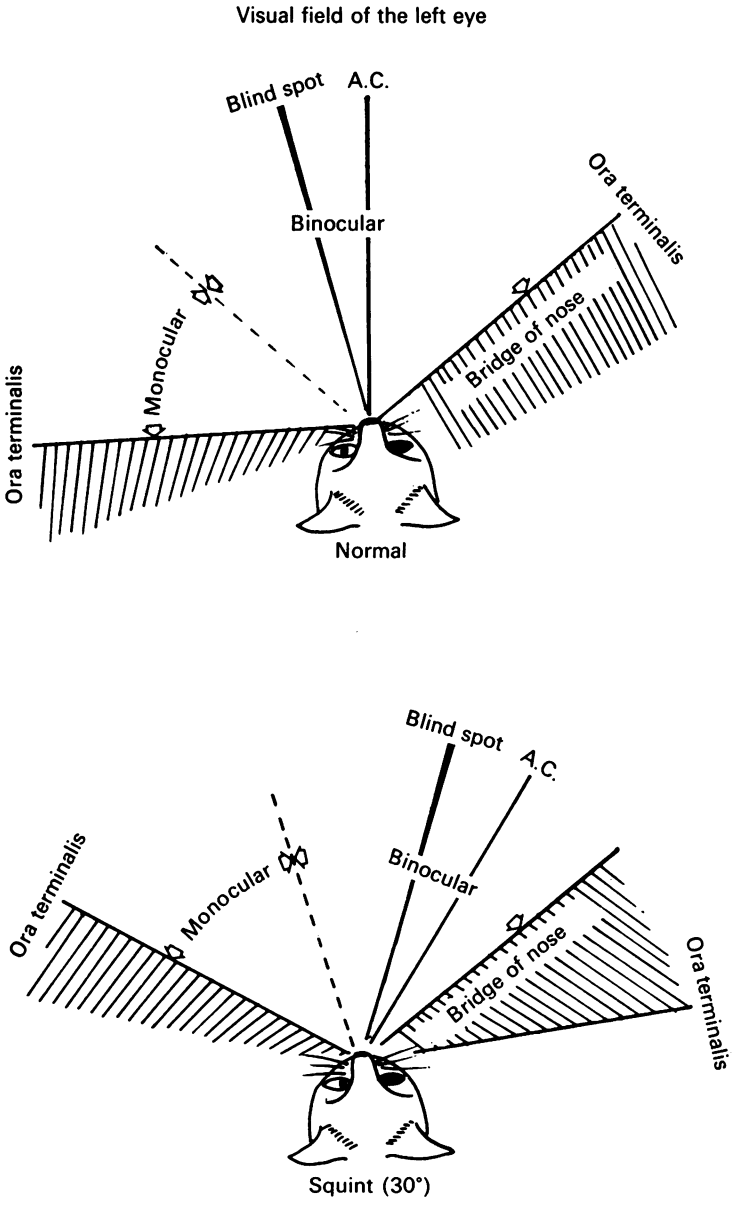
The analysis of perikaryal size distribution (Text-fig. 6) is consistent with a reduction in size of all classes of cell, and not necessarily a loss of large cells, as suggested for monocular deprivation (Sherman, Hoffman & Stone, 1972; Sherman, Guillery, Kaas & Sanderson, 1974). An additional comment can be made here, that the perikaryal size distribution of the LGN cells is unimodal, unlike that of retinal ganglion cells (Boycott & Wässle, 1974; Wässle, Levick, Kirk & Cleland, 1975). It is not possible therefore to distinguish on the basis of perikaryal size alone those cells projecting to area 17 and to area 18 (Gilbert & Kelly, 1975), which presumably represent principally 'X' and 'Y' cells, respectively.

As found for the functional loss, in our morphological studies the shrinkage of perikaryal size of cells fed by the squinting eye was again a graded effect. It was smallest in the zone fed by the temporal field, and greatest in the zone fed by the nasal field. The degree of shrinkage of cells in layer A, fed by the squinting eye, was much smaller than that reported by Guillery & Stelzner (1970), who studied only layer A of the LGN in monocularly deprived cats. The zone fed by the extreme nasal periphery of the squinting eye, on the other hand, showed shrinkage as great as that reported by Wiesel & Hubel (1963, 1965) for LGN cells fed by the monocularly deprived eye. However, it is difficult to compare our results with previous histological work which did not study the difference between LGN zones receiving projections from different parts of the visual field, apart from dividing the LGN into a 'binocular segment' and a 'monocular segment', particularly since the effect we found was a graded one within the 'binocular segment'.

How could a graded effect occur in the LGN of kittens raised with convergent squint? The simplest explanation for the changes we found in the LGN is that they are due to graded disuse of the retina.

As the upper diagram of Text-fig. 9 (adapted from Hughes (1976)) shows the extent of the visual field of the eye of a normal cat is limited by the margin of the retina, i.e. the ora terminalis. The edge of the temporal visual field coincides with the nasal end of the retina, whereas the temporal retinal edge coincides with the bridge of the nose which limits the extent of the visual field. The binocular segment is defined from the overlap of the projections of the retinae from the two eyes, which is approximately 90° (45° on each side of the area centralis projection line).

The lower diagram of Text-fig. 9 illustrates the visual field seen by the left eye with a convergent squint of 30° (this was the degree of convergent squint present in many of the kittens studied by us). It can be seen from



Text-fig. 9. The limit of the visual field of a cat seen by a normal left eye (upper diagram) and that seen by a left eye with a convergent squint of 30° (lower diagram). The diagram for the normal eye has been adapted from that shown by Hughes (1976). Note that the convergent squint of 30° results in approximately 30° of the temporal retina, which should receive inputs from the peripheral nasal field, being hidden behind the bridge of the nose, thus preventing stimulation.

Text-fig. 9 that the visual field of the squinting eye is, as a whole, rotated to the right, i.e. nasally. This results in the part of the temporal retina which normally receives an input from the peripheral nasal field being hidden behind the bridge of the nose, and thus losing the chance of being stimulated during the sensitive period of development. We suggest that this will cause disuse atrophy or arrest of cell growth at the LGN. The fact that the shrinkage of cells in the LGN for the zone receiving fibres from the hidden retina was equivalent to that found after optic tract section (Garey, Fisken & Powell, 1973) and after monocular deprivation (Wiesel & Hubel, 1963) is in accordance with this view.

Furthermore, the greater the angle of squint the greater the extent of the nasal field for which no functional cells were encountered and, of course, the greater the angle of squint the greater the portion of retina hidden behind the bridge of the nose. This effect will be graded rather than sudden because the shadow of the bridge of the nose does not create a sudden occlusion; its effect depends on the angle of incident light and on the degree of eye movement, though this was limited in these kittens. The disuse of the retina will result in degenerative change in the LGN. There is also evidence that a marked lowering of cytoplasmic and nucleolar RNA levels in the receptor and inner nuclear layers, and a reduction in mean nuclear volume and cytoplasmic area of cross-section of ganglion cells of the retina, occurs in cats raised in darkness and thus deprived of adequate visual stimulation (Chow, Riesen & Newell, 1957; Rasch, Swift, Riesen & Chow, 1961).

As for the amblyopia (reduction of the visual acuity and morphological changes in the cells fed by the area centralis of the squinting eye), we suggest that this effect is caused by inappropriate stimulation of the area centralis of the retina during the sensitive period of development (Ikeda & Wright, 1976*a*,*b*).

For the minor functional loss and minor shrinkage found in the cells receiving inputs from the temporal visual field any explanation is speculative. Whether retrograde degeneration, due to binocular competition at geniculo-cortical synapses as proposed by Wiesel & Hubel (1963), Hubel & Wiesel (1965), Guillery & Stelzner (1970), Sherman *et al.* (1972, 1974) and Yinon *et al.* (1975), or a tonic inhibitory influence from the visual cortex (Kratz, Spear & Smith, 1976; Duffy, Snodgrass, Burchfiel & Conway, 1976), or inadequate eye movement (Maffei & Bisti, 1976) is responsible for these minor effects remains problematical.

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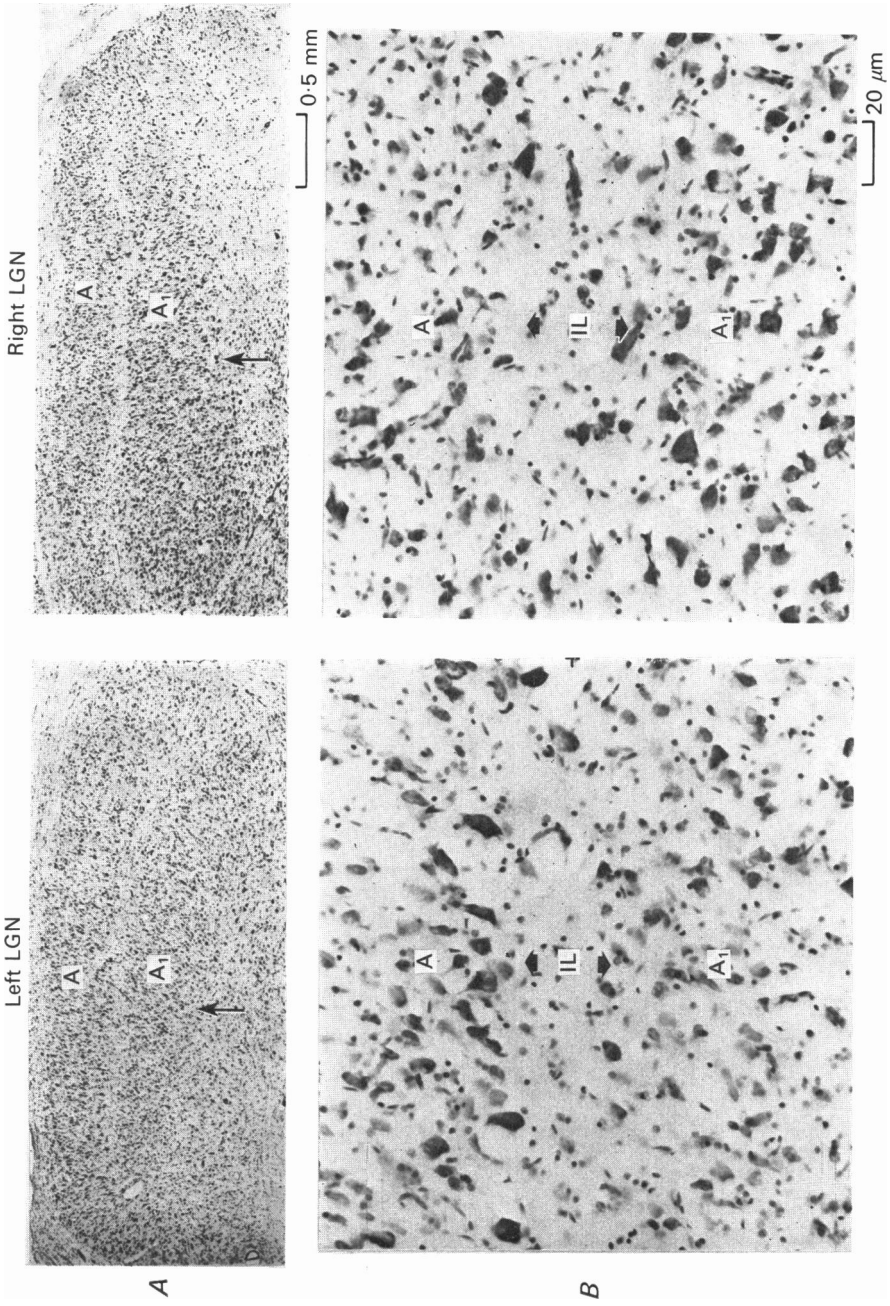
EXPLANATION OF PLATE

A, coronal section through the left and right LGN of a kitten raised with a convergent squint of 30° in the left eye. Layer A1 of the left LGN and layer A of the right LGN are fed by the squinting eye. *B*, comparisons of the same sections as shown in *A* at a higher magnification. The zones chosen are marked with arrows in *A*.


Right LGN
Left LGN
A
A1
IL
0·5 mm
20 μm
A
B