

NORMALITY OF SPATIAL RESOLUTION OF RETINAL GANGLION CELLS IN CATS WITH STRABISMIC AMBLYOPIA

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SUMMARY

1. A convergent or divergent strabismus was induced surgically in eight kittens and a cyclotropia of about 90 deg in two additional kittens.

2. Behavioural measurements were made of the visual acuity of each eye for square-wave gratings. All eight animals that were so tested displayed a reduction of acuity in one eye relative to the other of 1.3–2.5 octaves.

3. The activity of retinal ganglion cells was recorded within the amblyopic eye of six cats, three with a convergent strabismus, two with a divergent strabismus and one with a cyclotropia. Measurements were made of the spatial resolution of 215 on-centre cells for horizontal and vertical gratings.

4. In contrast to other reports, we found the spatial resolution of ganglion cells in the amblyopic eye of the strabismic animals to be comparable to those of normal cats at all retinal eccentricities. In particular there was no evidence for a loss of resolution in the vicinity of the area centralis.

5. Measurement of the cross-sectional area of cells in the lateral geniculate nucleus (l.g.n.) revealed no evidence of cell shrinkage in laminae receiving a projection from the amblyopic eye.

6. Together, these findings lead to the conclusion that the neural deficit responsible for the strabismic amblyopia in these animals did not lie in the retina but rather at more central levels of the visual pathway.

INTRODUCTION

Amblyopia is a relatively common disorder of visual development characterized by reduced acuity through one or occasionally both eyes that cannot be attributed to a residual optical error or a disease state. The most common form of amblyopia, strabismic amblyopia, is associated with a misalignment of the two eyes that usually arises within the first few years of life (Burian & von Noorden, 1974). Because of the gross disturbances of form vision and binocular functions and the relatively minor

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impairments of increment thresholds and dark adaptation (Wald & Burian, 1944; Burian & von Noorden, 1974; Duke-Elder & Wybar, 1973), it has long been thought that the major neural deficits in amblyopia lie central to the retina. A number of physiological investigations of cats and monkeys, in which a strabismus has been induced shortly after birth by surgical section of one or more extraocular muscles, have in fact revealed substantial deficits in the visual cortex (Hubel & Wiesel, 1965; Baker, Grigg & von Noorden, 1974; Crawford & von Noorden, 1979).

More recently, Ikeda and colleagues have investigated the effects of surgically-induced strabismus on more peripheral structures in the visual pathway of cats (Ikeda & Wright, 1976; Ikeda & Tremain, 1979). Surprisingly, they found that in some strabismic animals the spatial resolution of brisk-sustained cells was reduced in the region of the area centralis of the deviating eye (Ikeda & Tremain, 1979). These retinal anomalies were observed only in cats that always fixated with the non-operated eye. Additional evidence for an effect of strabismus on retinal ganglion cell properties has been provided by Chino, Shansky & Hamasaki (1980). Hence, earlier observations of a reduction in the resolution of brisk-sustained cells in the lateral geniculate nucleus that receive a projection from the deviating eye (Ikeda & Wright, 1976), and of a behavioural amblyopia through this eye (Jacobson & Ikeda, 1979) could then simply follow as a natural consequence of these retinal defects.

The reduced spatial resolution in retinal ganglion cells of strabismic cats is somewhat surprising in view of the lack of similar changes in the retinae of cats monocularly deprived by eyelid suture, a form of visual deprivation that on the surface would appear to be far more severe than a simple misalignment of the visual axes (Sherman & Stone, 1973; Kratz, Mangel, Lehmkuhle & Sherman, 1979; Cleland, Mitchell, Gillard-Crewther & Crewther, 1980). However, since strabismic animals with alternating fixation showed no retinal abnormality (Ikeda & Tremain, 1979), something other than a discordant visual input to the two eyes may be involved. To understand better the conditions which result in retinal changes, we have investigated the spatial resolution of retinal ganglion cells in the amblyopic eye of a number of strabismic cats. Despite a behaviourally severe loss of visual acuity through one eye, the visual acuities of retinal ganglion cells within the eye were normal at all retinal eccentricities.

METHODS

Animals and rearing conditions. Ten kittens were raised from about the time of natural eye opening (Table 1) with a strabismus induced by section of the tendon of one or more of the extraocular muscles of one eye. The surgery was performed under halothane anaesthesia in a 2:1 mixture of nitrous oxide and oxygen after prior sedation with 1 mg xylazine. The nictitating membrane was first removed, and the conjunctiva cut in the region of the appropriate muscle which was then located and sectioned. To prevent infection topical antibiotics were administered to the eyes following surgery. A convergent strabismus (esotropia) was induced in five of these kittens by section of the lateral rectus muscle in one eye, while a divergent strabismus (exotropia) was produced in three others by section of the medial rectus. Cyclotropia was induced in two kittens by section of all the extraocular muscles in one eye and gentle intortion of the globe by about 90 deg. Although there was some disturbance of the motility of the operated eye of all kittens for a few weeks following surgery, by 3-4 weeks of age substantial motility had returned. With the exception of two animals, con. 2 and div. 2, the operated eye remained very obviously deviated throughout life. In the case of div. 2, the strabismus appeared far less pronounced at the time of

the behavioural testing than immediately following surgery so that it was difficult to identify the deviating eye. All animals were reared in cages with mothers until 8–10 weeks of age at which time they were permitted to run free in a large colony room until the time of physiological recording. The complete rearing histories of all ten animals are summarized in Table 1.

Behavioural training. The visual acuities of the two eyes of each animal were assessed using the jumping stand and procedures that have been described extensively in earlier publications

TABLE 1. Rearing history of all ten strabismic kittens

Cat	Age (days)	Operation		Age at physiological recording (months)
		Nature of operation	Strabismus produced	
Con. 1	8	l.l.r.s.	Esotropia	—
Con. 2	7	l.l.r.s.	Esotropia	16
Con. 3	7	l.l.r.s.	Esotropia	12
Con. 4	13	l.l.r.s.	Esotropia	—
Con. 5	13	l.l.r.s.	Esotropia	11½
Div. 1	12	l.m.r.s.	Exotropia	—
Div. 2	11	r.m.r.s.	Exotropia	4½
Div. 3	11	l.m.r.s.	Exotropia	4½
Rot. 1	9	l.e.i.	Cyclotropia	—
Rot. 2	10	l.e.i.	Cyclotropia	12

l.l.r.s., left lateral rectus section; l.m.r.s., left medial rectus section; r.m.r.s., right medial rectus section; l.e.i., left eye intorted by 90 deg.

(Mitchell, Giffin & Timney, 1977). Although in many cases behavioural shaping of the animals was begun at 2 months of age, measurements of the acuities of the two eyes were not attempted for several more months when the acuities of normal kittens have approached or reached adult levels (Mitchell, Giffin, Wilkinson, Anderson & Smith, 1976). Briefly, each animal was trained to discriminate between a square-wave grating and an adjacent uniform field of the same space-average luminance situated beneath the animal on the jumping stand. The animals were trained to jump toward the grating by rewarding them with petting and with a small amount of highly palatable food. Incorrect responses (jumps toward the uniform stimulus) brought about a simple denial of these rewards. The grating was switched from side to side between trials according to a quasi-random schedule. After extensive training with gratings of low to medium spatial frequency under binocular viewing conditions, the animal's acuity for gratings was assessed in the following manner. The animal was first presented with a block of five trials with a grating of a low spatial frequency. If criterion performance was achieved (five consecutively correct) the spatial frequency of the grating was increased by a small amount (about 1/8 of an octave, where an octave represents a change in spatial frequency by a factor of two) for the next block of trials. This procedure was repeated until a spatial frequency was reached where the animal could not achieve criterion performance. At this point the spatial frequency of the gratings was reduced to a value that permitted the animal to re-establish criterion performance without any obvious signs of difficulty or distress. The spatial frequency of the gratings was then progressively increased as before until the animal again failed to perform correctly. While attempts were made to obtain two similar consecutive estimates of the highest spatial frequency that could sustain criterion performance within a single session, this was not always possible since some animals became agitated once they failed the discrimination.

The grating stimuli were photographic reductions or enlargements of commercially obtained materials (Mecanorma transfers) pasted on cardboard. The stimuli measured 25 x 25 cm and so subtended a visual angle of approximately 20 deg to the animal when poised on the platform prior to its leap. A graded series of gratings was available in order to change the spatial frequency in small steps. Further small refinements of the spatial frequency could be achieved by changes in the height of the platform from which the animal was required to jump. The uniform fields were

matched by eye to the mean luminance of the gratings (about 150 cd/m²). A set of uniform stimuli having luminances that were either 0.1 log units above or below that of the exact luminance match were randomly included in some blocks of trials on all animals in order to ensure that they were not employing a small but consistent luminance cue to aid the discrimination. No evidence for the utilization of luminance cues was ever found.

Measurements of thresholds were first made with binocular viewing. Once several identical estimates of the binocular thresholds were obtained on successive sessions, the animal was tested monocularly with a large opaque contact lens occluder covering the originally operated eye. With the exception of one animal (div. 2), the monocular performance of the animal using its non-operated eye was identical to that obtained with binocular viewing of the stimuli. A day was allowed to elapse following monocular testing of one eye before monocular testing of the vision of the other eye was initiated to avoid the effects of corneal distortions that may result in a previously occluded eye for a few hours following removal of the contact lens occluder. Tests of the vision of the operated eye were always immediately followed by a block of binocular trials with a grating of a slightly greater spatial frequency than the highest which allowed the animal to sustain criterion performance when employing the amblyopic eye alone. This was done to ensure that the poorer acuity that was achieved when the animal was forced to employ its amblyopic eye was not simply due to general reduction in performance on that particular day. Measurements of the acuity of the amblyopic eye were discontinued after identical thresholds had been achieved on three successive sessions.

On conclusion of behavioural testing the refractive state of the animals was assessed by retinoscopy both before and following instillation of a cycloplegic (2% homatropine or 1% atropine). None of the animals exhibited a significant anisometropia. The greatest difference between the refractive state of the two eyes of any animal (observed in div. 3) amounted to only 1 dioptre.

Physiological recording. Anaesthesia was induced with 3–4% halothane in a 2:1 mixture of nitrous oxide and carbogen. The halothane was then reduced to 1–2% for surgical procedures. During the experimental phase the animal was maintained on a 3:1 mixture of nitrous oxide and carbogen. The trachea and right cephalic vein were cannulated and, to reduce residual eye movements, the vago-sympathetic trunk was cut on the same side as the experimental eye. Two small craniotomies were made for bipolar stimulating electrodes to be stereotaxically placed in each optic tract slightly anterior to the dorsal lateral geniculate nucleus (l.g.n.).

The eyes were protected with clear plastic contact lenses. Phenylephrine hydrochloride (10%) was instilled into the experimental eye to retract the lids and nictitating membrane. To enable physiological recording from within the eye a ring was firmly attached to the eyeball, encircling the globe just behind the limbus. An attachment to this ring supported a hydraulic manipulator which entered the posterior chamber and was used to introduce tungsten-in-glass micro-electrodes (Levick, 1972). The pupil of the experimental eye was dilated with atropine eye-drops (1%) which also paralysed accommodation.

Animals were paralysed with an initial i.v. infusion of gallamine triethiodide (10–20 mg) and thereafter maintained with an infusion of gallamine triethiodide (5 mg/kg.h) and tubocurarine (0.4 mg/kg.h) in a glucose solution. The end-tidal P_{CO₂} was measured with a gas analyser and maintained near 4%. The e.c.g. was recorded through leads attached to the front limbs, and then displayed on a c.r.t. and amplified over a loudspeaker. The subcapular temperature was maintained at 37.5 °C by means of an electric blanket. The arousal state of the animal was carefully monitored by listening for sudden changes in the heart rate and observing the pupil of the unoperated eye, both of which were useful indicators of the level of anaesthesia of the animal (Dubin & Cleland, 1977) and could suggest the need to increase the proportion of nitrous oxide in the gas mixture.

Neural activity could be elicited by handheld targets or by projecting patterned stimuli onto a tangent screen 114 cm from the eye. An artificial pupil of 3 mm diameter was centred in front of the eye together with a spectacle lens chosen to optimize the spatial resolution of recorded brisk-sustained cells.

The electrode was initially directed toward a point in the retina that was on the vertical meridian (defined as the border separating brisk-sustained cells that project to the ipsilateral optic tract from those that project to the contralateral optic tract) and slightly below the area centralis. Cells were then sampled as the electrode was systematically moved along the vertical meridian and through the area centralis. The area centralis was operationally defined as the region with the smallest receptive fields and with local increase in antidromic latency. Functional classification of individual neurones was first determined with hand-held stimuli. The spatial resolution of all

on-centre cells was then assessed using computer-driven drifting square-wave gratings projected onto the screen. Gratings subtended 15 deg at the eye and their spatial frequency ranged from 0.5 to 8.0 c/deg in $\frac{1}{3}$ octave steps, i.e. increments of approximately 25% in spatial frequency. As in the earlier studies (Cleland, Harding & Tulunay-Keeseey, 1979; Cleland *et al.* 1980) the Michelson contrast $(L_{\max} - L_{\min}) / (L_{\max} + L_{\min})$ was 0.84 and the space average luminance of the gratings was 150 cd/m². Horizontally and vertically oriented gratings were presented to each cell at a drift frequency of 4 Hz. The peak-to-peak amplitude of the cell's response was then determined for two or more gratings which produced a response close to auditory threshold. From this the cut-off spatial frequency was calculated for each orientation by interpolating between data points, or extrapolating in those cases where the cell clearly responded to the highest frequency. The details of this can be seen in Fig. 2 of Cleland *et al.* (1980). For brisk units the response characteristic at high spatial frequencies falls rapidly once the amplitude is below 80 spikes/s. The slope on a double logarithmic plot is generally in the range -3 to -9. The cut-off spatial frequency was defined as that frequency which produced a mean peak-to-peak response amplitude of 25 spikes/s, a value previously judged to be at auditory threshold for a brisk unit at this drift frequency (Cleland *et al.* 1979). This produces clear modulation in the peri-stimulus histograms used for evaluation of response amplitude. For selected units Fourier techniques were used to determine the coefficient of the fundamental frequency. Applying similar methods of interpolation and extrapolation and a criterion value in this case of 20 spikes/s our estimation of cut-off spatial frequency using the two methods was always within 4%. (The need for a different criterion value is a result of the higher harmonics present in the response). Recordings were usually made over a 36 h period with initial emphasis being placed on the area centralis.

Histology. At the end of each recording session the animal was killed with an overdose of sodium pentobarbitone and perfused with normal saline followed by 4% formaldehyde in normal saline. Following perfusion the eyes were immediately enucleated, opened at the ora serrata and the posterior segments separated from the vitreous bodies. Each eye cup was washed in slightly hypotonic saline and the retina gently detached from the tapetum and choroid epithelium. After mounting on slides the retinae were stained for Nissl substance with Cresyl Violet. The top of the skull was removed and the head left overnight in formol-saline.

Before the brain was removed from the skull, stereotaxically determined coronal cuts were placed around the l.g.n. The blocks of tissue containing the l.g.n. were then placed in 10% sucrose in formol-saline. The sucrose concentration was then gradually increased to 30% over the next 48 h until the tissue equilibrated. 40 μ m frozen sections were later cut and stained for Nissl substance with Cresyl Violet. Quantitative analyses of l.g.n. soma size were made from sections on which the central visual field was represented (approximate Horsley-Clarke co-ordinates, frontal 5.5). Sections were magnified (625 \times) under a microscope and, if the nucleus and nucleolus were clearly visible, cell perimeters were traced by means of a drawing tube attachment onto a graphics tablet linked to a microcomputer (Apple II plus). Areas were automatically computed and stored for later statistical analysis. Soma areas were measured for laminae A and A₁ of both the right and left l.g.n. of four strabismic cats (con. 2, rot. 2, div. 2 and div. 3).

RESULTS

Behavioural tests

All eight strabismic animals that were tested behaviourally exhibited an amblyopia in one eye. This was just as true for animals that manifested a divergent strabismus as it was for animals with a convergent strabismus. The performance of two representative strabismic animals on the final few sessions under both binocular and monocular viewing conditions are shown in Fig. 1. The upper set of results shows the performance of div. 3, an animal that possessed a divergent strabismus and which was 3½-months of age at the time these results were obtained. The results shown underneath (*B*) were obtained from an older animal (con. 3) with a convergent strabismus and which was 11 months old when its visual performance was assessed. While the performance of both animals when using their right eye was virtually

identical to that achieved with binocular viewing, the performance mediated by the deviating (left) eye was much inferior. With their right eye both animals achieved comparable performance to that of normal animals in earlier studies (e.g. Giffin & Mitchell, 1978; Mitchell *et al.* 1976, 1977). By contrast, div. 3 using its left eye was unable to identify gratings having spatial frequencies higher than 0.85 c/deg. The left

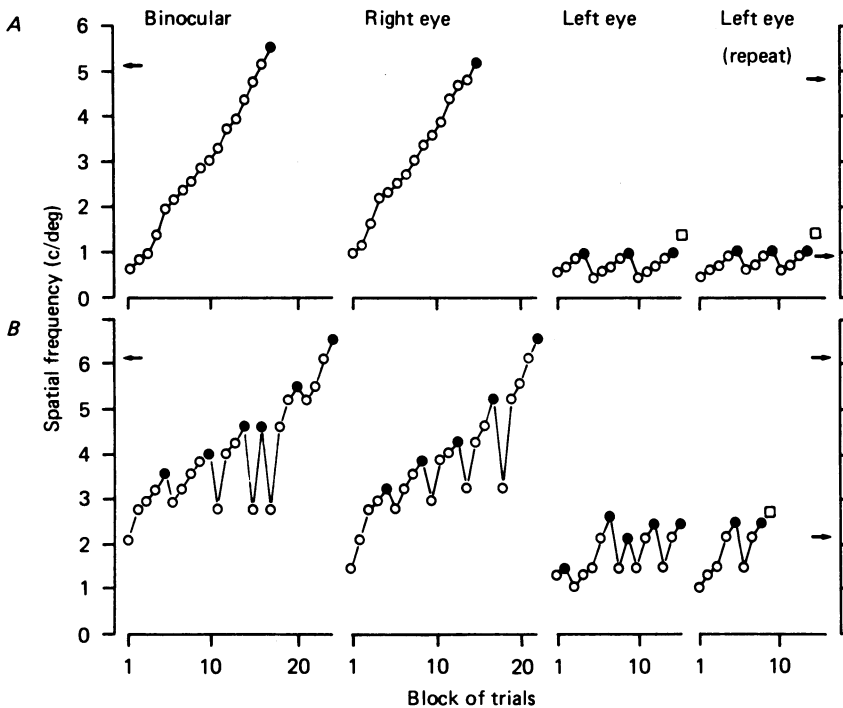


Fig. 1. Performance of two strabismic animals (div. 3 and con. 3) on four consecutive sessions, spaced one or two days apart, in which estimates were obtained of the binocular visual acuity, and the acuity of the right and left eyes. The abscissae indicate successive blocks of five trials. Open symbols define blocks of trials on which criterion performance was achieved, while filled symbols indicate those blocks of trials on which the animals failed to attain this level of performance. The horizontal arrows indicate the visual acuity, defined as the grating of highest spatial frequency for which criterion performance could be obtained under each viewing condition. *A*, performance of div. 3. With its right eye the animal achieved an acuity (4.8 c/deg) that was only slightly lower than the binocular acuity (5.2 c/deg). With its left eye, it manifested an acuity of only 0.83 c/deg on two consecutive sessions. The open squares indicate trials on which the animal was tested binocularly within minutes of the removal of the contact lens occluder from its right eye. *B*, performance of con. 3. The acuity of the right eye was identical to the binocular acuity, but with its left eye it was unable to sustain criterion performance with gratings above 2.1 c/deg.

eye of the other animal, con. 3, although severely amblyopic as well, was able to mediate a slightly better performance, 2.1 c/deg.

The behavioural results from all eight animals that were tested are summarized in Table 2, which lists first the grating of highest spatial frequency for which each animal sustained criterion performance with one or both eyes, followed by the spatial

frequency on which the animal was next tested and failed. The depth of the amblyopia, expressed in octaves, that was uncovered in each animal is also indicated. For this calculation the visual acuity was defined as the highest spatial frequency that permitted the animal to maintain criterion performance. In all but one case, div. 2, the amblyopic eye was the eye that had been caused to deviate by surgery early

TABLE 2. Visual performance mediated by each eye of eight strabismic cats

Cat	Age when visual tests completed (months)	Visual performance			
		Binocular (c/deg)	Non-operated eye (c/deg)	Operated eye (c/deg)	Visual deficit (octaves)
Con. 1	6	6.10-7.40	5.72-7.00	1.90-2.49	1.59
Con. 2	10	5.57-6.83	5.57-6.83	1.79-2.35	1.64
Con. 3	11	6.10-7.49	6.20-7.49	2.09-2.41	1.64
Con. 4	11	-	6.10-7.49	1.90-2.17	1.68
Div. 1	5½	5.72-6.10	5.72-6.10	2.16-2.30	1.40
Div. 2	3½	-	1.87-2.04	4.72-5.11	1.34
Div. 3	3½	5.19-5.57	4.81-5.19	0.83-0.96	2.53
Rot. 1	7	5.11-5.49	5.11-5.49	1.55-1.69	1.72

The first number refers to the highest spatial frequency of grating for which the animal could sustain criterion performance, while the second gives the next highest spatial frequency on which the animal consistently failed.

in post-natal life. This particular animal manifested the least obvious strabismus at the time of the behavioural tests. The magnitude of the acuity loss of the amblyopic eye of each animal, when expressed in octaves, were quite similar. This was so despite some differences in the absolute visual acuities, which were particularly evident in the case of the two youngest animals, div. 2 and div. 3, which manifested significantly lower visual acuities than the other animals. The lower absolute acuities of these particular animals could be attributed to the fact that the behavioural measurements were concluded prior to the age at which normal animals attain adult levels of visual acuity as assessed by the same behavioural technique (Mitchell *et al.* 1976).

Intraocular recording

Single unit recordings were made from retinal ganglion cells of two cats with a divergent strabismus, three with a convergent strabismus and from one of the cyclotropic cats. As an aid to classification of cells and location of the naso-temporal division the antidromic latency to electrical stimulation of the optic tract was measured on all cells. For on-centre cells the cut-off spatial frequencies were determined for both vertical and horizontal gratings. A total of 215 on-centre cells were recorded and these were classified as brisk-sustained (144), brisk-transient (42), sluggish-transient (10) and sluggish-sustained (19) (Cleland & Levick, 1974). None of the cells showed any evidence of immature receptive field properties such as described by Rusoff & Dubin (1977) or Ikeda & Tremain (1979).

In both groups of strabismic animals the antidromic latencies of the brisk-sustained and brisk-transient cells at a given eccentricity were clearly separated and comparable to the values found in normal animals (Cleland & Levick, 1974; Stone & Fukuda, 1974; Cleland *et al.* 1979). There was an initial fall in latency on moving from the

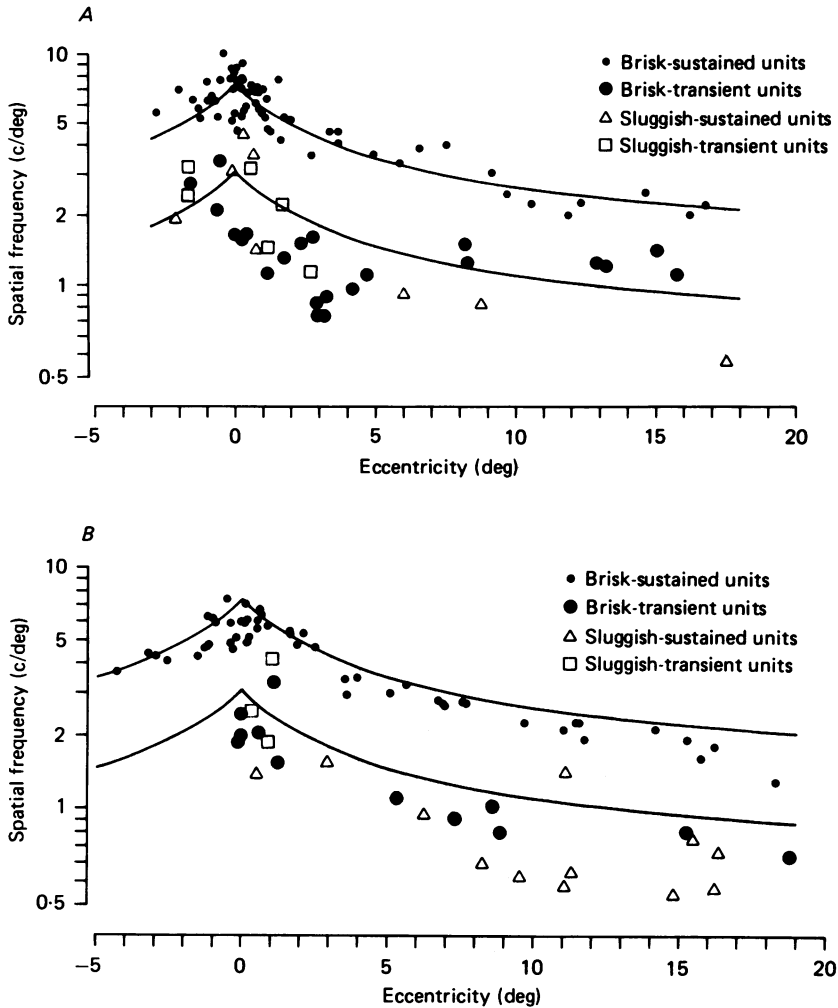


Fig. 2. Plot of the cut-off spatial frequency for on-centre retinal ganglion cells as a function of vertical eccentricity from the area centralis. Positive eccentricity is down in the visual field and up in the retina. The continuous curves represent the best fit to data obtained by Cleland *et al.* (1979) from normal adult cats. *A*, pooled data from three animals (con. 2, con. 3 and con. 5) reared with a convergent strabismus. *B*, pooled data from two animals (div. 2 and div. 3) reared with a divergent strabismus.

centre of the area centralis followed by a gradual increase at greater eccentricities. The latencies of the sluggish units were always greater than for brisk units, in agreement with results from normal animals.

As in our earlier study of retinal ganglion cells in monocularly deprived cats (Cleland *et al.* 1980), the cut-off spatial frequencies for vertical and horizontal gratings were frequently different. In general, the cut-off spatial frequency was slightly higher for horizontal than for vertical gratings. The mean cut-off spatial frequency of on-centre cells for horizontal and vertical gratings is plotted as a function of vertical eccentricity from the area centralis in Fig. 2. Pooled data from the three animals with

convergent strabismus are displayed in Fig. 2A while Fig. 2B shows data from the two divergent strabismic cats. The continuous line represents the average cut-off spatial frequency of on-centre cells as a function of eccentricity for normal animals (Cleland *et al.* 1979). In order to highlight the consistency between animals, individual data for each animal are provided in Table 3, in which is tabulated the spatial

TABLE 3. The highest cut-off spatial frequencies encountered amongst the sample of brisk-sustained cells recorded in the area centralis of each of six strabismic cats

Cat	Best cell (c/deg)	Best cell, any grating orientation (c/deg)	Average of four best cells	Number of cells
Con. 2	9.0	11.0	7.8	15
Con. 3	8.2	9.2	7.5	26
Con. 5	10.0	9.8	8.5	28
Div. 2	7.4	7.6	6.4	28
Div. 3	7.1	7.8	6.6	27
Rot. 2	7.4	10.0	7.0	13

The spatial resolution of each cell was measured for both vertical and horizontal gratings. Except where indicated, the figures quoted are the mean of the values obtained for the two grating orientations. Also shown are the number of brisk-sustained cells encountered in the area centralis of each animal.

resolution of the 'best' brisk-sustained cell in the area centralis of each animal, the average of the 'best' four cells, the total number of brisk-sustained cells recorded, and the age of the cat at the time the retinal recordings were made.

The cut-off spatial frequencies of cells from the cats with a convergent strabismus were comparable to data from normal animals (Fig. 2A). The acuities of cells recorded in the two animals with a divergent strabismus were very slightly lower than normal (by 10–20%) at all eccentricities. These particular animals were considerably younger than the others and when tested behaviourally manifested slightly lower visual acuity with their non-amblyopic eye (Table 2). However, even in these animals, some brisk-sustained cells were able to resolve gratings having spatial frequencies as high as 8 c/deg. Thus in none of the animals was there any evidence of a loss of resolution of brisk-sustained cells in the vicinity of the area centralis (see Table 3).

Histology

Microscopic examination of the retinal whole mounts obtained from each of the strabismic cats following intraocular recording revealed no detectable differences between the retinas of the amblyopic and non-amblyopic eye, a finding consistent with the physiological results.

To identify any morphological effect of a strabismus in the l.g.n., the cross-sectional area of 4063 cells was measured in four strabismic cats. Cells were sampled from the A and A₁ laminae of both the right and left l.g.n. of each animal. Histograms showing the distribution of cell areas throughout the binocular segments of laminae A and A₁ of one animal (con. 2) are displayed in Fig. 3. There was no evidence of cell shrinkage in laminae receiving an input from the amblyopic left eye (lamina A right l.g.n., lamina A₁ left l.g.n.). On both sides cells in lamina A₁ were larger by about

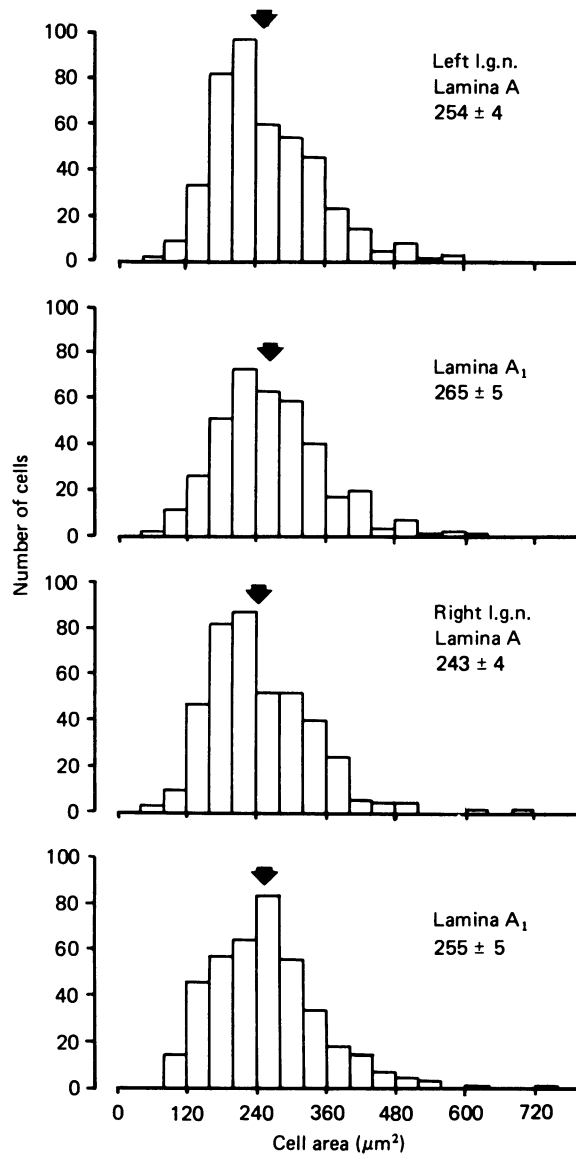


Fig. 3. Histograms showing the distribution of cell cross-sectional areas in the binocular segments of laminae A and A₁ from both the right and left l.g.n. of a cat (con. 2) reared with a left convergent strabismus. The numbers beside each histogram represent the mean cross-sectional areas (also indicated by the arrows) and the s.e. of the mean. Shrinkage of cells in lamina A of the left l.g.n. should be confirmed by a reduction in the cross-sectional areas of cells in lamina A₁ of the right l.g.n., if the shrinkage were due to a reduction in the efficacy of a proportion of the ganglion cells from the deviating eye.

7% than those in lamina A, a difference comparable to that observed in normal animals (Guillery, 1973). Close examination of the medial portion of the l.g.n. on which the central visual field is mapped also revealed no local evidence of cell shrinkage in any of the four cats studied in detail. Table 4 shows for all four animals the mean cross-sectional areas of cells in the portion of the l.g.n. which represents

TABLE 4. Mean cross-sectional cell areas from the l.g.n.s. of four strabismic cats, two reared with surgically-induced esotropia and two with exotropia

Cat	Mean cell areas (μm^2)			
	Left l.g.n.		Right l.g.n.	
	Lam. A	Lam. A ₁	Lam. A	Lam. A ₁
Con. 2	251 ± 6	255 ± 6	233 ± 5	234 ± 5
Con. 5	241 ± 7	264 ± 7	245 ± 8	264 ± 8
Div. 2	233 ± 9	260 ± 6	261 ± 7	278 ± 7
Div. 3	257 ± 5	279 ± 6	261 ± 5	289 ± 6

Cells were sampled in the binocular segments of laminae A and A₁ from both the right and left l.g.n. of each cat. With the exception of div. 3, cells were only sampled from the medial portion of the nucleus on which the central 5 deg of visual field is represented. In the case of div. 3 measurements were made from cells throughout the binocular segment. At least 400 cells were measured from each animal. Values are means ± s.e. of the mean.

the central visual field. Again there was no evidence of shrinkage of cells receiving afferents from the strabismic eye.

DISCUSSION

The important finding of this study is that while all our strabismic animals were amblyopic to behavioural tests the spatial resolution of individual retinal ganglion cells was normal. Thus, in contrast to a conclusion (Ikeda, 1979, 1980) drawn from an earlier study (Ikeda & Tremain, 1979), our findings indicate that strabismic amblyopia is not associated with a loss of spatial resolution of retinal ganglion cells.

All eight strabismic cats that were behaviourally tested demonstrated reduced visual acuity in one eye relative to the other of 1.3–2.5 octaves. Since all animals tested demonstrated a substantial amblyopia in one eye it is reasonable to assume that the two cats that were not behaviourally tested (con. 5 and rot. 2) were also amblyopic. Although the absolute acuities of the two eyes of our animals were generally higher than reported by others, the relative magnitude of the visual loss in the amblyopic eye was comparable to that exhibited behaviourally by strabismic cats in earlier studies (Jacobson & Ikeda, 1979; von Grunau & Singer, 1980). Jacobson & Ikeda (1979) reported an acuity deficit of 1.68 and 1.77 octaves in the deviating eye of the two cats in which divergent strabismus had been induced surgically at 3 weeks of age. A smaller deficit was reported in another study of two cats reared from 3 weeks of age with a surgically-induced divergent strabismus (von Grunau & Singer, 1980). At present it is unclear whether the difference in magnitude of the deficits reported in these two previous studies can be attributed to the nature of the strabismus (esotropia *versus* exotropia), or to the different surgical procedures by which they were produced. However, in this context it is noteworthy that the degree of amblyopia

exhibited by the exotropic cats in our study was no less severe than that exhibited by the esotropic cats. The single cat tested with a cyclotropia (rot. 1) demonstrated an amblyopia that was comparable in severity to the remainder of our strabismic cats and to cats reared with a similar degree of cyclotropia by other investigators (Timney & Peck, 1981).

Despite the presence of amblyopia that was as severe as that experienced by cats rendered strabismic by the procedure adopted by Ikeda and colleagues (Jacobson & Ikeda, 1979; Ikeda & Tremain, 1979), ganglion cells in the retina of the amblyopic eye of each of our strabismic cats were both morphologically and physiologically normal. Particular attention was placed on the physiological properties of retinal ganglion cells in the vicinity of the area centralis, the approximate location of which was first visualized by means of an ophthalmoscope and then located more precisely on the basis of decussation pattern, and the latency and spatial resolution of individually recorded ganglion cells. In every animal in which the area centralis of the amblyopic eye was systematically explored, several brisk-sustained cells were found that could resolve gratings having spatial frequencies of 7 c/deg or higher. Thus the visual acuities of retinal ganglion cells in the vicinity of the area centralis of the amblyopic eye were comparable to values obtained from normal animals (Cleland *et al.* 1979). On this basis it must be concluded that the neural deficit responsible for the severe behaviourally measured loss of visual acuity in our animals must lie at sites in the geniculostriate pathway beyond the retina.

The absence of any observable loss of spatial resolution of retinal ganglion cells in any of our animals is at variance with findings of Ikeda & Tremain (1979) who found that a large proportion of the behavioural amblyopia measured on their strabismic animals (Jacobson & Ikeda, 1979) could be accounted for by a loss of resolution of brisk-sustained retinal ganglion cells. In the area centralis of the deviating eye where the loss of resolution was greatest, the visual acuities of ganglion cells were reduced by an octave relative to values observed in normal animals or the non-deviating fellow eye of the strabismic cats. However, these retinal deficits were observed only in the deviating eye of strabismic cats that always fixated with the non-operated eye, as diagnosed by a cover test, a common clinical test for strabismus. In all of the animals so diagnosed, the strabismus had been induced by removal of the body of the lateral rectus muscle from one eye and usually the superior oblique muscle as well. In the case of the two animals that were diagnosed as having alternating fixation the strabismus had been induced by either section of the tendon of one extraocular muscle or removal of the medial rectus muscle. Because we found it extremely difficult to train even normal animals to maintain fixation without distraction for a sufficient length of time to perform a cover test, we did not attempt this test systematically on our strabismic cats. However, a number of observations suggest indirectly that the majority of our animals did not show alternating fixation. With the exception of two cats (con. 2 and div. 2), the misalignment of the visual axes of the two eyes was substantial and quite obvious on casual inspection. Over the course of many months the eye that appeared to deviate in all animals, apart from con. 2 and div. 3, was constant; there was no hint that the animals alternated fixation. More importantly, each of these animals exhibited an amblyopia with the deviating eye that was as severe as that manifested by animals reared with a strabismus induced

in the manner described by Ikeda and colleagues (Jacobson & Ikeda, 1979; Ikeda, 1979). On the basis of common clinical experience it is most unlikely that animals that exhibit such severe amblyopia would show alternating fixation. It is equally unlikely that the discrepancy between our findings and those of Ikeda & Tremain (1979) can be accounted for solely in the terms of the presence or absence of alternating fixation. Parenthetically, the strong likelihood that our animals did not show alternating fixation also poses difficulties for the theory (Ikeda & Wright, 1972) that links the loss of spatial resolution in retinal ganglion cells (Ikeda & Tremain, 1979), or in cells of the l.g.n., to habitually defocussed images in the deviating eye, since both their animals and ours would be equally susceptible to this loss of visual detail. Further doubt is cast on this theory by the observation of normal acuities of ganglion cells in animals monocularly deprived by lid-suture (Cleland *et al.* 1980), in which situation the retinal image would be blurred to an even greater extent.

In addition to the lack of any obvious morphological or physiological abnormality in the retina of the deviating eye, the findings from our strabismic animals differed from those of Ikeda and colleagues in another respect. Possibly as a consequence of the retinal deficits observed in their strabismic animals, they found the mean cross-sectional area of cells in laminae of the l.g.n. that received a projection from the deviating eye to be smaller by as much as 39% than those in laminae that received inputs from the non-deviating eye (Ikeda, Plant & Tremain, 1977). We could find no evidence of such cell shrinkage in our strabismic animals (Fig. 3 and Table 4).

The very obvious discrepancies between the findings from our strabismic and those of Ikeda (Ikeda & Tremain, 1979; Ikeda *et al.* 1977) indicate a fundamental difference between the two groups of strabismic animals. In fact, it is possible to identify a number of potentially important factors associated with the procedures employed to induce strabismus in the two groups of animals.

As already noted, Ikeda & Tremain (1979) induced strabismus in the majority of their kittens by removal of the body of two extraocular muscles (the lateral rectus and superior oblique) and the tissue on the lateral side of the eye. By contrast, the surgical procedure employed on our animals, simple section of the tendon of one muscle, was far less drastic. One of many potential consequences of the former procedure may be a severe and long-lasting impairment of the ocular motility of the deviating eye, which by itself may have effects on the developing visual system. In addition to the different surgical procedures employed, the ages at which the strabismus was induced in the two groups of kittens were quite different. Ikeda & Tremain's (1979) kittens were rendered strabismic at 3 weeks of age, at a time of heightened cortical plasticity. By contrast our animals were operated on much earlier; at or shortly after the time of natural eye-opening. The importance of these and other factors surrounding the surgical procedures employed to induce strabismus are currently under investigation.

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