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# IMAGE QUALITY OF THE CAT EYE MEASURED DURING RETINAL GANGLION CELL EXPERIMENTS

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SUMMARY

1. The modulation transfer function (MTF) of the dioptrics of fifteen cat eyes was determined. The *aerial* image, formed by the eye of a standard object (a 0·5–1·0° annulus), was photographed. The transmission of the film negative was measured with a scanning microdensitometer to yield the light distribution within the aerial image. Correcting for the double passage, this experimentally determined light distribution and the known object light distribution were used to obtain the MTF, applying Fourier methods. Each MTF was used to calculate the light distribution within the *retinal* image of stimuli of various geometry used in experiments on retinal ganglion cells in the same eye.

2. When the eye was equipped with an artificial pupil of the same size as that used in the neurophysiological experiments (4·0–4·8 mm diam.) the MTF had fallen to 0·5 at 2·43 c/deg. When the pupil was removed the MTF had fallen to 0·5 at a much lower spatial frequency (1·0 c/deg). This shows that even when one uses an artificial pupil too large to provide optimal image quality there is a vast improvement over using no pupil.

3. These image quality measurements were prompted by the need to know the *actual* stimulus image in experiments on the functional organization of the receptive field, a need exemplified in this paper by a few specific physiological results. The full neurophysiological results appear in the next two papers.

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## INTRODUCTION

In many experiments on the cat's visual system the stimulus to be imaged by the eye is carefully selected with the *intent* to expose some restricted portion of the receptive field of a ganglion cell to an illumination of some very specific spatial distribution. Now, the light distribution within a retinal image is never an exact replica of the object light distribution. Rather, the image quality (degree of blur) depends both upon the properties of the optic apparatus of the eye and upon such factors as the pupil size and the correcting lens that the experimenter has chosen. As work on retinal receptive field properties progressed in this laboratory (Cleland & Enroth-Cugell, 1968, 1970; Pinto, Enroth-Cugell & Gray, 1969; Enroth-Cugell & Pinto, 1970*b*) it became increasingly clear that there are many conclusions which cannot be drawn safely from the experimental results unless the *actual* light distribution on the retinal receptors is known. Hence the light distribution within the retinal image of the stimulus was determined during a series of experiments concerned with the properties of the surround response mechanism and with the interaction between the centre and the surround mechanisms. The results upon physiological optics are reported in this paper; the full neurophysiological results will be presented in the next two papers (Enroth-Cugell & Pinto, 1972*a*, *b*).

Our measurements do not represent the highest degree of accuracy that could be obtained with very refined methods and they are not valid for the image-forming apparatus as a whole. But we do feel that these measurements represent an improvement over not determining the image light distribution at all, for they give an estimate of the properties of that very portion of the dioptrics through which the light travelled when forming an image within the receptive fields whose functional properties were studied. For each such restricted portion of the image-forming apparatus we have assumed isoplanatism (spread function invariant of position) and isotropy (radial symmetry of point spread function). It is worth pointing out that much of the equipment required probably already exists in many laboratories engaged in visual research and the actual procedural steps that had to be added to a retinal ganglion cell experiment, already long and demanding, are *relatively* easy and hence likely to be successfully completed while both the cat and the experimenters still are in acceptable functional condition.

Fourier methods can be used to *calculate* the retinal light distribution from the known light distribution within an object provided that the modulation transfer function (MTF) of the eye that does the imaging is known (Gubisch, 1966). The MTF of each eye used in a retinal ganglion

cell experiment was obtained as follows: one annular object was imaged upon the cat retina and the light distribution within the *aerial* image formed in front of the cat's eye by light emanating from the retinal image was determined photographically (Flamant, 1955). From this measurement and the known light distribution within the object, Fourier methods yielded the MTF of the dioptrics under investigation. Once that was known the quality of the image formed upon the retina by stimuli of a variety of configurations, chosen during the neurophysiological experiment, could be calculated. It will be shown that imagery through the fully dilated pupil of the cat eye is very poor compared to imagery through an artificial pupil, even when this is too large to yield optimum image quality (4·0–4·8 mm in diameter). Results from one ganglion cell experiment exemplify that it is necessary to know the light distribution within the retinal image to reach certain conclusions regarding receptive field properties.

## METHODS

The neurophysiological results and the optical spread functions were obtained from the same cats. They were in light general anaesthesia (urethane; see Enroth-Cugell & Pinto, 1972*a*), or a pretrigeminal section of the brain stem (Batini, Moruzzi, Palestini, Rossi & Zanchetti, 1957) had been performed. In either case the eyes were immobilized with a muscle paralysing agent, the pupil dilated and the accommodation paralysed with atropine. Best possible general condition of the animal was maintained throughout the experiment, including the photography of the aerial image which was done either right after work on one cell had been completed or at the end of the neurophysiological experiment. Action potentials were recorded stereotaxically from single axons in the optic tract and stored on magnetic tape. Responses to many individual stimuli were averaged with a smoothing network and a digital memory oscilloscope to yield pulse density tracings. This is the form in which the ganglion cell responses of Fig. 5 are presented. (For details see next paper, Enroth-Cugell & Pinto, 1972*a*.)

A mask containing a 0·5–1·0° diam. annulus, located just in front of an extended source (xenon arc lamp illuminating a diffuser) was imaged by the cat eye (Fig. 1). The length of the light path from the annulus to the cornea (and from all stimuli used in ganglion cell experiments) was 125 cm. From a collection of contact lenses made of black opaque plastic with a central clear pupil (4–4·8 mm diam.) that power was chosen which, judging by direct ophthalmoscopy, would give the sharpest focus of the annulus on the retina. Next an experimenter positioned his eye where the camera lens is shown in Fig. 1; through the cube, mounted on an adjustable arm, he observed the image formed by the annulus on the cat retina. Assuming that (1) the optical system of the cat eye like that of the human eye (Westheimer & Campbell, 1962; Campbell & Gubisch, 1966) is reversible and (2) that the contact lens is close to the optimal one, then the rays emanating from the retinal image have, as they leave the eye, acquired a convergence that would have brought them to a focus 125 cm in front of the cat's eye had they not been intercepted by the observer's eye. They entered it through a $-0{\cdot}82$ dioptre lens and a telescope previously focused for infinity, which meant that the emmetropic eye of the observer received parallel rays. Spectacle lenses (in 0·5 dioptre steps) were then held between the cat's eye and

the cube and the observer decided whether or not the annular image on the cat fundus could be made sharper. If it could, then a contact lens of higher or lower power, as indicated by the spectacle lens, was chosen. The reason for using an annulus was that the degree of darkness in the middle of its retinal image turned out to be a much more reliable clue than the perceived degree of sharpness at the edge of the retinal image of a slit. The telescope with the minus lens mounted in front provided magnification, but, most important, it seemed to help the observer not to accommodate. The entire procedure described above was repeated until, from a series of contact lenses the (nominal) powers of which varied in 0·5 dioptre steps, the best available one had been selected. This was the lens with which the ganglion cell experiment and the photography of the aerial image was carried out. Both cat eyes were equipped with a contact lens in the manner described.

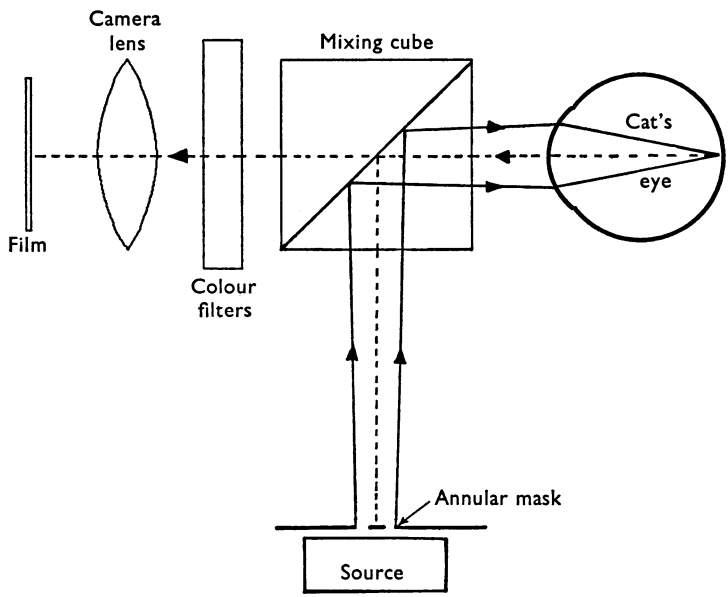


Fig. 1. Set-up for measuring the modulation transfer function of the cat dioptrics. Contact lens with artificial pupil has been omitted. Luminance of source $2{\cdot}5 \times 10^3$ cd/m$^2$.

For photography the standard 0·5–1·0° diam. annular mask was inserted in front of the source (Fig. 1) with the cube positioned such that the retinal image fell in the vicinity of the receptive field(s) that had been studied. In some cases, when the electrode was still recording action potentials from a cell that had just been studied, the annulus could be centred quite precisely on the receptive field by listening to the discharge. Both the quality of the aerial image used in the computations of the MTF and the quality of the image that the receptive field 'saw' thus depended upon passage of light through the same portion of the dioptrics of the cat eye. With two colour filters (Wratten 52 and 2A) attached to its lens mount, the camera was oriented to receive the rays from the retinal image. They were focused on to a sensitive film (Kodak 2475) whose spectral sensitivity was constant from 350 to 700 nm and whose spatial frequency response curve was flat up to 10 c/mm or about 20 c/deg of subtense on the cat retina. Each filmstrip was exposed for 200 sec to (1) the aerial

image of the 0·5–1·0° diam. annulus and (2) a calibrated neutral density step tablet (silver) spanning 4 log units in 20 steps. For this the colour filters were not used and a different, larger, uniform source was used to transilluminate the tablet. The film was developed for 8 min in Kodak DK-50 with agitation every 15 sec to reduce adjacency effects. The over-all performance of the system was determined by replacing the cat eye with a plane mirror while otherwise measuring the MTF as just described. It was flat to 6 c/deg.

An appreciable proportion of the energy content of the light that formed the image on the cat's fundus was at wave-lengths shorter than 475 nm (see curve for $S_2$ in Fig. 1*B* of Enroth-Cugell & Pinto, 1972*a*). To the experimenter looking into the cat's eye the image appeared sharp when light of wave-lengths which dominate his visibility curve were in best focus. But at that power rays in the wave-length range below 475–450 nm were out of focus. The film is relatively much more sensitive in the short and long wave-length regions than the human or cat eye. Hence, if unfiltered light reflected from the fundus had fallen on the film much of the blur of the aerial image would have been due to bluish light which was never perceived by the cat and thus of no physiological significance. The same would have been true about the deep red light but not to the same extent because the unfiltered light had less relative energy there than in the blue region. To assure that such 'false degradation' of the retinal image did not occur the two coloured filters were attached to the camera lens. The curve for the spectral composition of the filtered light is shown in Fig. 2.

In some instances the photographic image of the aerial image of the annulus contained irregularities which were caused by retinal vessels or pigment lumps and visible to the naked eye (see Fig. 3*A*). On each film negative one or two radii free of obvious retinal irregularities were chosen and the relative transmission measured along them. The measuring equipment consisted of instruments used for other purposes when not combined into a 'self-plotting scanning microdensitometer'. The film negative, emulsion up, was clamped between two microscope slides on the movable stage of a Leitz Laborlux microscope. One of its eye pieces held an adaptor carrying a fibre optics light pipe which collected from a 20 $\mu$ diam. area of the film. The adaptor accepted a thicker light pipe 'seen' by the photomultiplier of a photometer (Gamma Scientific, Model 2020). The shaft of a motor-driven potentiometer was mechanically coupled to the microscope stage; electrically the potentiometer was connected to the $X$-input of an $X$–$Y$ recorder whose $Y$-input received the output from the photometer. In this way the transmission was automatically plotted on the $X$–$Y$ recorder (Fig. 3*B*). Along each radius forty points per degree were read from the plot and the gross fog density subtracted from each value. Using the step tablet calibration, transmission at each point was converted into relative film illumination (Fig. 3*C*).

Thus, knowing the light distribution within the *object* imaged by the cat's eye and having determined the distribution within the *aerial* image formed in front of the eye, the Fourier–Hankel transform of both of these light distribution functions were obtained (Digital Computer CDC 6400) (Fig. 3*D*). Since the light that formed the aerial image had passed the dioptrics of the eye twice the (positive) square root of the ratio: image/object transform yields the modulation transfer function (Fig. 3*E*). To perform this computation one must convert from c/deg (of retinal subtense), which is how spatial frequency is expressed in the Fourier transform of the object, to c/mm (of film), which is how spatial frequency is expressed in the aerial image transform. The scaling factor for converting between these two units of spatial frequency depends upon (1) object-to-cat-eye distance, (2) the cat-eye-to-film distance, (3) the posterior nodal distance of the cat eye, which according to Vakkur, Bishop & Kozak

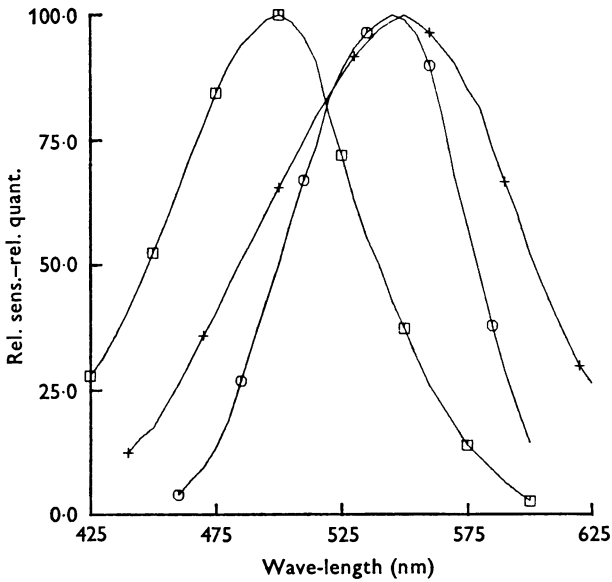


Fig. 2. The curve identified by circles was drawn through measurements every 12·5 nm. It gives the relative energy (in quanta) versus wave-length in the light which in our experiments fell on the camera film. The curve identified by the squares is the scotopic sensitivity curve averaged from four retinal ganglion cells (Granit, 1949). The curve identified by crosses is from Daw & Pearlman (1969). It is the average photopic sensitivity curve for 64 ganglion cells.

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Legend to Fig. 3.

Fig. 3. *A*. Photograph of the aerial image showing three vessels at approximately 11, 2, and 6 o'clock. The noticeably greater luminance between 6 and 11 o'clock is not due to non-uniformity of the source. The line shows the diameter along which densitometric measurements were made. *B*. Plot of the output of the densitometer ($y$) against distance ($x$) along the line in *A*. *C*. Distribution of illumination within the aerial image. The ordinate has been corrected for the characteristic exposure curve of the film. The abscissa represents radius along the left half of *B*. *D*. The Fourier–Hankel transform of the aerial image (triangles) and object (stars); each has been scaled to unity. *E*. Triangles: plot of the square root of the ratio of the two functions given in *D*. Stars: third-order polynomial fitted to the data in *E* after omitting points at discontinuities (see text, p. 390). This curve which is displaced upward by 0·2 was used as the MTF in computation of inverse transforms. *F*. Calculated distribution of the retinal illumination that results from imagery of the annular test object. This was obtained by inverse Fourier–Hankel transformation of the product of the object transform and the MTF in *E*.

(1963) varies by 16% from cat to cat. Of these the first was always constant, the second varied somewhat from experiment to experiment. Hence the ratio of cycles/deg to cycles/mm was different in each cat and this had to be taken into account. The third factor (the variation in nodal distance) was taken care of by the method outlined below. To convert from c/deg to c/mm we made use, in the following manner, of the unit *angular* magnification that exists between rays entering and leaving the cat's

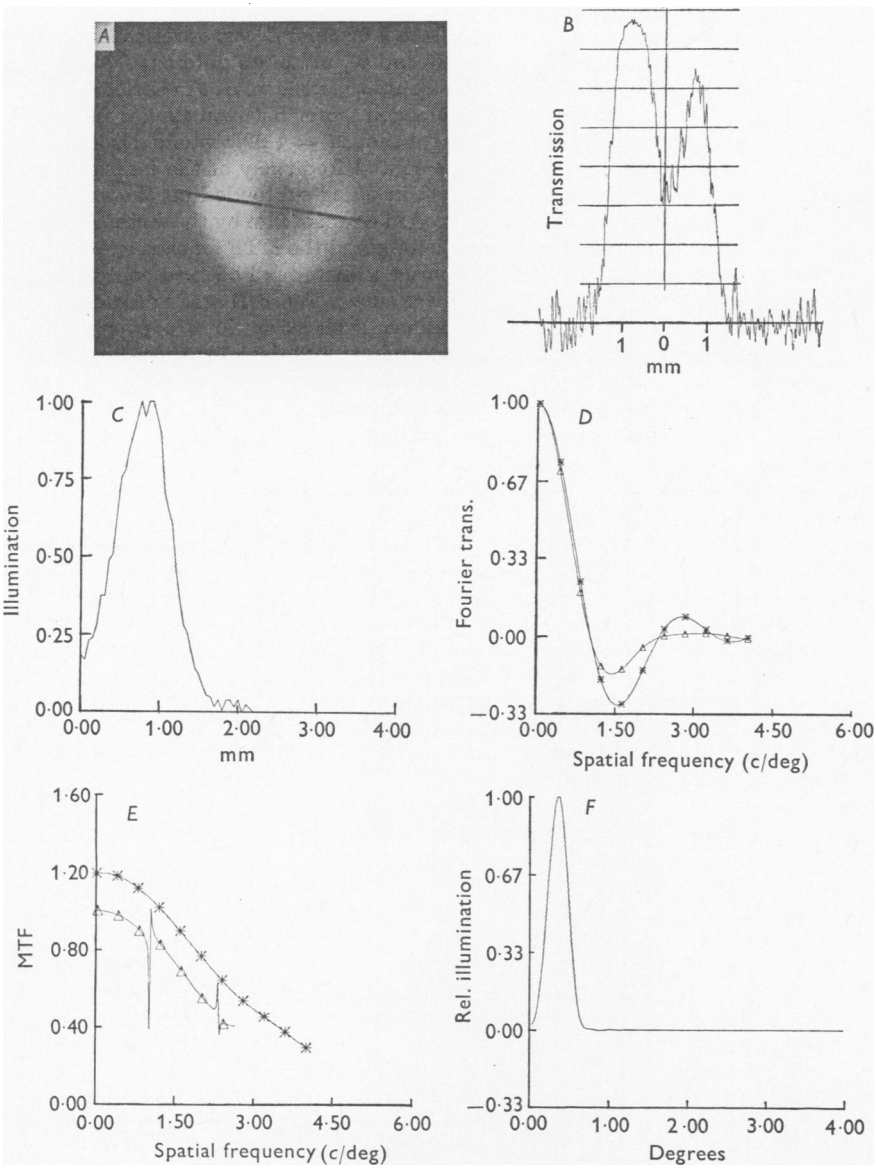


Fig. 3. For legend see opposite page.

eye; the lowest spatial frequency, expressed in c/deg, for which the Fourier transform of the *object* annulus (Fig. 3*D*) has zero content is 1·02. An angular magnification of unity means that the Fourier transform of the *aerial* image of the object annulus also has zero content at an angular spatial frequency of 1·02 c/deg. Moreover, this must be the *lowest* angular spatial frequency at which the transform of the aerial image has zero content, for there was no gross error in focusing on to the retina, nor any extreme aberrations in the dioptrics of the eye. It follows that the lowest *linear* spatial frequency, i.e. number of cycles per *millimetre*, at which the transform of the aerial image assumed zero value corresponds to 1·02 c per *degree* of retinal subtense. The linear spatial frequency (which we shall call $x$), although different from eye to eye could in each case be identified on the Fourier transform of the aerial image of the annulus. If we designate by $M$ the conversion factor between spatial frequency in c/deg and spatial frequency in c/mm, we get that $M = 1{\cdot}02/x$ where $x$ is known in each case. In other words, for each eye the spatial frequency of the Fourier transform of the aerial image could be expressed in c/deg subtended on the retina by multiplying the spatial frequency, $x$, expressed in c/mm of film by the factor $M$.

Once the conversion factor $M$ had been determined the MTF for each eye could be plotted. Near frequencies at which the Fourier transforms had zero content there were always discontinuities in the MTF (lower curve, Fig. 3*E*) arising from the low signal to noise ratio in these regions. To eliminate such discontinuities (upper curve, Fig. 3*E*) the RMS noise of the film was determined and data for those spatial frequencies where the Fourier transform content was less than the film RMS value were discarded. A third-order polynomial was then fitted to the remainder of the data and linearly extrapolated to zero content by means of a tangent to the curve at the highest usable spatial frequency. This extrapolation was necessary because abrupt truncation of the MTF resulted in severe oscillations of the inverse transforms.

To obtain the distribution of retinal illumination for any object we computed the inverse Fourier transform of the product of the object transform and the MTF. For (radially symmetric) spots and annuli this is most easily accomplished by means of the inverse Fourier–Hankel transform (Papoulis, 1968). Examples of computed retinal images appear in Fig. 3*F* and Fig. 5*C–E*. The small oscillations in the curves representing the retinal illumination distribution arise from unavoidable truncation errors which occur during numerical computation of the inverse transforms and do not represent real variations in retinal illumination.

The position of each receptive field within the visual field was estimated in the following manner. A cardboard screen with a Cartesian co-ordinate system was placed parallel to the stereotaxic Horsley–Clarke frontal plane about $\frac{1}{2}$ metre in front of the cat. The axes were labelled in degrees with the origin located on the line perpendicular to the cardboard and passing through the centre of the artificial pupil of the appropriate eye. The receptive field middle was located and its distance in degrees up or down and laterally was noted. All receptive field positions were then similarly plotted in a co-ordinate system identical to that on the cardboard, together with the position of the presumed area centralis, which according to Vakkur *et al.* (1963) lies 13·5° up and 3·5° lateral. The distance in degrees from each receptive field middle to the presumed area centralis was then measured on that plot. It ranged from 2 to 33°, with the majority of them being between 10 and 30°. If we relate the position of our receptive fields to the tapetal extent indicated by Pl. 1 in Bishop, Kozak & Vakkur (1962), then, in our best judgment, all fields were located within the tapetum, two or three perhaps in the border area. We estimate the reliability of the measurement to be within 3·5°.

## RESULTS

The optical quality of a total of fifteen eyes was determined. Typical MTFs are presented in Fig. 3*E* and Fig. 4*E* (upper curve). Among the fifteen eyes the mean of the spatial frequencies at which the MTF had fallen to 0·5 was 2·43 c/deg; the range was 1·7 to 3·6 c/deg. However, measurements above 3 c/deg are somewhat unreliable because (1) the content of the Fourier transform of the *object* (the 0·5–1·0° diam. annulus) was quite low at 3 c/deg (see Fig. 3*D*), (2) at 3·0 c/deg the double passage through the cat dioptrics attenuated this low content of the object transform on an average by $0{\cdot}41^8$. This caused the signal to noise ratio to be unacceptable above 3 c/deg. This was not a serious limitation of the technique for whenever a modulation transfer function was extrapolated beyond 3 c/deg (see p. 390) it was always true that the resulting curve led to an *under*-estimation of the sharpness of the retinal image.

Perhaps the most significant finding of this investigation was the image degradation incurred when no artificial pupil was used. One (atropinized) eye was refracted as described in methods except that the artificial pupil was not part of the contact lens itself; it was mounted on the surface of the lens. With the *retinal* image centred on the line passing through the middle of the pupil (perpendicular to the frontal plane) the *aerial* image was first photographed with artificial pupil; this was then removed and the aerial image again photographed while the cat wore the same contact lens in the same position. (The exposure time without pupil was 5 sec.) The photographs of the two aerial images were used to obtain the MTF with and without pupil. The results of this experiment appear in Fig. 4. It is clear that the image quality suffered seriously from removal of the 4·8 mm diam. pupil. When the artificial pupil was not in place, light entered the cat eye through the peripheral parts of the contact lens which were not involved in image formation when the artificial pupil was used. The MTF of the contact lens alone was therefore determined. All parts of the lens that passed light into the cat eye when no artificial pupil was employed were included. The MTF of the lens had not fallen to 0·5 at 4 c/deg. Thus, the difference in the quality of the retinal image formed with and without artificial pupil can clearly not be explained by the incorporation of more peripheral parts of the contact lens.

Fig. 5 is included to give a concrete example of the effect that image quality may have on the character of retinal ganglion cell responses, and, as a consequence, upon the interpretation of electrophysiological experiments on retinal ganglion cells. We will here define a ganglion cell response as a stimulus induced *change* in its discharge frequency. In the cat most ganglion cells are of centre-surround organization and usually their

responses result from the activity of both functional entities, the centre and the surround response mechanism (Rodieck & Stone, 1965). Such responses will be called *mixed*. The two response mechanisms are mutually

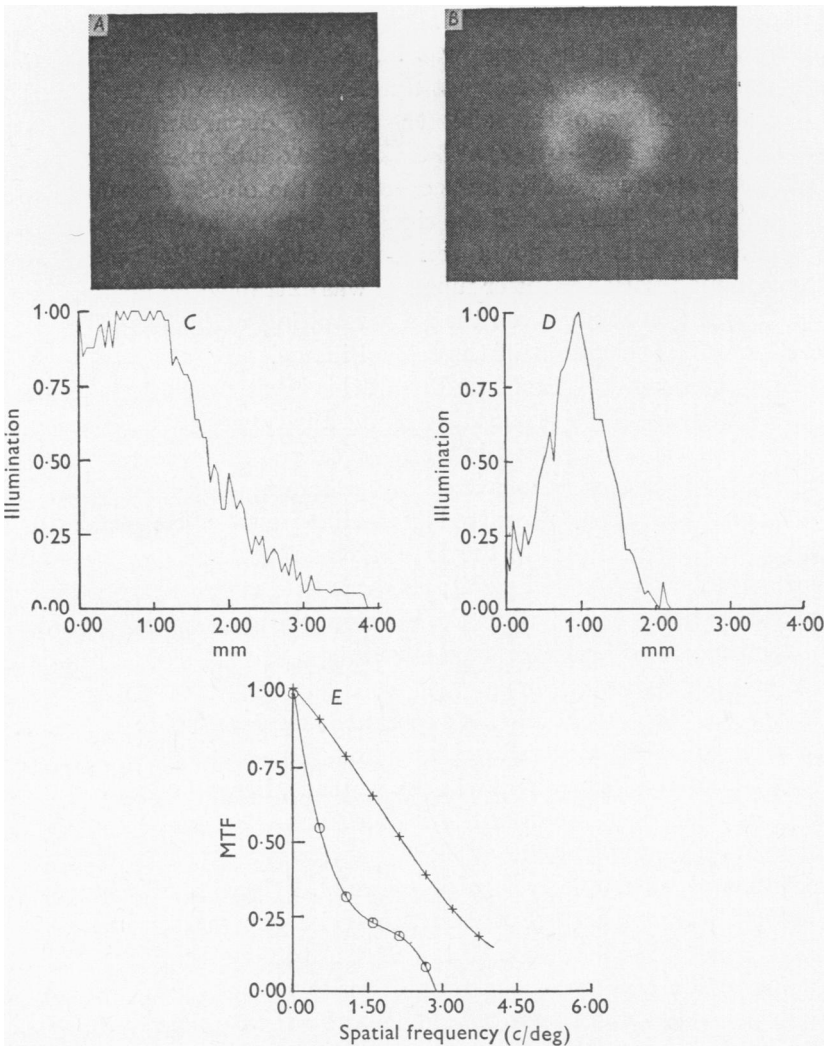


Fig. 4. Comparison of the optical quality of a cat eye without and with a 4·8 mm diam. artificial pupil. The contact lens is the same in both cases. The cat's pupil was fully atropine dilated in both cases. *A* is a photograph of the aerial image without, *B* with the pupil. Both photographs printed to equal peak density. *C* and *D* are microdensitometer plots of *A* and *B* respectively and corrected for the characteristic exposure curve of the film. Ordinates scaled to unity. *E* is the MTF of the optics of the cat's eye without (circles) and with (crosses) the artificial pupil.

antagonistic. That is, if at onset of light one provides an excitatory input to the ganglion cell (strives to increase spike frequency) the other produces an inhibitory input (strives to decrease spike frequency). At offset, the two mechanisms exchange roles. Responses elicited from either one of the mechanisms in isolation will be called *pure central* or *pure surround* responses. Such responses exhibit a far simpler behaviour than if the two mechanisms are allowed to interact to yield a mixed response (Stone & Fabian, 1968; Cleland & Enroth-Cugell, 1968; Pinto *et al.* 1969). It is quite easy to elicit a pure central response, for the central mechanism is relatively more sensitive than the surround mechanism over large parts of the receptive field (e.g. Rodieck & Stone, 1965). It suffices to keep the stimulus flux small and concentrated primarily on the central parts of the receptive field. For the same reason that pure central responses are easy to elicit, pure surround responses are difficult to obtain. The relative sensitivities of the two mechanisms must be reversed by some 'trick'. One such 'trick' is to apply a steady light to the middle of the receptive field where the sensitivity of the central mechanism is high and the surround sensitivity presumably low (Bishop & Rodieck, 1965). This will depress (adapt) the centre without substantially affecting the surround mechanism provided that the flux contained in the *actual* retinal image of the adapting light does not fall upon surround regions of appreciable relative sensitivity.

It is the last point that is exemplified for one on-centre cell in Fig. 5. In the presence of a steady adapting spot, centred upon the receptive field, a concentric flashing annulus (*A*) evoked a response, which although definitely dominated by the surround, was mixed. For there was an unequivocal transient increase in discharge rate not only at offset but also at onset of the light. No other combination of luminance and geometry of the annulus resulted in a pure surround response until (*B*) the steady adapting spot was made smaller (luminance unchanged). At this decreased diameter of the adapting spot, a flashing annulus of the same geometry as in *A* was applied at three different luminances (the same as in *A* and a higher and a lower one) and now the cell gave pure surround responses. This was evidenced by the lack of a spike burst at 'on' and by the fact that when these three responses were superimposed they all had the same time course (Pinto *et al.* 1969; Enroth-Cugell & Pinto, 1972*a*). Next let us consider the light distribution within the retinal image of the adapting spot when the cell did *not* yield pure surround responses (*A*, *C*) and when it did (*B*, *E*). For this particular cell the sensitivity of the *central* response mechanism was maximal and uniform over an approximately circular central area of 0·4° radius. (Sensitivity profile determined as described in Cleland & Enroth-Cugell, 1968.) *C* and *E* of Fig. 5 show the relative retinal illumination as a function of distance from the mid-point of the retinal image (and

of the receptive field) of the two equiluminous adapting spots. Within the retinal image of the larger spot (*C*) the illumination remained maximal as distant from the middle of the image as 1°. Knowing how retinal illumination varies with distance from the centre of the image one can calculate the spatial distribution of the *flux*. In *A*, where the cell did not yield a pure surround response, 20 % only of the total adapting flux fell within the area

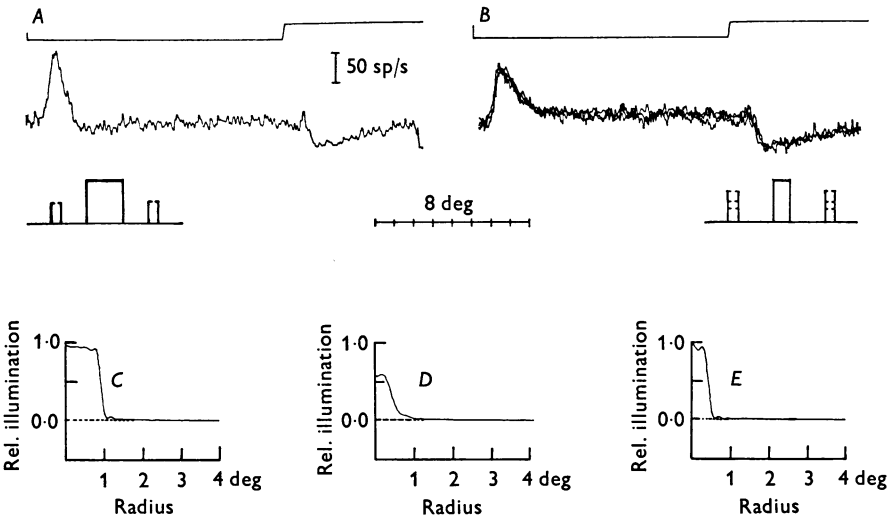


Fig. 5. On-centre cell. Upper tracings in *A* and *B* show stimulus time course; deflexion downward indicates offset of light. The lower traces are responses obtained with a 4·8 mm diam. pupil and shown in pulse density form. In *A*, the response is *mixed*. *B* shows the superposition of three responses elicited by annular stimuli of three different luminances. The superposition was achieved by vertical scaling and horizontal and vertical translation and provides evidence that these are *pure* surround responses. Profiles of the radially symmetric adapting lights and stimuli are shown in the diagrams immediately beneath the pulse density tracings. Unmodulated (adapting) lights have a solid, modulated an open top. The heights in the profiles are proportional to log relative luminance. The adapting lights in *A* and *B* are equiluminous, three different annulus luminances are indicated in *B*; the highest one was $9{\cdot}9 \times 10^{-2}$ cd/m$^2$; range 0·6 log units.

*C* shows the *calculated* relative retinal illumination as a function of distance from the centre of the image of the adapting spot used in *A*. The MTF used in the calculations was obtained with a 4·8 mm pupil from the very eye whose responses are shown in this Figure. *D:* the MTF for no artificial pupil shown in Fig. 4 was used to calculate the relative retinal illumination (plotted as in *C* and *E*) which would have resulted from imagery through the fully dilated cat pupil omitting the artificial pupil. Unity relative retinal illumination in *C–E* was taken as the illumination of the ideal retinal image. Note that unity retinal adapting illumination would have been 8·5 times larger in *D* than in *E* (and *C*) due to the larger pupillary area in *D*. *E:* relative retinal illumination for the adapting spot used in *B*.

of maximal central sensitivity. The rest fell upon receptive field regions where central sensitivity decreased quickly with distance from the field middle, and the *relative* sensitivity of the surround mechanism thus may not have been insignificant. Within the retinal image of the smaller adapting spot (*E*) maximal relative illumination extended only about 0·5° from the centre of the image. Calculation of the flux distribution showed that in this case when the cell *did* yield pure responses, as much as 75% of the total adapting flux fell within the region of maximal sensitivity of the central mechanism. Hence it is likely that only a small proportion of the total adapting flux fell upon areas of appreciable *relative* surround sensitivity. Apparently this 'withdrawal' of steady adapting flux to within a smaller central area affected the balance between the centre and the surround so that in the response to the flashing annulus in *B only* surround inputs (transient ones) could be identified. Now, it could be argued that it was not the difference in the retinal *distribution* of the adapting flux but the smaller *total* flux in *B* that enabled the cell to produce pure surround responses. We do not believe that this is so for in no case have we noted that increasing the adapting flux by luminance (diameter constant) would render a previously pure surround response to become mixed (see Enroth-Cugell & Pinto, 1972*a*).

The reason for including *D* of Fig. 5 is to illustrate the quite considerable improvement in image quality that follows the application of an artificial pupil although its diameter was larger than optimal. The extent to which the image of the smaller adapting spot (the same as in *B*, *E*) would have been smudged, had the 4·8 mm pupil not been used, can be judged by comparing the profiles of *E* and *D*. The total adapting flux would have been 8·5 times larger in *D* than in *E* and from the retinal illumination curve in *D* it was calculated that 46% only of the flux would have been restricted to within the area of maximum uniform sensitivity for the central response mechanism. A substantial amount of adapting flux may thus again, like in *A* where the cell did *not* yield a pure surround response, have fallen upon receptive field areas of possibly significant *relative* surround sensitivity. Hence, if the artificial pupil had not been used in this experiment, then this particular cell *may* never have yielded a pure surround response. It also seems safe to assume that unsharp imagery due to other causes may have prevented the cell from producing a pure surround response.

## DISCUSSION

The validity of the experimental procedures used requires that a number of conditions were reasonably well fulfilled.

First, to measure the light distribution within the fundal image, whose

sharpness the experimenter judged by looking into the cat eye, would be to no avail unless that light distribution and the light distribution across the outer segments of the receptors of the cat were the same, or at least closely related. The image observed by the experimenter must have been formed on the vitreal surface of the tapetum from which the distance to the proximal end of the outer segments is maximally about 50 $\mu$ (Prince, Diesem, Eglitis & Ruskell, 1960). Since this distance constitutes less than 0·5 % of the posterior nodal distance in the cat, the dioptric separation between the effective light distribution across the receptors and the measured light distribution within the fundal image should have been small and consequently the two light distributions closely related.

Secondly, the properties of the reflecting surface of the cat fundus, the tapetum, is of considerable significance. Reflexion should be diffuse, not specular as from a mirror, in order to guarantee that the ingoing and the outgoing light travel through the same portion of the dioptrics and that no coherence arises. Weale (1953) measured the variation in tapetal reflectivity in the cat at two angles of reflexion, 0 and 35°, and he concluded that the reflexion was diffuse. One of our findings supports this view. If the tapetum acted as a concave mirror and not as a diffuser, then it should have been possible for the experimenter to see a good replica of the test annulus cast on the viewing screen of the camera, *even* if the contact lens chosen for the cat was not the correct one for sharpest retinal focus. For each 'incorrect' contact lens one should have been able to find one location for the camera screen where the aerial image was sharp. The size of the aerial image, and the cat to screen distance, but not the quality of the image, would have depended upon the power of the contact lens chosen for the cat. In several experiments, 'incorrect' contact lenses were deliberately placed upon the cat's eye; the quality of the image on the viewing screen was never as good as with the correct lens in place, even when the camera was in best focus. We also determined (in the manner described by Campbell & Gubisch, 1966) the proportion of the reflected light that retained its polarization, removing the contact lens just before the measurement. Seventy-five per cent of the light had retained its polarization. The evidence cited above thus points to the fundus of the cat eye acting as a diffuse reflector although it retains polarization, much like an aluminized projection screen, as concluded for the human fundus by Campbell & Gubisch (1966). This is entirely compatible with the recent findings of Coles (1971). He showed that the cat fundus is 'composed of multilayer reflectors (domains) each being less than 6 $\mu$m in diameter' (less than 0·03° retinal subtense). The individual domains would be expected to retain polarization while the tapetal surface as such would appear as a diffuser.

Thirdly, the system should be isotropic. Two circumstances make it

clear that strict requirements for isotropy were not fufilled by our system (1) the irregularities of the fundal image due to pigment lumps and vessels which were mentioned on p. 387, (2) in some cases the light distribution within the fundal image was obviously asymmetric even when comparing two radii void of irregularities due to retinal structure. This can be seen in Fig. 3*B* where the densitometer output (= transmission of film negative) is plotted versus position along the line in Fig. 3*A*. This line was drawn so as to avoid obvious irregularities due to retinal vessels and pigment lumps, and yet the curve in *B* is quite asymmetrical. In fact it is the most asymmetrical among all such plots from the fifteen eyes studied. A

TABLE 1. Linespread function of the living cat eye as measured in three studies

| Investigator | Half-width for half-height | Half-width for tenth-height | Diameter of artificial pupil | *N* |
|---|---|---|---|---|
| Morris & Marriott (1961) | 12 min | 32 min | None used, atropine dilated pupil | 1 |
| Westheimer (1962) | 4–8 min | 12–24·5 min | 6 mm | 5 |
| Bonds, Enroth-Cugell & Pinto | 2–4 min | 6–10 min | 4·0–4·8 mm | 15 |
| | 15 min | 36 min | None used, atropine dilated pupil | 1 |

For this comparison, our Modulation Transfer Functions have been converted to linespread functions by Fourier methods.

total of five aerial images exhibited this kind of asymmetry. It may have arisen from astigmatism of the cat eye (which we made no attempt to correct) and/or from astigmatism due to oblique incidence. We have assumed that (1) because transmission was not measured through irregularities caused by regional variations in retinal structure, and (2) because we claim validity of the MTF only for that restricted portion of the dioptric apparatus through which the image forming light travelled, this lack of isotropy had only a small effect upon our results. In some cases the actual fundal image may have been less sharp than the one arrived at by correcting for double passage but no worse than the double passage image (*F* and *C* respectively in the example of Fig. 3).

To our knowledge, all measurements reported thus far involving the optical quality of living eyes have used either gratings or a self-luminous line. To enable direct comparison of our results with those of others, our MTFs were Fourier transformed to yield linespread functions. Table 1

shows the half-width at half and tenth-height of the linespread functions from the living cat eye as measured by Morris & Marriott (1961), Westheimer (1962) and ourselves. As a further check on the overall reliability of our method, a measurement was taken on one human eye using a 6 mm diam. artificial pupil; the MTF had fallen to one half at 4·35 c/deg which is not far out of line with the results of Campbell & Gubisch (1966). In their case the MTF had fallen to one half at 4·5–5·5 c/deg.

If one selectively desensitizes the central response mechanism of cat retinal ganglion cells with a small steady light spot while applying a flashing annular or spot stimulus to the outlying portions of the receptive field, then some cells willingly yield a pure surround response but others do not (Enroth-Cugell & Pinto, 1972*a*). These two kinds of behaviour may reflect genuine differences in receptive field organization. But, as clearly evidenced by the results in Fig. 5, the differences between individual cells could just as well be accounted for by differences in image sharpness from experiment to experiment. Here then is an example of how difficult it can be to draw safe conclusions about the functional organization of centre-surround ganglion cells unless one can trust that the stimulus image is sharp. The situation is comparable to that in Rushton & Westheimer's (1962) experiment on the bleaching adaptation pool. They could not have made such a strong argument as they did for adaptation depending upon the total bleach of a rod cluster, had they not known the quality of their retinal image of the bleaching light. The image quality measurements presented in this paper will be used in the next one together with neurophysiological results to show that the reason why some cells do, others do not yield a pure surround response probably reflects differences in functional organization of the receptive fields, not differences in the sharpness of the stimulus image.

It may seem surprising that the difference between the image formed through a 4·8 mm pupil and that formed through the maximally dilated natural pupil of the cat eye is so large. Admittedly, only one eye was tested with and without artificial pupil in this study. However, we feel confident that this striking degradation of image quality with omission of artificial pupil is a real one, because our results have now been confirmed (Bonds, A. B., unpublished) on two more cat eyes using the same method as Campbell & Gubisch (1966). (The aerial image of the cat's fundal image of a thin line was scanned by a photomultiplier and its output serially loaded into the addresses of an averaging computer.)

From experiments where a 4·5 mm diam. pupil was used Cleland & Enroth-Cugell (1968) concluded that the signal summation and the field adaptation pool of the central response mechanism of on-centre cells are coextensive. Sakman, Creutzfeldt & Scheich (1969) have also compared,

in the cat, the area over which visual signals are summated with that over which adaptive effects are summated. They elicited a ganglion cell response with a square wave stimulus of fixed size, luminance and modulation frequency which they applied to the receptive field middle. At the same time a 3–8° diam. annular, unmodulated light, concentric with the central stimulus, was directed on to the receptive field periphery. They observed how increasing annular luminance affected the amplitude of the response elicited by the fixed central stimulus and concluded that light falling on the receptive field 'surround alone' influences the adaptive state of the *central* mechanism. In the experiments of Sakman *et al.* (1969) the pupil was 'pharmacologically dilated' and no use of an artificial pupil is indicated. We have used the MTF obtained without an artificial pupil from an eye whose pupil was atropine dilated (Fig. 4*E*), to estimate the retinal light distribution within the image formed of such a 3–8° diam. annulus through the dilated natural pupil. According to our calculations the retinal illumination has fallen to 8 % only of its maximum value at the very centre of the annulus. At a point 1·5° out from the centre, that is, at a point where the object luminance theoretically is still zero, the retinal illumination is approximately 25 % of its maximum. Considering now (1) the larger sensitivity of the central response mechanism and (2) that it is stimulus *flux* (luminance × area), not luminance alone, that sets the state of adaptation of the central mechanism (Cleland & Enroth-Cugell, 1968) it seems reasonable to suggest that it might have been light 'that spilled over' from the annulus on to the central response mechanism, rather than light that fell on the 'surround alone' (Sakman *et al.* 1969) that influenced the adaptive state of the central mechanism in the experiments of Sakman *et al.* This, to us, would be a satisfying explanation of their results for Enroth-Cugell & Pinto (1970*b*) also have experimental evidence that supports the view that the surround does not set the adaptive state of the centre mechanism; their results on centre-surround interaction suggest that the activity within one response mechanism does not influence the properties of the other mechanism.

Several investigators have studied the spatial response characteristics of the human visual system with psychophysical methods (see e.g. Campbell & Green, 1965). Sine- and/or square-wave grating patterns were formed on the retina (1) after normal imagery by the optics of the eye and (2) without prior modification by the optics (interference fringes). In both situations the contrast sensitivity was determined. Comparison of the two sets of results showed that the optics of the human eye contribute less to the overall high-frequency cut-off (only about half) than does the 'retina-perception' part of the visual system. It is quite possible that the situation in the cat is similar; i.e. that the dioptrics contribute relatively little to the

high-frequency attenuation of the over-all 'dioptrics-brain system'. This is suggested by the spatial frequency characteristics of individual retinal ganglion cells, lateral geniculate and cortical units determined with grating patterns. These were formed on the retina after normal imagery by the cat optics, through a 3·5 or 4 mm diam. pupil (Enroth-Cugell & Robson, 1966; Campbell, Cooper & Enroth-Cugell, 1969). The contrast sensitivity of the single neurone with the *highest* spatial resolution (a lateral geniculate cell) had fallen to one half of its maximum at 2 c/deg which is close to the frequency at which, in this study, the MTF with the *most pronounced* high-frequency attenuation had also fallen to one half.

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