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RETINAL GANGLION CELL LAYER

OPTIC NERVE FIBRE COUNTS:


VANEY, D.I. and HUGHES, A. (1976). The rabbit optic nerve: fibre diameter spectrum and comparison with a retinal ganglion cell count. J. comp. Neurol. 170, 241-252. ... ... ... ... ... ... ... 246

HUGHES, A. and WÄSSLER, H. (1976). The cat optic nerve: fibre total count and diameter spectrum. J. comp. Neurol. 169, 171-184. ... ... ... ... ... ... ... 257

RETINAL GANGLION CELL IDENTIFICATION AND DISTRIBUTION:

HUGHES, A. (1975). A quantitative analysis of the cat retinal ganglion cell topography. J. comp. Neurol. 163, 107-128. ... ... ... ... ... ... ... 271


HUGHES, A., CAILLE, D. and VIBERT, J.F. (1980). A statistical analysis and comparison of some diameter spectra for classical neurones from different regions of the cat retinal ganglion cell layer. Pflüger Archiv. 388, 239-244. ... ... ... ... ... ... ... ... 330

IDENTIFICATION OF A LARGE MICRONEURONE POPULATION IN THE RETINAL GANGLION CELL LAYER:


HUGHES, A. and WIENIAWA-NARKIEWICZ, E. (1980). A newly identified population of presumptive microneurones in the cat retinal ganglion cell layer. Nature 284, 468-470. ... ... ... ... ... ... ... ... 355

Retinal Organisation:

Sampling Theory and Retinal Organisation:


The Terrain Theory:


Hughes, A. (1981). One brush tailed possum can browse as much pasture as 0.06 sheep which may indicate why this 'arboreal' animal has a visual streak: some comments on the 'terrain' theory. Vision Res. 21, 957-958.

Are Retinal Non-concentric Units Universal?


A Diversion: The Philosophy of Unit Nomenclature:

Hughes, A. (1978). A rose by any other name. On 'Naming of neurones'. Brain Behav. & Evol. 16, 52-64.
HUGHES, A. (1981). In praise of the pair tree... A final comment on 'Naming of Neurones'. Unpublished. ... ... ... 407

EYE MOVEMENTS

VERGENCE AND VERSION:


VISUAL CORTEX

THE PHYSIOLOGY OF PRIMARY VISUAL CORTEX:

HUGHES, A. and VANNEY, D.I. (1982). The organisation of binocular cortex in the primary visual area of the rabbit. J. Comp. Neurol. 204, 151-164. ... ... ... 468

VANNEY, D.I. and HUGHES, A. (1982). Single unit receptive fields of the rabbit primary binocular cortex. Exp. Brain Res. ... ... ... 482


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HUGHES, A. (1978). The neural representation of visual space. Nature 276, 422. ... ... ... 509

PROLEGOMENA

AN ATTEMPT AT SYNTHESIS FOR ONE SPECIES:

HUGHES, A. (1971). Topographical relationships between the anatomy and physiology of the rabbit visual system. Documenta Ophthal. 30, 33-159. ... ... ... 510
RETINAL GANGLION CELL LAYER
OPTIC NERVE FIBRE COUNTS
The Pigmented-Rat Optic Nerve: Fibre Count and Fibre Diameter Spectrum

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ABSTRACT A well distributed electron microscopic sample of 52,000 fibres from the pigmented rat optic nerve cross section indicates a total count of 120,000 ± 1,600 (S.E.M.) fibres ranging from 0.437 μ to 5.2 μ in diameter. The fibre diameter spectrum is essentially unimodal with a peak at 1.0 μ with a well developed “tail.” These results are identical to those available in the literature for the albino rat.

The presumed retinal ganglion cell population of the mammalian retina usually exceeds the optic nerve fibre count by no more than 20% (cat: Hughes and Wässle, ’76; rabbit: Vaney and Hughes, ’76; hamster: Tiao and Blakemore, ’76). Complete enumeration of optic nerve fibres in several rhesus monkeys (Potts et al., ’72) suggests that variation between individuals may account for discrepancies of up to 15% between ganglion cell and optic nerve fibre total counts obtained from different subjects. A discrepancy between ganglion cell and optic nerve fibre populations is also commonly accepted as incorporating the erroneous identification of “displaced amacrine” (Gallego, ’71) as ganglion cells. So far, however, a substantial excess of presumed ganglion cells over optic nerve fibres has been reported only in birds (70%, pigeon: Binggelli and Paule, ’69) which, in contrast to mammals, are classically described as containing a large population of “displaced amacrine cells” (Cajal, ’55) in the retinal ganglion cell layer.

A recent estimate (in preparation) of the total retinal ganglion cell population of the pigmented rat, which was based upon the integration of neurone density maps of the ganglion cell layer, indicates the presence of some 190,000 neurones—presumed to be ganglion cells—somewhat fewer than the 260,000 reported by Lashley (’32). However, this number substantially exceeds the published electron microscopic optic nerve fibre total counts for the albino rat of 117,000 (Forrester and Peters, ’68) or 109,000 (Treff et al., ’72) fibres and thus calls into question the criteria employed for ganglion cell identification in this animal.

The presence of a greater ipsilateral projection in the pigmented than in the albino rat (Lund, ’65) suggested the possibility that the total optic nerve fibre population of the pigmented animal may also be greater than that of the albino. The optic nerve count of the pigmented rat has in fact been reported to be 7% greater than that of the albino animal (Bruesch and Arey, ’42) but light microscopy was employed. If this is true then the neuronal population of the ganglion cell layer of the pigmented rat could be accounted for without postulating an unusually large population of displaced amacrine cells or doubting the criteria for the discrimination of neurones from glia. The subsequent optic nerve fibre count was thus undertaken on the same strain of pigmented rat as that used for ganglion cell total counts.

METHODS
Optic nerves of adult dimensions (Matheson, ’70) were obtained from 3-month-old D. A. agouti rats weighing no less than 230 gm. The animals were anaesthetised with 1 cc of urethane injected intraperitoneally and an eye enucleated with its attached length of intra-orbital optic nerve whose orientation was noted before it was cut away and fixed by immersion. The procedures for fixation, preparation of sections and measurements were similar to those previously reported for the cat (Hughes and Wässle, ’76) and rabbit (Vaney and Hughes, ’76).

The thin section employed for this study was taken between 1 mm and 2 mm posterior to the lamina cribrosa and, although slightly oval, showed no sign of oblique cutting. Thir-
Fig. 1 Diagram of the rat optic nerve thick section. The regions of the thick section corresponding to those obscured in the thin section by the supporting grid bars are indicated. Sample areas of 0.00265 mm² on the thick section are marked by a circle or disc whose adjoining number indicates the fibre count for the sample. The variation in fibre count per sample is small and thus the fibre packing density across the nerve is relatively uniform; its range is indicated by three isodensity contours between 490,000 and 570,000 fibres/mm². Samples from the six points marked with an open circle were subject to a fibre diameter survey and the results pooled as data for figure 2.

ty-nine sample photographs, each representing 0.00237 mm² on the thin section, were spaced along three vertical, two oblique and one horizontal axis; the distribution was influenced by the support bars of the grid. The sample positions were noted on a photomicrograph of an adjacent thick section and final prints of the sample photographs made at a linear magnification of × 5,135.

The compression of the thin section along the direction of cutting (Vaney and Hughes, '76) was determined by quantitative comparison between photomicrographs of the thick and thin sections and amounted to a linear and areal compression of some 11%.

The relative thickness of the myelin sheath is somewhat greater for small than for large optic nerve fibres of the rat (Matheson, '70) but whether the outside fibre diameter or axon diameter should be measured remains a vexed question with differing conclusions apparent in the literature (Ogden and Miller, '66; Tapp, '73). The common use of the outside fibre diameter for estimation of conduction velocity (Ogden and Miller, '66) and reconstruction of the compound action potential (Bishop et al., '69) as well as the more ready availability of this information for other species led to its use in this study. The fibre diameter spectrum was established by matching the area of each measured fibre to a series of circular holes in a plastic sheet. The equivalent mean diameter of the fibre was taken as that of the smallest circle whose area was equal to, or larger than, that of the fibre including its myelin sheath. No correction was made for possible compression of the fibres or for shrinkage during processing.

RESULTS

The criteria employed for the identification of axons were similar to those presented else-
where (Hughes and Wässle, '76; Vaney and Hughes, '76); no unequivocal nonmyelinated fibres were encountered although nonmyelinated figures concluded to be nodal sections amounted to some 0.15% of the sample.

The matrix of sampling areas and the number of fibres counted in each sample are shown within the outline of the thick section of the nerve in figure 1. The unsampled region masked by the grid support bars is outlined after correction for the compression of the thin section. The total area of the thick section was found to be 0.236 mm² compared with 0.21 mm² for the thin section. Each E.M. photograph representing 0.00237 mm² on the thin section thus represented 0.00265 mm² on the thick section and 88.9 such sampling areas would be required to completely cover the thick section. The 39 sample photographs thus survey 0.1035 mm² of the thick section or some 44% of the nerve cross sectional area.

The mean fibre count for the 39 0.00265 mm² photographs was 1,349 ± 18.7 S.E.M. and thus the total count for the 0.236 mm² area of the nerve is estimated to be 120,060 ± 1,660 S.E.M. fibres.

The mean fibre density in the nerve is some 509,000 fibres/mm²; this ranges from a low of 450,000 fibres/mm² in the central region to a high of 590,000 fibres/mm² in the inferior region. A set of iso-density contours are shown for the nerve of figure 1. The rat fibre density distribution is relatively homogeneous unlike that of the cat.

The fibre diameter spectra of sampled regions of the nerve were not markedly different from each other. There was an impression of slightly increased frequency of larger fibres in the upper region of the nerve. In view of the similarities of the different regions, the six
samples or about 300 measured fibres taken from areas marked with open circles on figure 1 were pooled to give a total sample of 1,808 fibres; the diameter histogram for these is shown in figure 2. The fibre diameters range from 0.43 \( \mu \) to 3.9 \( \mu \) with the modal diameter at 1.0 \( \mu \); the distribution is essentially unimodal.

**DISCUSSION**

The total optic nerve fibre count of 120,000 obtained for the pigmented D.A. agouti rat is close to the count for albino animals of 117,000 (Forrester and Peters, '68) and exceeds the 109,000 of Treff et al. ('72) by only 10%. In agreement with Forrester and Peters ('68), Treff et al. ('72) and Schloote ('70) no significant unmyelinated fibre population was observed even with examination of thin sections at high E.M. magnifications. The absence of unmyelinated fibres is consistent with results from most other mammals (cat: Hughes and Wässle, '76; rabbit: Vaney and Hughes, '76; monkey: Potts et al., '72; man: Cohen, '67) although, curiously, Tiao and Blakemore ('76) describe the hamster optic nerve as containing 58% unmyelinated fibres. In this latter instance it is possible that the section was taken very close to the lamina cribrosa where myelination may not be complete.

The presence of some 120,000 fibres in the rat optic nerve indicates that the corresponding retinal ganglion cell population must be of very similar magnitude. The excess of retinal neurones over optic nerve fibres encountered in the presumed ganglion cell total count which precipitated this study must therefore represent a population of "displaced amacrine" or arise from confusion of glia with neurones. A recent Nissl criterion study (Fukuda, '77) claims the presence of some 115,000 neurones in the rat retina in good agreement with available optic nerve fibre counts and thus does not support the presence of any substantial population of Nissl staining "displaced amacrines." However, detailed examination of these results suggests that the situation is not so clear. Fukuda's count is a minimum estimate obtained by summing the product of the area lying between each pair of adjacent retinal isodensity contours with its lower bounding density value. A more accurate integration of Fukuda's ganglion cell map (fig. 3: Fukuda, '77) would sum the products of the area between each pair of isodensity contours with the mean of the high and low bounding values. An enlarged copy of Fukuda's map was therefore cut up and areas between isodensity lines measured by weighing. Integration by the first method confirms Fukuda's results but the second indicated a total ganglion cell population of 146,000 cells. Fukuda's claim of agreement between the total neurone count and the optic nerve population is thus subject to doubt because of the excess of neurones over the optic nerve count in the more accurate integration. In addition, the retinal areas shown (figs. 1, 3: Fukuda, '77) are between 50% and 70% of the adult value (60 mm²: Hughes, '77); if this discrepancy arises from shrinkage greater than the 10% claimed then it will not influence the total count but if the retinas are incomplete then the true population must be further increased.

It is of interest to note that Bunt et al. ('74) describe a large population of the small neurones in the rat retinal ganglion cell layer as failing to take up horseradish peroxidase when it is injected into the lateral geniculate nucleus or superior colliculus; under similar conditions no such cells were observed in the cat retina (Kelly and Gilbert, '76). Of course, these small neurones may project to other regions of the brain but, alternatively, they may arise from the retina when passing to the periphery (Fukuda, '77; author's unpublished observations). However, in view of the doubts concerning ganglion cell identification the trimodality must be of questionable significance. The relatively uniform fibre packing density and regional fibre diameter
spectra of the rat nerve contrast with the radial variation encountered in the cat nerve (Hughes and Wäske, '76); this contrast parallels the difference between the retinal ganglion cell density gradients of the two species. However, it is apparent from the rabbit optic nerve (Vaney and Hughes, '76) that the regional fibre diameter spectra and packing density distribution of the nerve do not invariably retain the topography of the corresponding features of the retinal ganglion cell layer.

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The Rabbit Optic Nerve: Fibre Diameter Spectrum, Fibre Count, and Comparison with a Retinal Ganglion Cell Count

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ABSTRACT This electron microscopic study indicates that the rabbit optic nerve contains 304,000 ± 20,000 (p < 0.05) nerve fibres, of which at least 98% are myelinated. The fibre diameter spectrum of the nerve is unimodal and ranges from 0.25–7 μm with its peak at 0.75 μm. The projection of the visual streak fibres is not apparent in local diameter spectra near the optic nerve head. Integration of a ganglion cell density map of the retina from another rabbit indicates a total ganglion cell count lying between 455,000 and 547,000. The optic nerve fibre and ganglion cell counts are both substantially greater than the maximum fibre count previously reported.

An estimate of the number of ganglion cells in a retina may be obtained by counting neurons in the ganglion cell layer. The accuracy of the total count then depends upon the proportion of displaced ganglion cells (Bunt et al., '74) and the histological criteria used to discriminate either glia from neurons, or ganglion cells from both displaced amacrines and intraretinal association neurons (Binggeli and Paule, '69; Hughes, '75). In practice the count is made by integrating a density distribution map of the presumed ganglion cells (Stone, '65; Hughes, '75).

Counting the number of fibres in the optic nerve provides an alternative means of estimating the ganglion cell population. If there is a significant proportion of centrifugal fibres in the nerve such a count overestimates the number of ganglion cells. In addition, the failure of light microscopy to resolve small fibres in the optic nerve is well established and larger total fibre counts have been obtained for anurans (Maturana, '59), pigeon (Binggeli and Paule, '69), rat (Forrester and Peters, '67), and cat (Hughes and Wässle, '76) using electron microscopy.

The very large visual field and extensive visual streak of the lateral eyed rabbit (Hughes, '71) distinguish it from frontal species such as cat and monkey, and this contrast makes the rabbit an important subject for visual neurophysiology and anatomy. Brüssel and Arey ('42) estimated from light microscopy of the rabbit optic nerve that it contains 206,000 myelinated fibres. There has, however, been no quantitative electron microscopic study of the nerve, and a retinal ganglion cell total count is not available for the rabbit. Yuri ('60) and Narva ('61) have made cursory, electron microscopic observations on the rabbit optic nerve.

This paper provides a fibre count and a fibre diameter spectrum for one rabbit optic nerve. A second estimate of ganglion cell number is obtained by integrating a ganglion cell density map of the enucleated retina of another rabbit.

MATERIALS AND METHODS

Rabbit optic nerve

A 3-month-old laboratory rabbit was anaesthetized with urethane and the right eye, with 6 mm of the optic nerve attached, was enucleated. The dural sheath was quickly dissected from the nerve, and the eye immersed in the primary fixative (2% glutaraldehyde/1% formaldehyde in 0.12 m phosphate buffer). The orientation of the optic nerve was marked by passing a silk suture through the nerve 4 mm from the globe and knotting it over the dorsal surface. The nerve was then supported in a trough of plasticine and cut transversely 1 mm behind the globe.

The optic nerve was fixed in the glutaraldehyde solution for two and one-half hours, postfixed in 2% OsO₄ for two hours,
stained in 2% uranyl acetate for one-half hour, dehydrated in an ethanol-acetone series, and flat embedded in Durcupan with the dorsal suture knot on one side. The sections used in this study were cut 0.5 mm from the distal end of the embedded tissue. Subsequent examination of the Durcupan block showed that the cutting face was within 1°–3° of being perpendicular to the long axis of the optic nerve, as defined by the nerve fibre.

Thick sections (1 μm) and thin sections (deep gold) were cut with glass knives on an L.K.B. ultramicrotome, with the knife edge parallel to the dorsal-ventral axis of the optic nerve, and the direction of cut from the temporal to the nasal margin. The thick sections were stained for light microscopy with an equal parts mixture of 1% Azure II and 1% methylene blue in 1% borax. The thin sections were supported on uncoated, 400 mesh copper grids, stained with 0.4% Venable’s lead citrate for two minutes, and photographed in a Philips 200 electron microscope.

The nerve fibre counts and fibre diameter spectra are extracted from data based on the uniform sampling of a single, complete thin section. Two hundred and sixteen electron micrographs were taken on a (63 μm)² sampling matrix, one in the bottom-left corner of each window of the grid. A micrograph of a calibration grating replica was included on each 35 mm film and used to print the survey micrographs at a uniform magnification of × 16,100. A square, representing an area of 119 μm² on the thin section, was marked off on each micrograph print, and the nerve fibres within the square and those touching the right or bottom margins were identified and counted.

The outside diameters of the counted fibres in 94 of the 216 survey micrographs were measured by matching the cross sectional area of the fibre, including the myelin sheath, to the area of one circle from a series of concentric circles printed on sheet film. The concentric circles corresponded to diameters of 0.25–5.0 μm in 0.25 μm steps on the thin section.

Rabbit retina
A cresyl-violet stained whole mount of a rabbit retina was prepared using methods which have already been described (Hughes and Whitteridge, '73). Neurons in the retinal ganglion cell layer were counted by direct observation under the light microscope. Counts were made along parallel tracks spaced at 2 mm intervals and running obliquely to the visual streak. The distance between sampling points along a track depended on the local neuron density gradient and ranged from 0.25–1.0 mm. A uniform sample size of 50 to 100 neurons was maintained by increasing the area of the counting field at lower neuron densities. The criteria used to identify the neurons, the presumed ganglion cells of the rabbit retina, were similar to those reported for the cat (Hughes, '75), although a lower density of retinal glia in the rabbit made discrimination easier. Isodensity contours in steps of 1,000 ganglion cells/mm² were drawn on the density map of the retina without correction for shrinkage. The area of each region between adjacent contour lines was determined.

RESULTS

Nerve axon identification
The preservation of axoplasm ultrastructure was variable in quality and parts of the sheath of most myelinated fibres showed some breakdown of lamellae. Tissue preservation was not improved when perfusion was substituted for immersion in the fixative.

Over 98% of the counted axons were overtly myelinated and this feature provided an adequate single criterion for identification of these fibres, figure 1a. Under closer examination, the myelinated fibres also satisfied the primary criterion that the axoplasm contain both microtubules and filaments (Vaughn and Peters, '68).

The remaining processes were classified at the survey magnification of × 16,100. The fibrous astrocytes, with their characteristic paucity of microtubules and high density of fine filaments, figure 1a, were qualitatively different from nerve axoplasm (Vaughn and Peters, '68; Hughes and Wässle, '76) and little difficulty was experienced in differentiating between the two. The apparently unmyelinated axons, figure 1b, were few in number and not grouped together in bundles (Maturana, '69; Binggeli and Paule, '69). This suggests that many of these axons are myelinated fibres sectioned at the node of Ranvier, and for some processes this was confirmed by the presence of sub-axolemmal granular ma-
Fig. 1a–c  Electron micrographs of axons in the rabbit optic nerve. Over 98% of the counted axons were myelinated at the section plane, figure 1a left and right, and this distinctive feature provided an adequate criterion for identification. Other processes were classified as axons if their cytoplasm contained both microtubules and filaments. Some of these axons were apparently unmyelinated, figure 1b left, lacking either the subaxolemmal granular material, figure 1c, or the paranodal sheath cytoplasm characteristic of myelinated fibres sectioned at the node of Ranvier. The cytoplasm of fibrous astrocyte processes contained many closely packed filaments and very few microtubules, figure 1a centre, and figure 1b, right.

Fig. 1d  Transverse thick section of the optic nerve 1.5 mm from the globe. The presence of circular fibre cross sections immediately adjacent to elliptical fibres indicates that the apparent obliquity was not a cutting artifact.

Optic nerve cross sections

The thick section of the optic nerve, a light micrograph of which is shown in figure 1d, had a mean diameter of 1.2 mm and a cross sectional area of 1.11 mm². The section was slightly oval with the long axis of symmetry running from the dorsal-
temporal to the ventral-nasal surface. The great majority of nerve fibres did not differ markedly from circular form, and there was no evidence at this level in the optic nerve for either general obliquity of the fibres or local obliquity of fibre bundles. Light microscopic examination of the thick section revealed a relatively high proportion of large diameter fibres in the middle of the nerve, and this appeared to be correlated with a lower fibre density. The middle region was also characterized by a higher density of darkly stained glial perikaria.

Reconstruction of the optic nerve profile from the distribution of survey micrographs indicated the probability of significant compression of the thin section along the direction of cut on the temporal-nasal axis. A photomosaic of the thin section was made from a series of low power electron micrographs, and the nerve boundary interpolated over the grid bars. The resulting nerve profile had an area of 0.87 mm² which is 78%–79% that of the thick section. The separation between the dorsal-ventral axis and seven landmarks, and between pairs of landmarks, were measured on micrographs of both thick and thin sections. These measurements were consistent with a relatively uniform linear compression of 0.79 along the temporal-nasal axis. A second check, a stylized outline of the thick section was compressed by a factor of 0.80 perpendicular to the dorsal-ventral axis.

Fig. 2 Nerve fibre counts are shown for 205 of the 216 sample points on the thin section of the rabbit optic nerve, figure 2a. The positions of the 11 survey micrographs which had nerve sheath within the 119 μm² counting square are marked with asterisks; their combined count was 377 fibres in an area of 814 μm². A measure of local fibre density around each sample point was derived by summing the fibre count with its adjacent counts and calculating the mean. Isodensity contours have been drawn through the resulting smoothed density distribution above mean counts of 43, 53, and 60 fibres. These counts correspond respectively to local densities of 365, 449, and 507 nerve fibres/1000 μm² on the thin section. The 449 contour divides the optic nerve into three higher density regions and an unbroken lower density region of equivalent area. Within each of the higher density regions, the 507 contour delineates areas of peak density occupying one-eighth of the nerve. The 365 contour encompasses an equivalent area comprising two distinct regions of low fibre density.
The resulting profile, used in figures 2 and 3, closely matched the nerve boundary on the thin section photomosaic. The compression may be a consequence of the great width of the block cut in this study.

In order to investigate the organization of optic nerve more proximal to the brain, serial thick sections were cut from the same tissue block, 4 mm back from the thin section cutting face. A relatively high proportion of large diameter fibres in the middle of the nerve was also evident in these sections. However, unlike the sections cut near the optic nerve head, the proximal sections showed marked obliquity of fibre bundles, especially in the middle and nasal-ventral regions of the nerve. That this obliquity was not a cutting artifact is demonstrated by the presence of circular fibres immediately adjacent to elliptical fibres, figure 1c. Reconstruction of elliptical fibres from their serial sections indicated that many were S-shaped, with the oblique component almost perpendicular to the local anatomical axis.

Nerve fibre total count and density distribution

The distribution of the 216 sampling points across the thin section is shown in figure 2a; asterisks mark 11 peripheral survey micrographs which had nerve sheath within the counting square. The positions of the remaining 205 survey micrographs are marked with their respective fibre counts. Since each count is the number of nerve fibres in a square of area 119 μm², figure 2a can be regarded as a point density map of the thin section.

The 216 samples contained 11,279 nerve fibres in an area of 25,206 μm². Taking the cross sectional area of the nerve on the thin section as 0.88 mm², the above figures give a fibre total count for the rabbit optic nerve of 39,400 nerve fibres. The mean of the sample counts is 53.2 ± 4.9% (2 x s.e.m.), and these confidence limits applied to the fibre total count give a possible error of ±19,500 (p < 0.05). To the statistical error should be added an estimated maximum error of ±1% in the calculation of the area of the nerve.

Direct presentation of the distribution of fibre counts, as in figure 2a, provides only an indefinite map of fibre density because the samples are small and may be influenced by the occurrence of blood vessels and neuroglia within the counting square. In order to smooth the density data, each fibre count was summed with its eight adjacent counts, and the mean of the nine assigned to the middle data point as an index of local density. Three isodensity contours, figure 2b, have been drawn through the smoothed data, and the major feature of the fibre distribution is the U-shaped region of minimum density in the middle of the nerve section. Considering its complexity, the density distribution is remarkably symmetrical about the dorsal-ventral axis of the nerve.

Nerve fibre diameter spectrum

The equivalent, outside diameters of 4,692 nerve fibres in 94 of the survey micrographs were measured in 0.25 μm steps. To increase the subsample size, these micrographs were grouped in sets of four, uniformly distributed within the sampling matrix as shown in figure 3. The number of fibres measured in each subsample ranged from 120 to 292. No corrections were made for the known compression of the thin section, or for possible volume changes of the fibres during processing of the tissue.

![Fig. 3 Distribution within the sampling matrix of the 94 survey micrographs from which fibre diameter measurements were taken. To increase the subsample size, adjacent micrographs were grouped in 20 sets of four and 3 sets of three, which are numbered consecutively from 1 to 23.](image-url)
A fibre diameter spectrum for the whole nerve was constructed by summing the counts in corresponding diameter bins for each of the 94 samples. Figure 4 shows the percentage of the total fibre population in each 0.25 μm diameter range, together with the 95% confidence limits on the mean sample count for that bin. The confidence limits embody both the variation between diameter spectra in different parts of the nerve section and the variation in sample size.

The fibre diameter spectrum of the rabbit optic nerve is unambiguously unimodal, with a peak at 0.75–1 μm, and a mean diameter of 1 μm. Analysis of the spectrum indicates that those two values would not be significantly altered by corrections which could be made for the 21% compression of the thin section, since at the lower end of the spectrum the bin size is course relative to the fibre diameters. The nerve fibres range in diameter from less than 0.25 μm to 7 μm, as measured on the thick section. A light microscopic survey of the thick section showed approximately 150 fibres of diameter greater than 4.5 μm, and this is close to the number predicted from the fibre percentages in the last two bins of the e.m. diameter spectrum.

Separate diameter spectra were constructed for each of the 23 subsamples, and are presented topographically arranged in figure 5. The individual spectra are similar in form and their modal diameters are comparable. X² analysis does indicate, however, that nine of the subsamples were drawn from populations significantly different (p < 0.01) from that of the whole nerve. The extremes in the diameter distribution are represented by those spectra with a high proportion of either small diameter fibres (No. 14, No. 16, No. 19) or large diameter fibres (No. 7). The differences between these spectra and the fibre diameter spectrum of the whole 4,692 fibres measured.

Fig. 4 Fibre diameter spectrum of the rabbit optic nerve. The outside diameters of the counted fibres in the 94 micrographs indicated in figure 3 were measured by matching the cross sectional area of each fibre to the area of one circle in a series with diameter increments of 0.25 μm. The 95% confidence limits on the mean value for each bin are marked with a vertical line.
nerve are highly significant ($p = 0.001$).

Retinal ganglion cell count

An isodensity map of the distribution of presumed ganglion cells in the rabbit retina is shown in figure 6. The visual streak, as defined by the 2,000 ganglion cells/mm² contour, is a narrow band of high density.

Fig. 5 Topographical presentation of the fibre diameter spectra for the 23 subsamples whose local distribution is indicated in figure 3. The secondary modes apparent in the subsample spectra occur mainly in diameter bins containing only a small percentage of the measured fibres and, even then, are quite variable in position. This suggests that many of these modes represent sampling errors arising from the comparatively small number of fibres (n) measured in each subsample. However, spectra No. 8 to No. 11 show secondary modes with peak values between 10.5% and 16.5% of the subsample, and this bimodal form is the most visible feature of the diameter distribution in the middle of the nerve. Spectra No. 8, No. 10, and No. 11 have a secondary mode at 1.5 μm diameter, while that of spectrum No. 9 is at 1.25 μm.
whose peak runs horizontally across the retina about 2.8 mm below the centre of the optic nerve head. The organization of this retina has been described previously (Hughes, '71) and it is only necessary to consider the integration of the isodensity map required for an estimation of the total ganglion cell population. A minimum estimate of the ganglion cell population for each inter-isocount region is provided by the product of its area with the lower bordering density, and a maximum estimate by the product of its area with the mean of the lower and upper bordering densities. Limiting estimates of the ganglion cell population for each inter-isocount region and for the whole retina are given in table 1, and the total population lies between

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**Table 1**

<table>
<thead>
<tr>
<th>Density range (gang. cells/mm²)</th>
<th>Area (mm²)</th>
<th>Estimate of mean density</th>
<th>Estimate of ganglion cell No.</th>
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<td>Maximum</td>
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<td>16.2</td>
<td>3,000 3,500</td>
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<tr>
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<td>457 995</td>
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455,000 and 547,000 presumed ganglion cells.

**DISCUSSION**

**Optic nerve fibre count**

The estimated total count of 394,000 fibres for the rabbit optic nerve greatly exceeds Bruesch and Arey's (742) maximum light count of 265,000 fibres. The ratio of counts obtained with the two techniques is comparable to those found for other mammalian optic nerves (Forrester and Peters, '67; Hughes and Wässle, '76). The fibre diameter spectrum indicates that the lower figure is consistent with Bruesch and Arey having counted all fibres greater than 0.8 \( \mu \)m; a value close to the limiting resolution of the light microscope. Thick sections and close packing of fibres in the optic nerve would prevent the theoretical limit being attained in the resolution of individual fibres (Hughes and Wässle, '76).

It is likely that a centrifugal fibre projection to the rabbit retina exists, but there are no estimates of its magnitude (Cragg, '62; Ogden, '68; Luciano et al., '71). Such fibres would preclude a precise correspondence between the fibre total count and the ganglion cell number. However, estimates of the number of centrifugal fibres in the cat optic nerve are available and range from zero (Brindley and Hamasaki, '66) to less than 1% of the fibre population (Brooke et al., '65). It is thus probable that the fibre total count, within statistical error, provides an accurate estimate of the number of ganglion cells in the rabbit retina.

The ratio of the fibre total count to the number of presumed ganglion cells lies between 0.72 and 0.87 in the rabbit. This range is based on the minimum and maximum estimates of ganglion cell number, and does not accomodate the calculated statistical error in the fibre count. The apparent excess of neurons in the ganglion cell layer could support Râmon y Cajal's (55) suggestion that there is a small population of displaced amacrines in the ganglion cell layer of the mammalian retina.

The discrepancy between the fibre and cell body counts may reflect individual variability of ganglion cell number. In most studies individual variation within a species is masked by the statistical error arising from limited sampling of the total population of retinal ganglion cells or optic nerve fibres. This error has been eliminated by Potts et al. (72a) who have obtained replicable counts of all the fibres in two rhesus monkey and two human optic nerves; they reported individual variation of 13.5% for the rhesus counts and 8.5% for the human counts. The possibility of confusion between glia and neurons further reduces the significance of the fibre/neuron ratio.

As a result of these difficulties the cell body count is a less satisfactory measure of ganglion cell number than the fibre count.

**Fibre topography in the optic nerve**

The diameter spectra of ganglion cells in the area centralis and periphery of the cat retina exhibit pronounced differences (Fukada and Stone, '74) which are reflected in the axonal spectra of the two regions (Stone and Holländer, '71). Comparable differences between the diameter spectra of axons from the visual streak and periphery of the rabbit retina would be expected because the central and peripheral ganglion cell spectra (Hughes, '71) are as dissimilar as those of the cat. However, local fibre diameter spectra are comparatively uniform across the rabbit optic nerve. figure 5, and this suggests a breakdown of retinal topography in the fibre projection to the optic nerve head.

Both the cat area centralis and the primate macula are characterized by a high density of small ganglion cells in the temporal retina, and this pattern is reflected in the topography of the fibres in the optic nerve proximal to the globe (Potts et al., '72b,c; Hughes and Wässle, '76). The radial flow of fibres converging on the optic disc of these animals results in the maintained association of fibres from the circumscribed area centralis (Stone and Holländer, '71; Ogden, '74). By contrast, in the rabbit, axons from the inferior retina pass vertically in small bundles to join one of two bands of myelinated fibres which flow into the optic disc from the temporal and nasal retina (Hughes, '71). These bundles contain the outflow of visual streak and peripheral ganglion cells which lie on the same retinal meridian. If this association continues along the myelinated bands
then the topographical relationships of the fibres in the optic nerve would not reflect that of their cells of origin.

The apparent absence of a topographical projection into the nerve, as suggested by the local fibre diameter spectra in sections taken close to the globe, contrasts with the report of Brouwer (23) that the quadrants of the rabbit retina project discretely in the nerve, with the fibres from the upper retina lying in a crescent over those from the lower retina. The large number of oblique fibres encountered in the optic nerve 5 mm from the globe, figure 1e, could lead to a reorganized topography consistent with Brouwer's results. Unfortunately Brouwer did not specify the level of his optic nerve sections.

It is not implied that fibre organization proximal to the globe is random. The central region of the nerve is qualitatively different from its periphery. Nerve fibre densities are lower; the proportion of large diameter fibres is higher; the density of glial perikarya is greater; and there is some evidence for a secondary mode in the fibre diameter distribution.

Reconstruction of the compound action potential from the whole nerve fibre diameter spectrum was attempted on the assumptions that spike amplitude is proportional to fibre cross-sectional area and that conduction velocity is proportional to fibre diameter. Both the complex geometric reconstruction of Gasscr and Erlanger (27) and the pragmatic arithmetic approximation of Landau et al. (68) were used, but neither method provided a result comparable to the compound action potentials recorded by Granit and Marg (58) in the rabbit optic nerve.

ACKNOWLEDGMENTS

We thank Mrs. J. Ward and Mr. T. Spaight for their secretarial assistance, Mr. R. Westen and his team for photographic services, and D. I. V. thanks Doctor G. Schoeffl and Mr. C. McLachlan for their patient tutoring in electron microscopy.

LITERATURE CITED


Hughes, A. 1971 Topographical relationships between the anatomy any physiology of the rabbit visual system. Documenta Ophthal., 30: 33-159.


The Cat Optic Nerve: Fibre Total Count and Diameter Spectrum

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ABSTRACT An electron microscopic examination of two cat optic nerves indicates a mean total count of 193,000 fibres ranging from 0.5 µ to 13.5 µ in diameter. This count, although nearly double any previously reported, supports recent minimum estimates of the retinal ganglion cell population of the cat eye. A radial gradient of packing density exists across the nerve close to the globe; a high density "core" with a trimodal fibre diameter spectrum may be identified as the area centralis outflow and a peripheral low density region with a unimodal diameter spectrum contains the projection of the peripheral retina. Division of the peripheral fibre spectrum suggests the percentages of a, b and γ ganglion cells in the peripheral retina to be 5%, 42% and 53% respectively.

The total number of axons in the cat optic nerve has been estimated with fair conformity by several investigators who employed light microscopy (119,000 fibres, Bruesch and Are, '42; 120,000, Bishop et al., '53; 63,000 Van Crevel and Verhaart, '63; 86,000, Donovan, '67). In agreement with these counts Stone ('65) reported the total retinal ganglion cell population of the cat eye to be some 90,000 cells. By contrast, however, recent independent integrations of the cat retinal neuron population have suggested minimum totals of 190,000 (Wassle et al., '74) and 217,000 (Hughes, '75) neurones which are considerably in excess of previous estimates of either retinal ganglion cell or optic nerve fibre populations. If the excess of these recent retinal neurone counts over that of Stone ('65) is not made up of misclassified glia, displaced amacrine cells or retinal association neurones, then the cat optic nerve fibre population must have been seriously underestimated.

That light microscopy (Bruesch and Are, '42) may lead to underestimation of the optic nerve fibre population because of its failure to resolve small myelinated and unmyelinated fibres has been established from the larger total counts obtained with electron microscopy in anurans (Maturana, '59), pigeon (Binggeli and Paula, '69) and rat (Forrester and Peters, '67). Electron microscopy has, however, not so far provided a total fibre count for the cat and even the fibre diameter spectra obtained by its use are in conflict. According to Friede et al. ('71) the fibre diameter spectrum obtained by electron microscopy of the cat optic nerve has the same 2.5 µ modal diameter as that based upon light microscopy (Donovan, '67). In contrast, Bishop et al. ('69) report a modal diameter of about 1µ based upon electron micrographs of the cat optic nerve (both groups measured the smallest diameter of non-circular axons). The subsequent electron microscopic investigation of the cat optic nerve was undertaken in an attempt to resolve these conflicts and has already been reported in preliminary form (Hughes and Wassle, '75).

MATERIALS AND METHODS

Optic nerves were obtained from adult cats, weighting more than 3.0 kg, at the termination of three to four day neurophysiological experiments in which nitrous oxide had been employed as an anaesthetic. After the administration of a dose of nembutal adequate for deep anaesthesia the optic nerve was cut close to the rear of the orbit and the eye enucleated. The nerve was laid in a groove in plasticine and cut perpendicular to its long axis into 2 mm lengths; immediately afterwards it was immersed in ice-cold 2.5% glutaralde-
hyde, phosphate buffered (pH 7.4) and made up in Eadle’s saline (Cohen, ’67). Fixation for several hours in glutaraldehyde was followed by one hour in 2% osmium tetroxide, staining in 1% uranyl acetate, dehydrated in alcohol and acetone, and embedding in Durcupan.

Thick sections for light microscopy, orientation and area measurement were taken at 1 μ and stained with Azure II/methylene blue in borax solution. Ultra-thin cross sections of the whole nerve were then cut for electron microscopy, mounted on a square mesh copper grid bearing a formvar film and counterstained with 1% lead citrate. The thin sections were examined in a Phillips EM301 electron microscope at magnifications ranging from 300 to 6,000.

Three sections were employed for this study, each coming from a different cat. Section A was taken immediately behind the globe, section B at a distance of some 2mm, and section C some 7mm away. Section B was sampled along four diameters centered on the middle of the nerve and spaced at intervals of 45°. Section C was sampled more crudely along its major and minor diameters. Each sampling photograph was located on a light photomicrograph of a thick adjacent section by means of visible glial and connective tissue elements. A calibration grid ruled with 2,160 lines/mm was included on each 35 mm film from the electron microscope and established that the fibre counts and diameter measurements were made on prints which represented 0.00138 mm² of section B and 0.0092 mm² of section C; sample photographs for B and C were printed at an overall linear magnification of 4,630 and 4,847 respectively. A detailed survey of section A was not conducted. Higher magnifications were employed in certain areas of the nerve to make detailed investigation of its structure.

Few fibres had major to minor axis ratios of more than 2:1 so that their effective mean diameter was swiftly and accurately obtained by matching the total fibre cross sectional area, on photographs of magnification 4,630, to that of a series of holes drilled in a plastic sheet. The mean diameter was taken as that of the smallest circle whose area was equal to or larger than that of the fibre including its myelin sheath.

The nerve area in the thin sections was measured on a plot of the sheath inner margin drawn from successive readings of the calibrated electron microscope traverse micrometers as the border was followed in the viewing system. An estimate of the section area based on a square by square survey of a calibrated grid agreed within 2%. The compression of the thin relative to the thick section was established from measurement of the thick section area by tracing its outline enlarged × 100 onto transparent graph paper and counting 1 mm squares. A similar method was employed to determine the area of regions in the subsequently described isodensity contour maps.

RESULTS

Criteria for axon identification

Preservation of the nerve was not perfect after either immersion in, or perfusion by, the fixative; the myelin sheaths of some fibres revealed small patches of disorganisation and the cristae of glial mitochondria were frequently disrupted. The preservation was completely adequate for the identification of small processes at high magnification, with the exception of a few small patches at the periphery of the nerve.

The criterion for the identification of an axon cross section is that its axoplasm contains both microtubules and filaments (Peters, ’66). The great majority of the cat nerve fibres are myelinated, figures 1a,b, so that the lamellated sheath provides a straightforward additional criterion; few such axons possessed less than four lamellae.

Amongst the myelinated fibres were occasionally encountered apparently unmyelinated axons, figs. 1c,d,e; the presence of filaments amongst the microtubules serves to differentiate such axons from oligodendroglial processes, possibly figure 1g. (Peters, ’66; Vaughn and Peters, ’69) which are reported to be common in the post-laminar region of the cat optic nerve (Blunt et al., ’65). The rare and solitary appearance of such axons suggested them to be myelinated fibres sectioned at the node of Ranvier and this was confirmed in many instances by the presence of paranodal cytoplasm and sub-axolemmal electron-dense granular material (Peters, ’66), figures 1d,e; very rarely both of these fea-
Fig. 1 Criteria for axon identification in electron micrographs of the cat optic nerve. a and b, myelinated axons with distinctive sheath, neurotubules and filaments; c, unmyelinated axon with tubules and filaments but lacking the sub-axolemmal electron dense deposit and paranodal cytoplasm which permits the identification of a myelinated fibre sectioned through the node of Ranvier. d and e, f, process of a fibrous astrocyte containing numerous filaments, g, process lacking filaments but containing microtubules, possibly oligodendrogial. The bar scale on all photographs is 0.5 μ long.

structures were absent, figure 1c, and the element was accepted as an unmyelinated fibre (<0.1%). But even under high magnification survey no clusters of unmyelinated nerve fibres of classic appearance (Maturana, '60; Binggeli and Paule, '69; Coggeshall et al., '74) were observed.

The space between the axons is packed with the glial perikaria, processes and blood vessels. The fibrous processes of the astrocytes with their dense and irregularly packed filaments lack microtubules and are readily distinguished, figure 1f, from axons (Cohen, '67; Vaughn and Peters, '69; Hogan et al., '71). The proportion of elements of uncertain identification under high magnification electron microscope examination is less than 1%.

Section planes

Examination of the cat optic nerve was confined to its S-shaped intra-orbital division. Although the sections were somewhat oval there was no sign of either universal obliquity of axon profiles or of symmetrical, diametrically opposed density variations.
SECTION B

SECTION C

Figure 2
The plane of section is thus assumed to have been effectively perpendicular to the local longitudinal axis of the fibre bundles and nerve.

Several regions of the cat optic nerve may be differentiated by light microscopic examination of its sections. In the immediately post-laminar region, section A, the nerve is much narrower than elsewhere with a mean diameter of 1.2 mm and area of 1.13 mm$^2$. Some 2–3 mm behind the globe, section B, it is possible to discern a temporally off-centre core of high fibre density, a peripheral region containing many large fibres and the vestigial central retinal artery. The section employed from this region had a mean diameter of 1.6 mm and an area of 1.9 mm$^2$. Five to seven millimeters distal to the globe the central retinal artery penetrates the optic nerve and runs towards the eye (Davis and Story, '43); regions more distal to the globe are thus readily identifiable under the light microscope and in addition appear to have a more uniform distribution of large fibres and no obvious high density core. The section C was taken some 7 mm from the globe and found to have a mean diameter of 1.8 mm with an area of 2.6 mm$^2$.

Optic nerve fibre total count and density distribution

The distribution of sampling points and the fibre count at each is shown in figure 2 for the two thick sections B and C. Each sample position was marked on a large photograph of an adjacent thick section and located by means of glial and connective tissue cues during electron microscopy of the thin sections.

Landmark separations along different axes within the body of the nerve were compared on thick and thin sections. Little or no dimensional change was found in the thin section along axes parallel to the knife's edge but perpendicular to this there was linear compression, as reported by Vaney and Hughes ('76), of about 0.9 for section B and 0.85 for section C. Similar compression occurs in material from other species prepared by moderately and highly skilled microtomists. Compression appeared fairly uniform throughout the nerve. The compression factor was employed to adjust the sample photograph area to that which it represented on the thick section. The visual location of the sample points resulted in their being represented at their appropriate locations in figure 2 in spite of compression. The total fibre population of the nerve could then readily be obtained from the information given in table 1. The sample area differed between the two nerves, the most extensively examined was section B of which 8.2% of the area was photographed.

The total fibre count was computed in various ways for sections B and C. (1) a raw total was obtained as the product of the section area and the mean of all the pooled sample counts; (2) appropriate weighting was applied to allow for the greater area represented by the more eccentric samples and a pooled total then computed; (3) an isodensity map for the section was drawn and another total count formed as the sum of the products of the mean density and area of each of its regions. Neither light nor electron microscopy revealed sudden changes in fibre packing density, or fibre diameter distribution. In the unsampled areas of these sections so that it was justifiable to divide them by visual inspection into two or three arbitrary regions separated by isodensity lines. The redundancy inherent in the regularity of the density distributions makes acceptable the use of the relatively small samples employed above.

The retinas of cats 1, 2 and 3 were normal in appearance and certainly free from feline central degeneration. The most
<table>
<thead>
<tr>
<th>Cat 1: section A:</th>
<th>E.M. sample Area mm²</th>
<th>E.M. sample Area compression mm²</th>
<th>E.M. sample thickness mm²</th>
<th>E.M. sample compression mm²</th>
<th>No. of sample photos</th>
<th>% Age of area of nerve sampled</th>
<th>Total no. of fibres sampled</th>
<th>Mean No. of fibres per sample photo s.e.m.</th>
<th>Subregion total fibre count No. (P &lt; 0.05)</th>
<th>Total count for nerve region No. (P &lt; 0.05)</th>
<th>Mean fibre density in nerve or region fibres/mm²</th>
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<tbody>
<tr>
<td>Cat 2: section B: 2-3 mm behind globe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(a) All samples pooled</td>
<td>1.88</td>
<td>1.69</td>
<td>0.9</td>
<td>0.00198</td>
<td>0.0022</td>
<td>70</td>
<td>8.2</td>
<td>15,665</td>
<td>224 ± 7</td>
<td>191,400 ± 12,000</td>
<td>101,300</td>
</tr>
<tr>
<td>(b) 4 Quadrants; samples weighted for eccentricity</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>35</td>
<td>4.1</td>
<td>7,916</td>
<td>211</td>
<td>179,600</td>
<td>95,500</td>
<td></td>
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<td>(c) Isoquant map—core</td>
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<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>12</td>
<td>13.2</td>
<td>3,870</td>
<td>331 ± 11</td>
<td>20,100 ± 2,000</td>
<td>150,500</td>
<td>95,100</td>
<td>75,500</td>
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<tr>
<td>Intermediate</td>
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<td>40</td>
<td>8.9</td>
<td>8,712</td>
<td>218 ± 4</td>
<td>58,100 ± 3,600</td>
<td>75,500</td>
<td>95,100</td>
<td>75,500</td>
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</tr>
<tr>
<td>Periphery</td>
<td>0.69</td>
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<td>18</td>
<td>5.7</td>
<td>2,984</td>
<td>166 ± 6</td>
<td>52,100 ± 3,800</td>
<td>75,500</td>
<td>95,100</td>
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<td>Cat 3: section C: some 7 mm behind globe</td>
<td></td>
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<tr>
<td>(a) All samples pooled</td>
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<td>2.2</td>
<td>0.85</td>
<td>0.00182</td>
<td>0.00214</td>
<td>35</td>
<td>2.9</td>
<td>5,647</td>
<td>161 ± 6</td>
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<td>75,200</td>
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<tr>
<td>(b) 4 quadrants; samples weighted for eccentricity</td>
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<td>34</td>
<td>2.8</td>
<td>5,486</td>
<td>161</td>
<td>187,900</td>
<td>72,500</td>
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<tr>
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<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
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<td>4,826</td>
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<td>142,800 ± 8,200</td>
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<td>54,200</td>
<td>72,500</td>
<td></td>
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<tr>
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<td>811</td>
<td>116 ± 6</td>
<td>45,000 ± 4,600</td>
<td>187,900</td>
<td>72,500</td>
<td>72,500</td>
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Average total for nerve 2 & 3 pooled counts 193,200
straightforward estimate of the total fibre count, the pooled estimate of table 1, gives 191,400 ± 12,000 (p < 0.05) fibres in section B and 194,000 ± 14,500 (p < 0.005) in section C. The estimates of the total population computed by the three methods differ by less than 7% for one section, and when computed in the same fashion, by a similar amount between nerves.

The differences between the mean fibre densities in the isodensity map regions of sections B and C are highly significant (p < 0.001). In passing from the optic nerve head towards the brain it appears that this radial gradient of fibre density across the nerve decreases, as does the mean fibre density, from 178,000 fibres/mm² in the immediate post-laminar region, A, to some 72,500 fibres/mm² 7 mm distal to the globe, C.

Optic nerve fibre diameter spectrum

Cursory inspection of the light and electron microscope photographs, figure 3, revealed that the fibre diameter spectrum and packing density were not uniform throughout the nerve at the level of cross section B, 2-3 mm distal to the globe, although more homogeneous in section C. The mean diameter of 5,543 fibres, including the myelin sheath, was measured in photographs from section B. The diameter was recorded in steps of 0.22 μ up to 3.5 μ and 0.44 μ, or 0.66 μ, up to 13μ. The location of the sample photographs upon which diameter measurements were made is indicated on the nerve cross section of Fig. 2. No correction was made for shrinkage or compression of the fibres.

Separate diameter spectra were established for samples from each of the three fibre density regions of section B, figs. 4b,c,d. The 95% confidence limits on the mean percentage of fibres in a given diameter range is indicated for each region. About 20% of the confidence limit’s range was attributable to variation in the sample size; the remainder arose from variation in the spectrum between different parts of the nerve subregions.

Summation of the computed spectra for the three regions of the nerve, after appropriate weighting for their relative contribution to the total fibre count, supplied a fibre diameter spectrum for the whole nerve, Fig. 4a. This has an almost unimodal appearance with a mode of 1μ and range from 0.5 μ to 13.6 μ. Within the nerve there is a clear transition from a unimodal diameter spectrum for the nerve core, ranging from 0.5 μ to 8.0 μ and with a peak of 0.86 μ, through an intermediate spectrum to a bi- or possibly trimodal peripheral distribution of 1.19 μ modal value, Fig. 4d. The centro-peripheral gradient in the fibre diameter spectrum clearly indicates that the density regions of section B are not homogeneous; however, the samples taken from each are sufficiently well distributed to be representative.

DISCUSSION

The total optic nerve fibre count

The mean electron microscopic fibre counts reported above for two cat optic nerves, 191,300 and 194,500 respectively, are almost double the greatest value previously recorded, 120,000 (Bishop et al., '53), and even further exceed Donovan's ('67) complete light microscopic count of 86,000 fibres above 1μ in diameter.

The high fibre counts obtained by electron relative to light microscopy are commonly explained as arising from the failure of the latter technique to resolve small myelinated or unmyelinated fibres and such an explanation appears applicable in this instance. The 107,000 fibre excess of the above electron microscopic count over the light count of Donovan is predominantly composed of small fibres, Fig. 4a. Only 16% of Donovan's population is below 1.7 μ in diameter whereas this size range forms 48% of the above sample.

The majority of the excess fibres are greater than 1 μ in diameter which is surprising in view of the ability of the light microscope to resolve fibres of at least 0.6 μ diameter (Forrester and Peters, '67) or less under phase contrast, 0.25 μ (Potts et al., '72a,b). That light microscopy can be adequate for counting fibres of less than 1.8 μ diameter in the cat optic nerve is demonstrated by the results of Bishop and Clare ('55) who observed 50% of the population to be smaller than this in agreement with the above electron microscope spectrum. Clearly the conditions for counting even relatively large fibres when closely packed in thick sections may be critical with light microscopy.
Figure 3
The electron microscopic diameter spectrum presented above effectively confirms that of Bishop et al. ('69) but not that of Friede et al. ('71). Variations in shrinkage may influence comparisons between the fibre spectra obtained by different investigators but the range of diameters in this study, 0.5 μ to 13.5 μ, is similar to that of Donovan ('67), 1 μ to 10.5 μ, and any disparities may result from the more distal position of Donovan's section relative to the globe. It is of interest to note that, in spite of the differences at the small fibre end of the spectrum, the number and distribution of fibres greater than 3.5 μ diameter is almost identical in the above and Donovan's ('67) results (> P = 0.99). A comparison of the large-fibre populations of section B with those of Donovan is given in Table 2. The correspondence between the absolute numbers of large fibres, in spite of their different percentages, lends substance to the interpretation of the total count difference as arising from failure to resolve small fibres alone.

Donovan ('67) reports that an error of −4% to 10% occurs when only 10% of the area of the cat optic nerve is counted and argues that large samples are necessary in order to obtain a more accurate estimate of the total fibre population. Donovan's small samples were, however, rather localised yet the form of the sample distribution may be as important as its absolute size for economical sampling of a non-homogeneous nerve. The variations in fibre density and diameter spectrum across the nerve are systematic in the above results and enable smooth isodensity lines to be drawn; the total fibre count may thus be estimated from relatively small but well distributed sample areas. In view of the absence under high magnification survey of visually differentiable regions between the employed sampling rows it is apparent that a total count would offer little improvement in accuracy. The estimated 95% confidence limits of ± 6% of the mean total

**Table 2**

<table>
<thead>
<tr>
<th>Fibre size range</th>
<th>% of spectrum</th>
<th>Absolute number</th>
<th>Total count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donovan ('67) light</td>
<td>≥ 6.05</td>
<td>3.4</td>
<td>2,921</td>
</tr>
<tr>
<td></td>
<td>≥ 3.5</td>
<td>27.3</td>
<td>22,426</td>
</tr>
<tr>
<td>Bishop and Clare ('55) light</td>
<td>≥ 3.5</td>
<td>14.0</td>
<td>—</td>
</tr>
<tr>
<td>Section B, electron microscope</td>
<td>≥ 3.5</td>
<td>1.19</td>
<td>2,300</td>
</tr>
</tbody>
</table>

If Donovan is accepted to have counted all fibres larger than 3.5 μ then the total count for her nerve may be estimated from the E.M. diameter spectrum to be between (18.9118) × 2.921 = 245,460 and (16.1213) × 22,430 = 184,880 fibres, a mean total fibre count of 215,170 which is in good agreement with the total presented above.

**Table 3**

<table>
<thead>
<tr>
<th>Total fibre count</th>
<th>Fibre diameter range μ</th>
<th>Modal diameter μ</th>
<th></th>
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<tbody>
<tr>
<td>Teleost</td>
<td>200,000</td>
<td>0.26–9.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Frog</td>
<td>485,000</td>
<td>0.15–6.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Duck</td>
<td>1,500,000</td>
<td>0.3–6.0</td>
<td>1.15</td>
</tr>
<tr>
<td>Pigeon</td>
<td>2,370,000</td>
<td>0.1–7.0</td>
<td>0.76</td>
</tr>
<tr>
<td>Rat</td>
<td>118,000</td>
<td>0.4–4.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Rabbit</td>
<td>394,000</td>
<td>0.25–7.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Cat</td>
<td>193,200</td>
<td>0.5–13.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Rhesus</td>
<td>1,700,000</td>
<td>0.25–2.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Man</td>
<td>1,200,000</td>
<td>0.25–2.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Fig. 3 Overall view of a cat optic nerve thick transverse section adjacent to section B and showing the location of light and electron microscope photographe other of the core and peripheral regions. The differences in packing density and fibre size between the two regions are immediately obvious.
A. HUGHES AND H. WÄSSLÉ

TOTAL POPULATION
5,543 fibres measured

95% confidence limits on bin mean value

spectrum of Donovan scaled

FIBRE DIAMETER μ

CORE POPULATION
2,039 fibres measured

Fig. 4 A set of cat optic nerve fibre diameter spectra showing the percentage of fibres in size classes determined by the diameter of the circle which most closely matches the cross sectional area of the fibre. The 0.22μ bin width increases to 0.44μ and then to 0.66μ in passing towards the large fibre end of the spectrum. The 95% confidence limits on the mean value for each bin are indicated by a line. The percentage scale is increased ×10 at the diameter indicated by the dashed line. a. The mean fibre diameter spectrum for the total nerve indicates a second mode at about 2.5μ but the confidence limits suggest that a closer fit to a unimodal distribution is quite probable. The spectrum of Donovan (1967), shown hatched for comparison, is scaled in the ratio of its total population to that of the nerve in section B on the assumption that the light microscope failed to resolve the smaller fibres. It is clear, as discussed in the text, that the two distributions are a good fit above 2μ diameter but below this the population is greatly underestimated by light microscopy. b, c, d. The transition from the unimodal "core" spectrum to an, at least, bimodal peripheral distribution with a "tail" is readily apparent in these three histograms. The sample locations in each density region are indicated on figure 2 by black spots.
count for an 8.2% sample are similar in magnitude to the error range assessed by Donovan (’67) for equivalent sample sizes.

The above optic nerve fibre total count is in good agreement with recent estimates of the minimum retinal ganglion cell population of the cat as 190,000 (Wässle et al., ’74) and 217,000 cells (Hughes, ’75) rather than with an earlier report of 90,000 ganglion cells (Stone, ’65).

The ratio of cat optic nerve fibres to presumed retinal neurones thus lies between 0.9 and 1.0 on the basis of the minimum ganglion cell population estimates. The least probable, maximum, estimate of 260,000 retinal neurones (Hughes, ’75)
would lower the ratio to 0.75. It has been argued elsewhere (Hughes, '75) that confusion of glia with neurones could contribute significantly to this discrepancy. Arey and Gore ('42) found the ratio of optic nerve fibres to ganglion cells to be 0.94 in the dog but few other species have been investigated and most available results require confirmation with modern methods. Vaney and Hughes ('76) find the fibre/neurone ratio to lie between 0.72 and 0.87 in the rabbit so that the available mammalian results contrast with the value of 0.57 obtained for the pigeon (Binggeli and Paule, '69). In accordance with the histological evidence (Cajal, '55) the optic nerve fibre/retinal neurone ratio contra-indicates the presence of a large internuncial neurone population in the ganglion cell layer of the mammalian retina (cat, rabbit and dog) while substantiating it in the bird (pigeon).

It is improbable that the centrifugal fibre population of the cat optic nerve introduces significant error into the comparison between the total fibre and ganglion cell counts because it has at most been estimated to amount to only 1% of the fibre population of the nerve (Brooke et al., '65).

A comparison of the total fibre count, fibre diameter range and modal diameter between species so far investigated with the electron microscope is presented in table 3. The range of total counts is considerable, from 0.12 million in the rat to 2.4 million fibres in the pigeon, but has yet no teleological explanation. The range of fibre size and modal diameter vary much less between species in spite of the non-standardization of section planes.

Fibre topography in the optic nerve

The area centralis of the cat retina is distinctive because of the high density and small size of its ganglion cell population. The modal diameter and proportion of larger cells increases towards the periphery (Stone, '65). The intra-retinal diameter spectrum of the area centralis axonal outflow has been demonstrated by Stone and Holländer ('71) to have a lower modal diameter than that of a peripheral population: some 95% of the central axons, and only 50% of the peripheral, were less than 1 μ in diameter. The difference reported above between the diameter spectra of the core and peripheral nerve fibre samples are qualitatively similar to those between the intra-retinal area centralis and peripheral axonal outflows; they strongly indicate that the axonal outflow of the area centralis is represented by the core region in the nerve. This view is substantiated by the temporal situation of the core in intra-retinal sections close to the globe: an indication of the close correlation between the topography of the retina and proximal nerve sections in the cat. In both human and monkey optic nerve (Potts et al., '72c) the macular bundle is also found to be represented by a region of high fibre packing density which contains a large proportion of small fibres.

The cat retina has recently been described as possessing at least three major classes of retinal ganglion cell termed γ, β and α in order of increasing size and axon diameter (Boycott and Wässle, '74). The distinctive size ranges of these cell and axon types suggests that their proportions in the retina, which are difficult to arrive at from either Golgi whole mounts or Nissl stained material, might be obtained from the optic nerve spectrum. Unfortunately the fibre spectrum of the optic nerve is highly unimodal; however the peripheral distribution is essentially trimodal with a division at 2.25 μ and an extensive "tail," representing the larger axons, which is rather arbitrarily assumed to begin at 5 μ diameter. The more extensive sample than previously available (Hughes and Wässle, '75) indicates that 53% of the fibres range from 0-2.25 μ in diameter, 42% from 2.25-5 μ, and that 5% are larger than 5 μ; the proportions are similar for the "intermediate" and total population spectra and appear to represent the γ, β and α axons respectively. The proportion of small fibres, γ axons, is thus high in comparison with the results of Fukuda and Stone ('74) which suggested values of 39%, 52% and 9%, based upon division of the peripheral retinal ganglion cell diameter spectrum.

The action potential reconstructed from the fibre diameter spectrum of Bishop et al. ('69) indicates the three gross potentials of the cat optic nerve, T₀, T₂ and T₁ to correspond with the fibre diameter groups of <2 μ, >2 μ and >6 μ respectively. These three conduction velocity groups
have been identified with three retinal recep-
tive field classes (W, X and Y; Stone and Fukuda, '74; or sluggish, brisk-sus-
tained and brisk-transient; Cléland and
Levick, '74). Rodiek ('73) thus employed
these fibre diameter ranges to estimate
from Bishop et al.'s (69) spectrum the
true proportions of the retinal unit classes,
rather than their physiological encounter
rates (Cléland and Levick, '74; Wässle, et al., '75), as 52% W, 44% X and 4% Y.
These percentages are similar to those de-
rivcd for the y, p and a cells by division
of the above fibre diameter spectrum.
The whole-nerve fibre diameter spectrum
described above leads to a result similar
to that of Bishop et al. (69) when employed
in conjunction with their pragmatic tech-
nique for the reconstruction of the optic
erve compound action potential. The
need for a pragmatic approach to such re-
constructions may well arise both from the
non-homogeneity of fibre packing density
and diameter spectrum at one level of the
nerve and from changes in these factors
along its length.

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histological assistance and Ms. L. Speight
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RETINAL GANGLION CELL IDENTIFICATION
AND DISTRIBUTION
A Quantitative Analysis of the Cat Retinal Ganglion Cell Topography

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ABSTRACT A retinal ganglion cell distribution map has been prepared for the cresyl violet stained cat retina. It differs from previously published maps in revealing the visual streak to be more substantial and in showing a higher peak density of 9–10,000 ganglion cells/mm² at the presumed visual pole. The map was used to obtain a minimum estimate of the retinal ganglion cell population as 217,000 cells, more than double the total previously reported. The problem of classifying the cells of the ganglion cell layer is discussed in detail and examples of criterion cells illustrated. The paper also includes an account of retinal mensuration (dimensions, area, etc.) and a discussion of the visual streak orientation.

The cat has been variously described as possessing either a limited area centralis (Ganser, 1882; Chievitz, 1889, 1891; Krause, 1891; Zürn, '02) or a band of elevated ganglion cell density running across the retina (Slonaker, 1897). The presence of both structures was confirmed by Stone ('65) who provided topographic maps of cat retinal ganglion cell distribution.

Similar maps obtained in this laboratory during a survey of mammalian retinal ganglion cell topography are in agreement with those of Stone ('65) in so far as they also confirm the presence of a limited area centralis and an “arm” of elevated ganglion cell density. However, substantial quantitative differences are obvious; the ganglion cell density of the area centralis peak is 40% greater and that of the peripheral limit of the “arm” of elevated density is 440% greater than in Stone's results. These discrepancies are sufficiently large to be important in estimating the total number of ganglion cells in the cat's eye and when seeking correlations between morphology and physiology. Possible sources of discrepancy are analysed in the discussion.

The retinal ganglion cell distribution map which is presented below reveals the nasal arm of elevated ganglion cell density to be so well developed that it merits the description “visual streak” by analogy with the distribution so termed in a great variety of other species (Chievitz, 1889, 1891; Slonaker, 1897). The term “visual streak” is thus subsequently employed when referring to the “arm of elevated ganglion cell density.”

The above topographic findings are accompanied by a set of quantitative observations on the linear dimensions, area and orientation of the retina in the normal eye which are not at present available in the literature.

METHODS

The retinas used for the following investigations were obtained at the termination of neurophysiological experiments from the eyes of adult cats weighing more than 3 kg. A cut was made around the eye in front of the ora terminalis and the anterior segment removed with as much of the vitreous as possible. The retina was subsequently dissected from the eye cup immersed in saline; the optic nerve was severed by means of a scalpel blade cutting in the photoreceptor plane. The retina was lifted through the surface of the saline bath on a gelatinised slide whereupon it was flattened by surface tension; four radial cuts about the retinal periphery made this possible with a minimum of folding of the hemispherical organ. Surplus moisture was removed from the slide and the retina moved along to a relatively dry area. A mixture of 96% of absolute alcohol and 4% of 40% formalin was sprinkled on the retinal surface which was subsequently
covered with lint-free filter paper, topped with a clean glass slide and placed for 20 minutes under a 100 gm weight in a bath of the fixing fluid. The retina was washed and stained with 0.1% cresyl violet for about three minutes in order to ensure penetration through the whole ganglion cell layer and light staining of the cells of the inner nuclear layer. Preparations were dehydrated in alcohol, cleared in xylene and mounted in Xam.

The large number of cells to be classified required swift identification based upon direct microscopic observation. Cells of uncertain classification were re-examined with an oil immersion objective. The principal criteria employed will be described subsequently but it should be stated here that the not-uncommon difficult judgements depended upon detailed cues derived from colour and differential focusing. The identifications made would be considered unreliable if performed only on photographs after the manner of Stone ('63) and Fukuda and Stone ('74).

**RESULTS**

**Retinal ganglion cell distribution in the adult cat retina**

**Shrinkage during processing.** Measurements of cell densities obtained from dehydrated retinas have been subjected to large corrections for shrinkage during processing (e.g. Stone, '63) but direct inspection suggested shrinkage to be slight in retinas prepared by the above process.

To test this impression the coordinates of vascular landmarks around the retinal periphery have been determined before and after fixation, staining and mounting of the retinas of several species of animal including cat, rabbit, squirrel and rat (Hughes, '75). The distance between any two points within the body of the retina was reduced by usually less than 1-2% during processing; larger changes occurred adjacent to the retinal margins and radial incisions.

One such preparation is shown in its fresh state, figure 1A, and after dehydration and mounting, figure 1B; the various marks occasioned by adherent tapetum or regions of stripped receptors may be used for the estimation of peripheral and internal shrinkage. The magnification of the two prints differs by 0.3% and this is allowed for in table 1 which compares selected intervals of figure 1, A and B. During processing the retinal margins may undergo considerable distortion but in the main body of the retina the linear shrinkage is so small, about 1%, that the cell densities measured in this region require correction by a factor of between 1.0 and 0.98 which has been subsequently neglected.

The variability of peripheral shrinkage within one preparation and between preparations means that the total area change during processing cannot be derived from a linear scaling factor but must be measured from photographs of the flat mount taken before and after the histological procedures. Before dehydration the retina of figure 1A had an area of 497 mm² determined by counting the number of squares contained within its outline drawn on 1 mm squared paper; after dehydration and mounting, figure 1B, a reduction of 11% in area occurred to 439 mm². An extra round of processing for staining induced further shrinkage to 425 mm² peculiar to this preparation. For seven retinas of area ranging from 460 to 545 mm² when fresh the range of dehydrated retinal area was 430 to 508 mm²; this amounted to an average area shrinkage of 7%, range 1-12%. The magnitude of the area shrinkage is thus very variable but the average value is close to that for the smaller cat retina (11%) when subjected to similar procedures. It is to be emphasized that most of the retinal shrinkage is confined to the periphery and is especially concentrated at the radial cuts made during mounting.

**Criteria for cell classification in the ganglion cell layer of the retina.** The definitive criterion for the identification of a neurone in the retinal ganglion cell layer as a retinal ganglion cell is that it sends an axon into the optic nerve. Unfortunately neither the dendritic morphology nor the

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<th>TABLE 1</th>
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<td>Interval</td>
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<td>----------</td>
</tr>
<tr>
<td>a-b</td>
</tr>
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<td>1-2</td>
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<tr>
<td>3-4</td>
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Fig. 1. The retina from which the ganglion cell map of figure 7A was constructed is shown laid flat on the slide IA, before fixing and IB, after mounting and dehydration. Linear shrinkage during processing may be assessed by comparing measurements made on both photographs between a given pair of the numerous landmarks visible on the scleral surface. The suffixed arrows refer to measurements presented in table 1; the broad and curved arrows are explained in the text.
axon are visible in the cresyl violet stained material used in this study and the cells of the retinal ganglion cell layer must be classified by the appearance of their perikarya alone.

The presence of Nissl substance enables the differentiation of neurones of the retinal ganglion cell layer from glia and vascular elements; the reliability of this discrimination is discussed later. It is difficult, however, to assess the influence of displaced amacrine cells (Cajal, 1892; Gallego, '71) on the total count. If containing Nissl substance they will be miscounted as ganglion cells but if staining like cells of the inner nuclear layer they could be classified as glia. References to glia and ganglion cells must be thus understood to refer to presumed glia or presumed ganglion cells. The substantiation of the classification is taken up in the discussion (p. 122).

Some photographic examples of criterion cells have been presented in figure 2 in order to illustrate, to the extent possible.
may be differentiated from a larger somewhat more irregularly shaped class with light staining nucleoplasm, a very small nucleolus and obvious chromatin around the nuclear periphery. The largest of these nuclei are some 6 µm in diameter and the two groups may correspond with the dark and light oligodendrocytes of Mori and Leblond (’70) and Ling et al., (’73). Classification of glia was not attempted but characteristic types are shown in figure 2C.

Vascular cells offer little possibility of confusion with the neuronal elements because of their characteristic linear arrangement and in any case they are primarily capillary endothelial cells which are readily recognised.

The greatest potential error in the neuronal counts thus remains as the possible confusion between small neurones and glial cells; in most parts of the retina the glia greatly outnumber the neurones in the retinal ganglion cell layer and only a small proportion of them mistakenly identified as neurones would lead to large absolute errors in the ganglion cell distribution maps. A quantitative investigation was made in order to estimate the possible magnitude of this source of error.

Estimation of the percentage of cells of doubtful classification. In order to avoid the unconscious use of some absolute size threshold as a criterion for discrimination the following measurements were made on photographs of unknown magnification; the scale was added subsequent to the measurements. A region of retina near to the area centralis was randomly selected, photographed and printed, figure 3. Each cell in the photograph, regardless of classification, was matched in area to one of a set of holes drilled in a thin plastic sheet. The diameter of the smallest hole whose area was equal to, or greater than, that of the cell was used as an index of soma size and the count distributed amongst bins which represented increments of 1 mm in diameter. During this procedure it was noted when doubt as to the classification of the cell had arisen and this was almost invariably found to be associated with cells allocated to the 5th bin. Cells with an equivalent diameter which attributed them to bins 4 and below were invariably concluded to be glia; those attributed to bins 6 onwards were undoubtedly neurones.
Inspection by light microscopy of the retinal region shown in figure 3 revealed the presence of 130 neurones classifiable as ganglion cells according to the criteria outlined above, figure 3B. The area containing these cells was 66,700 \( \mu \text{m}^2 \) and the ganglion cell density was 1,950 neurones/mm\(^2\). The sampled area was found to be situated 1.1 mm above and temporal to the area centralis and close to the 2,000 cells/mm\(^2\) isocount line of figure 7A.

The histogram obtained by this procedure is shown in figure 4 accompanied by enlarged photographs of a selection of cells from figure 3 which are attributed to the bins corresponding to their equivalent diameters on the original print. Three modes are apparent; the first lies with the giant cells of greater than 20 \( \mu \text{m} \) diameter, the second between 12 and 16 \( \mu \text{m} \) and the third between 2.5 and 12 \( \mu \text{m} \). This last group is divided by the transition bin, 5, into glial and neuronal components. The identified glial cells are shown by the stippled area on the histogram; of the 319 cells counted in figure 3 some 57\%, or 181, were glia. Of the remaining 138 cells only 130 were accepted as neurones during the count under the microscope and the area corresponding to these on the histogram is shown hatched; the white region of bin 5 represents the eight cells which did not meet the criteria for ganglion cells. Given that the classification of cells of diameter greater or less than those in bin 5 is very reliable we may centre our consideration of the accuracy of the count on the 27 cells in this bin. If all eight doubtful cells are neurones then the count of 130 underestimates the total by 5%; if all 19 presumed neurones included in bin 5 are glia then the count is overestimated by 15%.

A second histogram of this kind was made for a region 10 mm nasal of the area centralis and selected randomly from the visual streak. The region counted is shown in figure 5A and the corresponding histogram in figure 6. A direct count by microscopy revealed the presence of 59 neurones, figure 4B, in the retinal region illustrated in figure 4A. The area was 0.0578 mm\(^2\) so that the neuronal density amounted to 1,020 neurones/mm\(^2\).

The histogram differs in some respects from that of figure 4; the cells in this region of lower density than that of figure 3A show four modes in their distribution,
CAT RETINAL GANGLION CELL DISTRIBUTION

Fig. 4  Histogram showing the number of cells in figure 3, which fall into arbitrary ranges of mean soma diameter, measured as described in the text. One hundred and thirty of the 319 cells in figure 2 were identified as neurones and they are represented by the hatched part of the histogram; the stippled region indicates the cells identified as glia. The white region of the histogram indicates cells whose identification was in doubt. The cell, 3, whose mean diameter in the original photograph led to its inclusion in the column bearing the unhatched region contains cytoplasmic Nissl substance and is classed as a neurone.

The large cells greater than 20 μ in diameter, a second group ranging from 12 to 18 μ, a third from 7 to 12 μ and the fourth from 3 to 7 μ. Inspection during measurement revealed the last group to be glia of slightly larger size than in the previous histogram; the extra mode arises because of the clear separation of the ganglion cells of the third mode from the glia. The cells of doubtful classification are thus contained in bin 6 of this distribution and, of its eight cells, three were identified as ganglion cells.

The hatched area of figure 6 indicates the 59 neurones identified by direct inspection; we may again obtain an estimate of the range of error of classification by adding the remaining five cells of bin 6 to the total so as to indicate a possible 8% underestimation of the neuronal count or subtracting the included three cells to give a possible overestimate of 5%.

The stippled region of the histogram again indicates the identified glia which amount to 139 of the 201 cells in the field or 69% compared with 57% in the previous region; the absolute count of glia is little changed from 2,800 mm² to 2,400 mm².

The retinal ganglion cell distribution. The results of the preceding section indicate that the error associated with mis-
classification of glia and ganglion cells amounts to no more than \( \pm 10\% \) of the total ganglion cell count near to the area centrals. This error may be smaller for samples from the visual streak and is probably negligible in the peripheral retina because the size range of the two cell types does not overlap.

A map of the outline of the retina and its major blood vessels was prepared by centering each desired point in the microscope field and noting its coordinates on a sheet of graph paper. An initial survey of ganglion cell distribution was made by assessing ganglion cell densities at the intersections of a 1 mm square lattice over the retina.

The ganglion cell densities below 500/\( \text{mm}^2 \) were sampled over an area of 0.178 \( \text{mm}^2 \), those below 5,000/\( \text{mm}^2 \) over an area of 0.0355 \( \text{mm}^2 \) and those above this density over an area of 0.0089 \( \text{mm}^2 \). The sample size was thus held at between 50 and 150 cells. In regions of rapid density variation some extra counts were made at intervals of 0.5 and 0.25 mm. Isocount lines were drawn through regions corresponding to the beginning of densities of 300, 750, 1,000, 2,000, 5,000 and 10,000 ganglion cells/\( \text{mm}^2 \). The resulting isocount line distribution is shown in figure 7A and related to the blood vessel distribution in figure 7B.

A finer counting matrix of 0.2 mm vertical and 0.25 mm horizontal intervals was employed to produce a more detailed map of the region about the area centrals. The corresponding isocount lines for density increments of 1,000 cells/\( \text{mm}^2 \) are displayed in figure 8.
The form of the map with its sharply localized area centrals and the visual streak extending across the nasal retina is similar to that described by Stone ('65). The isocount lines for the higher cell densities become progressively less elongated but in this preparation, unlike those of Stone ('65), the highest density isocount lines do not become circular but retain a major to minor axis ratio of at least 1.6:1. It is perhaps worth noting that the 1,000 and 2,000 isocount lines show superior, temporal and inferior bulges which correspond to those observed in the alpha cell distribution of Wässle, Levick and Cleland ('74) but are by comparison poorly developed. The area centrals and the visual streak are both almost free of the larger blood vessels and their major branches.

The relatively high ganglion cell densities of the temporal arm are best observed by comparison of the ganglion cell density profile passing through the area centrals and horizontally along the arm, figure 9A, with that for a vertical section through the area centrals, figure 9B. The logarithmic scale enables more detailed comparison of the lower density regions and readily reveals the more rapid decay of the vertical than the horizontal nasal profile.

The cat ganglion cell distribution illustrated in figures 7–9 is quantitatively very different from that of Stone ('65). The peak count in the area centrals amounts to 10,000 ganglion cells/mm² in this preparation but only 5,800/mm² in that of Stone ('65). Other preparations show peak counts of similar magnitude at 9,800 and 9,300 neurones/mm². In moving out along the visual streak we find the 159% discrepancy with Stone's ('65) result for the peak count increasing until, at the nasal limit of the retina, the peak count of the arm of high density is found to be about 800 cells/mm² compared with an estimated 180 cells/mm² in Stone's map, a 449% difference.

A direct comparison of the horizontal ganglion cell density profile of figure 9A with that of Stone (fig. 3, '65) cannot be made because it runs across the retina at a different orientation. Stone presents a density profile across the mean fixation plane of the paralysed cat (Vakkur et al., '63) which passes through the area centrals at an angle, θB 22.5° to the area centrals—optic nerve head axis. The small angle of intersection of the sampling axis and the long axis of the narrow iso-
density contours which define the visual streak makes the form of the profile obtained by this convention very sensitive to small changes of the angle of intersection. Identical contour maps would thus give different profiles.

In this paper the axis of the visual streak, defined by a straight line passing through the area centralis and extreme nasal and temporal peak counts, is presented as horizontal. The horizontal density profile of figure 9A is taken along this relatively invariant feature of the cat retina which is subsequently suggested to represent the fixation plane of the conscious as opposed to the paralysed cat. For comparison, estimates of the density profile along the arm of elevated density were derived from figure 2 of Stone (65) and plotted in figure 10 together with the corresponding data from the present work.

The difference between the two profiles is substantial and its origin is taken up in the discussion.

The number of ganglion cells in the cat retina. The revised ganglion cell isodensity lines for the cat retina which are presented in figures 7A and 8 may be employed to obtain minimum and maximum estimates of the total retinal ganglion cell population of this species.

The isodensity lines of figure 7A were transferred to 1 mm squared paper and the area between successive lines was determined. The product of each of these areas, with the value of its lower bordering isodensity line, was summed to give the minimum estimate of the total ganglion cell population. The region peripheral to the 300 cells/mm² line was estimated from the density profiles to have a mean density of 210 cells/mm². The breakdown of gan-
Cat retinal ganglion cell distribution shown in Table 2 was obtained with a minimum estimate of the total population as 217,000 cells.

The maximum estimate of the total count was obtained by taking the sum of the products of inter-isocount areas with the mean of the upper and lower bordering isodensity line values. By this means an estimated population of 260,000 cells was computed.

The mean used to compute the maximum estimate of the ganglion cell population does not take into account the rough circular symmetry of the isodensity lines. Its product with the area of each irregular ring thus underestimates the contribution of the low density "shell" and overestimates that of the high density "core." The resulting excess in the estimate of the ring's population is compounded by the overall concavity of the isodensity profiles. The magnitude of the consequent overestimate in the summed retinal ganglion cell population is difficult to assess so that in future discussion the minimum estimate of 217,000 ganglion cells has been employed with the knowledge that it represents at least 84% of the total population to be obtained in the limit by integration.

This minimum estimate of 217,000 ganglion cells as the total population of the cat retina considerably exceeds the figure of 90,000 provided by Stone ('65). The population of 190,000 ganglion cells indirectly computed from the total cell count of the cat retina by Wässle et al. ('74) is, however, within 12% of the minimum estimate.

Mensuration of the cat retina

Horizontal and vertical extent of the retina. Average dimensions of the cat retina have not so far been reported in the literature. It is unsatisfactory to simply assume those of the available flat mount
Fig. 10 The centro-peripheral ganglion cell density profile along the visual streak axis of the retina in figure 7A on a linear density scale for comparison with the corresponding profile derived from figure 5 of Stone ('65).

preparations because, in spite of the small central distortion, shrinkage near the periphery is great enough to significantly reduce the overall dimensions in some specimens. It also does not follow that the dimensions of even a fresh flattened retina are representative for the tissue when in situ in the globe under 20 cm H2O pressure. The dimensions of the retina have thus been tabulated in table 3 for various stages of preparation.

The horizontal extent of the frozen retina in situ was determined from photographs of sections through the frozen globe at the level of the optic nerve head and in a plane parallel to that containing the posterior ciliary vessels (thus slightly below and parallel to the long axis of the visual streak). The dimensions of the retina have thus been tabulated in table 3 for various stages of preparation.

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streak). The globe was frozen at an internal pressure of 20 cm H2O and the retinal width, when corrected for expansion of the vitreous upon freezing (± 1.03 for linear dimensions), is very similar to that determined from × 5.5 photographs of either the in situ retina in the flattened and pinned out eye cup or the excised retina laid out on a dry slide. The retina would thus appear to be under little or no tension in the normal globe under pressure.

The difference between the mean extent of the frozen retinas corrected for expansion and their excised companion retinas thus amounts to only about 1%. A somewhat larger select population of mounted and stained retinas is only 2% shorter overall than the fresh organ. These select retinas were followed through processing by photography and are known to be free from gross peripheral shrinkage. The larger population of 14 unselected retinas evidenced greater overall shortening of 6% relative to the in situ retinal mean length. The retinal dimensions increase with the weight of the cat over the range from 2.0 to 3.5 kg. The variation has not been thoroughly investigated but it would appear that the mean length at the bottom of this range is about 27 mm and at the top about 28.5 mm.

The vertical extent of the retina was measured along an axis through the optic nerve head and perpendicular to the posterior ciliary vessels. From table 3 it does not seem to be significantly different from the horizontal extent of the retina.

The scale accompanying Stone's figure 2 ('65) reveals the illustrated retina to be 34.6 mm wide which is readily seen to be some 5.6 standard deviations greater than the mean width of the fresh excised retinas of table 3.

The extent of the nasal and temporal hemiretinas. For some purposes it is convenient to have available the mean extent of the nasal and temporal hemiretinas determined with respect to either the optic nerve head or the area centrals. For convenience these measurements were made along the axis passing through the optic nerve head parallel to, although slightly below, the visual streak. The results are presented in table 3.

Distance between the peak count of the area centrals and the centre of the optic nerve head. An accurate mean value for this distance is important for the determination of the projection of the visual axis in neuro-ophthalmological experiments on the cat (Bishop et al., '62; Vakkur et al., '63). Earlier measurements of the separation of the blind spot, B, from the macroscopically estimated centre of the area centrals, A, in methylene blue stained retinas (Bishop et al., '62; Vakkur et al., '63) have been pooled (Nikara et al., '68) to give a mean value for BA of 3.37 ± 0.25 mm S.D. (n = 30). Examination of Stone's ('65) retinal ganglion cell maps was claimed to reveal (Nikara et al., '68) that the peak of the area centrals count is some 0.24 mm lateral of the geometrical centre of the area identified as "A": a correction of BA by this amount was subsequently adopted to bring the mean separation of the peak count and optic disc to 3.37 ± 0.24 = 3.61 mm. This round-about derivation was necessary because only one published map is available on which the distance may be directly measured (Stone, '65: fig. 2).

The flat mounts used in this investigation provide directly measured values for the area centrals peak/optic nerve head separation of 32 retinas in which the location of the peak count of the area centrals was determined macroscopically. The results are presented in table 4 where the mean separation is shown to be 3.35 mm. A 2% correction for shrinkage must be applied to this result in order to obtain an estimate of the distance in the unfixed and hydrated retina as 3.4 mm. This result is in very good agreement with the value of 3.37 mm presented by Nikara et al. ('68) and does not support the use of their 0.24 mm correction; macroscopic examination of the stained retina in the earlier papers

| Area centrals peak density — optic nerve head centre separation, mm |
|-------------------------|-----------------|-----------------|
| 3.55                   | 3.41            | 3.29            | 3.41 |
| 3.40                   | 2.95            | 3.08            | 3.45 |
| 3.33                   | 3.18            | 3.20            | 3.60 |
| 3.33                   | 3.32            | 3.41            | 3.44 |
| 3.35                   | 3.55            | 3.48            | 3.15 |
| 3.50                   | 3.35            |                 |     |

Mean separation 3.35 mm ± 0.16 mm S.D. (n = 32). Estimated separation for the fresh in situ retina (correction for 2% shrinkage) is 3.42 mm.
accurately defined the position of the area centralis peak count.

**Area of the cat retina.** The schematic eye for the cat (Vakkur and Bishop, '63; Vakkur et al., '63) does not deal with retinal mensuration; Stone's measurement of 730 mm² as the cat retinal area is alone available ('65). This is 150% greater than the 497 mm² reported for the retina of figure 7 when unfixed and quite outside of the range of unfixed excised retinal areas encountered in this series. The retinal areas were measured by transferring the 5.5 times enlarged photographic outline of either the fresh excised retina or of the stained and mounted organ to 1 mm squared paper and counting the number of squares contained within it. The results presented in table 5 after appropriate scaling. The range of area for seven fresh excised retinas was from 460 mm² to 545 mm² with a mean of 510 mm².

The in situ retina of the cat approximates a portion of a sphere so that its area may be computed as that of a spherical segment. From Vakkur et al. ('63) we have the posterior wall of the cat eye as of radius 12.5 mm and thickness 0.47 mm. The radius of curvature of the major portion of the photoreceptor layer is thus close to 12.0 mm and this is assumed to apply over its whole extent. Given the earlier result that the mean width of the fresh retina is 28.0 mm (table 3) we obtain by simple trigonometry that the height of the spherical segment is 7.28 mm whereupon the area of its surface, $2\pi rh$, is found to be 549 mm². This is only 69–73% of the areas computed by Stone ('65) in unspecified fashion from the schematic eye but is within the range encountered in this series and differs from the mean by only 8%. Thus neither Stone's ('65) measurement of the cat retinal area nor his corroboratory calculation were confirmed.

The representation of the in situ retinal area by that of the excised organ was supported in addition to the above calculation by measurements on the companion eyes to those providing the largest and smallest retinas. The in situ area was calculated from the retinal extent and radius of curvature, corrected for expansion, in photographs of a section through the plane of the optic nerve head of the frozen globe. The assumption of identical retinal extent along different diameters is supported by the measurements in table 3. The measured excised retinal area was within 8%
of that computed for the in situ organ of the companion eye, table 5, which is normally of very similar dimensions (Vakkur et al., '63).

The retina may be divided about a line through the area centralis either perpendicular to the axis of the visual streak to give nasal and temporal hemiretinal areas or along the streak to give upper and lower retinal areas. Mean values for a population of retinas are not available but the divisions have been presented for the typical retina of figure 7a in table 5. Its area of 497 mm² when fresh and unfixed is close to the mean value of 510 mm² obtained for seven retinas so that the absolute areas may be taken to be generally representative.

Orientation of the cat visual streak

The paralysed animal. Considerable effort has been expended by Bishop and his co-workers (Bishop et al., '62; Vakkur et al., '63) in the accurate determination of the position of paralysis of the cat eye which they assume to be close to the primary position of the eyes in the conscious animal. A fixation plane has been defined which intersects the retina as a horizontal line passing through the peak ganglion cell density of the area centralis when the eye is in the position of paralysis.

The orientation of the eye about the presumed visual pole is defined by the angle, $\Psi_B$, formed between a line joining the estimated centre of the area centralis to the optic nerve head centre and a line representing the presumed fixation plane (Bishop et al., '62). From Bishop et al. ('62) we have that $\Psi_B$ is $24.4^\circ \pm 4.26^\circ$ s.d. (n = 40) which is in agreement with a larger sample of Hammond (personal communication) for which $\Psi_B$ is $25.2^\circ \pm 4.3^\circ$ s.d. (n = 90). A more commonly employed result is that of Vakkur et al. ('63) which suggests $22.3^\circ$, range $15.0^\circ$ to $26.0^\circ$ and estimated s.d. $3.0^\circ$ (n = 12).

An estimate of the mean orientation of the cat visual streak with respect to the presumed fixation plane in the paralysed animal may thus be obtained if the mean angle, $\Psi_S$, between the long axis of the streak and the area centralis — optic nerve head reference axis is known. Measurements of this angle have been made for 12 flat mounted retinas on either complete ganglion cell density distributions or limited maps localised to the area centralis and to the terminations of the streak in the nasal and temporal retina. The results are shown in table 6.

The difference between the mean value for $\Psi_B$ of 22.2° in the position of paralysis and the mean value of 16.63° for $\Psi_S$ (table 6) is 5.57°, the standard error of this difference is 1.14°. The t-test indicated that the difference between the means is very unlikely to have arisen as a sampling error. Such an event is even more improbable for the 6.6° angle between the streak and the presumed fixation plane obtained from the larger samples of mean $\Psi_B = 25.2^\circ$. The visual streak thus lies at a mean angle of between 5.6° and 6.6° to the horizontal in the paralysed cat. Wässle et al. ('74) similarly estimate an angle of 6.0° from the cell distribution.

DISCUSSION

The origin of the discrepancy between the above results and those of Stone ('65)

The directly determined area centralis peak density in the dehydrated preparation of Stone's figure 2 ('65) was 8,700 ganglion cells/mm² which is in substantial agreement with the values of 9–10,000/mm² reported above and very close to the mean value of 8,800/mm² (n = 4) described by Wässle et al. ('74). Stone's application of a shrinkage correction factor of 0.67 to the peak density of the area centralis reduced its value to 5,800 ganglion cells/mm² and introduces a discrepancy with the result of this paper.

It was demonstrated in an earlier section that shrinkage in the region of the area centralis was very small for the preparatory technique employed above and Stone ('65) makes a similar claim for his
method. Stone did not, however, make a direct determination of shrinkage of the dehydrated preparation of his figure 2 but carried out control counts on five non-dehydrated wet mounts from which he obtained a mean peak density of only 5,800 ganglion cells/mm² rather than about 9,000 as in the dehydrated preparation. He concluded that the dehydrated retina had undergone shrinkage of 33% in area and thus arrived at his correcting factor of 0.67.

It is suggested that difficulty in observing all the cells of the double layered area centralis in the uncleared preparation led to a serious underestimate of the peak density in the control wet mounts and a considerable overestimate of the extent of shrinkage.

The validity of this interpretation of the discrepancy between the peak ganglion cell densities presented above and those of Stone ('65) is substantiated by consideration of the reported retinal dimensions. Stone reduced the linear scales of his illustrations relating to the dehydrated preparation by a factor of 0.84 in order to compensate for shrinkage of 19% linear. If shrinkage had not occurred then the dimensions of the retina should be excessive by this amount. It has already been pointed out (p. 19) that the retina of his figure 2, at 34.6 mm wide, is some 5.6 S.D. longer than the mean for retinas of this series. Elimination of the unnecessary shrinkage correction recovers the original dimension as 29.0 mm which, although large for a mounted preparation, is within the range of this series. Similarly, the retinal area quoted by Stone ('65) is 730 mm²; correction to the original dimensions by multiplying with a factor of 0.84² indicates an area of 515 mm² which is close to the mean value of 510 mm² reported and 549 mm² calculated in this paper. The question of criteria for ganglion cells does not appear to be involved in relating the two sets of results for the peak density of the area centralis.

In figure 11 the horizontal ganglion cell density profile along the peak count of the cat visual streak which was obtained in this study, A, is again presented for comparison with an equivalent profile of the streak derived from Stone's figure 2 ('65), B, but this time plotted on a logarithmic scale which reveals more clearly the differences in the lower density regions which were previously displayed in figure 10.

If the correction for shrinkage were the only source of discrepancy then a vertical shift on the logarithmic plot should be sufficient to make the two sets of results coincide. This does not occur as is shown by the hatched area separating lines C and D of figure 11. In curve D the separation of the sampling points derived from Stone's map has been corrected by the elimination of his shrinkage compensation so as to represent their original relative positions on the retina.

The ganglion cell densities reported in this paper are also invariably greater than those of Stone above and below the plane of the cat visual streak. Some 55% of the retina of his figure 2 ('65) is outside the 125 cells/mm² isocount line (188 cells/mm² corrected) whereas in the map of figure 7A only 30% of the retina bears the higher minimum density of less than 300 and more than 200 cells/mm². Thus, in regions away from the area centralis, a superficial shrinkage correction by no means accounts for the discrepancy between Stone's ('65) results and those presented above. The question thus remains as to whether the densities reported above exceed those of Stone as a result of the improper inclusion of glia and displaced amacrine cells or because of the inclusion of previously neglected ganglion cells.

Has the retinal ganglion cell population of the cat been overestimated in this study?

The discrepancy between the retinal ganglion cell densities described by Stone ('65) and those reported above manifests itself most conveniently, and independent of shrinkage, in the difference between the total ganglion cell populations obtained upon integrating the two isodensity maps. It has been pointed out that Stone ('65) estimates the total ganglion cell population of the cat retina to be 90,000 in contrast to 217,000 above and 190,000 reported by Wässle et al. ('74).

Stone supports his estimate of the total retinal ganglion cell population by indicating that it falls within the reported range of from 66,000 to 120,000 fibres in the optic nerve (120,000, Bishop et al.,
Fig. 11 The absolute discrepancy between the centro-peripheral ganglion cell density profiles along the visual streak axis of this paper, A, and of Stone ('65), B, revealed more clearly by comparison on a logarithmic scale. Shifting the two profiles so that the peak counts coincide and densities are expressed as percentages of their respective peak value, C and D, eliminates the differences which arise in Stone's use of an unnecessary shrinkage correction. The hatched region indicates the considerable residual discrepancy which must have its origin in other sources. A detailed discussion of this figure is given in the text.

This conclusion is supported by the results of an E.M. investigation of the cat optic nerve fibre population (Hughes and Wässle, '74) in which fifteen thousand fibres were counted in 70 photographs distributed so as to enable the isodensity map of the nerve to be constructed. The minimum total population estimated by the integration of this map was 200,000 fibres ± 5,000 s.e.m. which is in good agreement with the minimum total retinal ganglion cell count of 217,000 cells reported above and that of Wässle et al. ('74) of 190,000 cells. The excess of these two counts over Stone's total of 90,000 ganglion cells is thus not predominantly made up of mistakenly counted glia or displaced amacrine cells but represents true ganglion cells which send axons into the optic
nerve. The agreement between the optic nerve fibre count and the total ganglion cell count of the present paper is strong support for the criteria employed in the identification of ganglion cells.

It is improbable that the discrepancy arises in the identification of large and medium sized neurones of the retinal ganglion cell layer. The other possibility is that a very large population of small neurones has been left uncounted by Stone. An examination of figures 6 (Stone, '65) and 1c (Stone, '66) may convince the reader of a substantial basis for this interpretation.

The ganglion cell diameter analysis of Stone ('65) indicates a mean threshold diameter of about 8\(\mu\)m for the differentiation of neurones from glia. It is conceivable that over the majority of the retina there is a population of small cells of mean diameter falling below this criterion which would account for their being overlooked in the earlier study.

After elimination of the unnecessary shrinkage correction the relatively small excess of the above counts over those of Stone ('65) in the region of the area centralis may be accounted for in terms of his formal 8\(\mu\)m diameter threshold for ganglion cell classification. It is clear from direct measurement that this is an inappropriate value for the immediate vicinity of the peak count. Fukuda and Stone ('74) and Stone and Fukuda ('74) incorporate some 10\% of cells smaller than 8\(\mu\)m mean diameter at the area centralis and a similar proportion, ranging down to 6\(\mu\)m in diameter, has been reported by Wässle et al. ('74). In the region temporal of the area centralis sampled in the histogram of figure 4 the discrepancy with the normalised count of Stone ('65) is about 25\%, figure 13, and the percentage of cells of less than 8\(\mu\)m estimated mean diameter amounts to 20\% of the population.

These very small cells are, however, not particularly numerous in the sample taken from the visual streak some 10 mm nasal of the area centralis. In the corresponding histogram of figure 6 it is obvious that the retinal ganglion cells of less than 8\(\mu\)m mean diameter make up less than 5\% of the population. Their complete neglect by Stone ('65) would suffice to account for only 9\% of the 230\% difference in density remaining between his and the above sample for this region after their normalisation to eliminate the unnecessary shrinkage correction (C.D in figure 13). In peripheral retina the <8\(\mu\)m cells are even less common.

Thus, for the majority of the retinal area and of the ganglion cell population, an explanation other than small size must be sought to account for the earlier failure to identify some 50\% of the population. The size distribution of the previously neglected cells thus remains unspecified but small cells, probably corresponding to members of the \(\gamma\) classification described by Boycott and Wässle ('74) must predominate.

The peak density of the kitten retinal ganglion cell distribution

Stone ('66) reports the peak density of the area centralis in a dehydrated whole mount preparation of a seven week old kitten retina to be 8,500 ganglion cells/mm\(^2\) (fig. 6, Stone, '66). The excess of this value over the previously reported adult peak count of 6,000 ganglion cells/mm\(^2\) is said to be "due to the relatively small size of the eye" and is suggested as disappearing with the increase in kitten retinal area by a factor of 1.42 during growth to cathood. The discrepancy which gives rise to this suggestion would, however, be eliminated if the true adult peak count is accepted to be 8,700 cells/mm\(^2\) in Stone's ('65) preparation as was indicated in the earlier part of this discussion.

Stone's claims were reinvestigated by sampling in the central area of two 14 day old kitten retinas. The cells of the area centralis were well differentiated but more peripherally their identification was difficult and an isocount map has not been presented here. The peak count was between 10,000 and 11,000 ganglion cells/mm\(^2\) in both retinas, within 10\% of the peak counts reported earlier for the adult cat. The reported difference of 40\% between the kitten and cat peak densities (Stone, '66) was not confirmed. Rather, these counts suggest that the kitten's area centralis is well differentiated by 14 days of age, and that the peak ganglion cell density changes little during subsequent growth.
Does rolling of the eye occur in the anaesthetised and paralysed cat?

It has been shown that in flat mount preparations the visual streak lies at a mean angle of 5.6° to 8.6° below the presumed fixation plane of the paralysed cat. Distortion of the equatorial strip of retina which contains the visual streak and area centralis seems to be an unlikely explanation of the discrepancy in view of the good fit of the streak axis to a straight line when radial relief incisions have been placed before mounting. Again, the retinal vessels in a fundus photograph match the normalised flat mount vessel pattern almost perfectly thus suggesting minimal distortion. For the larger landmark separations this may amount to ±5% which could account for only a 1° deviation of the streak from the presumed fixation plane.

The question thus arises as to whether the visual streak deviates from the horizontal in the conscious as well as in the paralysed cat. It is teleologically unlikely that it does; in the more lateral eyed species the visual streak is normally held, horizontal (rabbit, Hughes, '71; goat, Hughes and Whitteridge, '73). A medial rotation of the upper part of the pupil during paralysis would account for the deviation of the visual streak from the horizontal in that condition.

Sanderson ('72) has reported the presence of rotation in two of six animals but it was opposite in sense to that suggested above. His method involves receptive field plotting to determine the borders of the homonymous hemifield but is rather indirect. His findings conflict with those of Hubel and Wiesel ('62) who describe the upper margins of the pupils as rotating inwards by 5° during anaesthesia and paralysis and of Bishop et al. ('62) who give a more detailed account. These authors find the angle formed between the upwardly converging pupils of the conscious cat to be 11.0° ± 2.5° S.D. (n = 12). In four anaesthetised and paralysed animals this angle increased to 21.0° because of inward rolling of the eyes. The rolling of each eye thus averages (21.0°−11.0°) / 2 = 5.0°, which is in good agreement with the estimate of 5.6° of rolling based on the assumption that the streak is horizontal in the conscious cat and is regarded as strong support for this hypothesis. The question of torsion of the eye under anaesthesia and paralysis is currently under re-examination.

General

Whitteridge ('73) and his co-workers have shown that the area of the cat lateral geniculate laminae (Seneviratne, '63), visual cortex (Bilge et al., '67) and superior colliculus (Vejaesy, '67) have respectively 33%, 42% and 40% devoted to the upper and 67%, 58% and 60% devoted to the lower field representation. Bilge et al. ('67) suggest that no such asymmetry appears in the retinal ganglion cell distribution.

The results presented in table 6 show, however, that when divided about the presumed fixation plane of the streak axis, the relative areas of the lower, 40%, and upper, 60%, retina are in similar ratio to the areas of the corresponding representations of the upper, mean 38%, and lower, 62%, visual field in the electrophysiological maps. Integration of the map of figure 7 reveals that the lower retina contained 96,700 ganglion cells and the upper 118,500 or 45% and 55% respectively.

The revised cat retinal ganglion cell distribution map, figure 7, reveals the visual streak to be a more prominent feature of the distribution than has previously been described: the 1,000 ganglion cells/mm² isocount line encloses 34% of the total retinal ganglion cell population within only 12% of the retinal area (p. 118). This result is in keeping with the reports of Whitteridge ('73) and his co-workers who describe the sectors of the visual hemifield, 150°−180° and 180°−210°, which adjoin the horizontal plane to the visual streak, as obtaining a disproportionate representation in the lateral geniculate nucleus (Seneviratne, '63; Whitteridge, '73), visual cortex (Bilge et al., '67) and superior colliculus (Vejaesy, '67). According to Vejaesy ('67) these two sectors occupy 53% (n = 5) of the mapped superior colliculus area rather than the 33% to be expected from the fraction of the visual field represented.

The elongated form of the isodensity profiles has been found to obtain even for those defining the region of peak count in the area centralis of three retinas (in
8 retinas Wässle has found all but one to possess oval peak isocount contours; personal communication). It is of interest that rod and cone peak isodensity contours are also oval (Steinberg et al., '73). In contrast, Stone ('65, '66) defines the peak count by circular isocount lines in all retinas and suggests that the transition from the oval form at the lower densities may signify a continuous qualitative change in the organisation between centre and periphery. The form of the peak density isocount line is of some significance in estimating the overlap of hemiretinal distributions in tract sectioned cats (Stone, '66).

The revised retinal ganglion cell distribution maps necessitate the re-examination of previously obtained correlations between the ganglion cell density profile of Stone ('65) and factors such as LGN magnification (Sanderson, '71) or receptor density (Steinberg et al., '73). Careful choice of the orientation of the selected ganglion density profile employed for such comparative purposes is also required. The determination of the centro-peripheral variation in the rod or cone convergence ratio onto the ganglion cells (Steinberg et al., '73) will be subject to the possibility of considerable error if the receptor densities are not compared with those of the functionally related ganglion cells. The ONH-AC axis is available for relating the profiles but if they are from different preparations then the range of γS alone is sufficient to ensure the possibility of substantial misalignment of the major axes of the two distributions and consequently erroneous convergence ratios.

Electrophysiological magnification factor profiles are commonly determined with the eyes in the position of paralysis. Such results may be compared with a ganglion cell density profile (Sanderson, '71) across the projection of the mean presumed fixation plane onto an individual retina (Stone, '65). An additional ganglion cell density profile has been presented in figure 12 for this purpose and is accompanied by the equivalent profile of Stone ('65) for comparison. Both profiles are at an angle, γB, to the ONH-AC axis of 22.5°, but, again, the range of ± 13° for this angle may make the profile quite unsuited to the

![Graph](image_url)

**Fig. 12.** Centro-peripheral ganglion cell density gradient. A, for a section running across the retina of figure 7A at an angle of 22.2° to the ONH-AC axis in the presumed mean fixation plane of the paralysed animal. The corresponding profile of Stone (fig. 3, '65) has been replotted on a logarithmic scale for comparison. B. These profiles do not run along the axis of the visual streak.
representation of the situation in the original experiment. The wisdom of seeking a correlation between the profile of magnification factor, at some central station and that of the total retinal ganglion cell density may be questioned. The retinal ganglion cell population has been demonstrated to contain three morphological classes, $\alpha$, $\beta$ and $\gamma$ cells (Boycott and Wässle, '74) each with different central projections established from the relationship between perikaryon size, axon diameter (Boycott and Wässle, '74) and the components of the compound action potential in the central visual pathways (Bishop et al., '69; Stone and Fukuda, '74). Only if the proportions of these classes are constant throughout the retina is it justifiable to substitute the total ganglion cell profile for those representing the specific classes projecting to a given destination.

The proportion of the different classes of cell in various regions of the retina has not yet been definitively established. Wässle et al. ('74) argue that the $\alpha$ cells form a relatively constant 3.3% of the retinal population, whereas Fukuda and Stone ('74) report them as varying from 1% centrally to 9% in the periphery. Failure of the latter workers to count the whole population of peripheral ganglion cells would lead to a discrepancy of this order in the periphery. In temporal retina Fukuda and Stone ('74) argue that the relative proportions of $X$ and $W$ cells remain constant over a range of 20° temporal of the area centrals at 60% and 40% respectively.

The cat has so far been behaviourally showed to achieve the resolution of gratings of only 11' period (Smith, '37) although the use of cortical evoked potentials suggests a minimum capability of 10' (Berkley and Watkins, '71). The peak ganglion cell density of 9–10,000 cells/mm² corresponds to some 440 cells/degree² (0.22 mm² on retina). Of these only the brisk sustained class, $X$ cells, are regarded as possibly subserving resolution (Cleland et al., '71). Provisional acceptance of the estimate that they form 60% of the area centrals population indicates that, at most, only 260 cells/degree² could be available to the cortex for tasks involving resolution. The corresponding mean angular separation of their receptive field centres is 3.7°. A theoretical minimum of 7.4° for the period of a resolvable grating in the stabilised retinal image is thus established and is close to the behaviourally demonstrated resolving power of the cat.

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I wish to thank H. Wässle for permitting some measurements on his preparations, for access to unpublished material and for extensive discussion. I am indebted to B. B. Boycott and H. Wässle, W. R. Levick and B. G. Cleland, and J. Stone and Y. Fukuda for allowing access to their respective papers prior to publication and to P. Hammond for providing unpublished optical data. My thanks are also due to Ms. L. Speight for secretarial assistance provided with characteristic equanimity.

LITERATURE CITED


1891 Über das Vorkommen der area


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Population Magnitudes and Distribution of the Major Modal Classes of Cat Retinal Ganglion Cell as Estimated From HRP Filling and a Systematic Survey of the Soma Diameter Spectra for Classical Neurones

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ABSTRACT A survey of diameter spectra in presented for classic neurones of the cat retinal ganglion cell layer. From these, with the aid of retrograde HRP filling for central retina, a set of density distribution maps has been prepared for each of the major modes of the neuronal diameter spectrum. The total population of classical neurones, the presumed ganglion cells of Hughes ('75), confirms published values with a minimum of some 207,000 comprising 5,600 cells in the α mode, 80,700 cells in the β mode, and 120,700 cells in the γ mode.

A proportion of classical neurones in the γ mode do not fill by retrograde transport of HRP from either optic nerve or superior colliculus. Their morphology is characteristic and includes a conspicuous basophilic nuclear bar or fold; they remain subsequent to ganglion cell retrograde degeneration and resemble some profiles of the amacrine layer. It is presumed that they represent a class of displaced amacrine cells.

Estimates based on ganglion cell identification by HRP filling indicate populations of about 80,000 cells in both the γ and the β modes and a total count of about 170,000 ganglion cells; a good agreement with Hughes and Wässle's ('76) optic nerve fibre count, but lower than the classic neurone count.

It is concluded that the distribution maps for ganglion cells in each of the three modes of the soma diameter spectra are similar in form and resemble that of the total neurone density map. The ganglion cell population of the γ mode in the visual streak is not found to increase in proportion relative to that of the β mode, as has been reported elsewhere.

Three major groups of ganglion cell may be identified by their characteristic dendritic trees in all regions of the cat retina (Boycott and Wässle, '74). These α, β, and γ cells have specific physiological classes of receptive field (Cleland and Levick, '74a,b; Fukuda and Stone, '74). Their distribution patterns would be of great interest but cannot be obtained from capriciously stained Golgi material.

However, the soma diameter of α cells is removed from the local mean and permits their being mapped by inspection in Nissl-stained retinas, where dendritic trees are not visible (Wässle et al., '75; Stone, '78). Outside a small strip of central retina, the β and γ cells also occupy predominantly separate modes of the soma diameter distribution, and the proportion of each class might be estimated from ganglion cell diameter spectra. Even in central retina the bimodal spectra may be provisionally subdivided by retrograde filling with horseradish peroxidase (HRP) from central injections (Kelly and Gilbert, '75) into three apparently class-related components which have the same distinctive projections as the modes of peripheral retina.

A survey has been made of local neuronal diameter spectra for various regions of the cat retinal ganglion cell layer. These have been employed to estimate the density distribution of cells in each of the three overt modes and, by the aid of HRP retrograde transport, in the
"latent" central modes. The modal density maps offer an indication of class distributions but will require correction to the extent that cross-modal contamination occurs.

Unfortunately, there is doubt whether all the small neurones of the y mode are ganglion cells. Integrations, which are confirmed below, of published classical neurone distributions (Wassle et al., '75; Hughes, '75—presumed ganglion cells) exceed available optic nerve fibre counts (Hughes and Wassle, '76; Stone and Campion, '78). However, retrograde transport of HRP from the superior colliculus enables identification of a confirmed ganglion cell population which amounts to some 170,000 cells, in agreement with the optic nerve count of Hughes and Wassle ('76), but exceeding even the revised, 116,000 mean, ganglion cell counts of Stone ('78). The excess of classical neurones over the optic nerve fibre population is attributed below to a morphologically distinct set of small non-ganglion cells which resemble certain profiles of the amacrine layer.

Similar attempts to estimate class distributions from modal proportions have been made by Fukuda and Stone ('74) and Rowe and Stone ('76). However, they divide their retinal ganglion cell population according to a 14-μ size criterion in all regions away from the area centralis, and equate the elevated "small" cell population of the visual streak with an increased population of "W" (γ) cells. Their conclusion is not supported by the subsequent results, which indicate the three modes of the retinal ganglion cell distribution, as defined by modal dips and axonal projections, remain in relatively constant proportion throughout the retina.

METHODS

Retrograde transport and development of HRP

The optic nerve was exposed at a distance of some 5 mm from the globe in animals anaesthetised with Nembutal. The sheath was opened, the fibres sectioned, and HRP crystals (Sigma VI) applied to the cut ends. The animals were maintained in a drowsy state by means of Nembutal for periods between 24 and 48 hours.

The superior colliculus of other cats was exposed by aspiration while avoiding damage to the lateral geniculate nucleus. 5 μL of a 20% solution of HRP (Sigma VI) were injected over a period of 30 minutes at several locations.

The eyes were enucleated under deep Nembutal anaesthesia after 36 hours, hemisected, and briefly prefixed by 15 minutes immersion in cold 2% formal-saline (Straus, '64). The retina was removed, and relief cuts inserted and laid on filter paper. It was presoaked in a benzidine dihydrochloride solution (BDHC1) and developed according to the procedure of Strauss ('64) for a blue reaction product. This procedure was modified by fixation and mounting between the development and stabilisation baths in order to eliminate shrinkage of the stabilisation bath without loss of filling or of fine dendritic trees. Sodium nitroprusside was dissolved in the fixative to initiate stabilisation. The reaction product visibility was intensified by cresylecht dye. No neurones but some microglia and pericytes revealed an endogenous capability to produce reacting product in control retinas.

All operations involving BDHC1 were carried out in plastic bags on a tray covered with bleach-soaked paper. Protective gloves were worn, and all utensils and bags were soaked overnight in bleach before disposal or washing.

Density maps

The mounted retinas were mapped with the stage micrometers at a magnification of X10. Local cell densities (Hughes, '75) were entered on a 1 mm square lattice or with finer steps at the area centralis and streak. Oil immersion was employed for counting, and several fields of view were averaged in low-density regions to increase the significance of the samples.

Cells were measured by means of a graticule and eyepiece micrometer or compared with a criterion profile viewed through a camera lucida.

Maps were integrated with a planimeter (Allbrit); the procedure was repeatable within 0.5%. No allowance was made for shrinkage, which was less than 2% overall (Hughes, '75).

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Cell diameter spectra

At each sample location, a series of photographs was obtained at different planes of focus with a Zeiss Photomicroscope III. Diameter spectra was established from the maximum cross-sectional area of the cells in the photographs after classification under oil immersion. Cells were matched in area to a series of circles on a plastic film, and their size expressed as the diameter of the area of the circle just containing them (Hughes, '75). Various magnifications of print have been used; these are subsequently indicated in terms of the bin-width for diameter increments or print magnification.

The modal percentages in all subsequent histograms were obtained by division at the troughs. When a trough was represented by one bin, the proportion represented was divided equally between the adjacent modes.

RESULTS

Nomenclature

Rowe and Stone ('76) classify ganglion cells smaller than 14 μ as "small," 14-20 μ "medium," and those greater than 20 μ as "large." The implicit and argued identification of these groups is with the W,X,Y classes of Stone and Fukuda ('74) and, thus, with the γ, β, and α cells. These criteria were employed in periphery and streak.

To avoid confusion with the classification of Rowe and Stone because of the contrasting results described below, any reference to small, medium, and large cells has been avoided. Instead, the components of the soma diameter spectra are referred to as the α Nissl mode, the β Nissl mode, and the γ Nissl mode (abbreviated γN). All that is implied is that the major component of the mode corresponds to the given morphological class, not that other classes are absent from each mode. Emphasis is thus put on the modal, rather than single cell, basis for estimating class proportions.

The modal-dip method of division is a relative measure and less influenced by shrinkage than absolute size criteria when employed to divide the population. It is more likely to reflect changes of significance in the underlying functional classes than a fixed criterion size, which may readily become arbitrary if not justified for each retina. Kelly and Gilbert ('75) and results below show that cell size in a given region may vary from retina to retina, even with the same preparatory techniques and the absence of overall retinal shrinkage, and that it certainly varies with the method of fixation.

Fixed and dehydrated Nissl-stained retinas

Criteria for neurones. Classic neurones were identified by published criteria (Hughes, '75). They possess substantial cytoplasm, obvious Nissl substance, no nuclear chromatin, and an obvious nucleolus. Published criterion photographs of streak and area are supplemented here by a region of peripheral retina of neuronal density 430 mm⁻², some 8 mm above the area centralis (Fig. 1A) and its soma diameter spectrum (Fig. 2). The profiles are illustrated at higher magnification because of the debate about criteria (Fig. 1B); none of these neurones has an equivocal appearance. The profile spectrum (Fig. 2) has four modes; the stippled region represents presumed glial cells which form 78% of the profiles, compared to 57% 1 mm above the area centralis (Hughes, '75); their density of 1,490 mm⁻² is about 65% of that near the area centralis or visual streak. The remaining 22% of profiles were identified as neurones and show an obvious trimodal distribution.

The neuronal character of central profiles is also illustrated (Fig. 3) for a temporal region near the 2,000 neurones mm⁻² isocount line. The three neuronal diameter modes described by Boycott and Wässle ('74) are obvious; the large cell (1) is equivalent to the α cell of a Golgi preparation; the heavily Nissl-staining, round to oval cells with central nuclei (2-12) predominantly correspond to the β cells, the smaller cells with eccentric nuclei and Nissl staining in their limited cytoplasm (13-25) comprise, at least, the γ cells.

The soma size spectrum of Figure 4 was accurately determined by weighing cut-out neuronal profiles to establish their area. The distribution independently established by subjective circle matching is shown hatched in Figure 4. The result justifies visual matching; jitter in placement of the cells was one bin, or 0.56 μ, between the two methods.

The three modes are apparent even in the small sample of Figure 4. The α cells are detectable by eye because they have diameters well-displaced from the local mean; the β cells are similar in form, but it is conspicuous that some 87% of the cells in the γN mode of Figure 4 have eccentric nuclei, compared to only 7% in the βN mode. That this is not a fixation artefact is indicated subsequently.

Neuronal diameter spectra. A survey of soma diameter spectra at 1.4 μ bin resolution was made for seven regions of an alcohol-formalin-fixed, Nissl-stained retina. (Fig. 5). Percentages for α cells are based on a very small
CAT GANGLION CELL MODAL DISTRIBUTION

A 1.4-μ bin has been employed for demonstrating the general trends in soma diameter across the alcohol-fixed, fresh, and HRP-filled retinas. However, the position and magnitude of the βnm/ynm dip are established more precisely with a less convenient oil immersion survey. Three sets of such spectra were produced: area centralis to peripheral retina (Fig. 6), along the streak (Fig. 7), and a vertical sweep through the visual streak perpendicular to its axis (Fig. 8).

Spectra from near the area centralis (Fig. 6), show the β mode progressively shifting away from the γ mode, with an increase in mean value from 11.7 μ at the 5,900 neurone mm⁻² spectrum to 15.3 μ in the 520 neurone mm⁻² spectrum. Over this range the γ mode mean only changes from 8.7 μ to 9.7 μ, a 10% rather than a 30% increase in mean diameter. In the regions which can be subdivided, there is an increase in the γ mode proportion from 41% at the area centralis to some 60% in the peripheral retina. The position of the βnm/ynm dip shifts relatively little until the periphery is reached.

These progressive changes in modal mean diameter with retinal position have been shown to be statistically significant (Hughes, Caille and Vibert, 1981).

Along the visual streak (Fig. 7), the βnm/ynm dip is weak and variable, and the trimodal distribution is hardly apparent until the nasal end is approached. It is not implied that division of the spectra at these βnm/ynm dips is an accurate means of designating the mode proportions. In passing down the retina, through the streak and into the periphery again (Fig. 8), the β mode moves nearer to the stationary γ mode and then separates again; concomitantly, the maximum size of the βnm cells and the magnitude of the βnm/ynm dip progressively reduces and then increases. The changes are symmetrical above and below the streak. In passing down from a local density of 390 neurones mm⁻² to 1,000 neurones mm⁻² isocount line, the spectra for regions other than central streak and area centralis are very similar, although the proportion of neurones in the γ mode appears to increase from about 50% near the 1,000 neurones mm⁻² isocount line to 60% or more in peripheral retina.

Fig. 2. Mean soma diameter spectrum for the profiles shown in the area of retina in Figure 1. Thirty-four of the 162 profiles were identified as neurones; the remainder were classified as presumed glia, whose diameter spectrum is shown stippled above. The modal spectrum is trimodal, and rough percentage proportions are indicated.

Of the profiles shown, a 10% rather than a 30% increase in mean diameter. In the regions which can be subdivided, there is an increase in the γ mode proportion from 41% at the area centralis to some 60% in the peripheral retina. The position of the βnm/ynm dip shifts relatively little until the periphery is reached.

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This area has a relatively high proportion of small neurones for peripheral retina. Each of the numbered profiles is enlarged and shown in its optimal focal plane in B. It is apparent that they all have characteristic neuronal appearance in terms of the classification of Hughes (75), based on Nissl staining.
Figure 3
comprise area centralis. This feature is eccentric, invaribly mode of the eccentric, invariably mode

\[ \text{SOMA DIAMETER } \mu \]

Fig. 4. Mean soma diameter spectrum for the neurones 1-28 of Figure 3. The outlines of the spectrum represent mean diameter measured by weighing cut-out profiles; the hatched regions represent the results measured by the process of area matching, as described in Methods. The two techniques generate a similar spectrum. It is noteworthy that even for a region close to the area centralis, the \( \gamma \)mm comprises 57%, the \( \beta \)mm, 39%, and the \( \alpha \)mm, 4% of the total. The majority (87%) of neurones in the \( \gamma \) mode have eccentric nuclei.

**Fresh retina; Nissl-stained but neither fixed nor dehydrated**

Neuronal appearance. Cresyl-fast-violet was dissolved in Tyrode's solution to stain an un-fixed and undehydrated retina in order to determine the range of modal diameters of fresh retina for computations related to physiological encounter rates, and to assess the effects of soma shrinkage associated with histological processing.

A region of the area centralis near the lower 1,000 neurones \( \mu \text{mm}^2 \) isocount line of such fresh retina is illustrated in Figure 9. The three classes of neuronal size are equally apparent, in proportions similar to those of fixed and dehydrated retinas. The cytoplasm of neurones in the \( \gamma \) mode is more abundant than in the fixed material, yet their nuclei remain almost invariably eccentric, in contrast to the central nuclei of the \( \beta \)mm neurones, thus indicating that this feature is not an artefact of histological processing.

Neuronal diameter spectra. Neuronal soma diameter spectra were produced for five regions of the fresh retina, (Fig. 10), with a bin width of 1.4 \( \mu \) for comparison with the dehydrated retina of Figure 5.

The relative proportions of the three modes are similar in corresponding regions of the fresh (Fig. 10) or fixed retina (Fig. 5). However, in fresh retina the peripheral \( \beta \)mm/\( \gamma \)mm dip is located near 15 \( \mu \), but in the processed tissue, shrinkage shifts it to about 12 \( \mu \). The \( \gamma \) and \( \beta \) modes range from 10-25 \( \mu \) in fresh retina, but from 7-20 \( \mu \) in the processed retina of Figure 5. The small areal shrinkage of the alcohol/formaldehyde fixation method (Hughes, '75) is thus accompanied by about 20% linear shrinkage of neuronal somata.

The samples of cells in the \( \alpha \) mode was too small for their size distribution to be ascertained. A diameter spectrum was thus produced for 1,500 fresh peripheral cells larger than 20 \( \mu \) (Fig. 11). The \( \alpha \)mm/\( \beta \)mm dip was found to be situated at 27 \( \mu \), and the distribution of 197 \( \alpha \) cells is apparent as a distinct mode, in contrast to the "tail" of histograms based on small samples. The mean diameter of fresh peripheral \( \alpha \) cells was found to be 35 \( \mu \), compared to about 26 \( \mu \) in alcohol/formaldehyde-fixed material. On the visual streak, in contrast to Rowe and Stone ('76), the fresh \( \alpha \) cells were found to be significantly reduced in diameter, by some 9%, to 32 \( \mu \).

The fresh peripheral \( \gamma \)mm mean diameter is 12.2 \( \mu \), the \( \beta \)mm, 18.5 \( \mu \), and the \( \alpha \)mm, 35 \( \mu \); the corresponding values for peripheral Golgi-Cox-stained cells are 13.5 \( \mu \), 20 \( \mu \), and 32.5 \( \mu \) (Boycott and Wässle, '74). The size range for \( \alpha \) cell somas in fresh material lies between those of Boycott and Wässle ('74) for Golgi Cox and Golgi-rapid processes. Fixation in hot formalin vapour (Rowe and Stone, '76) appears to cause similar shrinkage to alcohol/formaldehyde fixation; shrinkage in 5% formal saline or Carnoy is less marked.

The topographic variation in modal percentages

The mode percentages in Figures 5-8 are summarised in Figure 12, with supplementation from additional sites near to the line of each sweep. The samples were too small for the local \( \alpha \) cell percentage to be accurately assessed. The true value was thus established for each site by counting within a larger area, and the histogram values of \( \beta \)mm\% and \( \gamma \)mm\% then corrected by obtaining their product with \( \frac{100}{100-\alpha} (\beta \text{mm}\% + \gamma \text{mm}\%) \).
Fig. 5. A low-resolution survey, 1.4-μ bin, of soma diameter spectra for classic neurones in various regions of the retinal ganglion cell layer. In this and the following survey spectra, the histograms are accompanied by information on the mean neuronal density at the site, the number of neurones measured, and the number of sites sampled. The sample sites are indicated for each spectrum on an outline retina in which the 1,000 neurones mm⁻² isodensity line is drawn. The results were obtained by the area-matching procedure. Where possible, the spectra have been divided about the 1 nm/m and 3 nm/μm dips into their rough modal proportions. The significance of the spectra is discussed in the text.
Fig. 6. A high-resolution, 0.56-μm bin, oil immersion survey of neuronal diameter spectra within regions at various distances above the area centralis.
Fig. 7. A high-resolution, 0.56-μ bin, oil-immersion survey of neuronal diameter spectra for positions along the visual streak.

Fig. 8. A high-resolution, 0.56-μ bin, oil-immersion survey of neuronal diameter spectra for position along a vertical line passing from peripheral retina, through the visual streak, and out to peripheral retina again. Careful examination shows that the fin mode moves progressively towards the ym mode in passing onto the visual streak and then away again. This results in obscuration of the intermodal dip in the region of peak density on the visual streak.
CAT GANGLION CELL MODAL DISTRIBUTION

Figure 8

STREAK 4mm ABOVE PEAK
390 + 130 neurones/mm²
n=80 sample site
14 sites

STREAK 0.5mm BELOW PEAK
910 + 190 neurones/mm²
n=103
9 sites

STREAK 1.5mm ABOVE PEAK
770 + 260 neurones/mm²
n=130
10 sites

STREAK 1mm BELOW PEAK
750 + 200 neurones/mm²
n=108
14 sites

STREAK 0.5mm ABOVE PEAK
1,210 + 230 neurones/mm²
n=106
6 sites

STREAK 3mm BELOW PEAK
540 + 220 neurones/mm²
n=108
14 sites

STREAK PEAK
1,300 + 370 neurones/mm²
n=97
5 sites

STREAK 5mm BELOW PEAK
390 + 110 neurones/mm²
n=86
16 sites

MEAN SOMA DIAMETER μ

FIXED & DEHYDRATED 0.56 μ 8IN (OIL IMMERSION; x 1,780 PRINTS)
The modal percentages summarised in these diagrams are of varying reliability. The weakest region is the mid-range of the visual streak; no dip was apparent from 2–5 mm nasal. However, the vertical sweep through the end of this region clearly indicates the probable peak proportions from the trend in passing through its flanking values. The $\beta_{\text{mm}}/\gamma_{\text{mm}}$ ratio suggests a shift from a high $\gamma_{\text{mm}}$ percentage in the periphery to a larger $\beta_{\text{mm}}$ percentage in both visual streak and area centralis.

These changes of modal proportions in moving from the periphery to area or streak, (Fig. 12), are small but progressive and thus likely to be valid. However, a single model population of intermediate composition may be statistically fitted to neuronal spectra from all three regions and the differences in modal composition between the 22 samples of Figures 6, 7 and 8 do not satisfy a statistical test of significance (Hughes, Caille and Vibert, 1980).

**Vertical and horizontal profiles of modal density.** The modal proportions derived from the formalin/alcohol-fixed preparations (Fig. 12) have been used to weight the previously pub-
Fig. 10. A low-resolution, 1.4-μ bin, survey of soma diameter spectra for stained but fresh, unfixed, retina such as that shown in Figure 9. The modal proportions in different retinal regions are similar to those of Figure 5 for fixed retina, but the mean modal diameters are larger. The formalin/alcohol-fixed somata shrink some 20% in diameter, relative to fresh retina. The modal dimensions in these peripheral size spectra from fresh retina correspond well with those of Boycott and Wassle (74) for peripheral Golgi material, whose somata must therefore shrink relatively little in their procedures.

lished vertical and horizontal density profiles of the classic neurone distribution (Hughes, '75) to obtain βnn and γnn densities (Fig. 13). The alpha cell distribution was determined directly.

The unimodal distribution of soma size in the region of the area centralis peak density prevented directly estimation of the mode proportions. A region of density 6,000 neurones mm$^{-2}$ some 0.4 mm from the peak of the area contained some 56% of neurones in the βn mode; this is a similar value to that obtained by Kelly and Gilbert ('75) for cells projecting to the LGN from a region of density 5,800 ganglion
obtain modal distribution
the previously published
was assumed for modelling simplicity, the isopercentage density information count (Wassle et al., '75). This demonstrates above suggests a $\beta$nm peak density of some 6,600 neurones mm$^{-2}$ smaller cells at the region of peak density. If the majority of these are $\gamma$ cells, this suggests a $\beta$nm mode maximum possible density of some 7,000 neurones mm$^{-2}$ on the visual axis.

Modal isodensity maps. The density profiles of the various modes vary in similar fashion with increasing eccentricity from the area centralis (Fig. 12). This was emphasised by a series of estimated modal density distribution maps. For modelling simplicity, the $\alpha$ cell proportion was assumed uniform at 3% of the neurone count (Wassle et al., '75). The above mode percentage information was plotted on a previously published density map (Hughes, '75), and isopercentage profiles were drawn (Fig. 14A). This map was then employed to weight the previously published neuronal densities to obtain modal distribution maps. The model $\alpha$nm, $\beta$nm, and $\gamma$nm maps are shown in Figures 14B-D. The form is similar for all three maps; absolute densities are much lower for the $\alpha$ mode distribution. Away from the central area, the $\gamma$nm densities are higher than those of the $\beta$nm map, in accordance with the relative proportions indicated in Figure 14A.

The proportion map (Fig. 14A) was integrated to estimate the mean percentages of the modes for the whole retina as 58% $\gamma$nm, 39% $\beta$nm, and 3% $\alpha$nm. The separate mode maps were also integrated to give 5,500 $\alpha$nm cells, 85,000 $\beta$nm cells, and 115,000 $\gamma$nm cells; a result satisfactorily consistent with the proportions obtained by integration of Figure 14A.

Direct count and confirmation of the $\alpha + \beta$ mode population

That the 217,000 neurones of the retinal ganglion cell layer (Wassle et al., '75; Hughes '75) substantially represent ganglion cells has been questioned by Stone ('78), whose mean ganglion cell count is only 115,000 cells. Comparison of Stone's maps ('78, Fig. 4) with the estimated $\beta$nm map of Figure 13C reveals that outside the central area the $\beta$nm isodensity lines are similar in distribution to those of Stone's total count map. In addition, the $\gamma$nm map of Figure 14D alone shows higher peripheral densities than Stone's ('78) total ganglion cell map.

The estimated magnitude of the total ($\alpha + \beta$) nm population and its distribution was thus tested by counting all cells larger than the local $\beta$nm-$\gamma$nm criterion diameter in a formalin-fixed retina.

The local criterion diameter for the $\gamma$nm and $\beta$nm modes was determined from 25 spectra. These have been pooled (Fig. 15) to represent three major regions; their $\beta$nm and $\gamma$nm proportions were similar to those of previous studies. The criterion diameter was 14 $\mu$ m in the periphery and 12.5 $\mu$ m outside the 2,000 mm$^{-2}$ isocount line. Within this line, modal overlap is considerable, and each local density was divided in the assumed ratio ($\alpha + \beta$) mm 55%: $\gamma$nm 45%.

The density of ($\alpha + \beta$) nm cells larger than the local criterion diameter was determined for 650 sites. Conservative standards of acceptance were applied. The resultant isodensity map (Fig. 15) was integrated by summing the product of the area between isodensity lines and the mean density of ($\alpha + \beta$) nm cells for all sample sites contained within that area. The total count of 86,300 ($\alpha + \beta$) nm cells agrees with the model estimate of 95,000. The map is more cruciform than the $\beta$nm map of Figure 14C, but
CAT GANGLION CELL MODAL DISTRIBUTION

Fig. 12. The mode percentages of Figures 5-8 are summarised for various profiles of the retina. The published spectra were supplemented by other data. The $\alpha$ percentage was determined by direct local counting; the $\beta$ and $\gamma$ percentages of the original spectra were then corrected as explained in the text. The $\beta/\gamma$ modal ratio undergoes an obvious reversal in passing from periphery to streak or area centralis. But evidence from retrograde transport of HRP suggests that this may be accounted for by an increase in the relative proportion of non-ganglion cell neurones in the peripheral retina if the changes in proportion are significant (Hughes, Caille and Vibert, 1980).

Integration of the map in Figure 16 indicated a minimum total count of 297,000 neurones. Subtracting the $(\alpha + \beta)$ nm population of 86,300 cells, we estimate the $\gamma$ nm population to be 210,700 cells, compared to 115,000 in the map of Figure 14D. The earlier result is confirmed.

The $\alpha$ cell population was mapped directly for the original retina of Hughes ('75) and is presented as Figure 17. It is similar to that of Wässle et al. ('75), although its less cruciform density distribution is similar. The total $\beta$ nm population was thus found to be larger than the 46,000 suggested by Stone ('78).

A density distribution map for all classic neurones was established on a sampling matrix of 600 points (Fig. 16). The density profiles of this map are more cruciform than previously published (Hughes '75), but similar in form; the $1,000$ mm$^{-2}$ isodensity region gives rise to a very pronounced streak.

The $\beta/\gamma$ ratio is plotted against distance from area centralis along the horizontal axis in Figure 12. The $\beta$ percentage is shown for distances of 0 to 16 mm, with $\gamma$ percentages for distances of 0 to 4 mm.
Figure 13
Fig. 14. A modal proportion map (A) and a set of model or estimated modal distributions (B–D) based on the data in previous figures. The similar form of the modal distribution for the three classes is apparent. Integration of the maps suggests minimum totals of 5,000 αnm cells, 85,000 βnm cells, and 115,000 γnm cells in an overall retinal proportion of 3% αnm:39% βnm:58% γnm.

Fig. 13. The vertical and horizontal density distributions of neurones from previously published results (Hughes, '75) have been adjusted by the above local mode percentage to obtain modal density distributions. The α cell distribution was determined directly. The form of the profiles is similar for the three modes.
DENSITY MAP OF GANGLION CELLS
IN THE $\alpha$ AND $\beta$ MODES
OF LOCAL SIZE SPECTRA ($\alpha+\beta/\text{mm}^2$).

Figure 15
**Fig. 15.** A density distribution map of the directly counted ganglion cells in the (anm + β/m) of local size spectra. The proportion of a cells is small, so that it is effectively a β/m density map. The three small spectra summarise the information with which the criterion diameter for inclusion of a cell was determined for a given area. The hatched region of the spectra indicates accepted cells. Asterisks, triangles, and squares indicate the location of sample sites employed to obtain the corresponding spectrum. Sample sites at which a count of cells above the criterion size was made are indicated by black dots. The area centralis peak count is marked by a star and could only be estimated, as indicated in the text, to be about 6,000 ganglion cells per mm². Isocount lines define zones of (anm + β/m) density mm⁻².

The table gives the area of isocount zones and the mean (anm + β/m) ganglion cell density obtained by averaging all sample values within the zone. The product of these two factors determines the total (anm + β/m) count within the zone, and the sum for all zones gives the total (anm + β/m) population of 86,300 ganglion cells.

The overall shape of the isodensity contours is very similar to that published previously for the total presumed ganglion cell distribution (Hughes, '75); densities are lower, the cruciform distribution is more apparent. It should be noted that, in contrast to Rowe and Stone's ('76) suggestion, the visual streak is well defined in this predominantly β/m distribution map.

The density distribution pattern is very similar to that of the model estimate of Figure 14c, and the integrated population of 86,300–9,600 = 86,700 β/m cells is in good agreement with the model value of 85,000.
The α cell distribution for the retina of Hughes (‘75) was determined for comparison with the model map of Figure 14B. The density distribution is very similar, as would be expected. The form of the distribution is similar to that published by previous authors, and integration of the map indicates a total population of 5,500 α cells.

A comparison of the density profiles of the previously published retina (Fig. 12) and that of Figures 15 and 16 is presented for all the neurones and the βmm population. It can be seen from Figure 18 that the results are similar.

**Ganglion cells identified by HRP retrograde transport from the optic nerve**

Retinal ganglion cells filled with HRP transported retrogradely from the end of the transected optic nerve gave criteria for ganglion cells. This method has the potential advantage of filling cells regardless of their central projection. Complete retinal filling was never approached. Orientation of the photograph permitted small areas to be selected in which filling appeared complete, but these were too small to be confident that any non-filling classes of neurone were represented in true proportion. These results at least provide local size ranges for conclusively identified ganglion cells.

**The area centralis peak density.** It has been claimed (Hughes, ’75) that the peak density of presumed ganglion cells is 9,000 mm⁻² in the area centralis, rather than 6,000 mm⁻², as re-
Fig. 18. Continuous lines represent the density of neurones in the vertical and horizontal profiles of the retina described by Hughes ('75): retina A. In the upper diagram the original total count density profiles for retina A are contrasted with the equivalent profiles for retina B (small triangles), and a set of indirect estimates obtained from the local \( \beta \) \text{mm} count for retina B and the reciprocal of the local \( \beta \) mode percentage (squares). The vertical and horizontal profiles are very similar for both retinas.

The lower figure compares the \( \beta \) \text{mm} density profiles obtained by counting above the local criterion size and deducting 

...
prints. Subsequently, the retina was fixed and mounted, with no significant change in the peak density of 9,040 neurones mm$^{-2}$. Stone's peak count of 6,000 mm$^{-2}$ (65) for fresh retina was not confirmed.

Because the area centralis cells are small and uniform in appearance, there is little basis for the usual identification criteria. Yet the absolute ganglion cell density of this region is important because it determines the maximum behavioural resolution and is a reference value for normalising plots of the distribution of various cell classes and magnification factors. A peak density for ganglion cells, rather than neurones, was thus determined from two retinas with area peaks which had completely filled after application of HRP to the optic nerve. The peak density in these retinas was 9,500 and 9,880 ganglion cells mm$^{-2}$.

The peak density measurement in the area centralis of the fresh retina is within the reported range for fixed and dehydrated retinas (Hughes, '75; Stone '78) and indicates shrinkage at the area to be small, at most 5%. The mean peak density of 9,690 ganglion cells mm$^{-2}$ in the HRP study indicates the majority of these neurones to be ganglion cells and substantiates the presumed ganglion cell peak count (Hughes, '75).

Criteria based on HRP filling from the optic nerve

Photomicrographs of HRP-filled retinal ganglion cells are presented in Figure 19 for regions 2-5 mm above the area centralis.

Three categories of retinal ganglion cell size are apparent. Some of the HRP-filled profiles are very small and would fall in the "debatable" category of Nissl-stained material (Hughes, '75); the nucleus often overlays the HRP-filled cytoplasm, giving an impression of an erroneously low total volume.

The small ganglion cell profiles of Figures 19 A-E are very similar to those of neurones in Figure 3, 13-28. Two distinct types of small neurone—no smaller than some filled profiles—were not observed to take up HRP. Of 200 neuronal profiles, 12% were members of the two classes. Presumed glia were not observed to take up HRP.

Soma diameter spectra of ganglion cells filled with HRP from the optic nerve

Soma diameter spectra were constructed for six regions of two HRP-filled retinas. Shrinkage was reduced by addition of 2 vol% glacial acetic acid to the formalin/alcohol fixative to produce Carnoy's fluid.

The peripheral βnm/ynm dip lies near the 15-μm mean diameter for Carnoy-fixed material (Fig. 20), as in the spectra from fresh retinas. By contrast, the peripheral spectrum for a formalin/alcohol-treated retina (Fig. 20) shows considerable shrinkage, relative to Carnoy material, and the dip is at about 12 μm. Twenty percent of the γ cells are below 8 μm in diameter in this material. Obviously, the size of the cells is very sensitive to the technique employed. The spectra for HRP-filled ganglion cells are similar to those for neurones in corresponding regions of fresh and dehydrated retinas.

Criteria for the proportion of ganglion cells in the yn, relative to the βn mode, based on filling with HRP from the superior colliculus

Kelly and Gilbert ('75) have shown that the majority of neurones in the yn mode fill with HRP when it is injected into the corresponding region of the superior colliculus. However, they did not accurately specify regions from which samples were taken, and their criteria for neurones may not be the same as those employed here. Their work has thus been repeated by making massive injections of HRP into the superior colliculus so as to fill sufficiently large areas of retina to avoid the need for selective sampling and consequent error in modal proportions.

Soma diameter spectra were constructed for peripheral retina and for the visual streak in the region where the distribution is unimodal. A sample of HRP-filled peripheral ganglion cells is shown in Figure 21. The smaller cells correspond to the classical neurones described by Hughes ('75) and Stone ('78). Again, a substantial number of two types of classic neuronal profile, identical to the group in the optic nerve sample, did not fill. Such cells have a restricted cytoplasm, compared to cells of similar size which do fill. Their nucleus is very clear and usually contains at least one bar-like element which appears to be a nuclear fold filled with basophilic material and aligned with the major axis of the cell (Fig. 21A). One class of these cells has a circular nucleus with three major clumps of basophilic material about it; the other has an elongated nucleus with basophilic material at each end. In general appearance they resemble some of the larger cells of the amacrine layer (Fig. 21C), whose density exceeds 1,000 mm$^{-2}$ in peripheral retina and which are much too numerous to represent displaced ganglion cells. After chiasm section (see Acknowledgments), these cells remain in the ganglion cell layer in regions from which practi-
CAT GANGLION CELL MODAL DISTRIBUTION

...ally all neurones of the kind identified as ganglion cells by filling with HRP have become atrophied or have disappeared (Fig. 21B). Their cytoplasmic basophilia and the possession of a dendritic tree resembling that of some amacrines in supravital methylene blue-stained material (unpublished observations) suggests that these cells are not glia.

The total peripheral sample of classic neuronal profiles is divided into three groups in Figure 22A, B. The distribution of cells which filled from the superior colliculus is similar to that of Kelly and Gilbert, 75 (Fig. 22A). Below these are the diameter spectra of the unfilled cells, which comprise two components (Fig. 22B). One comprises unfilled cells similar to those filling with HRP. The other, indicated by the hatched spectrum, represents a population of classic neurones which do not fill with HRP from the optic nerve or superior colliculus. In peripheral retina they comprise some 29% (85 in 356 cells), which is greater than in the selected areas filled from the optic nerve and suggests either a substantial bias in the sampling of that study or that the cells fill from a site other than the superior colliculus. Careful examination of the cells which fill from the optic nerve suggests, however, that neurones with prominent nuclear folds are not included unless the reaction product changes their appearance markedly. It is concluded that the selection of small, filled regions has introduced bias against these cells, and accounts for the difference in their proportions in the two instances. It is evident, by comparison of the hatched spectrum of unfilled cells with that of the gamma mode ganglion cells above, that the unfilled cells overlap the lower end of the ganglion cell diameter spectrum.

Figure 22D shows the distribution of all neuronal profiles, and it is apparent that the γn mode proportion, like that of the above peripheral neuronal spectra, is about 62%. Below this (Fig. 22E) is presented the percentage distribution for profiles, excluding the non-ganglion cell category, whether they are filled or unfilled. In this spectrum the γ mode forms only 49% of the total population.

The cell number (Fig. 22C) and percentage distributions (Fig. 22F) are also shown for a population of fillable cells from visual streak some 5 mm from the area centralis. The distribution of filled and unfilled cells in this unimodal envelope is very similar to that in peripheral retina if the β mode were to about the γ mode. The effective "dip" or natural division in this distribution lies at the 13 μ diameter; division here shows the "latent" γn HRP mode to be some 47% of the total and not significantly different in its proportions to that of the peripheral ganglion cell spectrum.

Within 0.4-mm of the area centralis peak, the proportion of ganglion cells in the γ mode at 42% when the distributions of Kelly and Gilbert (75) are divided about the μn/δm dip. At the center of the area, it has been reported above that the proportion may be lower still; filling from the superior colliculus has not exceeded 30%. Some 5° eccentric, however, the results of Kelly and Gilbert (75) show that 46% of their population lay in the γ mode when division is made about the μm/δm dip.

It is apparent that deviation from the peripheral γn mode proportion of 48% ganglion cells occurs only in a limited region of the area centralis, and, although of functional importance, this is unimportant when estimating the total ganglion cell population.

Accepting that the proportion of 48% of ganglion cells in the γn mode of local spectra applies over most of the retina, we then estimate the minimum total ganglion cell population as 86,000/(1-0.48) = 165,000 cells. The true value is probably somewhat larger.

**Another estimate of the peripheral γn ganglion cell proportion from the optic nerve fibre diameter spectrum**

The fibre diameter spectrum of the peripheral optic nerve differs from those for the central regions (Hughes and Wässle, '75). The topography of the retinal cells of origin appears to be retained in their projection into the optic nerve. The peripheral optic nerve fibre spectrum contains three modes in the proportions 53%, 42%, and 5%, in order of increasing size. These modes probably represent the γn, βn, and μn modes of the retinal ganglion cell distribution. In Figure 23 the fibre diameter and the neuronal spectra for peripheral fresh retina have been plotted against one another by employing features such as largest and smallest element, dips and peaks of modes, etc. The points have been fitted by a regression line, which indicates the similarity between the two populations.

It has long been suggested that there is proportionality between the size of a nerve cell and the diameter of its axon, as is indicated in Figure 23. Only recently has this become directly demonstrable for individual neurones (Cullheim, '78). Figure 23 indicates that the same regression coefficient is adequate for the three modes.

The peripheral small fibre mode of 53% lies between the overall 59% for the neuronal γn
Fig. 20. A low-resolution survey, 1.06-μ bin, of soma diameter spectra for select small areas of retina whose neuronal population was well-filled with HRP transported retrogradely from the optic nerve. The results give overall size ranges for ganglion cells identified by HRP-filling, but the sample sites were so small and oriented so selectively for photography that the proportions in the various modes cannot be assumed to be representative of all retinal neuron classes. However, the overall pattern of change in the spectra when passing across the retina from the area centralis to either streak or periphery is similar to that of the neuronal population shown previously. The peripheral spectrum of the formalin/alcohol-fixed material reveals marked shrinkage relative to that of the Carnoy-fixed retina.

Fig. 19. Photomontage of select, well-filled areas of retina in which the majority of neuronal profiles contain HRP transported retrogradely from the transected optic nerve. The sample regions were located 2-5 mm above the area centralis in an alcohol/formalin-fixed retina. The three size categories of ganglion cell are apparent and are indicated by large, medium, and small arrows. Some particularly small profiles containing HRP granules are indicated by "s," and other unfilled neuronal profiles, by "n." The large proportion of very eccentric nuclei in the small cell group should be noted. Compare these small ganglion cells to the neuronal profiles of Figure 3, 13-28. Unidentified profiles are presumed glia.
mode, or the higher values obtained in far periphery, and the peripheral $\gamma$ mode proportion of 48% of ganglion cells. The optic nerve fibre distribution was based on a large sample of 1,040 measured fibres and probably represents the overall peripheral proportions accurately. In combination with the estimate of the total $\alpha + \beta n$ mode population given above, we obtain from the optic nerve peripheral fibre proportions an estimate of 86,000 (1-0.33) = 189,000 ganglion cells, the same as Hughes and Wässle's (75) estimate of 180,000 fibres for the total optic nerve fibre population.

Map of the minimum density distribution for gamma mode ganglion cells

The minimum proportion of HRP-fillable ganglion cells in the ym mode is thus almost equal to that of neurones in the $\alpha$ and $\beta n$ modes of peripheral and streak retina. Only at the region of peak density in the area centralis does the proportion fall, possibly to 30% at the very center of the area. These changes in the area will have little influence on the total magnitude of the ym population.

Given the map of $\alpha + \beta n$ mode distribution, we may now draw an isodensity map of gamma mode ganglion cells by estimating their relative proportion from HRP work. This is presented in Figure 24. The distribution clearly deviates from the map of the ym neurones presented earlier (Fig. 14D) as the periphery is approached. The implication is that the proportion of non-ganglion cell neurones of classic appearance increases in passing from center to periphery, or that an increasing proportion is included in the neurone maps.

Integration of this map gives a total minimum population of gamma mode ganglion cells of some 78,000; when combined with the $\alpha m$ and $\beta nm$ populations, this suggests a minimum total of 165,000 ganglion cells.

**DISCUSSION**

**Total classic neurone and ganglion cell counts**

A minimum count of 217,000 neurones was obtained for the ganglion cell layer of the cat retina, according to the criteria of Hughes (75). These neurones were provisionally defined as presumed ganglion cells (Hughes, '75; p.110), but their number exceeded a previous total count of 99,000 cat retinal ganglion cells (Stone, '65). This published neurone count has been confirmed above by a count of 207,000 neurones on a different retina, while Wässle et al. (75) estimate some 190,000 neurones.

Stone ('78) reports a mean total of 116,000 ganglion cells (range 105,000-140,000); this is substantially lower than the neurone counts of Hughes (75) and Wässle et al. (75), although it exceeds Stone's earlier total by up to 56% (Stone, '65).

A neurone is definitively identified as a retinal ganglion cell if its axon projects into the optic nerve. The chromatolytic response to optic nerve section (Stone, '65) shows the neurones of the $\alpha + \beta n$ modes to be ganglion cells. Retrograde filling with HRP from the LGN (Kelly and Gilbert, '75) and superior colliculus confirms all neurones in these modes to be ganglion cells. The similarity between the morphologically derived $\alpha m + \beta nm$ map of Figure 15A and the map of $\alpha m + \beta nm$ neurones filled with HRP from the LGN (Cooper and Pettigrew, '79) confirms the criteria employed above. The modal percentage map for neurones of the ganglion cell layer (Fig. 14A) enabled production and integration of the separate modal isodensity distributions (Fig. 14B-D). The neuronal population of the original retina (Hughes, '75) was estimated to contain 85,000 $\beta nm$ cells and 5,500 $\alpha m$ cells. This was confirmed by a count of $\alpha m$ and $\beta nm$ cells in another retina, which suggested the presence of 80,700 $\beta nm$ cells and 5,600 $\alpha m$ cells.

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**Fig. 21.** A) A photomontage bringing together nonrepresentative proportions a selection of smaller HRP-filled profiles, which have been identified as classical neurones. All of these cells were located in a region of density about 800 neurones mm$^{-2}$ above the area centralis. The cells with central nuclei "a" and "b" represent the alpha and beta populations of Golgi material, respectively. The cells "c" are confirmed ganglion cells of the gamma mode and, with the exception of one suffixed "u," are labelled with HRP from the superior colliculus. The suffix "e" indicates the eccentric nucleus, which is characteristic of many profiles in this mode. Profiles "n" are classical neurones, identified by cytoplasmic basophilia at the sites indicated by small arrows, with elliptical nuclei and two cytoplasmic poles. Cells labeled "nt" are similar but have three cytoplasmic poles and a circular nucleus. These neurones do not fill with HRP from either the transected optic nerve or superior colliculus. They are almost invariably characterized by a long basophilic nuclear fold, which is very conspicuous and not seen in ganglion cell nuclei. A large, but lightly staining nucleus is present. The diameter spectrum of such cells is shown in Figure 22. B) Both types of presumed non-ganglion cell neurones, "n" and "nt," are encountered in the ganglion cell layer some 9 months after chiasma section and degeneration or atrophy, of the recognised ganglion cell classes. C) The amacrine layer also contains profiles which are very similar in appearance to the non-ganglion cell classical neurones of both classes. This suggests that these profiles may thus represent a class of displaced amacrine. Scale, 10 μ.
Fig. 22. Soma diameter spectra for retina filled by retrograde transport of HRP from injection sites in the contralateral superior colliculus. The population of classic neuronal profiles from sampling sites in the upper peripheral retina has been divided into three classes and displayed in A and B. In A, the distribution of filled ganglion cells is presented. The unstippled population of B represents unfilled profiles whose appearance is otherwise indistinguishable from ganglion cells. The stippled spectrum of B represents unfilled cells not similar to the population of filled ganglion cells. These neurones have been illustrated in Figure 21, and were also encountered in the periphery of retinas filled from the optic nerve. They are assumed to be non-ganglion cells, but represent a population of classic neurones. The percentage spectra, thus, respectively show the modal proportions estimated for the total profile population of classic neurones (D) and those for the distribution of ganglion cells alone (E), identified by filling, large size, or their similarity to filled cells. Ganglion cell populations for a region of nasal visual streak are also presented as cell count (C) and percentage population spectra (F).

It is apparent that the peripheral ynm mode contains a large population of cells which project to the superior colliculus, and it amounts to about 49% of the total peripheral ganglion cell spectrum. Although the ynm mode is not apparent from the intermodal dip in the sample of streak population (F), it is evident that the projection pattern of this region corresponds to its proportions to that of peripheral retina (E), with the "latent" ynm mode amounting to a similar 47% of the total. These results confirm those of Kelly and Gilbert (’75) and are understood to indicate that the disappearance of the ynm/ynm dip in passing from the periphery to the visual streak (Fig. 8) is consistent with no more than a reduction in the mean diameter of the ynm mode, relative to a ynm mode of constant mean diameter.
Subtraction of the ann + βnn population from the total neurone count of their respective retinas reveals a population of from 121,000 to 126,000 ynm neurones. How many of these are ganglion cells? Kelly and Gilbert’s (’75) results show that a minimum of 40% to 47% of the local population of ganglion cells are contained in the ynm. In periphery and visual streak, which form the majority of the retina, the results above indicate 48% of ganglion cells to be in the ym mode. The ym mode population is thus slightly smaller than the combined ann and βnn count and amounts to some 79,000 to 83,500 cells in the two retinas considered.

The peripheral spectrum of the optic nerve fibre diameter distribution (Hughes and Wasse, ’76) suggests some 53% of the total fibre population to be small fibres which probably arise from cells in the gamma mode. From Hughes and Wasse’s most accurate optic nerve fibre count, this suggests a population of 95,000 ganglion cells in the gamma mode.

Summing the estimated ann, βnn, and ynm ganglion cell populations, we obtain a minimum total ganglion cell count of 174,000 for the retina for Hughes (’75), and 165,000 for that of Figure 16.

The original minimum presumed ganglion...
MINIMUM DENSITY MAP OF GANGLION CELLS
IN THE Ynm OF LOCAL SIZE SPECTRA

Fig. 24. A minimum-density map for ganglion cells in the ynm of the local size spectra. This was derived from the HRP spectral proportions and the count of omn + 6nm neurones. Because the results of retrograde HRP-transport indicate the ynm and ynm populations to be similar, their distribution maps are also alike and do not differ significantly in form from the map of total neurone density (Fig. 16). The star designates the area centralis peak count. There is no obvious ym mode in this region, and HRP fills no more than 30% of the neurones from the superior colliculus. If these correspond to the ynm population of peripheral retina, the peak count may be about 3,000 ganglion cells mm⁻² in the "latent" ynm mode defined by such projection. About 9.4 mm away from the peak count, however, the density of cells in the overt ynm amounts to some 2,500 ganglion cells mm⁻². Integration suggests a minimum population of 75,000 ynm cells.

The similarity of this estimated map to the ynm map (Fig. 15) and to the total neuronal distribution contrasts with the expectations of Rowe and Stone ('76), whose results would suggest substantial differences between the modal distributions.

cell counts of 217,000 and 207,000 neurones thus exceed the minimum ganglion cell count by some 42,000 neurones of classical appearance. These cells are most apparent in peripheral retina.

The mean minimum ganglion cell count of 169,500 cells for the above two retinas is in excellent agreement with Hughes and Wässle's ('76) most accurate integration of the fibre isodensity distribution to 180,000 fibres. It is subsequently argued that the excess neurones are unlikely to represent unfilled ganglion cells.

The optic nerve fibre total count in cat

Stone and Campion ('78) report cat optic nerve counts of 112,000 to 147,000 (128,000 mean) fibres. These range from 62% to 82% of Hughes and Wässle's ('76) count of 180,000-
190,000 fibres and support Stone's ('78) lower ganglion cell counts. Stone and Campion ('78) thus question the corresponding results of Hughes and Wassle ('76) and, implicitly, the estimated ganglion cell count above.

Is the 18%–38% difference between the two sets of counts significant? Three publications on rat optic nerve obtained counts within 5% of one another (see Hughes, '77), and Potts et al. ('72) record individual variation in monkey optic nerve counts to be about 13%. The range in electron microscopic counts of the cat optic nerve fibre population appear to be rather large.

Hughes and Wassle ('76) presented a fibre-diameter spectrum for the whole nerve which has recently been confirmed by Freeman ('78). This suggests that any error in Hughes and Wassle's ('76) count would have to arise in its computation from the nerve and sample areas, but all measurements were cross-checked in several ways and allowance was made for compression in cutting (Vaney and Hughes, '76). The range of nerve areas reported by Stone and Campion ('78) is very similar.

Stone and Campion ('78) suggest that Hughes and Wassle ('76) neglect the glial network when integrating. However, the nerve capsule and the core surrounding the central vessel were not included in the computation of cross-sectional area, and were shown black in their Figure 2 (Hughes and Wassle, '76) for this reason. Their sampling matrix was locally randomised and accepted glial profiles which appeared in the photographs. Measurement of the larger glial processes in the electron micrographs employed for the count indicates them to occupy a minimum 14.6% of the total area, within and excluding the capsule, which is within the range of Stone and Campion ('78). The discrepancy between the two sets of results did not arise from failure to allow for the area of glia and capsule in Hughes and Wassle's ('76) computation.

Freeman's ('78) confirmation of Hughes and Wassle's ('76) whole nerve fibre diameter spectrum is important. Donovan ('76) counted all the large fibres in a cat optic nerve by light microscopy. Given confirmation of Hughes and Wassle's ('76) spectrum, we may more confidently accept the total fibre count, estimated from the proportion of large fibres and their number in Donovan's count, as from 160,000 to 220,000 (Hughes and Wassle, '76), values which support the counts of Hughes and Wassle ('76) rather than Stone and Campion ('78).

Reconsideration of Hughes and Wassle's ('76) results thus reveals no technical reason for doubting their validity, independent of Stone and Campion's ('78) observations. It should be noted that Stone and Campion selectively exclude glial processes when measuring sample area, to obtain a glia-free axon density which is multiplied by a macroscopic estimate of glia-free section area to derive the total count. It is possible that error is introduced by this procedure, which involved arbitrary criteria in a manner avoided with the random sampling of Hughes and Wassle ('76).

The basis of differences between published cat ganglion cell counts

Differences between counts could arise from varying techniques of integration. However, the planimetric integration of all published maps (Table 1) shows that the presumed ganglion cell counts of Hughes and of Wassle et al. consistently exceed the ganglion cell counts of Stone by some 100,000 neurones (Table 16). Even the minimum ganglion cell estimate of Hughes (Table 11b) exceeds Stone's mean total by some 57,000 ganglion cells (Table-ID).

Presumably, both groups of authors count all the cells in the ann + βnn total. The excess of the above minimum ganglion cell count of 169,500 cells over Stone's most recent mean count of 116,000 cells must then represent cells in the ym mode. The ym mode in Stone's diameter spectra would therefore be predicted to be smaller, relative to the βnn mode, than in either the neuronal or minimum ganglion cell spectra above.

HRP-filling suggests that the 169,500 ganglion cell total may be roughly divided by the modal proportions of Table 2A to give the counts in section B of Table 2. If the 53,500 difference (Table 2C) between this and Stone's ('78) 116,000 total count is located entirely in the ym population, its subtraction from the total ym mode count of this paper (Table 2B) gives the predicted ym mode counts for Stone et al.'s results (Table 2D) and the corresponding modal percentages (Table 2E). Stone's ym mode would then contain the low proportion of 25% of his ganglion cell population, much lower than Rowe and Stone's ('76) "small" or γ cell percentage. A similar but independent result is obtained by subtracting the α + βnn mode cell population estimated for the neuronal population of the first retina from Stone's total ganglion cell count, 116,000-90,500 = 25,500, a ym proportion of 22% of the total population identified as ganglion cells.

Fukuda and Stone ('74) regard the peripheral spectra as possessing similar modal ranges from 1.5 mm eccentricity. Rowe and Stone's ('76) peripheral spectra 3A, 6A, and 11A are therefore pooled in Figure 25. It is apparent
that the \( \text{yn} \) mode does appear to be small when compared to the minimum ganglion cell spectra above (Fig. 22) or to that of Kelly and Gilbert (75).

The \( \beta \text{mm/ynm} \) dip of these spectra is located at a somewhat smaller diameter than Rowe and Stone's (76) 14-\( \mu \) criterion. The consequence is that the "small" cell population appears to be generated from the \( \text{yn} \) mode inflated by incorporation of part of the \( \beta \) mode. Rowe and Stone's (76) own division of their spectra to obtain a 33% "small" cell proportion thus does not confute the above prediction of a 25% \( \text{yn} \) mode proportion. A generous division about the \( \beta \text{mm/ynm} \) dip in Figure 25 indicates the \( \text{yn} \) mode proportion to be 29%, close to the predicted 25%

Rowe and Stone (76) thus reject as non-ganglion cells not only neurones which do not fill with HRP in this study, but also a substantial proportion of those which do. Such relative conservatism is less obvious in work of Fukuda and Stone (74), who obtain a 49% \( \text{yn} \) mode, but if characteristic of Rowe and Stone's (76) and Stone's (78) mapping criteria, would account for the difference between their total counts and the minimum ganglion cell count above.

In terms of this paper's populations, Stone (78) effects his dichotomy within the small ganglion cell group, although his own classification would not recognise this, because the non-ganglion cells are presumed to be basophilic glia. What criteria justify this division? Stone (78) emphasises undefined properties based on visual comparison between normal and nerve-sectioned retina as the foundation of his classification. The non-ganglion cells resemble profiles remaining 6 months after optic nerve section; ganglion cells resemble those which disappear. Experience gained in deciding whether or not the unfilled profiles above are similar to filled profiles in the same field of view indicates that such comparisons are difficult to make consistently and objectively, even when morphological criteria have been overtly defined.

Small ganglion cells, according to Stone (78), possess Nissl granules "in a more or less complete ring around the nucleus." But some 87% of neurones in the \( \text{yn} \) mode of fixed retina above (Fig. 4) are characterized by an eccentric nucleus, which results in their cytoplasm and Nissl-staining being restricted around one.

### TABLE 1. Integrations of published ganglion cell and neurone maps

<table>
<thead>
<tr>
<th>Source</th>
<th>Published Minimum</th>
<th>New Integration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>A. Total neurone count</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wässle et al. (75)</td>
<td>190,000</td>
<td>217,000</td>
</tr>
<tr>
<td>Hughes (75)</td>
<td>217,000</td>
<td>240,000</td>
</tr>
<tr>
<td>Hughes (81)</td>
<td>207,000</td>
<td>231,000</td>
</tr>
<tr>
<td>Mean</td>
<td>204,000</td>
<td>206,000</td>
</tr>
<tr>
<td>B. Minimum ganglion cell count</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hughes (75)</td>
<td>174,000</td>
<td>195,000</td>
</tr>
<tr>
<td>Hughes (81)</td>
<td>165,000</td>
<td>185,000</td>
</tr>
<tr>
<td>Mean</td>
<td>169,500</td>
<td>169,500</td>
</tr>
<tr>
<td>C. Ganglion cell count</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stone (78)</td>
<td>80,000</td>
<td>104,000</td>
</tr>
<tr>
<td>Rowe and Stone (76)</td>
<td>141,000</td>
<td>141,000</td>
</tr>
<tr>
<td>Stone (78)</td>
<td>114,000</td>
<td>121,000</td>
</tr>
<tr>
<td>Stone (78)</td>
<td>105,000</td>
<td>121,000</td>
</tr>
<tr>
<td>Mean</td>
<td>112,500</td>
<td>112,500</td>
</tr>
<tr>
<td>C. Difference (A-C)</td>
<td>91,500</td>
<td>103,200</td>
</tr>
<tr>
<td>D. Difference (B-C)</td>
<td>57,000</td>
<td>67,000</td>
</tr>
</tbody>
</table>

The map for Wässle et al. (75) was obtained by reconstruction from their set of total neurone count profiles. Hughes (78) refers to this paper.

### TABLE 2. Predicted modal composition of somas in Stone's total count population

<table>
<thead>
<tr>
<th></th>
<th>( \text{ynm} )</th>
<th>( \beta \text{mm} )</th>
<th>( \alpha \text{mm} )</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Hughes (81)</td>
<td>48.5%</td>
<td>48.5%</td>
<td>2%</td>
<td>100%</td>
</tr>
<tr>
<td>B. Hughes (81)</td>
<td>82,150</td>
<td>82,150</td>
<td>5,200</td>
<td>169,500</td>
</tr>
<tr>
<td>C. Hughes (81)</td>
<td>-53,500</td>
<td>-53,500</td>
<td>-53,500</td>
<td>-53,500</td>
</tr>
<tr>
<td>D. Predicted</td>
<td>28,650</td>
<td>82,150</td>
<td>5,200</td>
<td>116,000</td>
</tr>
<tr>
<td>for Stone's (78) total count (B-C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. Stone</td>
<td>24.7%</td>
<td>70.9%</td>
<td>4.5%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Neurones of classical appearance in the ganglion cell layer which appear not to be ganglion cells

The difference between the minimum total population of neurones of classical appearance in the retinal ganglion cell layer (297,000-217,000) and that estimated for either ganglion cells (169,500) or optic nerve fibres (150,000) amounts to possibly more than 40,000 cells.

HRP-filling from optic nerve and superior colliculus has suggested that the excess of non-ganglion cell neurones is predominantly located in the peripheral yam mode, and accounts for the difference between its proportion of 58% in the neuronal spectrum and 48.5% in the ganglion cell spectrum identified by projections to the superior colliculus.

A morphologically distinct subpopulation of ganglion cell layer yam mode profiles has been described above as failing to fill with HRP applied to either optic nerve or superior colliculus. It could be suggested that these are simply ganglion cells which have failed to fill, however, the distinctive form, low-ratio of cytoplasmic-to-nuclear-volume and prominent basophilic nuclear fold, (Fig. 21A), are not encountered in any ganglion cells filled with HRP from the optic nerve. These, however, are features of profiles in the amacrine layer, (Fig. 21C), whose total population may be some 500,000 cells, as estimated from a minimum peripheral density of 1,000 mm², which is too great for it to be suggested that they represent displaced ganglion cells. Cytoplasmic basophilia and a prominent nucleolus suggest that such profiles, whether in the amacrine or ganglion cell layers, are classical neurones rather than glia.

The dendritic trees of these "bar-cells" in supravital methylene blue-stained preparations (unpublished observations) are very similar to those of the similar amacrine population.

Their vital staining is further evidence suggesting the neuronal nature of these cells. Examination of a retina 9 months after chiasm section shows that the "bar-cells" remain unchanged in regions where cells of the type known to fill with HRP, and thus identified as ganglion cells, have become either unrecognisably atrophied or have disappeared entirely (Fig. 21B). The corresponding class of cell in the amacrine layer remains unchanged under such conditions.

Although such cells form a significant proportion of peripheral neuronal spectra, they appear to be relatively uncommon in central retina, which suggests that neither their den-
sity nor proportion remain uniform. Their distribution will be dealt with elsewhere (in preparation).

These non-ganglion cell neurones might be displaced amacrine (Gallego, '71; Hughes and Vaney, '80; Perry, '79) or association neurones of the ganglion cell layer (Gallego and Cruz, '65). The suggestion that such cells are basophilic glia (Stone, '78) is unlikely to be true (Hughes and Vaney, '80); indeed, it has recently been indicated that a substantial proportion of the presumed glial population of cat retina may be made up of microneurones. (Hughes and Wiernawa-Narkiewicz, '80).

Modal proportions and distributions

The local soma diameter spectra of classic neurones has been shown (Fig. 6, 8) to change progressively from trimodal to bimodal, in passing from periphery to area centralis or visual streak. The ynm mean diameter remains quite constant, but that of the βn mode becomes smaller as the regions of higher density are approached, and the βnm/ynm dip disappears as the modes merge.

In passing from periphery to area centralis, the results of Boycott and Wässle ('74) show the soma diameter of identified γ cells to remain relatively constant, but the α and β cells become progressively smaller, until the size ranges of the β and γ cells overlap. The mean modal diameters of Nissl preparations thus reflect the soma size of the cell class regarded as forming their predominant component. Peichl and Wässle ('79) report preliminary results for regions from periphery to area centralis, which suggest that the proportions of identified α, β, and γ cells undergo changes very similar to those of the modes in this study.

Golgi criteria are not available for comparison with the Nissl-stained soma diameter spectra on the visual streak. However, there is no apparent anatomical reason to regard the changes in the Nissl-spectra upon passing from periphery to streak (Fig. 8) as different in principle from those in passing towards the area centralis (Fig. 6). It is concluded that the progressive shift of the βnm/ynm dip arises because the βnm cells progressively reduce in mean diameter with increasing density, until their mode merges with the ynm mode of relatively unchanged y cell mean diameter. The conclusions of this study are summarised in Figure 26, where a simple model is presented of how the peripheral modes could be changed to generate the spectra of central and streak retina.

Retrograde filling from HRP injections into the superior colliculus (Fig. 19) shows that the proportion of ynm cells which can be identified as ganglion cells forms a constant 48% in both peripheral retina and visual streak. Even near the centre of the area centralis, the y mode contains at least 45% of the local ganglion cell population (Kelly and Gilbert, '75). The proportion of ganglion cells in each mode is thus relatively constant over the majority of the retina, and the modal density distribution maps for ganglion cells are thus similar in appearance (Fig. 17—ynm; Fig. 15—βnm; Fig. 24—γnm), to the total density map (Fig. 16).

The results of Kelly and Gilbert ('75), the above results, the data of Cooper and Pettigrew (79), and of Wässle and Iling ('80) show the majority of ynm cells to be filled by retrograde transport from the superior colliculus, and the majority of βnm cells from the LGN A laminae. In streak, area, and periphery, the proportion of cells in each mode which fill from superior colliculus or LGN is quite constant, so that not only modal proportions, but also these components of their projection, appear uniform across the retina.

To the extent that the distinctive modal projections represent the predominant proportions of the α, β, and γ cells which make them up, they also indicate the relative uniformity of these gross morphological classes across the retina. Boycott and Wässle ('74) proposed a correspondence between the α, β, and γ cells and certain classes of electrophysiologically encountered ganglion cells. Studies by Cleland and Levick ('74a, b) and Fukuda and Stone ('74), based on the draft suggestions of Boycott and Wässle ('74), were in general agreement in accepting the α, β, and γ cells to correspond to brisk-transient, brisk-sustained, and the sluggish/rarely encountered classes, or the Y, X, and W cells, respectively. It may thus be concluded that these functional groupings are also distributed throughout the retina in relatively similar proportions, which are represented by the modal density maps. However, it must be accepted that some degree of cross modal "contamination" exists.

Rowe and Stone ('76) came to quite different conclusions. At the area centralis they accept Fukuda and Stone's ('74) use of a progressively smaller criterion diameter for dividing between the anatomically defined "X" and "W" cells as density increases. However, they employ the same 14-μ absolute diameter criterion on the visual streak that Fukuda and Stone ('74) used to discriminate between peripheral "X" and "W" cells.

It automatically follows that the reduction in the mean soma diameter on the streak will result in an increase of the relative proportion of "small" to "medium" cells. The results above
are consistent if this division is accepted. However, Rowe and Stone ('76) equate "small" with W and "medium" with X, and conclude that the relative proportion of W to X cells increases on the streak.

Rowe and Stone ('76) do not consider either the progressive shift of the intermodal dip in approaching the streak, or Kelly and Gilbert's ('75) HRP-filling studies. They do not demonstrate the suitability of their criterion size for the given retina. In order to justify the division of modes in a region which is apparently a morphological extension of the area centralis by a different means to those employed at the area centralis, they rely primarily on physiological evidence. Their anatomical argument for a
fixed-size criterion is that they observe no reduction in the size of "large" cells on the streak, and thus the "medium," or "X," cells may also be unchanged relative to peripheral retina. But the lack of correlation between ynm and fmm diameter changes in passing to the area centralis (Boycott and Wässle, '74) indicates the weakness of this justification.

However, an cells were found to be some 9% smaller on the visual streak in this study. Now it has been suggested that, for a given class of cell, there is a proportional relationship between soma diameter, dendrite tree diameter, and receptive field diameter (Cleland and Levick, '74a, b; Fukuda and Stone, '74). Rowe and Stone ('76) report that the W cell receptive field diameters are similar in periphery and streak, but that on the streak the receptive fields of X and Y cells are smaller by 0.7 and 0.77, respectively. If the correlation between soma diameter and receptive field diameter is valid, these results are at odds with Rowe and Stone's ('76) own interpretation but are in agreement with that advanced here.

The similarity in form of the α, β, and γ mode maps to the total neurone distribution contrasts markedly with the suggestion of Rowe and Stone ('76) that the "medium," X, cells have an area-centred circular isodensity distribution, and that "small," W, cells are concentrated in an elliptical distribution in the visual streak. Rowe and Stone ('76) see the combination of these two distributions as necessary to explain the cruciform total receptive field maps, but the "large" cell, Y or alpha, map (Wässle et al., '75) possesses this form without such a special basis. In this study the three modal cruciform distributions are regarded as arising from common morphogenic determinants.

**Encounter rates**

Rowe and Stone's ('76) primary argument for a predominance of small, "W," cells in the visual streak is electrophysiological. They compared the relative frequencies and absolute encounter rates of the different functional classes for two regions of equivalent density in peripheral area and visual streak. The W cells were recorded with greater frequency on the streak (47.6%) than in the area periphery (20%). X cells were more commonly recorded in the peripheral area (51%) than in the visual streak (36.4%). The absolute encounter of W cells was higher in streak, and that of X cells, lower. These interesting results now require confirmation.

Rowe and Stone ('76) interpret their findings as indicating W cells to be more common on the visual streak, but their results are equally consistent with the possibility that X cells are harder to record on the streak. This would be true if they shifted towards the smaller diameter range suggested above and by their reduced receptive field diameters (Rowe and Stone, '76). Their absolute encounter rate and relative percentage might then be expected to fall.

However, no quantitative theory is available for variations in encounter rate between different regions of retina, or between classes of cells. Fukuda and Stone ('76) were unsuccessful in accounting for their relative central and peripheral encounter rates with a variety of models. Electrode impedance has been suggested to be of systematic importance (Stone, '73), and this has been denied (Levick and Cleland '74); many factors must contribute, apart from the obvious one of neuronal target area, to alter the sample proportions. Differences in the fibre layer overlaying the two sample regions of Rowe and Stone ('76) may act as mechanical determinants. No current analysis of the results of Rowe and Stone ('76) could be quantitatively profitable. It suffices that their results are qualitatively compatible with the interpretation provided in this paper as well as with their own hypothesis.

**Mode and class proportions for the entire retina**

The spectra of classic neurones were integrated over the retina to obtain 89% ynm, 37% fmm, and 3% omm; HRP-filling indicates the modal proportions of identified ganglion cells to be about 48.5% ynm, 48.5% fmm, and 3% omm for the entire retina. The small size of the area centralis and similarity between the modal proportions of streak and peripheral diameter distributions mean that the overall mode proportions are similar to those of peripheral retina. Rowe and Stone ('76) have made no similar integration of their analogous, but not corresponding, groups of "small," "medium," and "large" cells.

Instead, they derive the proportions of W, X, and Y units from estimates based on the contribution of the tα, tβ, and tγ groups to the optic nerve compound action potential; they accept values ranging between 52% W, 44% X, and 4% Y (Rorieck, '73) and 60% W, 39% X, and 5% Y (attributed to Bishop et al., '89). But the anatomical W cells (Fukuda and Stone, '74) and the "small" cells (Rowe and Stone, '76) represent only 35-45% of the total in regions outside the streak, and thus fall short of the minimum assumed 55% W cell population (Stone, '78). Rowe and Stone ('76) suggest that the "small"
cells at the streak peak compensate for the low peripheral proportion and bring the population percentage up to the assumed value. Their assumed population proportions are satisfactorily consonant with those of this paper without any ad hoc hypotheses.

THEORETICAL IMPLICATIONS

The availability of the above $\alpha$ and $\beta$ mode density distributions makes it possible to determine whether the local array densities of these classes and the physiological properties of the corresponding single units are related in the manner expected from sampling theory. The results of such a comparison and their implications with respect to the mechanisms underlying visual grating acuity have been presented elsewhere (Hughes, 1980).

ACKNOWLEDGMENTS

I am grateful to Dr. G. Berlucchi, Dr. K.P. Hoffman, and Dr. Heinz Wässle for the loan of a retina from a chiasm-sectioned cat from which the photographs of Figure 21B were obtained, and to Mrs. B. Inglis for determining the fresh $\alpha$ cell diameter spectrum.

LITERATURE CITED


A Statistical Analysis and Comparison of Soma Diameter Spectra for Classical Neurones from Different Regions of the Cat Retinal Ganglion Cell Layer

A. Hughes, D. Caille, and J. F. Vibert

Abstract. Multimodal soma diameter spectra for neurones of the cat retinal ganglion cell layer have been represented by three subpopulations of independent, normal diameter distribution. Recurrent computation according to the technique of Vibert and Caille (1978) has extracted best fit populations for samples from various regions of central and peripheral retina. The model subpopulations from all these regions did not differ significantly in their relative proportions or variance. Significant progressive variation between subpopulations representing different regions of retina were observed only in the mean diameter of the $\alpha$ and $\beta$ mode cells. The parameters of the $\gamma$ mode population were statistically uniform across the retina. The cat retina thus appears to be more homogeneously organized than has been suggested elsewhere.

Key words: Statistical model — Cat — Retina — Ganglion cell layer — Neuronal diameter spectra.

Introduction

The soma diameter spectra of classical neurones in the cat retinal ganglion cell layer change from trinodal to bimodal in passing from periphery to the area centralis. Boycott and Wässle (1974) have demonstrated in Golgi preparations that the soma diameter range of $\gamma$ cells remains relatively constant over the retina but that the $\beta$ and $\alpha$ cell somas become smaller as the area centralis is approached. The changes in the central soma diameter spectra have thus been interpreted (Rowe and Stone 1976; Hughes 1980) as resulting from the progressive shift of the $\beta$ cell mean diameter towards the $\gamma$ cell mean as the area centralis is approached, with the eventual fusion of the $\beta$ and $\gamma$ modes. With the possible exception of the very centre of the area (Hughes 1980), the proportions of cells in the $\beta$ and $\gamma$ modes appears to be relatively similar in peri-centralis and peripheral regions; this conclusion is supported by HRP filling (Kelly and Gilbert 1975).

A similar change from a trinodal to bimodal diameter spectrum occurs for classic neurones of the retinal ganglion cell layer in passing from peripheral retina to the high density region of the visual streak. However, Golgi material is not available to guide interpretation. In contrast to the treatment of the area centralis spectrum (Fukuda and Stone 1974), Rowe and Stone (1976) divide the streak bimodal distribution into small, medium and large cell modes by means of the same criterion diameters which they employ for the peripheral population. The shift to a population of smaller mean diameter in the visual streak thus inevitably results in an increase in the proportion of the small cell population and a reduction in the proportion of medium diameter cells. They interpret this to indicate the $\gamma$ cell population to increase on the visual streak and the $\beta$ cell proportion to decrease. However, it will be equally apparent that application of a constant mean criterion diameter to a spectrum in which the $\beta$ mode simply decreased in mean diameter would result in similar observations on the cell size modes but with no change in the underlying proportions. In this situation Rowe and Stone (1976) would classify the smaller cells of the $\beta$ mode as additional cells in the $\gamma$ mode.

Hughes (1980) has presented soma diameter spectra for classical neurones of the retinal ganglion cell layer in the form of three series of histograms which run from area centralis to the superior peripheral retina, from area centralis along the visual streak, and perpendicularly through the visual streak from superior to inferior retina. These spectra permit the progressive shift in modal proportions and mean diameters to be followed. It was concluded, in contrast to Rowe and Stone (1976), that the unimodal distribution on the
visual streak simply arises from a reduction in the $\beta$ mode mean diameter and consequent fusion of the $\beta$ mode with the relatively stationary $\gamma$ mode in similar proportions to peripheral samples.

The analysis of the subpopulations in the soma diameter histograms seemed ideally suited for the application of Vibert and Caille’s (1978) statistical technique for the identification of subpopulations by recurrent analysis of multimodal distributions. The results of such an analysis were statistically consistent with the interpretation put forward by Hughes (1980).

Methods

The analysis was performed on digitized versions of the 22 histograms which form Fig. 6, 7 and 8 of Hughes (1980).

During computer analysis the multimodal soma diameter histograms were treated as composed of three subpopulation, the $x$, $\beta$, and $\gamma$ modes, which predominantly but not exclusively contain $x$, $\beta$, and $\gamma$ ganglion cells. The recurrent method of Vibert and Caille (1978) was employed to reconstruct the population sample of a histogram by adding computed subpopulations assumed to be independent and normally distributed. Each subpopulation may thus be uniquely defined by its mean, variance and relative weight.

Recurrent adjustment of subpopulation parameters then enabled the difference between the synthesized and experimental populations to be optimally reduced. This difference was evaluated by the quadratic distance $\chi^2$ between computed and experimental populations.

Given the best-fit mean diameter, variance and percentage of the three subpopulations for each histogram model, it was possible to determine whether the mean soma diameter for each subpopulation is significantly different from one site to another. In the same manner their variance and percentages were compared between sample sites. Means were computed by Student’s $t$-test, variance by Fisher’s $F$ test and relative weights by the percentages comparison test (Snedecor and Cochran 1976).

Results

Computer analysis of the 22 histograms gave straightforward results; a detailed presentation is unnecessary. With the assumption of 3 normally distributed subpopulations, and without further human intervention in their extraction by recurrent computation, it was found that:

1. The differences between subpopulation percentages for peripheral, area and streak populations were not statistically significant ($P > 0.05$). The mean percentage of the 22 samples is summarized in Table 1 for each subpopulation.

2. No statistical significance could be attributed within each subpopulation to the differences between the diameter variances of samples from peripheral, area and streak retina ($P = 0.05$). The mean diameter variance of the 22 samples is summarized in Table 1 for each subpopulation.

3. The only statistically significant differences between the computed subpopulations of a given mode were found to be those of their mean diameters. Table 1.

- a) Although differences between the mean diameters of the $\gamma$ subpopulations from different retinal regions were significant at the $P = 0.05$ level, they were small and not progressive with change of location along the track of a given sample series.

- b) By contrast, when analyzed as first order differences from the smallest to the largest mean of each series, the bigger and more significant ($P = 0.001$) differences between the mean diameters of the $\beta$ subpopulations were substantially progressive for all three tracks from periphery to area or streak (Fig. 1).

- c) The samples from which the $\alpha$ subpopulations were computed are too small for reliance to be placed on the tabulated means.

Discussion

The above analysis began by representing the classic neurone population of the cat retinal ganglion cell layer...
with three independent subpopulations of normal diameter distribution. The subpopulations of the resultant best-fit models of soma diameter spectra from all parts of the retina did not differ significantly in variance or percentage. Even the mean diameter of the γ mode remained essentially constant; only the α and β mode mean diameters showed significant and progressive change with location.

The corollary of these results is that the available data statistically justifies a model of the soma diameter distributions from all parts of the retina which are comprised of the same three, independent, normally distributed diameter subpopulations of fixed proportion and variance. Matching to a given local sample would require change of only the mean β and α mode diameters; that of the γ mode may remain constant. Although not computed, this model is closely represented by the mean values for the measured subpopulations in Table 1.

Statistical analysis is thus consistent with the conclusion of Hughes (1980), that the three major modes of the soma diameter spectra for classical neurones of the cat retinal ganglion cell layer vary little in their proportion throughout the retina and have similar forms for their density distribution maps. Rowe and Stone (1976) view that the three modal classes have density distributions of different form and that their relative proportions differ significantly across the retina does not arise as a best-fit outcome of the above analysis.

In particular, the analysis supports the possibility that the bimodal distributions of area centralis and visual streak both arise from a shift, when passing from the peripheral trimodal distribution to regions of higher density, in the mean β mode diameter towards the constant, but lower, γ mode mean diameter. This interpretation is strongly substantiated by the finding that the relative proportions of the non α mode population which projects to the lateral geniculate nucleus and to superior colliculus is relatively unchanged between peripheral and streak retina (Kelly and Gilbert 1975; Hughes 1980).

Of course, the above analysis only indicates that it is not statistically justifiable to claim a significant change in the relative proportions of the α, β and γ modes on the basis of the measured spectra; further data may establish the significance of differences in their relative proportions. More probable is the presence of several components in the modes treated here as independent homogeneous subpopulations. Thus, the proportion of non-ganglion cell classic neurones in the γ mode (Hughes 1980) may vary from region to region. In addition Rowe and Stone (1976) have pointed out, and are confirmed by Hughes (1980), that streak β mode cells are significantly smaller than those of the area centralis at the same density. The essence of Rowe and Stone’s (1976) thesis of reduction in the β cell population on the streak might therefore be retained by arguing that the number of β cells in the β mode is progressively reduced as the peak density of the streak is approached but that some large γ cells form a small diameter component of the peripheral β mode population which symmetrically increases in proportion at the streak. The reduction in β mode mean diameter on the streak would thus be accounted for without loss of the independent γ mode. However the relative proportions of the β and γ modes which project to the lateral geniculate nucleus and superior colliculus re-
main quite constant between streak, periphery and area centralis (Kelly and Gilbert 1975; Cooper and Pettigrew 1979; Hughes 1980) so that this interpretation seems improbable.

References


Hughes A (1980) Population magnitudes and distribution of the major modal classes of cat retinal ganglion cell as estimated from HRP filling and a systematic survey of the soma diameter spectra for classical neurones. J Comp Neurol in press


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IDENTIFICATION OF A LARGE MICroneURONE POPULATION IN THE RETINAL GANGLION CELL LAYER
Coronate Cells: Displaced Amacrine
of the Rabbit Retina?

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ABSTRACT The rabbit retinal ganglion cell layer contains a soma population which is morphologically distinct from the demonstrated ganglion cells. These "coronate cells" (Vaney, '80) have many features in common with classical neurons and are qualitatively different from typical glial cells. Detailed investigation by specific staining, ribonuclease treatment, and electron microscopy indicate that coronate cells are microglia. The coronate cells contain somatic subsurface cisternae typical of rabbit amacrine cells, and are similar in appearance to some neurons of the amacrine cell layer. It is suggested that they represent, at least in part, a population of "displaced" amacrine cells.

The discrepancy between a previous estimate of 394,000 optic nerve fibers (Vaney and Hughes, '76) and the maximum presumed ganglion cell count of 471,000 cells in the rabbit retina (Vaney and Hughes, '76) has been ascribed in a preceding paper (Vaney, '80) to the inclusion in the ganglion cell count of cells which do not send axons into the optic nerve. Vaney ('80) identified a morphologically distinct subset of these soma which he named "coronate cells."

In this paper, those features such as cytoplasmic basophilia which led to the coronate cells being originally classified as ganglion cells (Hughes, '71) are examined in detail in order to establish whether this small cell should be regarded as a neuron or a glial cell. The findings indicate that coronate cells are neurons and probably represent, at least in part, a population of "displaced amacrine" cells comparable to those described in other mammalian retinas (Cajal, '55).

Current interest in the quantitative topographic distribution of retinal ganglion cells in various species (see Hughes, '77a) has necessitated reinvestigation of the criteria for distinguishing neurons from glia, and displaced amacrones from ganglion cells (Bunt et al. '74; Hughes, '75; Stone, '78). Density maps for the various cell classes of the ganglion cell layer can only be obtained from Nissl-stained material in which the soma alone provides cues for identification. The need for classificatory criteria applicable to such material is emphasized by the large proportion of coronate cells and other potential microglia which are found in the ganglion cell layer of the rabbit retina (Vaney and Hughes, '76; Vaney, '80).

METHODS Retinal whole mounts

Freshly dissected retinas were mounted on gelatinized slides and fixed in an alcohol/formalin solution according to a procedure for minimizing areal shrinkage (Hughes, '75).

Nissl staining

The mounted retinas were brought back to water and stained either with cresylecht-violet or a galloycyanin lake. Cresylecht-violet (0.1%) (10485; Chroma, Stuttgart), unbuffered and pH-unadjusted, was found to give specific Nissl body staining although this is possibly aided by the differentiating procedure (Stenram, '54). If the pH of the cresylecht-violet was adjusted to 3.5 with acetic buffer the staining became metachromatic and less specific with glial nuclei showing residual staining after ribonuclease treatment (Powers and Clark, '55). Enzymatically treated retinas and untreated material were stained both metachromatically and nonmetachromatically with the cresylecht-violet. The control retinas in the ribonuclease series were also stained with a galloycyanin lake (10780; Chroma, Stuttgart) prepared according to the method of de Boer and Sarnaker as described in Pearse ('61). Although the results were very similar to those obtained with cresylecht-violet, there is substantial evidence that galloycyanin lake, in contrast to cresylecht-violet, gives specific staining to the soma of a minority of neurons of the retina of the rabbit, as identified in the study of Vaney and Hughes ('80, '82).

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A. Hughes and D. I. Vaney

echt-violet, also stains non-RNA, Nissl-associated material (Terner and Clark, '60; Clark, '69).

Ribonuclease treatment

The following procedure was employed in order to establish that RNA is the common source of basophilia in the Nissl bodies of ganglion cells and in the corresponding cytoplasmic granules of coroanate cells. Retinas fixed with Carnoy's fluid (Pearse, '61) were divided and the two pieces mounted separately. One half was incubated at 37°C in a solution of 0.5% pancreatic ribonuclease in distilled water according to the method of Pearse ('61). The control half was incubated with distilled water alone to ensure that the disappearance of basophilia in the enzymatically treated material was ribonuclease-specific.

In vivo, methylene blue staining

The eye of a urethane anesthetized rabbit was injected with 0.5 ml of 0.05% methylene blue vital stain (Michrome 418; Gurr, London) after a relief needle had been inserted at the opposite pole. The retina was removed 20 minutes later and blued by immersion in 0.5% H₂O₂ in saline solution, made permanent by immersion in cooled 10% ammonium molybdate solution for 20 minutes, fixed in an alcohol/formalin solution on a gelatinized slide, dehydrated, cleared and mounted in Xam (Gurr, London).

Retrograde axonal transport

Horseradish peroxidase (type VI; Sigma, St. Louis) was applied to the distal end of an optic nerve sectioned intraorbitally in a urethane anesthetized rabbit. The retina was removed after 24 hours, fixed in a 3.5% formalin solution, and developed with diaminobenzidine diHCl (Sigma, St. Louis) and H₂O₂ using the conventional procedure described by Chan-Palay ('77).

Cell identification and counting

Material prepared in this study was examined with a Zeiss Photomicroscope III at a magnification of 1250. Regions of the HRP retina which were well filled with reaction product were photographed under oil-immersion, each micrograph covering an area 94 µm by 141 µm. Every cell in each micrograph was classified under the microscope and the presence or absence of filling with HRP recorded. The equivalent diameter of each soma was measured by matching the area of the cell to one of a set of circles printed on sheet film. Each increment of 1 mm in diameter represented 0.63 µm.

Electron microscopy

The preparation of the block of retina from which semithin and ultrathin sections of the coroanate cells were cut has been described by Vaney ('79).

RESULTS

Nissl staining

Neuroglia. Certain cells in the ganglion cell layer of the rabbit retina correspond in their appearance to classically described micro-
Fig. 1 Light micrographs 1-2 illustrate glial nuclei (g) from the vitread surface of the retinal ganglion cell layer; 3-4 show glial nuclei found among the ganglion cells. The nucleus 4g contains less dense chromatin than that of 3g and is a typical member of an apparently separate class. The series of illustrations 5-9 shows a range of ganglion cells which respectively decrease in size. Long arrows point to basophil nuclear invaginations and the short arrows to basophil Nissl granules lining the nuclear wall at the cytoplasmic pole of the cells. Micrographs 10-18 illustrate coronate cells. The cell of 10 possesses a subnuclear ring of Nissl-staining material (long arrow) and a conspicuous nucleolus (open arrow); additional Nissl-staining granules are present in the cytoplasm distal to the nucleus (short arrow). This cell could be an incorrectly classified ganglion cell and lies at the extreme range of coronate cell appearance. More typical coronate cells are illustrated in frame 11: one (C) shows, like 13C, a simple ring of juxtanuclear basophil material with short nuclear invaginations arising from it; the other contains a faint nucleolus (open arrow), pronounced juxtanuclear Nissl staining (long arrow) and some free cytoplasmic Nissl substance such as is also visible in cell C3 of frame 12 (short arrow). Features similar to those described are visible in frames 12 and 13, which show the same region at different levels of focus. Frames 14-15 and 16-18 show the same radially oriented coronate cells at different levels of focus to illustrate the ring of subnuclear basophil; this organization is not dissimilar to that in a radially oriented ganglion cell, 19-21, whose cytoplasm is more extensive. These illustrations are discussed in more detail in the text.
macroglia. Their nucleoplasm ranged from light to dark with the procedures employed and contained intensely staining, granular chromatin, but the cytoplasm of the glial cells remained unstained so that it was outlined only by birefringence. The retinal nerve fiber layer contained interfascicular cells comparable in appearance to oligodendrocytes (figures 1, and 3). Lying among the retinal ganglion cells were two main types of macroglia. One was relatively small and oval, the other larger and less chromatin-dense; both had quite lightly staining but granular nucleoplasm which often contained several nucleoli (figures 1, 1). A distinctive glial class, which forms a regular array in a sharply defined region at the boundary between the ganglion cell and inner plexiform layers, had an oval to circular nucleus some 5 μm in diameter which contained considerable chromatin (figures 1, 3). These nuclei correspond in form and position to the microglial (or Hurtig cell) nuclei described by Marchessani (26) in silver-impregnated material.

Classical neurons. The majority of cell bodies in the ganglion cell layer are neurons of classic appearance. Vaney (80) has described the distinguishing features of these cells, but certain aspects of their appearance now require emphasis. The great majority of the cells possessed an eccentric nucleus. The nucleoplasm stained very lightly and was often penetrated by long invaginations of the nuclear wall originating at the boundary of the cytoplasmic cap. These processes were filled with basophilic material (long arrows, figure 1,1) and appeared in many cells to point towards the nucleus, itself a well-defined organelle. The cytoplasm of the classical neurons was well provided with basophil Nissl granules (figure 1,1). In the smaller neurons this material packed the juxtanuclear zone at the cytoplasmic pole of the cell (short arrows, figure 1, 1, 1).

Quantitative evidence was presented in a previous paper (Vaney, 80) that the population of classical neurons is identical to the population of retinal ganglion cells, and further evidence for this equation will be provided in this paper.

Coronate cells. Vaney (80) described a class of cell body in the ganglion cell layer of the rabbit retina as coronate cells because many had the appearance of a head bearing a crown. Although distinctive in appearance, coronate cells possess characteristics which are typically neuronal. For example, the "crown" consists of a ring of basophil material adjacent to the nucleus in the cytoplasmic cap, from which basophilic nuclear invaginations arise. Representative coronate cells (labelled C) are shown in figure 1, 1, 1, 1, 1.

The somas of coronate cells are at the lower end of the neuronal size scale and, therefore, it is not surprising that they have a high ratio of nucleoplasm to cytoplasm. Three-dimensional profiles of the nucleus indicate that the limited cytoplasm is usually cupped within the nucleus, sometimes incorporating a central mass of subnuclear basophil material (figures 1, 1, 1, 2). The staining of the small nucleolus varied from light to intense when it was identifiable (open arrows, figures 1, 1, 1, 8, 8, 8). Coronate cell nuclei occasionally contained a chromatin mass but were relatively agranular in appearance, unlike glial nuclei, and similar to small ganglion cell nuclei in staining density. In heavily stained preparations their nucleoplasm was decidedly more chromophilic than that of ganglion cells, although still agranular.

In addition to the stained subnuclear material and nuclear invaginations which together form the basophil crown (figures 1, 1, 1, 1, 1), the cytoplasm often contained small basophil bodies dissociated from the nucleus and extending into the main dendrite of the cell (short arrows, figure 1, 1, 1). Thus, the distinctive pattern of the basophilia in coronate cells is qualitatively comparable to the Nissl staining seen in small ganglion cells. The dendritic process penetrated the inner plexiform layer either obliquely (figure 1, 1, 1) or, less typically, radially (figure 1, 1, 1, 1, 1). In the latter case, the subnuclear basophil material might be followed down to a ring of varying size (figure 1, 1). A comparable morphology can be observed in those ganglion cells whose nucleus lies above the cytoplasm, except that the subadjacent basophilia is more extensive (figure 1, 1, 1, 1). Occasionally the perinuclear cytoplasm contains basophil granules which may be embedded in the outer nuclear wall (figures 1, 1, 2).

The essential features of the coronate cell soma are shown diagrammatically in figure 2. The pear-shaped cell body contains a cupped nucleus and around the border of the nuclear encapsulated cytoplasm (nc) there is a rim of Nissl substance punctuated by large pieces of subnuclear Nissl material (smn). The process of the major dendrite contains free Nissl material (fn) and there are some perinuclear Nissl granules (pnm). Invaginations into the nucleus from the cytoplasmic cap are packed with basophil material (ni) and appear to approach the nucleolus (nl). The major dendritic process (td) leaves the cell body opposite the nuclear indentation.
Fig. 2 1: Light micrographs at sequential focus planes of a methylene blue-stained coronate cell. The outline of the dendritic tree is delineated in ld. 2: A schematic drawing of a coronate cell showing the cupped nucleus. The rim of Nissl substance around the encapsulated cytoplasm (nc) is interspersed with large blocks of subnuclear Nissl substance (snn). The major dendritic process (td) contains free Nissl material (fn) and a perinuclear Nissl granule is apparent (pnn). Invaginations into the nucleus from the cytoplasmic cup are packed with basophil material (ni) and appear to approach the nucleolus (nl). 3: Camera lucida sketches of coronate cells stained in vivo with methylene blue showing the primary branches of the dendritic trees.
Methylene blue in vivo staining partially filled the dendritic tree of some corona cells but they were so faint as to preclude adequate photographic reproduction. In figure 2 the basal processes have therefore been inked in or sketched. The main features of the class are illustrated in figure 2, and 2a. Arborization appears to begin relatively close to the soma. The longest main dendrite observed is illustrated in figure 2a. The orientation of the nuclear cap normally indicates the direction of the main process, which commonly penetrates at a slightly oblique angle into the inner plexiform layer where it begins branching well before reaching the level of the amacrine cells.

In no instance did in vivo methylene blue staining reveal the presence of an axon hillock or of an axon leaving a corona cell, although in the same retinas axon hillocks and stained axons could be readily observed to arise from ganglion cells.

The large group of cells described as unclassified by Vaney (80) were similar in several ways to the corona cells, except that the nucleus was not cupped around the cytoplasm and subnuclear basophilia was correspondingly reduced.

Ribonuclease treatment

Nissl staining of neurons is established as reflecting the basophilia of the RNA organized in the neuronal cytoplasm (Pearse, '61). It has not been demonstrated that the apparent Nissl staining of subnuclear and free cytoplasmic material in corona cells can be equated with RNA staining. One half of a retina was subject to ribonuclease treatment as described in Methods. The other half was incubated in distilled water without the enzyme as a control for the specificity of the enzyme.

After ribonuclease treatment a nonmetachromatic cresylecht-violet stain found no binding sites, and the basophil material of corona cells was digested as effectively as the Nissl substance of the ganglion cells (figure 3). The outlining of the cells is due almost entirely to differences in refractive index. A metachromatic stain bound to the nucleoli of cells but reacted no better with the Nissl bodies (figure 3). This dye did stain the glial nuclei in the fiber and inner plexiform layers (figure 3). By contrast, the control retina revealed a substantially normal staining pattern both to the nonmetachromatic cresylecht-violet and to the gallocyanin lake (figure 3, 3).

It is thus confirmed that the basophilia of cytoplasmic material in corona cells is due to RNA, and that there is no reason to distinguish between the corona cell basophil pattern and that of the Nissl staining in the retinal ganglion cells.

Electron microscopy

Semithin sections (1 μm) were serially cut from a block of glutaraldehyde-fixed retina (Vaney, '80) until an apparent corona cell was encountered. The cell was provisionally classified as corona on the basis of its appearance in the last semithin cut prior to ultrathin sectioning: It had a maximum width of 7 μm and its cytoplasm was restricted beneath the nucleus which was deeply cupped (shown diagrammatically, figure 4.). An electron micrograph of a proximal ultrathin section is shown in figure 4a, and already the cell is narrowing and the cytoplasm becoming restricted indicating that the previous sections had not been cut from one side of a larger ganglion cell. The cell was wholly contained within 9 μm of section thickness and, thus, its dimensions were typical of corona cells in glutaraldehyde-fixed material (Vaney, '80).

The cytoplasm of the corona cell exhibited an equivalent intensity of staining to the large neurons on the same ultrathin sections, and was certainly not darker. The nucleus was diffusely filled with chromatin and somewhat darker than the cytoplasm—a feature common to smaller neurons such as granule cells (Chan-Palay, '77). Under electron microscopy, the nuclei of larger neurons appeared slightly lighter than their cytoplasm.

All the features of the schematic diagram of the corona cell were identifiable in the electron microscopy material. The complex form of the nucleus with multiple invaginations was also typical of the larger neurons. The invaginations were filled with granular endoplasmic reticulum and RNA granules, as was the subnuclear region indicated by the curved arrow in figure 4. Patches of this Nissl substance occurred under the nucleus at various points and isolated from it as free, stacked endoplasmic reticulum. The stub leaving to the left of the soma in figure 4, corresponds with the dendritic shaft seen by light microscopy in the semithin section (figure 4).

At the point marked with the numbered arrow (2) in figure 4, and enlarged from another section in figure 4a, there occurs a contact between a neural process and the cell soma. The inner wall of the process is darkened and below the area of contact or embedding there is a stacked collection of agranular subsurface cisternae. At other specialized regions of contact between neural processes and the soma, limited numbers of small vesicular profiles were noted but the material did not permit identification of synapses.
Fig. 3  The appearance of ribonuclease-treated and control retina after RNA-specific staining. The basophil material of coronary cells is removed by ribonuclease treatment but not by water incubation.
Fig. 4  Panel 1: Electron micrograph of a longitudinal ultrathin section of a coronate cell; the nucleus is outlined. Stacked granulated endoplasmic reticulum is apparent in the cytoplasm (broad arrow) and more is present adjacent to the nucleus at the entrance to the nuclear invagination. At the bottom left of the cell is a specialized junction (numbered arrow), which is shown in enlargement in figure 4, panel 2. Beneath the area of contact is a stack of sublamellar cisternae.
Ganglion cells in the rabbit retina were filled with horseradish peroxidase (HRP) by retrograde axonal transport at the distal end of the cut optic nerve and counterstained with cresylecht-violet. The standard DAB development process ('80). Figure 7 illustrates representative filled regions of visual streak and peripheral retina, with the HRP-containing neurons indicated by dark arrows and two unfilled profiles by hollow arrows. In all regions of the retina, no soma identifiable as a corona cell by other criteria contained any HRP granules, even though ganglion cells of similar diameter filled in the same region (figure 7, 7a).

Soma diameter spectra were constructed for 117 cells sampled within 0.4 mm of the peak of the visual streak and for 137 cells sampled between 5 and 7 mm below the streak. The sampled regions were almost directly under the optic nerve head. For comparison with the results of Vaney ('80), the cell bodies were classified as glia, corona, and unclassified, ganglion cells filled with HRP granules, and as cells designated as ganglion cells on other criteria (Vaney, '80) but not filled with HRP reaction product. Classification was performed by one of the authors (A.H.) independently of the author responsible for establishing the criteria. The relative numbers and size distributions of the cell groups in visual streak and peripheral retina are shown in figure 6.

It is evident from comparison of the diameter histograms with those presented by Vaney ('80: figure 5) that the cells measured in this study are more shrunken. For optimal Nissl staining the HRP retinas were fixed with a formalin/alcohol mixture as described by Hughes ('75) and not with the glutaraldehyde fixative employed by Vaney ('80). Moreover, Vaney's cells were measured under microscopic examination using a camera lucida, whereas in this study the cells were measured on photographs following individual classification under the microscope. The size distribution of somas on the visual streak ranged from 3.5 to 14 μm compared with 4-22 μm in Vaney's material; soma size in peripheral retina ranged from 3.5 to 17.5 μm compared with 4-31 μm in the glutaraldehyde-fixed retina. Cells larger than 25 μm in diameter in Vaney's ('80) peripheral sample represent only 0.35% of all somata and, thus, the equivalent cells could easily have been missed in the small sample used in this study. Although the size of glial nuclei varies little between the two fixation procedures, ganglion cells and corona cells both undergo substantial shrinkage during alcohol/formalin fixation. The ganglion cell mode in the peripheral sample is reduced from 13 μm to about 9 μm.
Fig. 5 Areas of rabbit retina on the visual streak and in the inferior periphery after application of horseradish peroxidase to the severed optic nerve. Retrograde transport of HRP has filled all ganglion cells marked with a small arrow. Empty profiles are indicated with hollow arrows; g, glial cell; c, coronate cell. No coronate cells filled with HRP.
As described by Hughes ('71) and Vaney ('80), cell size increases in passing from the visual streak to peripheral retina. The relative proportions of the cell groups in both streak and periphery are similar to those reported by Vaney ('80), and both sets of data are listed in Table 1. Of those cells classified in this study as ganglion cells, using the criteria of Vaney ('80), only 3% in the streak and 12% in the periphery failed to fill with HRP in the sampled regions, which had of course been selected for their high percentage of filled cells. The coroneate and unclassified cells did not fill with HRP and formed a distinct distribution. Unfortunately, the shrinkage increased the overlap of the coroneate/unclassified group with the smaller ganglion cells, abolishing the separation between the two groups apparent in Vaney’s peripheral distribution. These results are consistent with Vaney’s ('80) criteria for ganglion cells.

**Other observations**

In most regions of the retina, whether central or peripheral (figure 7., 7.), the coroneate cells were free of HRP reaction product and only ganglion cells were filled, usually in the ganglion cell layer but occasionally displaced in the inner nuclear layer (figure 7.). In a few patches, however, coroneate cells were observed which at first sight appeared to contain HRP granules. More detailed examination showed that the granules lay on the surface of the cell (figure 7.) and did not fill the cupped cytoplasm. In certain instances, the coroneate cell appeared to have either a dendritic or axonal terminal on its surface (figure 7.). Some of the amacrine cells
RABBIT CORONATE CELLS

TABLE 1. Profile composition

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Visual streak</th>
<th>Periphery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hughes and Vaney</td>
<td>Vaney ('80)</td>
</tr>
<tr>
<td>Glia</td>
<td>5%</td>
<td>8%</td>
</tr>
<tr>
<td>Coronate and unclassified</td>
<td>14%</td>
<td>19%</td>
</tr>
<tr>
<td>Ganglion cells</td>
<td>81%</td>
<td>73%</td>
</tr>
</tbody>
</table>

below these patches received similar terminals or arborizations (figure 7a, 7b). HRP granules on the surface of coronate cells were sometimes seen to be arranged in a line which could be traced back to a branching, HRP-filled process (figure 7c, 7d). While it is not possible to establish the origin of these processes, the branching one shown in figure 7c, 7d appeared to be parallel to the axons in the nerve fiber layer.

DISCUSSION

Coronate cells are not identifiable as gli

Marchesani ('26) recognized three types of glial cell in the rabbit retina: Müller cells which impregnated with astrocyte staining procedures, typical microglia revealed by the silver carbonate process in the ganglion cell and inner plexiform layers, and the remaining cells of the ganglion cell layer which Marchesani described as oligodendroglia with a dark round nucleus and a bright halo of cytoplasm. No typical astrocytes were identified. Berliner ('31), Lessell and Kuwabara ('63), Blunt et al., ('65) all reported oligodendrocytes in the myelinated bands but not in other parts of the retina. Berliner ('31) describes the ganglion cell layer of the rabbit retina as containing both astrocytes and microglia. Lessell and Kuwabara ('63) were unable to demonstrate typical astrocytes in the retina but, using astroglial staining, they were able to outline bipolar-shaped cells in the nerve fiber, ganglion cell, and inner plexiform layers which they labelled "lemmocytes": a term used by Wolter ('55) to describe a modified form of astroglia in the fiber layer of human retina. Recently Ogden ('78) has given a detailed description of astrocytes in the primate retina. For example, Vrabec ('70) found microglia distributed throughout all layers.

All of these reports are based on specific metallic stains; even greater confusion arises if descriptions based only on Nissl-stained, nuclear morphology are taken into account. The variety of oligodendrocyte nuclei and the possibility of their confusion with microglia has been described by Smart and Leblond ('61), and confusion in the identification of glial classes has been documented by Kuhlenbeck ('70).

In Nissl-stained material we have tentatively identified both oligodendroglial nuclei (nerve-fiber layer) and microglia (ganglion cell and inner plexiform layers). We also find in the ganglion cell layer a larger, pale glial cell with a granular nucleus which is round but lacks the dense chromatin typical of oligodendroglia. Macrogial cells in this location have previously been identified as either astrocytes or oligodendrocytes.

The coronate cell cannot be confused with any of the classical glial classes. The relatively pale nucleus might suggest an astrocyte but the basophil cytoplasmic inclusions, granular endoplasmic reticulum, and absence of cytoplasmic fibrils argue against this view. Alternatively, the basophil cytoplasmic inclusions and the light nucleus might suggest the cell to be a light oligodendrocyte (Mori and Leblond, '69).

However, the coronate cell soma is pear-shaped, which suggests a unipolar rather than a multipolar cell, a conclusion supported by methylene blue staining; it does not reveal granular nuclear chromatin with Nissl stains and its nuclear form is complex and invaginated in a manner unusual of oligodendroglia. Under the electron microscope, the cytoplasm of the coronate cell is similar in density to that of larger neurons and appears lighter rather than darker than the nucleolus (Peters et al., '76; Chan-Palay, '77).

The Nissl material in coronate cells could be readily visualized using standard procedures of fixation and staining which typically do not lead to obvious basophilia in glial cells. The suggestion that Nissl basophilia is not an unequivocal criterion for identification of a neuron has been made on the basis that glial cells possess granulated endoplasmic reticulum, which might be expected to bind Nissl stains (Stone, '78). Electron microscopy does reveal that oligodendrocytes possess a well-developed granulated endoplasmic reticulum
but it is only poorly developed in astrocytes (Peters et al., '76). However, it is well established that neither form of macroglia shows obvious cytoplasmic basophilia after formalin or alcohol fixation in contrast to their prominent nuclear staining. Mori and Leblond ('69) report that defatted material shows no oligodendroglial cytoplasmic basophilia although light oligodendrocytes show light basophilia in Epon sections. König ('61) describes the RNA of oligodendrocytes as binding with Nissl stains only after treatment with both deoxyribonuclease and nitrous acid to unmask its basophilia; astrocytes do not even stain after this treatment. Such heroic procedures are not required to elicit coronae cell basophilia.

Following recognition of the neuronal nature of the granule cells in the cerebellum, a small but expanding body of literature has been concerned with the structure and function of microneurons in the nervous system (Altman, '67). These studies emphasize that ambiguous neuron-like cells in nervous tissue may be microneurons (Altman and Bayer, '77; Kaplan and Hinds, '77). An open mind should even be kept with respect to apparent glia: Large populations of "oligodendroglia" in frog and toad dorsal horns have now been identified as microneurons in spite of their very dense nuclei and poor basophilia (Palkovits, '68; Sasaiki, '76). The coronae cell does not even look like a typical glial cell and, with its small Nissl granules and their associated basophilia, is closer to classical neurons in appearance.

Coronae cells appear to be neurons

In lightly stained Nissl material the pale nuclei of coronae cells appear similar in density to those of small ganglion cells; nuclear basophilia is apparent in more heavily stained preparations although the nucleoplasm remains agranular in appearance. An obvious nucleolus is often lacking; it is sometimes rather faint when visible; in many cells it may be represented by what appears to be a chromatinn mass on the nuclear membrane at the tip of a nuclear invagination. Difficulty in recognizing the nucleolus under light microscopy, and nuclear basophilia, are characteristic of some microneurons such as cerebellar granule cells (Palay and Chan-Palay, 1974). Both cupping of the nucleus and the intricate folding of the nuclear wall to form cytoplasmic invaginations packed with RNA lead to nuclear morphology with a complexity characteristic of neurons rather than glia (Smallwood, '06; Smallwood and Rogers, '08; Peters et al., '76; Chan-Palay, '77). The prominent subnuclear and free cytoplasmic basophil material, identified above as RNA and corresponding in position to the stacked granulated endoplasmic reticulum, is again characteristic. Electron micrographs of coronae cells indicate a somewhat darker nucleus than is found in adjacent larger neurons but this is common to other microneurons. The sublammellar cisternae stacks found under the somatic contacts are typical of neurons (Pappas and Waxman, '72; Raviola and Raviola, '69). The major process leaving the cell body at the pole bearing the cytoplasmic cap is similar to that found in ganglion cells of the same layer. In all of these features the coronae cell is characteristically neuronal and merits the necessary light and electron microscopic criteria (Burri and Schneider, '73; Schneider et al., '71).

Several specialized junctions between processes and the coronae cell soma have been observed in the limited electron microscopic material so far examined but only a few small, vesicle-like profiles have been located near the region of contact; the material did not permit identification of the junctions as synapses. These contacts may correspond to those observed in HRP material (figure 7, 7, 7, 7,). The similarity between these regions of contact with their associated subsurface cisternae, and structures observed in conventional amacrine cells is taken up subsequently.

Coronae cells are not retinal ganglion cells

From the above evidence we conclude that the coronae cells of the retinal ganglion cell layer are neurons. Vaney's ('80) results indicate that only a fraction of all the neurons in the retinal ganglion cell layer can send their axons into the optic nerve and suggest that the neurons of classical appearance are the retinal ganglion cells. Indeed, we find that it is these cells which are filled when HRP is applied to the sectioned optic nerve. In select regions up to 95% of the classical neurons filled with HRP and it is thus not possible that the population of cells described as coronae and unclassified could make a significant contribution to the optic nerve fibre population. The classical neu-

![Fig. 7](image-url)
rons account for nearly all the optic nerve fibers. The HRP study indicates that the excess of neurons over optic nerve fibers (547,000 compared with 394,000; Vaney and Hughes, '76) must consist of coroane and unclassified cells rather than classic neurons.

Are coroane cells intraretinal association neurons or displaced amacrine cells?

It is possible that those neurons which are not retinal ganglion cells also possess axons which only project locally within the retina. Such axons have been described for intraretinal association neurons (Marenghi cells) in the ganglion cell layer of human and dog retina (Marenghi, 1900; Gallego and Cruz, '65). Similar association neurons have been identified in rabbit retina but are described (Dawson and Lieberman, '78) as possessing large somata in contrast to coroane and unclassified cells. We have never identified an axon or axon hillock originating from a coroane or an unclassified cell in methylene blue or HRP material.

Although the absence of an axon is characteristic of the amacrine cells of the inner nuclear layer, we depend almost entirely upon the work of Cajal (55, 59, '73) for evidence that the ganglion cell layer of vertebrate retinas contains small neurons which lack an axon and are similar in appearance to amacrine cells. Such "displaced amacrine" of the retinal ganglion cell layer are described by Cajal ('55) as being "in abundance" or "very numerous" in reptiles, amphibians, and birds. Several reports have subsequently confirmed the presence of displaced amacrine in the chick retina (Castro, '66; Nishimura et al., '79), not as a rarity, but in substantial numbers (Galvez et al., '77). Displaced amacrine were encountered frequently in the mammalian retina by Cajal ('73) and were illustrated only for the newborn kitten (Cajal, '59). Such "displaced" or "inferior" amacrine are not to be confused with the "interstitial" amacrine (Cajal, '73) demonstrated in the mammalian inner plexiform layer.

The coroane cell is quite unlike the multipolar interstitial amacrine of the mammalian retina. It does not resemble the multiprocessed amacrine cell demonstrated in the retinal ganglion cell layer of dog and cat by Gallego ('71). The distinctive somatic form of the coroane cell is more characteristic of the nonmammalian displaced amacrine cells (Cajal, '73). Like them, it appears to send a major basal process into the inner plexiform layer where it branches on the vitread side. The terminal branches of methylene blue-stained cells were not impregnated, so it is not known whether the typical delicate tree of Cajal's nonmammalian displaced amacrine is present.

Very recently, however, evidence has accumulated of the presence of a unipolar displaced amacrine in the mammalian retina (West, '76; Perry, '79). In rat these cells are small "with a single primary dendrite passing a short distance into the inner plexiform layer before branching on a plane" (Perry, '79). It is possible that such neurons are typical of those in the ganglion cell layer of the rat which survive optic nerve section (Eayrs, '52; Perry, '79), do not fill with HRP (Bunt et al., '74), and comprise the excess of retinal ganglion cell layer neurons over the optic nerve count (Hughes, '77b). It thus appears very likely that the coroane cell represents an amacrine-like cell common to the retinal ganglion cell layer of all classes of vertebrate. A substantial excess of ganglion cell layer neurons over optic nerve fibers has also been reported for the cat (Hughes and Wasse, '76).

The presence of both presynaptic and postsynaptic regions on the processes of the amacrine cells of the inner nuclear layer (Dowling and Boycott, '66) is definitive for these cells and provides a functional explanation for their lack of an axon. Such synaptic organization has yet to be demonstrated in displaced amacrine and the processes of coroane cells remain to be examined by electron microscopy.

Little is known about displaced amacrine but the coroane cells and amacrine of the inner nuclear layer have many features in common. The coroane cells are usually slightly larger than amacrine of the inner nuclear layer (figure 7,b,e) but the organization of their Nissl substance is very similar to that in some amacrine cells (fig. 8). In primates the lobulated nucleus has been described as distinctive of amacrine (Dowling and Boycott, 1966) but Dubin ('70) has shown that this feature varies from species to species and, moreover, it is a feature common to neurons in general (Peters et al., '76). However, the similarity between some human amacrine cells and coroane cells is quite striking (Hogan et al., '71: figures 9-62, 9-65, 9-67). Like amacrine cells of the inner nuclear layer, coroane cells are rapidly destroyed by low concentrations of kainic acid (Hughes and Narkiewicz, unpublished observations).

Of particular significance is the observation that amacrine cells in man (Missotten, '65), monkey (Dowling and Boycott, '66) and rabbit
RABBIT CORONATE CELLS

(Raviola and Raviola, '69) possess subsurface cisternae which often underlie a junction between a process and the cell soma. Subsurface cisternae are typically associated with nerve cells (Rosenbluth, '62) and are apparent in the electron micrograph of the identified coronate cell (fig. 4). Rabbit amacrine cells possess cupped nuclei like coronae cells and have somatic subsurface cisternae said to be rarely encountered in other classes of rabbit retinal neurons (Raviola and Raviola, '69). The subsurface cisternae of the coronate cell in figure 4 are distinctive, like those of rabbit amacrine cells, in being free of ribosomes on all elements (Raviola and Raviola, '69). The processes contacting coronate cell somata contained small vesicles but our preparation did not permit the identification of synapses. These contacts were similar to the axosomatic contacts on amacrines as described by Meller and Eschner ('65) in various species; in none have such junctions yet been identified as synaptic (Missotten, '65; Dowling and Boycott, '66).

The available evidence thus suggests that the coronae cell lacks an axon but, more importantly, it has several positive features in common with the amacrines of the inner nuclear layer. We tentatively conclude that it is an amacrine-like cell, a "displaced-amacrine," rather than an association neuron. Proof of this requires demonstration that the coronae cell makes synapses and that the same process bears pre- and postsynaptic sites.

CONCLUSIONS

There can be little doubt that coronae cells are neurons although their formation of synapses has yet to be demonstrated. Current anatomical interpretations of retinal ganglion cell function take no account of the presence of either displaced amacrine cells or association neurons. The results of Vaney ('80) show that the number of coronae cells in the ganglion cell layer of the rabbit retina is substantial and it is thus worthwhile comparing their density with the limited data on displaced amacrine cells.

The capricious staining of the Golgi method cannot provide an accurate indication of the relative numbers of each cell type but the high impregnation frequency of displaced amacrines in the bird peri-fovea suggests that they are present in large number (Cajal, '55). This has been independently substantiated by Binggeli and Paule ('69), who found that there are 1.8 million neurons in the ganglion cell layer of the pigeon retina which do not send an axon into the optic nerve: This is equivalent to a mean density of 5,400 displaced amacrines per mm².

In the rabbit the density of coronae cells is much lower, ranging from 360/mm² on the visual streak to 180/mm² some 6 mm below the peak of the streak (Vaney, '80). The presence of about 153,000 coronae and unclassified cells in the ganglion cell layer of the rabbit retina (Vaney and Hughes, '76; Vaney, '80) indicates that there are up to 360 neurons/mm² which do not project into the optic nerve. Similarly, Hughes (77b) has suggested that there is a substantial population of displaced amacrines in the rat retina which amounts to some 1,000 cells/mm². This conclusion is supported by earlier reports (Eayrs, '52; Bunt et al., '74), and the results of Perry (79) are consistent with the presence of such cells.

The presence of substantial numbers of displaced amacrines in the ganglion cell layer of the mammalian retina, although contrary to the current conception of its anatomy which is based on capricious Golgi and methylene blue staining, would harmonize the organization of this region of retina with that accepted for other vertebrate classes in which large populations of such cells are commonly accepted to exist. Moreover, the large numbers of displaced amacrines indicated by Nissl studies on pigeon, rat, and rabbit retinas suggest that the retinal position of these cells is not a morphogenic accident and, therefore, the label "displaced" may be inappropriate. It is difficult to accept the implication of the discussion by Hinds and Hinds (78) that mammalian displaced amacrines accidentally fail to complete their migration to the amacrine cell layer, in view of the situation in reptiles and birds, rather than being "puzzling" that displaced amacrines are present in the mature mammalian retina (Hinds and Hinds, '78) it should be puzzling if they were not present.

Cajal ('55) has suggested that no significance should be attributed to the location of the displaced amacrine soma because it is the layer of the inner plexiform layer at which it synapses which is functionally important. However, it has recently been demonstrated that another "displaced" cell, the displaced ganglion cell of the pigeon retina, forms a discrete population which projects specifically into the accessory optic system (Karten et al., '77). It seems most probable that the soma location of the displaced amacrine also reflects a distinctive retinal connectivity and thus the ubiquitous distribution of displaced amacrines throughout the vertebrate classes suggests that a unique functional role should be sought for them.
Fig. 8. Illustrations 1–12 show select neurons of the amacrine layer which have features similar to coronae cells of the ganglion cell layer (C in frames 13–20). Short arrows indicate the characteristic ring of juxanuclear Nissl substance, long arrows show RNA-filled nuclear invaginations, and open arrows indicate the location of the nucleolus or its out-of-focus image. Demonstration of these cells requires delicate staining; if achieved in the ganglion cell layer then the amacrine layer neurons show rather low contrast. Cells looking like coronae cells are rarer in the amacrine layer than in the ganglion cell layer. They are commonly arranged radially, with the nucleus below the "crown" of Nissl granules which are thus often seen in a separate plane of focus; 1, 2 and 3, 6 show different focal planes of the same cells. Absolute densities have not been estimated for these cells but it is often possible to obtain two or three in a 100-μm square which might contain 60 other profiles in the same focal plane. Cells looking like coronae cells may thus be found in the amacrine layer. It is possible that this simply arises from the presence of common structures in small neurons of different functional type but it is not inconsistent with the suggestion that coronae cells are "displaced" amacrine. The select neurons of the amacrine layer are quite unlike the class of larger neurons with prominent Nissl substance which have been identified as displaced ganglion cells by their filling with HRP applied to the sectioned optic nerve (figure 7).
LITERATURE CITED


RABBIT CORONATE CELLS


A newly identified population of presumptive microneurones in the cat retinal ganglion cell layer

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A large population of microneurones has recently been discovered in the rabbit retinal ganglion cell layer1,2. These 'coronate' cells may represent a class of displaced amacrine cells3,4. Like conventional amacrine cells4 they are swiftly and selectively destroyed5 by low concentrations of kainic acid, a neurotoxin6. No similar cell has been described in the cat retina, which is reported to contain 217-260,000 neurones of classical appearance7. These outnumber the 128-180,000 optic nerve fibres8 but it has been suggested that the excess comprise Nissl-staining glial cells9. We report here that, using the neurotoxic effects of kainic acid to test and confirm the neuronal nature of the classic neurone excess, a large additional population of at least 730,000 presumptive microneurones was revealed. They resemble rabbit coronate cells, do not project into the optic nerve and have been previously identified as presumed glia6,9. Various lines of evidence for the neuronal nature of these cells is presented below, but synapses have not been demonstrated; subsequent reference to microneurones must therefore be regarded as presumptive.

The vitreous humor of cats anaesthetised with Nembutal was injected with either 12 or 120 nM of kainic acid in 10 μl of saline. After 3 h the retina was removed, mounted and stained6.

As shown in Fig. 1, near the area centralis, α, β and larger γ ganglion cells10 seemed to be normal at the lower dosage but, apart from one microglial cell, none of the remaining non-vascular profiles resembled those of normal retina, and all showed kainic-induced necrocytosis; the nuclei of many were pyknotic6. Small γ-mode neurones seemed scarce but the number of necrotic profiles was far in excess of their possible

Fig. 1 Cells in the ganglion cell layer of the cat retina after an intraretinal injection of 10 nM kainic acid. They are situated near the 2,500 classic neurones per mm² isodensity line some 1.5 mm temporal of the area centralis, α (a), β (b) and the larger γ (g). Nissl-stained somas appear in relatively unchanged form. One γ cell near the centre of the figure has begun to degenerate and shows the characteristic nucleus with clumped chromophic material. The large, medium and small arrows indicate profiles in progressively more advanced stages of degeneration. The final product is the pyknotic profile designated by the smallest unlabelled arrows. The open arrow indicates a classic glial cell. The degenerating profiles of this region must represent small cells because the densities of large and medium-sized cells are normal. The number of kainic-sensitive cells far exceeds the possible number of degenerate γ cells. Alcohol/formalin-fixed material. Scale bar, 10 μm.

Fig. 2 a–i. Cells from the untreated cat ganglion cell layer resembling those which are destroyed by kainic acid and have previously been presumed to be glia. Small arrows: RNA-specific basophilia in free cytoplasmic granules; medium arrows: similar material at the juxtanuclear region of the cell's cytoplasmic pole; large arrows: basophilic nuclear inclusions. The nucleus is obvious in cells of a and c, in b it was below the nucleus, j, k Cells of the amacrine layer with features resembling those of kainic-sensitive profiles in the ganglion cell layer (a–i). L Two types of glial nucleus from the nerve fibre layer; one shows clumped, the other dispersed, chromatin. These are from an eye injected with 120 nM of kainic acid and enucleated after 3 h, but their appearance is within the normal range. Cells such as those in a–k were destroyed by this treatment although they lie deeper within the retina. Alcohol/formalin fixed. Scale bar 10 μm.
contribution; the surprisingly large population of pyknotic cells was particularly obvious in the periphery, where ganglion cells are less common.

A 0.013-mm² area near that shown in Fig. 1 contained 75 non-vascular profiles. Of these, 2 were classic glia with obvious nuclear chromatin, 2 were \( \alpha \)-mode cells, 15 \( \beta \)-mode cells and 8 \( \gamma \)-mode cells; the remainder were unlike any profiles of normal retina. Because this region in untreated retina has a \( \gamma \)-cell population equal to the combined \( \alpha \) - and \( \beta \) -mode populations, it is estimated that nine of the novel profiles represent degenerate classic neurones of the \( \gamma \) mode. Thus, 34 profiles (45\%) represent classic neurones; 2 (3\%) were classic glia and the remaining 39 (52\%) represent cells not previously included in the neurone count but which have been destroyed by a neurotoxic compound.

In normal retina this area (Fig. 5A of ref. 6) contains a similar proportion (43\%) of classic neurones, but the remaining 57\% of profiles are presumed to be glia. The most common of these 'presumed glia' lack the conspicuous nuclear chromatin of classic glia and often possess a prominent nucleolus. In kainite-treated retina only 3\% of profiles in the ganglion cell layer were recognisable as glia; the remaining 54\% of presumed glia were missing. There was instead a corresponding proportion of 52\% residual kainic-damaged profiles which we conclude represents the necrotic presumed glia of the normal cat retina; their absolute densities are similar at 2,800 mm⁻² and 2,960 mm⁻² (ref. 6).

Kainic acid does not seem to be gliotoxic, early gial response to kainic acid resembles that to glutamate and is reported to involve swelling but not necrosis and pyknosis. The exposed glial cells of the fibre layer and perivascular glia were unchanged in appearance even at the 10 times higher dose of kainic acid (Fig. 2A, as were glia in the ganglion cell and inner plexiform layers. Müller cell bodies and feet seemed normal, without the damage we observe with DL-aminoacidic acid, a known gliotoxic compound. On this basis, we conclude that the majority of the presumed glia in the normal cat ganglion cell layer are, in fact, neurones.

Figure 3 shows a lightly stained region of normal cat retina; the majority of profiles correspond to the presumed glia of previous reports. The pear shape of some cells (Fig. 2), the juxtanuclear basophilia, bisphosphil nuclear invaginations and

![Image](image_url)

**Fig. 3** A lightly Nissl-stained region of normal retina somewhat more peripheral than that of Fig. 1. The somas of Nissl-stained \( \alpha \) (a), \( \beta \) (b) and \( \gamma \) (g) mode neurones are apparent. The small arrows indicate the profiles of cells previously identified as presumed glia. A large number of these cells are present, as would be expected in a region of normal retina similar to that shown in Fig. 1. However, no such profiles with relatively clear nuclei and juxtanuclear basophilia can be seen in Fig. 1. A quantitative analysis (see text) shows the majority of the glia-sensitive profiles to correspond to the small, pale profiles shown here. These presumed glia are thus sensitive to the influence of a neurotoxic compound not known to have gliotoxic effects. Alcohol/formalin-fixed material. Scale bar 10 \( \mu \)m.

**Fig. 4** The soma diameter spectrum illustrates the size range of non-vitally stained cells in the various classic neurone modes of peripheral cat retina subject to supravital methylene blue staining and non-vital counterstaining. In addition, the stippled regions represent the presumed microglioneurone spectrum previously identified as presumed glia. The location in the soma size spectrum of some supravital methylene blue-stained microglioneurones is indicated in black. The partially stained unequivocal neurones \( h \) to \( k \) occupy the lower range of the microglioneurones. A medium-sized \( \gamma \) soma is shown at \( a \) for comparison. Neurones occupying a region of the diameter spectrum significantly outside the \( \gamma \) mode of classic neurones have not previously been reported. Their presence shows that cells in the class labelled 'microglioneurones' are not too small to be neurones of the cat retinal ganglion cell layer.
cytoplasmic granules, size range and restricted cytoplasm are similar to those of rabbit corona cells. Some possess the 'crown' of juxtanuclear basophilia which led to the term 'coronae' in rabbit (Fig. 2d, f, i); its basophilia to Galloccyanin-lake is confirmed to be RNA-specific in the cut by its elimination with prior ribonuclease incubation, a result similar to that seen in the rabbit. The complex nuclear form is, its basophilic invaginations and cytoplasmic RNA basophilia are characteristically neuronal.

These cells are smaller than any neurons previously described in the retinal ganglion cell layer and are different from reported cat displaced amacrine cells. Supravital methylene blue-stained retinas were examined for examples of their dendritic trees. Figure 4 shows the diameter range of soma profiles for neurons in each preparation. Dendritic trees are shown only for cells with very small, 5-7 μm, somata well displaced from the y-mode population. None are completely stained but the variety of form suggests considerable heterogeneity; their vital staining offers additional evidence for their neuronal nature. Many possess one main dendrite which branches close to the soma. No axons or axon-hillocks were observed and the cells do not fill with horseradish peroxidase from the optic nerve.

In peripheral retina the presumptive microneurons form 75% of the profiles in the retinal ganglion cell layer but their density is lower, 1,550/mm². In a 470-mm² cat retina this suggests a population of at least 730,000 microneurons. Adding the 217,000 classic neurons we estimate a population of up to 10⁶ neurons in the cat ganglion cell layer. This is so far in excess of even the largest optic nerve fibre counts that it precludes the possibility of a significant proportion of the microneurons being ganglion cells. That such a large population has remained undiscovered for so long suggests that they are, like the interplexiform cell, only rarely impregnated by the capricious Golgi stain.

Two classes of neuron which do not project into the optic nerve are recognized in the retinal ganglion cell layer—cells of Marenghi and displaced amacrine cells. Cells of Marenghi, unlike the microneurons of cat and rabbit retina, possess an axon. However, cells similar to microneurons do occur in the amacrine layer (Fig. 2/ k) of both species. Rabbit corona cells, cat microneurons and non-displaced amacranes are all sensitive to kainic acid. It is concluded that the microneurons represent a class of 'displaced' amacrine cell. There are additional reasons for assuming this in the rabbit.

Displaced amacranes are regarded as common in non-mammalian species but have only occasionally been identified in mammalian retina. Their large number in the bird retina has been well demonstrated. Now cat, rabbit and rat are also known to possess a large population of neurons in the ganglion cell layer which are not ganglion cells. No distinctive role has been postulated for either displaced amacranes or the micro-neurone population. The relative uniformity of microneuron density across the retina suggests that these cells function in relation to a fixed-size module of inner nuclear layer neurons.

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A simple technique for the electron microscopy of retinal cells previously identified by Nissl staining and light microscopy

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Key words: electron microscopy — light microscopy — identified cells — Nissl stain

A stage of Nissl staining has been introduced into the routine procedure by which retinal flat mounts are prepared for electron microscopy. This permits photomicrographic survey of areas which are subsequently removed, embedded and thin sectioned. The technique is employed to positively identify members of a given cell class by light microscopy before detailed analysis of the same cells by electron microscopy. The method may be applicable to tissue slices.

The retinal ganglion cell layer of rabbit (Cattaneo, 1922; Vaney and Hughes, 1976; Vaney, 1980; Hughes and Vaney, 1980; Hayden et al., 1980; Vangy et al., 1981; Hughes and Wieniawa-Narkiewicz, 1982), cat (Hughes and Wieniawa-Narkiewicz, 1980; Hughes, 1981) and rat (Eayrs, 1952; Hughes, 1977; Perry and Walker, 1980), has been demonstrated to contain large populations of cells which appear to be displaced amacines. The identification of the more equivocal forms as neurones and the analysis of their connections now requires electron microscopy of serial sections. However, these new classes have so far been characterized by light microscopy alone and transfer of their identifying morphological criteria to the electron microscope is very difficult.

The method of examining glutaraldehyde-fixed, Golgi-impregnated retinas by light microscopy and then embedding selected cells for serial sectioning and electron microscopic examination (Stell, 1965; Blackstad, 1968) is potentially very useful under such circumstances in spite of its technical difficulties (Blackstad, 1970). Unfortunately, the majority of the presumptive displaced amacines do not impregnate readily which may in part account for their belated discovery. It is alternatively possible to cut thick and thin sections from unimpregnated material, examining the former under light microscopy after Nissl staining and the latter with the electron microscope. However, even the transfer of criteria from whole mounts to the 0.5 μm ‘thick’ sections is not straightforward as indicated by the difficulties encountered.
when using this method to identify the electron microscopic features of microglia (Vaughn and Peters, 1968). To make this easier some workers (Hollander, 1970; Grimely, 1965; Cox and Seely, 1974) cut very thick sections, 20-40 µm, in order to visualize more of each cell and then re-embed selected areas for thin sectioning. However, the complexity of the procedure increases and dye penetration into very thick plastic-embedded sections is poor (Berkowitz et al., 1968).

Methods

The following is a very simple technique which introduces Nissl staining between the initial fixation of the retinal whole mount and osmication. A photographic survey of cells in an area of the retinal ganglion cell layer can then be made immediately after staining. Subsequent embedding of blocks of up to 3 mm long for serial sectioning and electron microscopy permits a detailed analysis of each photographically identified cell.

Adult rabbits (3-4 kg) were anaesthetized with Nembutal. The posterior chamber of an eye was injected with 1 ml of a fixative comprising 2% glutaraldehyde, 0.03 M dextrose and 0.1 M phosphate buffer at pH 7.2. A relief needle was introduced at the opposite pole of the eye while the fixative was introduced over a 2 min period. The eye was immediately enucleated and hemisected with a cut 2 mm behind the limbus; the anterior segment and vitreous were removed. The eyecup was immersed for 1.5 h in the same fixative at room temperature and the retina subsequently detached either in saline or fixative, floated onto a gelatinized slide, covered with lint-free filter paper (Whatman-50) and left to adhere to the slide for 0.5 h under a weighed slide immersed in fixative.

The retina was rinsed in saline and stained with 0.1% methylene blue in Dulbecco’s Modified Eagle’s Medium (Flow Labs) or saline. Better results were obtained by this means that if the dye was added directly to the glutaraldehyde. The wet mount was examined under a coverglass by light microscopy until ganglion cells (Vaney, 1980; Hughes and Vaney, 1980) and coronate cells (Vaney, 1980; Hughes and Vaney, 1980) were well stained. The dye was washed from the tissue with fresh fixative and the cover glass replaced. A suitable area about 3 x 5 mm was chosen for photography, the coverslip removed and a razor blade used to cut out the form of an irregular trapezoid for orientation. A small cut on one side provided a landmark for identification of the first cells during microtomy.

The isolated block of retina was placed in fixative on a fresh slide and covered. The selected region was photographed at several planes in sequential overlapping fields at a magnification of ×375 on a Zeiss Photomicroscope III. Exposure to a high light intensity prior to photography bleached the methylene blue background staining more than that of the cells and improved contrast. The result, with an air objective and uncleared tissue, was a delicate and detailed staining which revealed intranuclear folds, invaginations and other important morphological features for cell identification. In some preparations the Müller cell feet begin to break down during photography. This is readily detected if the contrast diaphragm is closed because
A: this illustrates the ganglion cell layer of an uncleared region of a retinal flat mount. The nerve cells were stained by methylene blue. The cells 'g' are ganglion cells, those labelled 'c' are coronate cells (Vaney, 1980; Hughes and Vaney, 1980). A line indicates the plane of radial thin sections cut on the ultramicrotome. B: this low-power electron microscopic photomontage shows a radial section of the ganglion cell layer along the line in A. The comparison of cells in the 2 photographs enables this to be readily confirmed; begin with the two large ganglion cells on the left whose nuclei are just grazed by the plane of section in A and appear as patches of lighter nucleoplasm in the radial sections of B. Arrows indicate cross-sections of coronate cells. The scale represents 50 μm.

Fig. 1.
small bubbles may be observed to form at their margins.

The tissue was stored in fixative while the adequacy of the film was confirmed. It was then processed for electron microscopy in the manner described by Hughes and Vaney (1980). A Reichert microtome was employed to cut several 0.5 µm thick initial sections, which were stained for light microscopy (Richardson et al., 1960). Comparison of these sections with the photographic survey montage made it possible to identify their position and orientation. When this had been achieved, silver serial sections were cut with glass or diamond knives, supported on parlodian and carbon-coated slot grids, stained for 45 s with 0.4% lead citrate (Venable and Coggeshall, 1965) and finally examined in a Philips 301 electron microscope. By using 3 mm-long sections, a large number of cells may be examined simultaneously during serial sectioning and the nature of each identified by reference to the light microphotographs.

Results

Fig. 1A illustrates an area of methylene blue stained ganglion cell layer in which a line indicates the plane of radial sectioning. Fig. 1B is a photomontage of low-power electron microphotographs of a thin section taken along this line. In Fig. 1A the ganglion cells are labelled 'g' and the coronate cells 'c'. The thin section cuts through several ganglion and coronate cells. The arrows in Fig. 1B indicate the EM cross sections of the coronate cells cut by the section plane shown in A. The cells of B may readily be identified with those of A. The basophilic nuclear processes of coronate cell 'c', can be seen to be folds of the nuclear membrane in B. In definitive confirmation of Hughes and Vaney (1980) it is apparent that the cytoplasm of ganglion cells is darker staining than the nucleoplasm whereas there is little difference between their intensity in coronate cells. Such criteria accumulate with observation and subsequently enable confident identification of these and other cell classes without light microscopic identification. The quality of the EM material after this procedure is satisfactory and it is possible to trace processes into the plexiform layer and identify synapses for the ultrastructural analysis of connections (Hughes and Wieniawa-Narkiewicz, 1982).

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References

RETINAL ORGANISATION
SAMPLING THEORY AND RETINAL ORGANISATION
Cat Retina and the Sampling Theorem; the Relation of Transient and Sustained Brisk-Unit Cut-off Frequency to $\alpha$ and $\beta$-Mode Cell Density

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Summary. The relationship of brisk transient and brisk-sustained unit cut-off frequencies to their respective array cut-off frequencies has been calculated by means of sampling theory from the corresponding $\alpha$ and $\beta$-mode cell densities at various retinal eccentricities. Interpretation of the results depends on whether on and off cells of each class are functionally homogeneous or heterogeneous populations. In agreement with expectations from sampling theory for a system involved in resolution tasks, it is found that the brisk-sustained system has less potential for undersampling and spurious imagery than the brisk-transient system, which is thought to play a different role. The brisk-sustained array could avoid such aliasing altogether if it is functionally heterogeneous. The differences between the two systems are similar to those between insect eyes respectively optimised for high resolution vision or the detection of high velocity image motion.

Key words: Retina - Sampling theorem - Brisk, X and Y, ganglion cells - Cat - Visual acuity

Of some nine classes of ganglion cell encountered in cat retina, only brisk-sustained, X, units have been suggested to mediate form recognition and resolution tasks (Enroth-Cugell and Robson 1966; Cleland et al. 1971; Ikeda and Wright 1972; Stone et al. 1979). The non-linear behaviour, low peak density and other properties of brisk-transient, Y, units have rather suggested their involvement in orientation reflexes or movement detection (Enroth-Cugell and Robson 1966; Cleland et al. 1971; Ikeda and Wright 1972; Stone et al. 1979). The receptive fields of both brisk classes form two-dimensional arrays which sample the retinal image and relay some of its information to the brain. The potential performance of such arrays in resolution tasks has intrinsic bounds which are defined by sampling theory. Because of the marked difference between the roles often attributed to the two brisk systems in the current consensus, it is interesting to compare the extent to which they are organised and perform in accord with sampling theory.

Background to Sampling Theory

Any retinal image can be treated as if composed from the images of a set of independent sinusoidal gratings with differing frequencies, phases, and amplitudes; these are its Fourier Components (Bracewell 1965). Measurement of retina or visual system performance in terms of the highest frequency of grating which it can faithfully transmit is thus of general importance because it specifies the highest frequency of Fourier Component available at the output and thus defines the complexity and quality of image that can be relayed.

Helmholtz long ago pointed out that at least one relatively unstimulated element must be interposed between each of those activated by bright bars if a sampling array is required to resolve a grating pattern (Helmholtz 1924). In modern parlance, a grating so matched to a sampling array is said to be at the cut-off frequency, fc, because it is the highest frequency which the system can accurately transmit. A grating of lower frequency than this is oversampled but nevertheless generates a unique set of output values which define the original stimulus with no loss of information and enable its accurate reconstruction, fc/2 Fig. 1. A grating of frequency higher than fc, however, is undersampled and does not generate a unique array output. The original stimulus thus cannot be reconstructed and masquerades as a spurious lower frequency signal. Loss of information by this means is known as 'aliasing' and is readily understood in its simplest form by considering a grating of frequency 3fc sampled by an array at the rate 2fc, Fig. 1. The array output wrongly suggests the stimulus to be of the critical frequency fc, Fig. 1, and although its presence is detected, it is not resolved.

These ideas now have a formal basis in the sampling theorem (Cauchy 1841; Gabor 1946; Shannon and Weaver 1949, Yen 1956). Given an array of n elements per degree, the component of highest spatial frequency, fc, in a one-dimensional brightness distribution which can be relayed by the array without loss of
Application of Sampling Theory to Brisk Units

The sampling theorem has been applied to the insect eye (Barlow 1965; Snyder et al. 1977), to the relationship between human acuity and foveal photoreceptor density (Green 1970) and to the estimation of the upper limit on the behavioural resolution of sheep (Hughes and Whitteridge 1973) and cat (Hughes 1975) from the peak density of ganglion cells in the area centralis. However, the latter procedure may lead to gross overestimation if several independent populations are present in unknown proportions.

Recently, the separate distributions and density profiles of the α and β-cell populations of the cat retina have been estimated (Peichl and Wässle 1979; Hughes 1981). Brisk transient units have been identified as α-cells (Cleland et al. 1975) and brisk sustained units are considered to correspond to the β-cells (Boycott and Wässle 1974; Cleland and Levick 1974; Fukuda and Stone 1974; Levick 1975; Peichl and Wässle 1979); density distributions are thus available for each functional class. With increase in eccentricity from the area centralis, the density of each cell class decreases, the receptive field diameters increase and the corresponding cut-off frequencies are therefore reduced. Now that Cleland et al. 1979) have described the decline in grating cut-off frequency of brisk-transient and brisk-sustained units as a function of eccentricity, it is possible to compare single unit cut-off frequencies with those computed from the local cell density of the corresponding morphological cell class over a wide range of densities.

If brisk-sustained cells really are the substrate of grating resolution, and if they are organised in accord with the requirements of sampling theory, then their local cut-off frequency and density might be expected to be related by a formula like (1) or (2) above. By contrast, the brisk transient cells are unlikely to be involved in high resolution tasks so that there is no reason to expect their cut-off frequencies and densities to be related by the above equations. Indeed, it may be advantageous for movement detecting cells to pass frequencies above that of the array cut-off (Snyder 1979).

Comparison of Array and Element Cut-off Frequencies Over a Range of Eccentricity

The mean spatial cut-off frequencies of brisk transient and sustained units at various midline eccentricities, Figs. 2A and 3A, respectively, are plotted after Cleland et al. (1979). The continuous curves were computed from their fitted relationships and indicate the element cut-off frequency, fc, as a function of eccentricity.

The density distribution of α-cells in the corresponding regions of retina, Fig. 2C, was obtained by

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Fig. 1. Three grating patterns of different frequencies are illustrated in cross section to the left of the figure. One, of frequency fc, is optimally matched to a sampling array symbolised by the row of arrowheads at frequency 2fc. The two values sampled by the array in each grating cycle suffice for reconstruction of the original signal, fc, with no information loss, as demonstrated to the right hand side. Frequencies which are lower than the critical value fc defined by sampling theory, such as fc/2, are oversampled but reconstructable. Frequencies such as fc, which are higher than the critical value fc, are undersampled and 'alias' as lower frequencies, fc in this instance, which becomes apparent when the output of the array is reconstructed.
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Information is n/2 = fc. Given a two-dimensional array of density d = n² then the highest spatial frequency which can be unambiguously specified in a two-dimensional brightness distribution is,

$$f_c = \sqrt{\frac{d}{4}}$$

for a square array or,

$$f_c = \sqrt{\frac{d}{3.46}}$$

for a hexagonal array after Snyder and Miller (1977).

If a sampling array is to be free from spurious imagery then it must not be presented with a significant signal energy above the cut-off frequency, fc, determined by its density. In man-made systems this is commonly achieved by interposing a filter before the array. Whether, or not, a system is organised to be free from aliasing may thus be determined by comparing the cut-off frequency calculated from its array density, fc, with that above which the output modulation of its individual elements is negligible, fc., when presented with a range of spatial frequencies. Only if fc is equal to, or higher than, fc, is the potential for spurious imagery absent.

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Application of Sampling Theory to Brisk Units

The sampling theorem has been applied to the insect eye (Barlow 1965; Snyder et al. 1977), to the relationship between human acuity and foveal photoreceptor density (Green 1970) and to the estimation of the upper limit on the behavioural resolution of sheep (Hughes and Whitteridge 1973) and cat (Hughes 1975) from the peak density of ganglion cells in the area centralis. However, the latter procedure may lead to gross overestimation if several independent populations are present in unknown proportions.

Recently, the separate distributions and density profiles of the α and β-cell populations of the cat retina have been estimated (Peichl and Wässle 1979; Hughes 1981). Brisk transient units have been identified as α-cells (Cleland et al. 1975) and brisk sustained units are considered to correspond to the β-cells (Boycott and Wässle 1974; Cleland and Levick 1974; Fukuda and Stone 1974; Levick 1975; Peichl and Wässle 1979); density distributions are thus available for each functional class. With increase in eccentricity from the area centralis, the density of each cell class decreases, the receptive field diameters increase and the corresponding cut-off frequencies are therefore reduced. Now that Cleland et al. 1979) have described the decline in grating cut-off frequency of brisk-transient and brisk-sustained units as a function of eccentricity, it is possible to compare single unit cut-off frequencies with those computed from the local cell density of the corresponding morphological cell class over a wide range of densities.

If brisk-sustained cells really are the substrate of grating resolution, and if they are organised in accord with the requirements of sampling theory, then their local cut-off frequency and density might be expected to be related by a formula like (1) or (2) above. By contrast, the brisk transient cells are unlikely to be involved in high resolution tasks so that there is no reason to expect their cut-off frequencies and densities to be related by the above equations. Indeed, it may be advantageous for movement detecting cells to pass frequencies above that of the array cut-off (Snyder 1979).

Comparison of Array and Element Cut-off Frequencies Over a Range of Eccentricity

The mean spatial cut-off frequencies of brisk transient and sustained units at various midline eccentricities, Figs. 2A and 3A, respectively, are plotted after Cleland et al. (1979). The continuous curves were computed from their fitted relationships and indicate the element cut-off frequency, fc, as a function of eccentricity.

The density distribution of α-cells in the corresponding regions of retina, Fig. 2C, was obtained by
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Fig. 2. A The physiologically measured cut-off frequencies for cat brisk-transient units at various vertical midline eccentricities are denoted by filled circles and fitted by a bold continuous line (Cleland et al. 1979); vertical lines indicate estimated S.E.M. The strip of horizontal hatching denotes the range bounded by cut-off frequencies computed from the β-mode cell density on the assumption of either a hexagonal array, h, or a square array, s, if the on and off outputs are employed in a single heterogeneous system. The vertical hatching and bounding lines indicate similar cut-off frequencies computed on the assumption that the α-mode cell population is made up of separate homogeneous on and off systems. The theoretical and physiological curves are of similar form but it is apparent that the cell cut-off frequencies exceed either array cut-off frequency at all eccentricities and that the potential for aliasing is unavoidable if significant energy is present in the image above the array cut-off frequency. B α-cell frequency derived from the curve of C. C α-cell density as a function of vertical midline eccentricity (Hughes 1981). The theoretical curves were calculated from this data by the use of equations (1) and (2) above.

Fig. 3. A The conventions for this figure are similar to those of Fig. 2A, but the physiological cut-off frequencies are those measured by Cleland et al. (1979) for brisk-sustained cells at various eccentricities. It is apparent from the theoretical curves that the heterogeneous hexagonal and square arrays bound a region which is quite a good fit to the physiological results. This indicates that the brisk-sustained, β-mode, array can avoid aliasing. If, however, it functions as separate, homogeneous on and off populations then the potential for aliasing is inevitable because the physiological cut-off frequencies of the individual cells would be substantially above the local array cut-off frequency. B Estimated β-mode cell frequency derived from the density curve of C. C β-mode cell density as a function of vertical eccentricity above the area centralis (Hughes 1981). The discs represent estimates made from spectral proportions; the squares, an estimate from another retina obtained by direct identification according to morphological criteria.
A. Hughes: Cat Retina and Sampling Theory

direct mapping (Hughes 1981). The β-mode density distribution was determined from the local ganglion cell densities and the proportion of ganglion cells in the β-mode of the local neuronal diameter spectra of noncentral retina, Fig. 3C discs. A confirmatory distribution was also obtained by direct identification according to morphological criteria, Fig. 3C squares. The peri-central proportion of 60% β-mode cells was extrapolated into the central area, where the soma diameter spectrum is unimodal and precludes direct assessment of the β-mode percentage from the diameter spectrum, to obtain an estimated peak β-mode density of some 6,000 cells mm² compared to the 6,500 mm² directly identified by Pfeihl and Wässle (1979). The continuous curve is fitted by eye to the mean of the two sets of results, Fig. 3C, and provides a basis for calculation of the array cut-off frequency, fc.

Retinal distances and densities were converted to angular terms by the use of a retinal magnification factor of 0.2 mm/deg (Hughes 1976) without correction for 1-2% shrinkage (Hughes 1975).

It is well known that brisk units possess either on or off centre receptive fields. When the retina is explored systematically these types are encountered in equal frequency (Cleland and Levick 1974). Correspondingly, the equivalent morphological classes are divisible into on and off populations which branch at different depths in the inner plexiform layer (Nelson et al. 1978). The α and β populations are approximately comprised of nearly equal proportions of the two classes (Wässle et al. 1981; Wässle et al. 1981). Calculation of array cut-off frequency is thus complicated by the need to consider two models for each class. For one it is assumed that the sampling array is comprised of a mixture of on and off cells which form a single functional system, a heterogeneous array, and the sampling density is then taken as equal to the local density of cells in the α or β-mode.

For the alternate model it is assumed that on and off cells form two homogeneous but independent sampling populations of equal density; their sum is then equal to the density of all cells in the α or β-mode. The local values of α or β-mode density, or half this for the homogeneous model, were substituted at one degree intervals of vertical eccentricity into equations (1) and (2) above in order to compute the theoretical array cut-off frequency for ideal heterogeneous and homogeneous systems with square or hexagonal arrays, respectively.

The heterogeneous models — on and off forming one system — are represented in Figs. 2A and 3A by a strip of horizontal hatching; the homogeneous models — on and off as separate systems — by vertical hatching. The continuous curves which define the upper and lower bounds of each of these strips indicate cut-off frequency for hexagonal and square arrays, respectively. They were fitted by eye to the calculated points.

It is apparent that the physiological cut-off frequencies are very similar to those independently calculated from the anatomical data. The curves for the physiological results fall-off as α or β-mode cell frequency, Figs. 2B and 3B, rather than density, Figs. 2C and 3C.

Before treating the implications of these results in detail it remains necessary to consider their reliability. The cut-off frequencies predicted by the heterogeneous and homogeneous models differ by only √2 and neither physiological curve is more than an octave displaced from a theoretical curve. Interpretation of the results would be very sensitive to systematic errors. However, gross errors in the data are unlikely. Improved fit between the physiological results and the theoretical models could be obtained only if the cell densities/deg are underestimated or the cut-off frequencies overestimated. The small conceivable errors operate in the opposite direction.

The accuracy of the retinal magnification factor is critical when converting retinal dimensions and densities to angular terms but its variation over the entire published range for central retina, from 0.19 mm/deg to 0.21 mm/deg (Vakkur et al. 1963; Cleland et al. 1975; Hughes 1976), causes only a 4% change in the estimated cut-off frequency. If some shrinkage is uncorrected, or there are other classes of cell in the β-mode of the diameter spectra, then correction would only increase the gap between physiological data and the theoretical models. The use of smaller pupils, increase in light intensity or improvement in sensitivity of the physiological cut-off criterion would result in similar effects.

Implications of the Results

For each class of brisk cell the Figs. 2 and 3 show the ratio between physiological and array cut-off frequencies to be relatively uniform across the retina. However, accepting the results to be free from substantial error, the brisk-transient and brisk-sustained systems appear to be differently organised:

1. The array cut-off frequency for the brisk-transient, α-system, cells functioning as either a heterogeneous or a homogeneous population is at least an octave below the physiological cut-off frequency of its individual elements at all eccentricities examined. The brisk-transient system must therefore be subject to aliasing.

2. By contrast, the physiological cut-off frequency of the brisk-sustained, β-system, cells is well matched to the area bounded by the square and hexagonal array models on the assumption of a single heterogeneous population of on and off cells. However, if the population functions as separate homogeneous on and off arrays then aliasing would be as inevitable as in the α-system.
In spite of differences in the region of retina explored, in the method of stimulus movement, in the technique of judging cut-off, the use of a 12-fold lower light intensity and of pooled rather than localised samples it is found that Peichl and Wässle's (1979) physiological and anatomical results are similar to those above outside the area centrals. However, the situation reverses at the area centrals where their brisk-sustained cut-off frequencies are consistent with separate homogeneous systems free of aliasing. The higher cut-off frequencies of Cleland et al. (1979) possibly reflect a closer approach to the centre of the area centrals which is facilitated by their technique of midline identification and search. The current diversity of anatomical and physiological results from the area centrals has led to its emphasis in this study and a greater reliance being put on comparison of data from a wide range of eccentricities.

Studies of the insect compound eye have revealed conspicuous correlations between the tolerance of potential aliasing and life style (Snyder et al. 1977; Snyder 1979). These parallel the relationship between the above results and the working hypotheses put forward earlier. The a-cell array, postulated to be involved in movement detection, resembles the eye of certain diurnal insects (Snyder et al. 1977) which appear to accept significant undersampling, and the potential for aliasing, in order to gain adequate contrast for dealing with images in motion at high angular velocities. The presence of aliasing has actually been demonstrated in Drosophila (Goetz 1965) but is presumably of little significance relative to the advantage accrued in the natural habitat (Snyder 1979).

By contrast, the β-cell array has been suggested to be involved in visual tasks requiring high resolution. If it is organised as a heterogeneous array then it resembles the eyes of photopic, hovering insect predators whose high acuity regions are free from significant undersampling (Snyder et al. 1977).

However, it is not inevitable that a resolution system should avoid undersampling and a pair of homogeneous arrays of β-cells is plausible. In the normal habitat there may not be significant energy at high frequencies or the spurious imagery may be insignificant to the species. A more sophisticated treatment of the sampling process, which involves optimising both resolution and contrast in the presence of noise to obtain a minimum for the number of potential 'pictures' transmissible by the array (Snyder et al. 1977; Snyder 1979), indicates undersampling to be optimal under certain conditions of illumination. Whether the visual system avoids significant aliasing in the β-cell system, or accepts it, primarily depends upon whether the on and off arrays are functionally heterogeneous or homogeneous in acuity tasks.

In principle, this might be determined from measurements of cat grating resolution. Postulating a maximum β-cell density of 7,000 mm² (Hughes 1981), then a hexagonal array could attain 9 cyc/° as a heterogeneous system or 6.4 cyc/° as two homogeneous systems. The behavioural results of many earlier workers indicate cat acuity to be about 6 cyc/° (Smith 1955; Mair and Mitchell 1973; Blake et al. 1974). However, the use of greater light intensities in more recent behavioural (Mitchell et al. 1977) and evoked potential (Harris 1978) experiments indicates a peak acuity of 8.5–9.5 cyc/°, in good correspondence to the brisk-sustained unit cut-off frequencies of Cleland et al. (1979). Although the results of Jacobson et al. (1976) also support the higher value, there are indications that aliasing may have occurred. Confirmation of a 9 cyc/° peak grating resolution would count against the possibility of homogeneous arrays, especially if demonstrated in an orientation discrimination task which reduces the possibility that aliasing enables grating detection at frequencies for which resolution has failed.

Whatever the outcome, the above results suggest that, if the two brisk systems are similar in their functional array organisation, the postulated high resolution β-system will always be subject to less potential aliasing than the α-system.

Wässle (1981) has recently described the matrices of shallow and deep branching β-cells which are claimed to correspond to the functional on and off populations (Nelson et al. 1978). They form independent correlated arrays with the nearest neighbour to each cell almost invariably of the opposite class. Such organisation is necessary for, and consistent with, a heterogeneous array but is not inconsistent with functional independence. The irregularity of the distributions (Wässle 1981) is not necessarily detrimental to their performance because sampling theory permits accurate reconstruction from all arbitrary distributions, albeit with impossible requirements of accuracy in extreme cases (Yen 1956; French et al. 1977; Hughes 1977).

The Variation of Visual Acuity with Eccentricity

The axons of retinal ganglion cells provide a final common path from the eye; if central processing does not further limit performance, then the anatomically defined variation of β-cell cut-off frequency with eccentricity and the corresponding physiological results of Cleland et al. (1979) could be regarded as
independent hypotheses of how behavioural grating acuity varies along the lower vertical meridian in the cat visual field. Grating resolution in this animal would thus be expected to vary with eccentricity as \( f \)-mode cell frequency. This obvious theoretical possibility is postulated by some (Frisen and Frisen 1976; Drasdo 1977; Rovamo and Virsu 1979) to pertain between human total ganglion cell frequency and acuity.

Elsewhere it has been suggested that human and monkey acuity vary as cortical magnification factor (Cowie and Rolls 1974; Daniel and Whitteridge 1961; Cowey and Ellis 1969). Thus, because central magnification factors have often been described as varying with ganglion cell density (Rolls and Cowey 1979; Jacobsen 1962; Hughes 1971; Sanderson 1971; Webb and Kans 1976) it pragmatically follows that acuity varies as ganglion cell density (Rolls and Cowey 1979; Hughes 1977; 1978). In this instance cat acuity would fall-off as \( f \)-cell or, more approximately (Hughes 1981), as ganglion cell density.

At present, the data available for man and other species is of inadequate quality to permit discrimination between the two hypotheses. However, the two models suggest quite different rates of fall-off in visual acuity as a function of eccentricity. Their predictions from the above results differ nearly sixfold at 20° in the cat's visual field. Unfortunately the peripheral acuity of this species has only been measured in individuals with diseased retinas (Blake and Bellhorn 1978). Hopefully this situation will soon be rectified.

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A. Hughes: Cat Retina and Sampling Theory

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THE TERRAIN THEORY
A comparison of retinal ganglion cell topography in the plains and tree kangaroo

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Retinal ganglion cell distribution maps were made from cresyl-violet-stained flat mounts of the retinas of (Fig. 1A) Megalagia rufa, the red or plains kangaroo, and (Fig. 1B) Dendrolagus doriana, Doris's tree kangaroo from the high-altitude rain forest of the New Guinea Western Highlands.

The red kangaroo is seen to possess a visual streak whose peak count lies at its temporal end in the presumed forward fixation area; above this area the low-value isodensity lines bulge upwards and serve the image of the ground in front of the mouth as in sheep (Hughes & Whitteridge, 1973) and rabbits (Hughes, 1971).

The tree kangaroo retinal ganglion cell distribution is more radially uniform; the low-value isodensity lines form horizontal ovals like those of man and monkey but it is curious that the high value isodensity lines which define the area centralis are vertical ovals.

The visual streak is as well developed in the grey forester, Macropus giganteus, as in the red kangaroo and yet the former never enters open country; the streak is thus not an exclusive feature of open-country species but rather correlates with certain classes of terrestrial as opposed to arboreal life-style. Although the visual horizon is not overt in forested country the same gradation of visual texture (Gibson, 1950) which defines it on the plains is present in the retinal image and it remains invariant in positional relationship with the retina. Under these conditions uniform sampling would be redundant and a species in either habitat which required panoramic surveillance might optimally match its peak retinal ganglion cell densities to the region of finest texture in the retinal image of the overt, or implicit, horizon. In the arboreal habitat there is no external feature which projects in fixed orientation on to the retina and those species which occupy it most commonly have a high degree of radial symmetry of the retinal ganglion cell isodensity lines about the fixation point.

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Fig. 1. A. The left retina of a red kangaroo, *Megalia rufa*. B. The right retina of a Doria's tree kangaroo, *Dendrolagus doriana*. The maps are based on a 1 mm square sampling matrix which was reduced to a 2 mm matrix for counting the peripheral regions. No blood vessels are visible on the retinal surface of these marsupials. Glial cells were readily discriminated from the neurones in the Nissl stained material. The isodensity lines indicate retinal ganglion cell density per mm², usually progressing in steps of 1000 cells/mm².
LETTER TO THE EDITORS

ONE BRUSH TAILED POSSUM CAN BROWSE AS MUCH PASTURE AS 0.06 SHEEP WHICH MAY INDICATE WHY THIS "ARBOREAL" ANIMAL HAS A VISUAL STREAK: SOME COMMENTS ON THE "TERRAIN" THEORY

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The "terrain theory" (Hughes, 1975, 1977) suggests that a visual streak is correlated with terrestrial, aquatic and aerial lifestyles in which the image of an extended terrain, whether open or partially obscured, is formed in the orientation on the retina for even part of the time; the arboreal species examined did not possess visual streaks and were attributed with more radially uniform ganglion cell distributions. However, the presence of a visual streak in the arboREAL brush tailed possum led Freeman and Tancred (1978) to conclude that its ganglion cell distribution, "does not support either of these hypotheses". This result has recently been cited as demonstrating failure of the terrain theory (DeBruyn et al. 1980).

Now possums do show arboreal specialisations, but an old Australian pseudadoxia epidemicus is that they interbreed with rabbits and this arose because possums thrive as well in rabbit wars as in trees (Wood-Jones, 1923). In Western Australia they are called "ground possums" because they live in rock crevices and other terrestrial homes. Even when they do live in trees, they browse clover and grass (Mason, 1958); pasture forms 30% of their diet in New Zealand so that 17 possums graze as much as one sheep (Gilmor, 1965; Harvie, 1973). Winter (1977) reports that 14% of the time away from the nest is spent on the ground and that 22% of feeding is terrestrial. At least 14 nights of the lunar month provide a moonlit terrain in their open canopy habitats so that much time is spent under conditions in which a visual streak would be appropriate according to the terrain theory.

Thus Freeman and Tancred (1978) unfortunately took no account of the available behavioral data before casting doubt on the terrain theory. The need for caution in categorising species as "arboreal", "terrestrial", "nocturnal" etc as if these are exclusive qualities is illustrated by Cartmill (1972) and discussed by Peterson and Ulinski (1979) in relation to the terrain theory.

It should be reassuring to the proponents of the terrain theory that possum retinal organisation is interpretable in this fashion. But all is not well! Hokoč and Oswaldo-Cruz (1979) describe the South American opossum as possessing a radial ganglion cell distribution. Perhaps this species spends more time in the trees? But Anthony (1928) writes, "Although the hind feet, with grasping great toes and the prehensile tail are arboreal specialisations, the Opossum is perfectly at home on the ground and may wander considerable distances in search of food without taking to the trees". Future theories about retinal ganglion cell distribution obviously need a basis in more extensive ecological and behavioural data.

DeBruyn et al (1980) raise two interrelated problems by describing the visual streak as a horizontal specialisation and noting their failure to account for vertical isocount bulges in terms of some feature of the image analogous to the horizon. But the terrain theory does not suggest that the visual streak is matched to the image of the visual horizon. The streak, or any other distribution, is rather seen as minimising redundancy only for a subset of the image, the information array, which contains those elements significant in the animal's lifestyle.

On an extended terrain the images of objects at increasing distance from the eye are formed at progressively reduced vertical eccentricity. An animal may thus minimise sampling redundancy by progressively increasing sampling density in passing from superior to equatorial retina (Hughes, 1977). Complete or partial compensation for the effects of the law of visual angle may then be achieved but the specialisation is vertical, not horizontal as claimed by DeBruyn et al. (1980). The horizontal extent of the streak, as determined by variation in the peak count and vertical gradients with increasing horizontal eccentricity, is a function of the weighting given by the animal to uniform treatment of the entire horizontal field of view and quite independent of adaptation to the horizon (compare dog and rabbit; Hughes, 1977).

In all mammalian instances known to me, the peak density of the vertical isocount bulges lies in the meridian which contains the central areas and intersects with the median sagittal plane when the eyes are in...
the primary position (Hughes, 1977). Now, upper and lower central field is important to all species during forward locomotion because it deals with the space through which body and head must pass and raised sampling densities would minimise excursions of fixation from the image of destination point. In grazing and cursorial species the superior density bulge can be very conspicuous—the anakatabatic area (Hughes, 1977)—to increase information capture over that important range of frontal terrain containing the destination, the ground to be encountered by the feet and food to be grazed. The ganglion cell distribution thus crudely defines a species' weighting on information from any region of its potentially spherical visual field. The common presence of several ganglion cells classes introduces the possibility that each may access “information arrays” of differing form. The z, β and γ cells of the cat retina are claimed to have different distributions (Rowe and Stone, 1976) but this has been contested (Hughes, 1977, 1981; Wässle, 1980).

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ARE RETINAL NON-CONCENTRIC UNITS UNIVERSAL?
THE RECEPTIVE FIELDS AND TOPOGRAPHICAL ORGANIZATION OF GOAT RETINAL GANGLION CELLS

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According to the descriptions of Schwalbe (1887), Chevitz (1891) and Zürn (1902) the sheep and goat have a limited circular or oval area centralis about 10 mm to the temporal side of the optic nerve head, but have no visual streak. In contrast, Slonaker (1897) and Johnson (1901) both describe a bandlike region extending across the retina, but not an area centralis.

As part of a more extensive study of mammalian ganglion cell topography we have constructed a ganglion cell density map for sheep and goat retinas. We find both groups of earlier investigators to be correct in their positive findings and that the two species possess a well developed circular area centralis at the temporal end of an extensive visual streak.

The investigation of retinal unit properties in the goat was undertaken in order to determine their distribution in different parts of the retina of a species with this mixed ganglion cell topography. It should be noted that we use the term "area centralis" to refer to the region of high count at the temporal end of the visual streak because this is conventional although strictly inappropriate.

METHODS

Preparation

Four goats weighing about 15-20 kg were anaesthetized with a dose of 400 mg of Veterinary Nembutal (Abbott Laboratories) which was injected into the jugular vein. The animal was immediately fitted with a tracheal cannula, carotid loops were placed in position and the eyelids were sutured together after treating the cornea with a 1% silicon fluid (Hopkin & Williams, M.S. 100) to minimize evaporation and wrinkling. A limb vein was catheterized for the administration of further doses of Nembutal and for the provision of a drip during the experiment.

The top of the skull was removed and the goat was decerebrated by suction while taking care not to damage the blood supply to the optic tract and chiasm. Residual bleeding after the tension on the carotid loops had been removed was controlled by Gelfoam and pressure. The ears were then "buttonholed", all wounds were treated with Polymyxin wide band antibiotic spray and dressed. Crystopan benzylpenicillin (0-6 g) was administered and the animal transferred to a stereotactic frame.

The body temperature was maintained by a homeothermic blanket equipped with a rectal thermometer. A polythene bag fitted to the nose and mouth collected the copious saliva. Fluid balance was established by a saline drip at a rate of about 500 cm\(^3\)/hr. Dextran was administered via the drip immediately after surgery (Macrodex, Pharmacia Ltd.) and then followed by saline or 4% glucose saline as appeared appropriate. Micturition occurred regularly throughout the ensuing 20-30 hr of the experiment.

The blood clots were removed from the optic tract and chiasm with the help of a Zeiss Ikon dissecting microscope. In the goat the optic nerves are not exposed within the skull cavity but appear only as they enter the chiasm. For one experiment the bony covering of the nerve was nibbled away but in the remainder the recordings were made from the optic tract as it left the chiasm. The dura covering the nerve was torn open with electrotyically sharpened tungsten wires and the cavity covered with liquid paraffin to a depth of about 1 cm.

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Each eyelid was fitted with a weighted thread so that the corneas could be protected from evaporation when the eye was not in use; further applications of the silicon liquid were made at intervals. The eyes were not saturated to a ring but two sutures were made in the cornea–scleral margin, one at the nasal and one at the temporal end, and these were used to anchor the eye to the stereotactic frame. Contact lenses were not available and no correction for viewing distance was made. The eyes were observed to be almost emmetropic.

Recording

Extracellular spike recordings were made from single fibres of the optic tract with 2-10 MΩ tungsten electrodes. Some of the electrodes were coated with four separately baked coats of bakelite varnish; others were given a single coat of Stoner–Mudge varnish. The electrodes were driven down vertically by a hydraulic drive which was mounted on a Prior micro-manipulator to allow displacement in the horizontal plane. Penetrations were made into the right and left optic tracts and these were shifted laterally during the course of the experiment so that samples of contralateral and ipsilateral units could be obtained.

The signals were recorded through an FEI input stage, fed to a Tetronix 122 preamplifier and then to a Tetronix 565 equipped with two 3A74 amplifiers. The raw data was fed to one channel of a Ferrograph tape recorder while the other channel was supplied with monitor information from photocells and the shutter which was recorded by a simple FM system.

Visual stimuli. The goat was placed upon a sturdy rotatable table facing a large steel tangent screen about 1 m distant upon which sheets of plotting card could be secured by magnets. After obtaining a unit it was thus possible to rotate the animal carefully, so that its (receptive) field fell on the tangent screen.

The position of each unit was recorded with respect to the sagittal plane passing through the eye and to the horizontal plane by means of a protractor and a thread extending from the eye to the receptive field plot. The distance of the plot was also recorded. The optic nerve head was observed ophthalmoscopically and its elevation and angle to the parasagittal plane noted frequently. In the majority of eyes the ascending blood vessels leaving the optic nerve head were vertical so that the eye was not rotated in the parasagittal plane and the visual streak was horizontal. If this was not the case the appropriate corrections were made in the subsequent calculations.

When mixing the data from the four experiments the above measurements were used to convert the unit positions to a cylindrical coordinate system with a correction applied to normalise any changes in the optic nerve head projection. The four sets of results were then nomographically transformed to bring the optic nerve heads to a standard position on the horizontal and projecting 50° temporal of the midline. The projection of the main ascending blood vessels was kept vertical in all cases.

The tangent screen could be illuminated by a 60W bulb fed from a variac transformer to provide simple background lighting control. A variety of prepared stimuli on 2 × 2 slides could be projected by a Leitz Prado slide projector which was fitted with a slide carriage, an iris diaphragm, a neutral density wedge of 2 log unit range and a shutter which could be remotely controlled. The output of this system passed to a gimbals–mounted front-surfaced mirror which could be rotated in two planes by hand or in one selected plane by a sturdy pen motor driven from the oscilloscope time base. The position of the stimuli was monitored by two photocells equipped with magnets which could be positioned anywhere on the tangent screen.

The iris diaphragm greatly facilitated the demonstration of a surround and the estimation of its magnitude in the units with a concentric organization. During expansion of the projected spot the increasing area and moving edge elicit a response from “on” areas, during contraction “off” areas are stimulated. The position of the beginning and end of firing is easily marked on the record card and overlap indicates the presence of an “on–off” area. Wands bearing various sizes of card disc and other card stimuli were also used in the mapping of the receptive fields.

Schematic eye data. The optical data reported subsequently was obtained from the enucleated eyes of one animal. After enucleation the sclera was cleaned and each eye was in turn connected via a hypodermic needle in the vitreous to a saline reservoir of adjustable height. The pressure in the eye was set to 30 cmH₂O. The eye was centred in a perimeter with the temporal border horizontal and the ascending blood vessel vertical. The extent of the visual field in the horizontal was established by noting the point on the perimeter at which the retinal reflex observed through a hand held ophthalmoscope ceased when passing to the nasal and to the temporal limits of the field. The angle observed was identical to that at which the transversely viewed retinal image of the ophthalmoscope light transferred to the iris outer surface.

The retinal magnification in the normal goat eye was determined by setting two pea bulbs 22 cm apart at 60 cm from the corneal vertex. The distance between the two transcollerally viewed images of the light sources was measured by means of a travelling microscope for temporal, central and nasal retina. The distance on the retina corresponding to 1° in the visual field is then readily calculated.

Retinal flat mount. The ganglion cell counts were carried out on the left and right retinas of one animal. These were dissected free under saline by gentle brushing with a small piece of cotton wool held in watchmaker's forceps. The retinas were removed as far as the ora serrata in order to ensure that they represented the whole organ in horizontal extent. The hemispherical retina was made to lie flat with minimal folding by means of four radial cuts in the periphery. While the isolated retina was floating in the saline bath a
The Receptive Fields and Topographical Organization of Goat Retinal Ganglion Cells

previously gelatinized slide was brought up and used to lift it through the surface when it was flattened by surface tension. The retina was treated with a mixture of 96% absolute alcohol and 4% of 40% formalin, covered with filter paper topped by another slide and pressed by a weight for twenty minutes in a bath of the fixing fluid. It was subsequently washed and stained in warm 0.1% cresyl violet (Hopkin and Williams), cleared, dehydrated and mounted in the usual fashion.

Counts of ganglion cell density were made at one mm intervals along lines running from top to bottom of the retina at 0.5 mm intervals along the horizontal. The sampling interval was increased to 0.25 mm in the area of the visual streak. Each point was the average of three counting fields and the magnification was adjusted so that about 60 cells occurred in a field. The counts were adjusted to a density per mm$^2$ but not corrected for shrinkage.

RESULTS

Concentric units

The receptive fields of the majority of the goat optic tract units were similar in organisation to the concentric units described by Kuffler (1953) in the cat retina. A complete protocol was available for 88 units; 84 of these were concentric types of which 49 possessed "off" centre and 35 "on" centre fields. The central region of the field was usually circular but was occasionally slightly elliptical along the horizontal axis. The response to stimulation of the receptive field periphery varied considerably; a small spot of light was adequate to elicit an excitatory response from some units whereas an entire annulus did not suffice in others. In all cases the surround clearly reduced or suppressed the response of the central area when simultaneously stimulated by a moving edge and no example of the "centre only" unit was encountered.

Sustained and transient units. A further division was made of the concentric unit receptive fields similar to the X, Y (Enroth-Cugell and Robson, 1966), I and II (Fukada, 1971) and the sustained/transient (Cleland, Dubin and Levick, 1971) classifications of cat retinal units. In the goat optic tract 31 units were transient and 46 sustained.

The presentation of a flashing light spot or an appropriate target in the receptive field centre of a sustained unit brings about a great increase in the firing rate which remains elevated with little decrement until the stimulus is removed or changes contrast. The continued presentation of a stimulus in the receptive field of a transient unit resulted only in a rapid return of the firing rate to the mean level.

The averaged response to the sudden presentation of a small, 1°, black target in the receptive field centre of a sustained (a), and a transient (b), "off" unit is shown in Fig.1, for comparison. The firing frequency of the transient unit can be seen to be higher than that of the sustained unit in the first 100 m sec but by contrast, it has fallen back to a much lower level within the next 100 m sec. The grouping of the initial discharge gives the transient unit a characteristic squeaky sound when heard over the monitor loudspeaker.

The location of transient units was most readily determined by moving the hand or its shadow about in the visual field at various speeds and attempting to elicit the characteristic sound of the grouped discharge. Maintained units were best sought with a large rectangular piece of black or white card. It was easy to move the card until it intercepted the receptive field with its vertical edge, either suppressing or enhancing the firing rate, and then to raise the card until the horizontal border passed out of the receptive field whereupon the change in firing rate located the receptive field at the corner of the card.

Many of the maintained units, as the searching procedure suggests, did not have powerful surrounds. To illustrate this, the response of a sustained "on" unit to the presentation of a small white disc against a black background is shown in Fig.1, (c). The response of the unit is, however, very similar when a large white card is presented instead, Fig. 1, (d).
A set of responses of a transient "off" centre unit with receptive field centre 2° in diameter is given in Fig. 1 for comparison with the high maintained firing rate of the previous sustained unit. The response to a small light spot 1° in diameter, Fig. 1, (e), reveals the characteristic transient form with an initial burst of firing followed by a much lower frequency of irregular maintained firing at "off" and no response at "on". An "on" response is revealed, however, when the surround is included by increasing the diameter of the light spot to about 8°, Fig. 1, (g). Step decreases and increases in the illumination of a 1°, Fig. 1, (f), and a 20°, Fig. 1, (h), spot of light elicit a burst of firing with each change of illumination. The grouping is more clearly defined for illumination of the central region alone. The ability of transient units to follow rapidly moving stimuli is clearly shown in
Fig. 1, (i) where a burst of firing is obvious for each finger shadow moving over the field and photocell when the stimulus velocity is well over 100°/sec.

Many of the sustained units did not show high frequencies of firing in their initial discharge to the presentation of a stimulus, Fig. 1, (k). Averaging confirms this finding. The rapid movement of a shadow through the field of such an “on” unit, Fig. 1, (m), clearly induces a transient inhibition of firing at even high velocities of stimulus movement, 100°/sec, but to the ear, the absence of a grouped discharge after the removal of the stimulus makes 1:1 correspondence between the unit response and the stimulus passages much less obvious than in the transient units, Figs. 1, (j) and 1, (l). Thus, as in the cat (Cleland et al., 1971), the ability to follow rapidly moving stimuli with a burst of firing for each passage under general illumination is present only in the transient units; at lower velocities of movement (10°/sec) similar responses can be obtained from the sustained units.

**Directional units**

The remaining four in the sample of 88 units were directional units of the “on-off” type which have been reported to be present in the retinas of the rabbit (Barlow, Hill and Levick, 1964) and ground squirrel (Michael, 1968). Firing could be elicited by black or white stimuli moving in a preferred direction across the receptive field but not when passing in the reverse, null direction (Fig. 1, n). The angle between the entry radii along which some firing could be induced was just over 180° in all four cases so that responses could be obtained for stimulus passage along both entry radii perpendicular to the preferred axis, Fig. 1; (o). The angle between the preferred entry radii for the goat directional units thus lies between the 300° of the rabbit and the 50° of the ground squirrel. Close examination shows the burst of firing for each passage of the stimulus spot through the directional unit receptive field to be biphasic, Fig. 1, (o). One part of the response corresponds to the entry of the spot into “on-off” receptive field and the other is the OFF response upon its exit.

**Receptive field sizes**

A considerable range of receptive field centre diameter was encountered. One sustained “off” unit in the region of the area centralis was completely inhibited, against a black background, by a small white spot subtending an angle of about 0.15° at the eye. The majority of the receptive fields were in the order of 1° in centre diameter but well away from the streak rather larger units were to be found. A field situated 45° above the optic nerve head had a centre 7° in diameter.

The periphery of the receptive field was investigated by the expanding spot test (see Methods) in which the moving edge of the light spot could usually elicit firing up to 10° out giving an upper limit for the surround diameter of about 20°

**Some goat optics**

The techniques outlined in the Methods section were used to make certain measurements necessary for relating the recorded location of the receptive fields in the goat’s visual space to the specialised areas of its retina.

The total horizontal field of the enucleated eye was found to be 190° (mean of two eyes). The optic nerve head projected 80° temporal of the nasal limit of the monocular field (mean of two eyes).
Measurements on central and peripheral retina gave a mean value of 0.22 mm/degree, or 4.5°/mm, for the retinal image magnification. This value indicates a posterior nodal distance of about 12.6 mm for the goat eye.

Pisa (1939) finds the monocular field of the sheep eye, which is very similar to that of the goat, to overlap the midline by about 30° on the horizontal, thus giving rise to a binocular field some 60° wide. Given this result and those above it is possible to estimate the separation of the various features on the retina. During fixation of a distant object the area centralis is assumed to project into visual space in a parasagittal plane.

If the monocular overlap of the midline is 30° then, because the optic nerve head is 80° from the nasal limit of the monocular field, the area centralis and decussation line will lie 50° nasal of the optic nerve head, which itself lies 110° nasal of the temporal limit of the field. Using the mean value of 0.22 mm/deg the retinal distances corresponding to the angles subtended in the visual field are as follows:

<table>
<thead>
<tr>
<th>Calculation</th>
<th>Calculated from optics (mm)</th>
<th>Measured on retina (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal limit to ONH</td>
<td>110°</td>
<td>24.2</td>
</tr>
<tr>
<td>ONH to middle of area</td>
<td>50°</td>
<td>11.0</td>
</tr>
<tr>
<td>Area c. to temporal limit</td>
<td>30°</td>
<td>6.6</td>
</tr>
<tr>
<td>Total horizontal extent</td>
<td>190°</td>
<td>41.8</td>
</tr>
</tbody>
</table>

Note: nasal limit of retina corresponds to temporal limit of field.

The results of the calculation from the optics are presented in Table 1 along with the corresponding measurements made, uncorrected for shrinkage, on the flat mounted retina displayed in Fig. 3. The difference between the theoretical and actual measurements of the distance between the optic nerve head (ONH) and the area centralis is probably accounted for by slight folding evident in the preparation.

The vertical extent of the monocular visual field was not measured optically but can be estimated from the retinal image magnification and measurements on the flat mounted retinæ. The vertical extent of the retina in the plane of the optic nerve head, see Fig. 3, is 41 mm but of this only 17 mm lie below the optic nerve head and 24 mm above it.

The upper limit of the monocular field, if not determined by the optic adnexa, will thus be 17/0.22 = 77° above the projection of the optic nerve head. The lower limit of the field would correspondingly be 24/0.22 = 109° below the optic nerve head. These measurements would be in a vertical plane set an angle of 50° to the sagittal plane when the eyes are fixed on infinity.

Now the normal sheep and goat hold the slit shaped pupil horizontal and the ascending main artery and vein nearly vertical; it follows from the structure of the eye and retina that the visual streak is horizontal under these conditions. Ophthalmoscopic examination indicates that the optic nerve head is held so as to project onto the horizontal plane as would be expected if the visual streak, which intersects it, is to accept the image of the animal’s visual horizon.

Under these circumstances there is a 19° overlap of the monocular lower field beyond the vertical in the plane of projection of the ascending blood vessels and optic nerve head.
Such an overlap would deal with the ground below the chin and it is worth noting that the ganglion cell densities in the region bordering the upper retinal limit, 700/mm², which deals with the visual field under the chin, are up to three times as high as those of the lower retinal limit, 250/mm², which deals with the extreme upper visual field.

**Normal position of the eyes**

Confirmation of the previous conclusions about the normal relationship of the projection of the areal centrales can be obtained from mapping the projection of the binocular visual field onto visual area I of the cortex. The following information is from unpublished results of such mapping experiments (HUGHES, KAHLIA, NASH and WHITTERIDGE). The results are for sheep cortex but the organization of the goat visual system is very similar.

The sheep were set up in a perimeter and the projection of the visual field onto the cortex was mapped with tungsten electrodes under nitrous oxide anaesthesia. In the binocular cortex two sets of points were obtained for each electrode insertion; one for the projection of the left and one for that of the right eye. The results were nomographically transformed to bring the projection of the optic nerve heads onto the 0° horizontal parallel and to fuse the most nasal point of the contralateral field representation with the corresponding point of the superimposed ipsilateral projection. Their projection was set on the central vertical meridian. The projections of the more temporal points of the binocular map simultaneously fused or approached fusion. The angle subtended between the projections of the optic nerve heads then indicates their normal relationship in an animal looking to infinity. Results obtained as a byproduct of three mapping experiments are shown in Fig. 2 where they are mapped onto a spherical coordinate system set so distant from the sheep's head that the 11.5 cm interocular distance may be neglected.

For three animals the mean angle between the optic nerve heads is thus determined to be 94°. Both areal centrales project onto the sagittal plane and the central vertical meridian so that an angle of 47° is subtended between the area centrais and the optic nerve head. This angle is very close to the 50° predicted from the visual optics.

**Retinal ganglion cell density map**

Figure 3 is a diagram of a flat mount of the goat retina prepared by the techniques described in the Methods section. The ganglion cell density isocount lines indicate the presence of both a visual streak running almost the whole length of the retina and passing through the optic nerve head as well as the small circular area centralis which lies on the streak 9.5 mm temporal of the optic nerve head. The major blood vessels of the retina avoid the regions with a ganglion cell density of more than 2000 cells/mm².

The dotted 1500 cells/mm² isocount line is L shaped, a feature missed by its neighbouring density lines. The horizontal part or streak region would, as explained earlier, take the image of the visual horizon with the eyes in their primary position; in this case the area centralis would be directed straight ahead to an infinitely distant fixation point near to the intersection of the sagittal plane with the horizon. The 20 mm upward extension of the 1500 cells/mm² line is directly above the circular centralis region which means that its projection reaches 90° below the horizontal and extends about 20° on either side of the central meridian. It includes within its projection only the binocularly viewed region of visual space determined by PISA (1939).

The tapetum obviously underlies the whole of the high ganglion cell density region.
Fig. 2. The visual field of a sheep at the centre of a sphere large enough to make the interocular distance insignificant. The dark line, PC, shows the intersection of the sagittal plane with the sphere to form the central meridian. For three animals, S₁, S₂, and S₃, arc shown those points in visual space which gave rise to a response at a single recording site in the left binocular region of visual area I via left and right eyes. There are thus two circles for each site which is indicated by a number. The normal relative positions of the two optic nerve head projections in an animal looking to infinity have been obtained by nomographically transposing the left and right projection maps for each animal until the optic nerve heads project onto the 0° parallel with the streak horizontal and then rotating the left and right maps along the parallels until the most nasal right eye projection for that animal has fused with the projection via the left eye. The mean angle (3 animals) subtended between the optic nerve heads is then 94° which, assuming they are symmetrically disposed in both eyes, leads to an angle of 47° as the mean value for the angle subtended between the optic nerve head and the region of peak count in the temporal retina.

(Fig. 3) and is of a brilliant greeny-blue colour. The remainder of the retina is backed by black pigment.

**Position of the single unit receptive fields in the goat’s visual space**

The receptive fields of all the units in the previously described sample have been plotted in a cylindrical coordinate system in which the optic nerve head projections are normalized, Fig. 4 (see Methods). The symbols of the accompanying legend designate the various classes of unit and indicate whether they were ipsilaterally or contralaterally projecting receptive fields. On the same plot are shown the temporal and nasal limits of the visual field, the plane of the optic nerve head, as well as the area centralis, visual streak and the outline of the 2000 g.cell/mm² isocount line projections.

The majority of the units recorded fall within the bounds of the 2000 g.cell/mm² isocount line and most of the contralaterally projecting fields are temporal of the area centralis projection. The ipsilateral fields are concentrated nasal of the line but there is considerable overlap into the temporal region.
Fig. 3. Retinal ganglion cell density map of a goat retina that has been flat mounted in the manner described in the Methods section. The ganglion cell isocount density lines are shown in black and are intersected by a number which indicates their value in thousands of ganglion cells per mm². The area covered by the tapetum is shown in green. Veins are drawn blue and arteries red. Note the manner in which the large blood vessels avoid the regions of highest count.
FIG. 4. The location in the visual field of the right eye of the receptive fields of the goat optic tract units described in this paper. A flattened cylindrical coordinate system has been used. The lines indicate the 0° parallel, H, the limits of the monocular field, O.S., the meridian containing the projection of the optic nerve head at its intersection with the 0° parallel, ONH, and the meridian containing the projection of the circular high count region of the temporal retina, PC. The dotted line outlines the projection of the 2000 g.cells/mm² isocount line into visual space. The symbols for the different unit classes are shown above and the two large arrows define the meridia between which the units were placed in the "centralis" class discussed in the text.

A more detailed breakdown of the positions of the receptive fields is interesting. In the following Table 2 receptive fields are described as being located in the area centralis when they lie within the two meridia 30° nasal and 30° temporal of the projection of the peak count; all other receptive fields are lumped together in the section labelled "remainder".

From Table 2 (a) it can be seen that whereas 68 per cent of the receptive fields located in the "centralis" region are of the sustained type, this group constitutes only 30 per cent

### Table 2.

<table>
<thead>
<tr>
<th>Category</th>
<th>Centralis (%)</th>
<th>Remainder (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Sustained</td>
<td>35</td>
<td>68</td>
<td>11</td>
</tr>
<tr>
<td>Transient</td>
<td>11</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Unclassified</td>
<td>4</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Directional</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100</td>
<td>37</td>
</tr>
</tbody>
</table>

(b) OFF

<table>
<thead>
<tr>
<th>Category</th>
<th>Centralis (%)</th>
<th>Remainder (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON</td>
<td>23</td>
<td>45</td>
<td>12</td>
</tr>
<tr>
<td>Directional</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100</td>
<td>37</td>
</tr>
</tbody>
</table>
of the "remainder" region. As a corollary, 54 per cent of the "remainder" area units are of the transient type compared with only 22 per cent of the "centralis" region. The sample of directional units is too small for conclusions to be drawn.

From Table 2 (b) it is clear that there are nearly equal numbers of "on" and "off" units, 45 per cent compared to 53 per cent in the "centralis" region but nearly twice as many "off" units in the remaining retina, 59 per cent compared with 33 per cent.

Examination of the original data reveals that the number of "on" and "off" maintained units in the "centralis" and "remainder" areas is about equal in both cases but whereas the same applies to the "centralis" transient unit population it does not apply to that of the "remainder" area where there are three times as many "off" as "on" transient fields. This excess accounts for the predominance of "off" units in this region.

It is to be noted that the so called "remainder" region contains the visual streak as well as the peripheral retina and that differences between the "centralis" and "remainder" areas might be even greater if the sample of units had been large enough to separate the streak units from the "remainder" group to form a pure "peripheral" sample.

DISCUSSION

The mammalian species that have so far been studied form a continuum with respect to the variety of their retinal ganglion cell receptive fields. At one end of the spectrum are the squirrel monkey (HUBEL and WIESEL, 1960) and rat (BROWN and ROJAS, 1965; PATRIDGE and BROWN, 1970; Hughes, in preparation) which appear to possess concentric units alone. Most workers put the cat in the same category (HUBEL, 1960; WIESEL, 1960; KUFFLER, 1953; ROBSON and STONE, 1965; ENROTH-CUGELL and ROBSON, 1966; Andrews and Hammon, 1970; Fukada, 1971) but there are a few reports of non-concentric units in this species (Stone and Fabian, 1966; Roeder, 1967). At the other extreme are those mammals that possess retinases containing a large percentage of non-concentric receptive fields. In the ground squirrel (Michael, 1968) and the grey squirrel (Robson and Cooper, 1966) the non-concentric population is made up of directional units but in the rabbit there are also at least six other classes of unit (Barlow et al., 1964; LEVICK, 1967).

The differences in receptive field organisation between the two extreme groups of mammals is paralleled by differences in their retinal morphology detectable with the electron microscope (DOWLING, 1968; DURBIN, 1970). The rabbit and ground squirrel have a much higher ratio of amacrine to bipolar synapses in the inner plexiform layer than do the monkey, cat and rat. The higher density of amacrine synapses is possibly the anatomical basis of the specialized receptive fields of the rabbit and ground squirrel.

Too little is known of the function of non-concentric units for an explanation of these species differences to be given. It is worth noting, however, that the retinal ganglion cell isocount lines are disposed with a greater degree of radial symmetry in the species with a pure concentric population, whereas horizontal ovality is most apparent in those well provided with non-concentric units. The ovality of the isocount lines and the variety of receptive fields are most prominent in the rabbit, inevitably suggesting a functional relationship between the two; perhaps the non-concentric units carry on an analysis of a kind specialised to detect movement or change in the image of the visual horizon.

The results from the goat suggest that it falls midway in our continuum of mammalian species. Non-concentric units form only 5 per cent of the above sample compared with 25 per cent in the ground squirrel and 60 per cent of the rabbit visual streak population. 5 per cent is, however, a very significant proportion in comparison with the value of
The Receptive Fields and Topographical Organization of Goat Retinal Ganglion Cells

0.001 per cent estimated from the literature for the cat and suffices to place the goat between the grey squirrel and cat. No E.M. results are available to relate this conclusion to the retinal histology.

In absolute terms the visual streak of the goat has a ganglion cell density per square degree of similar magnitude to that of the rabbit, although it is low compared with the area centralis. It might, therefore, be argued that the goat streak could be expected to contain a similar variety to the rabbit of non-concentric units for the analysis of its visual horizon. These specialized receptive fields are difficult to detect because of their limited spontaneous activity, selectivity and powerful surrounds (Levick, 1967). It seems unlikely that all of the other non-concentric unit classes should have failed to reveal their presence in a sample the size of the above, drawn predominantly from the region of high ganglion cell density. Biassed sampling resulting from electrode selectivity for large fibres would be a possible explanation for the absence of more sophisticated receptive fields but the Woods metal electrodes are known to record fibres as small as 0.1 μ (Maturana, Leitvin, McCulloch and Pitts, 1960) which is w.11 below the fibre diameter range of 0.8-11.0 μ which was observed in our E.M. photographs of the goat optic nerve. We provisionally conclude that the variety of units in the goat is much more limited than in the rabbit. There would thus appear to be a qualitative distinction between the visual streak of the goat and that of the rabbit. The morphologically defined visual streak appears to be a relatively common feature of the mammalian retina (Hughes, in preparation) and is usually secondary to a circular area centralis. Only in the rabbit is the area centralis peak count itself so elongated and coupled with such a limited provision of visual neocortex that perhaps precoding, reflected in the unit variety (Hughes, 1971), is entailed. This might be quite unnecessary in a large brained animal such as the goat.

The receptive fields of cells in the circular part of the goat area centralis are similar to those of the cat but the significance of the sustained and transient classes remains to be determined. In the cat the two groups maintain their individuality after passing through the lateral geniculate nucleus (Cleland et al., 1971) and in the rabbit the large field “off” transient fields pass through the LGN to the cortex (Takahasi, Levick and Oyster, 1969; Hughes, 1971). Clearly, in the cortex, the two classes may form the substrate of separate movement and maintained contrast detecting systems but evidence of this is lacking.

Retinal ganglion cell topography in the goat

The visual streak of the goat contains for the greater part of its length a maximum ganglion cell density of about 100 g.cells/cm², which is of a similar order of magnitude to that of the rabbit peak count of about 170 g.cell/mm². The goat streak is remarkable, however, for the presence of a superimposed region of high ganglion cell density in the region dealing with forward vision. In the rabbit there is no corresponding specialization of the temporal visual streak and the ganglion cell density falls off slightly in this region (Hughes, 1971).

Another feature of the goat ganglion cell topography which contrasts with that of the rabbit is the presence of the region of “vertical streak” or upward extension of the 1500 g.cell/mm² isocount line which projects to nearly 90° below the horizontal on the frontal vertical meridian. This bump in the isocount lines is much less obvious in the rabbit (Hughes, 1971) but in both animals it deals with the ground in front of, and near to, the animal’s mouth which is of considerable interest to grazing forms.
Given that both species tend to keep the visual streak horizontal, it seems likely that the difference in the magnitude of the temporal upswing of the isocount lines is related to the difference in the height of the heads above the ground. For viewing the ground at a given distance and resolution the angle subtended between the top of the required isocount bump and the horizontal plane would increase as a tangent function of the height of the eye.

The peak count in the centre of the area centralis of the goat is about 14,000 g.cells/mm² uncorrected for shrinkage. In absolute terms this is 14,000 × (0.2-22)² = 680 g.cell/deg². A 1° line on the retina would intersect \( \sqrt{680} = 26 \) ganglion cell receptive fields. We may thus estimate the visual acuity of the goat by the application of Shannon's sampling theorem. According to this the sampling density of a system is at twice the maximum frequency that the system can transmit. The estimated cut-off frequency is thus 26/2 = 13 cycles/deg² which corresponds to a grating with a minimum resolvable bar width of 2-3' compared with 5-5' for the cat; a sampling aperture transmitting this frequency would have to be about 4-6' in diameter which is of the same order of magnitude as the smallest receptive field, 10', recorded from the region of peak count.

Acknowledgements—The apparatus used in this investigation was provided by the M.R.C. to whom A.H. is also indebted for support.

REFERENCES


The Receptive Fields and Topographical Organization of Goat Retinal Ganglion Cells


Abstract—The receptive fields of 88 single units recorded from the goat optic tract have been investigated and found to be of three main classes; sustained (52 per cent), transient (35 per cent) and directional (5 per cent). The retinal ganglion cell density of the goat retina has been mapped and reveals the presence of both a circular area centralis and a visual streak. The positions of the recorded receptive fields in the visual space of the animal have been related to the retinal ganglion cell topography by means of observations on the optics of the goat eye. Sustained units were found to be more common than transient units in the neighbourhood of the area centralis (68%; 22%) but the reverse was true elsewhere (30%; 54%).

Résumé—On enregistre les champs récepteurs de 88 unités dans le tractus optique de la chèvre, et on obtient trois classes principale: soutenus (52%), transitoires (35%) et directionnels (5%). On trace de la carte de la densité des cellules ganglionnaires dans la rétine de chèvre; il y a en même temps une aire centrale circulaire et une bande visuelle. La position des champs récepteurs enregistrés dans le champ visuel de l'animal est reliée à la topographie ganglionnaire rétinienne par observation de l'optique de l'œil de la chèvre. Les unités soutenues sont plus abondantes que les transitoires au voisinage de l'aire central (68%: 22%) et c'est le contraire autre part (30%: 54%).

Zusammenfassung—Es wurden die rezeptiven Felder von 88 einzeln aus dem Schnerv des Ziegenauges abgeleiteten Zellen untersucht. Es fanden sich drei Hauptgruppen: dauerlicht- (52%), änderungs- (35%) und richtungsempfindliche (5%). Die retinale Ganglienzellendichte wurde graphisch dargestellt. Es zeigte sich sowohl die Existenz einer runden zentralen Zone als auch die eines Sehstreifens. Die Lage der abgeleiteten rezeptiven Felder im Sehraum des Tieres wurde unter Einbeziehung des Optik des Ziegenauges mit der retinalen Ganglienzellentopographie in Zusammenhang gebracht. Man fand, daß in der Nähe der zentralen Zone dauerlichtempfindliche Zellen häufiger auftreten als änderungsempfindliche (68%; 22%). Eine entgegengesetzte Verteilung (30%; 54%) konnte anderswo gefunden werden.
Directional units in the rat optic nerve

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Key words: rat — optic nerve — retina — directional unit — visual receptive field

Single units with directional receptive fields were recorded from the rat optic nerve and thus revealed, in contrast to earlier reports, the presence of at least one class of non-concentric retinal unit in this species. In confirmation of prior work, examples of concentric, surround-dominated and centre-dominated receptive fields were also encountered. In some respects their properties resembled those of either brisk or sluggish classes in the cat retina.

In spite of extensive searching in the rat optic nerve, Brown and Rojas\(^1\) encountered no single unit receptive fields which possessed other than a central on or off area with or without a surround. This led to the rat being grouped with cat and monkey and these species being contrasted\(^6\)\(^--\)\(^7\) to rabbit, pigeon and frog in which more complex receptive fields had been described. Since then nearly all classes of rabbit retinal receptive field have been reported in cat retina\(^8\)\(^--\)\(^16\)\(^--\)\(^17\). Reinvestigations of monkey\(^5\)\(^--\)\(^13\) and rat\(^14\) have not so far revealed receptive fields with other than radially symmetric properties. It thus seems worthwhile to make a brief report of some directional units encountered in the rat optic nerve during recording for another purpose\(^10\).

D.A. Agouti rats of between 115 and 130 days of age were anaesthetized with an i.p. injection of 1 ml of 25% urethane solution. A tracheal cannula was fitted and the animal mounted in a head holder. The skull was opened and anterior forebrain removed by suction to reveal the optic nerves. Their overlying blood vessels were left intact and paraffin introduced to prevent dehydration.

The animal was fitted with a rectal thermometer, wrapped in a homeothermic blanket and arranged facing a 1 m distant screen. The eyelids were retracted and a zero power contact lens fitted after the eye had been secured, by means of 3 equispaced sutures, to a ring at the corneo-scleral boundary.

Under the conditions of plotting with a high background intensity and bright stimulus spot, it was found that the pupil remained about 0.3 mm diameter. Because of the great depth of field under these conditions\(^10\) no attempt was made to correct for viewing distance.

Single units were recorded with 20–40 MΩ NaCl-filled micropipettes driven by means of a Kopf hydraulic microdrive.
Fig. 1. Single unit responses recorded from rat optic nerve. The on-off receptive field of the directional unit was mapped by means of a small light spot. Responses at on are represented by ‘+’ and at off by ‘—’; null response is shown by a circle of the same size as the light spot. The directionality of the unit is revealed by its responses to movement of the light spot through the receptive field along axes at intervals of 45°. The lower trace shows the output of a photocell placed at the positions indicated by a heavy circle on the map. DE denotes the dark edge and LE the light edge of a wide card strip passed up and down through the field. Both edges elicit firing for upward movement, neither for downward. Nasal, N, and inferior, I, directions in the visual field are indicated. Several centre-dominated, symmetrical, receptive fields were characterized by the similarity between their sustained or transient responses to an optimal, centre-sized, spot and to whole field flash. They resembled concentric receptive fields in demonstrating properties analogous to the brisk or sluggish units of the cat retina. The centre dominated on unit above was described as brisk-like because of its initial transient to an optimal flash and the inhibitory pause in maintained firing upon its cessation. The response to whole-filled illumination is similar. The very low maintained firing rate and unresponsiveness to rapid target movement of the centre-only, transient off unit was sluggish-like. The absence of an initial transient burst upon flashing of an optimal light spot and the immediate return to the previous maintained firing rate upon its cessation led to the sustained on unit also being classed as sluggish-like. The last series of responses is from a surround-dominated unit. The off response to an optimal spot flashed in the field centre is considerably weaker than the on response to an annulus in the surround. Simultaneous illumination of surround and centre results in reduction of the response.
Visual stimuli were presented by a modified Keeler ophthalmoscope which produced slits or spots of light and a Leitz Prado projector fitted with a diaphragm and N.D. wedge. Card stimuli were available.

The receptive fields were initially mapped out with a 30 min exploring spot of intensity 100 cd/sq.m against a background of 10 cd/sq.m.

Three on/off directional units with similar properties were encountered in a total population of 230 roughly classified units. The receptive field illustrated in Fig. 1 lay 100° temporal to the median sagittal plane and near to the 0° horizontal meridian. A light spot flashing anywhere within the receptive field defined by moving stimuli elicited on and off responses. The preferred direction of this unit was vertically upward. Both other directional units preferred horizontal temporal movements. The preferred direction was the same for black and white discs moved through a 4° receptive field. Firing could be obtained for entry angles up to 90° from the preferred direction but not at 135°. There was a clear null direction in which movement maximally suppressed the limited spontaneous discharge. A wide strip of card moved up and down through the field elicited a discharge similar to that of a small disc; dark and light edges were equally effective when moving up through the field and ineffective when moving down. There was no sign of inhibition from a suppressive surround when large stimuli were employed. Directionality was retained for all rapid movements (>100°/sec) which elicited firing.

Two-hundred and ten units were recorded with classic centre surround receptive fields whose responses were sustained or transient and corresponded to an earlier description of rapidly or slowly adapting units. However, it has not previously been reported that some of these cells showed the initial high frequency burst of impulses upon excitation of the centre, followed by an inhibitory pause in the maintained firing rate upon cessation of excitation, which is characteristic of the brisk units of cat retina. The maintained firing rate of other units was very low and they evinced the slow initiation of firing upon centre excitation which is considered characteristic of sluggish units in the cat. This group contained examples of surround-dominated units.

More unusual, but in confirmation of previous reports, were 17 units with surrounds which were either very weak or not demonstrable under these conditions of illumination. Some of them are illustrated in the lower part of Fig. 1 and it is apparent that their centre dominated responses, like those of classic concentric units, have properties which resemble those of cat brisk or sluggish units.

Thus, in contrast to previous reports, it is here concluded that the rat possesses retinal ganglion cells with non-concentric receptive fields. It is probable that the on-off directional units of the rat dorsal lateral geniculate nucleus receive the input of the corresponding retinal class.

The presence of a non-directional on-off class in the lateral geniculate nucleus has been confirmed and suppressed by contrast units (uniformity detectors) reported. Neither of these classes was encountered in the optic nerve but their presence in the lateral geniculate nucleus argues, by analogy with directional units, for the existence of the corresponding retinal populations. Non-directional on-off receptive fields and uniformity detectors have already been described in the retina of both rabbit and cat.
In confirmation of a previous report the concentric units of the rat optic nerve were found to be of the sustained or transient type. Examination of their responses to flashing of optimal light spots revealed examples of concentric and centre-only fields with properties resembling those of the brisk or the sluggish units of the cat retina. Examples of rat concentric units have thus been encountered which may be described as brisk-like or not-brisk-like, sluggish-like or not-sluggish-like but the examination was not systematic and these dichotomies cannot be concluded to be exclusive in the rat; the brisk-like/sluggish-like division is thus not established as a dichotomy. In so far that the brisk/sluggish, sustained/transient properties correspond to X, Y or W properties the report of representatives of X, Y and W-like concentric and centre-only fields in the rat lateral geniculate nucleus is in agreement with these findings.

Representative populations of retinal units cannot be expected when recording from the optic nerve. It is well established that even during direct retinal recording the encounter rates do not necessarily reflect the true proportion of a given cell type unless systematic exploration of the retina is carried out. At present this procedure appears to be precluded in rat because of the tiny vitreal space. However, the trend appears to be for the progressive discovery of receptive fields with qualitatively similar spatial properties in each species of vertebrate retina, although spectral sensitivity, conduction velocity and axonal projection may differ.

It is improbable that major qualitative distinctions will remain possible between the variety of receptive field classes in rat, on one hand, and cat, rabbit, pigeon and frog, on the other. Correlations, such as Dubin attempted, between species grouped on the basis of retinal anatomy and those grouped on single unit physiology will require quantitative rather than qualitative physiological support which must await development of recording techniques appropriate for small eyes.

SOME COMPARATIVE AND EVOLUTIONARY REFLECTIONS ARISING
OUT OF STUDIES ON RETINAL CODING IN THE WALLABY

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The retinal ganglion cells of cat and rabbit have some 12 classes of receptive field in common. A study by means of conventional intraocular recording techniques in the Tammar wallaby (*Macropus eugenni*) has revealed the presence of the full range of retinal concentric classes such as on and off, brisk and sluggish, sustained and transient and linear and non-linear as well as the majority of the non-concentric types including on-off (local edge), orientation selective (on-off adjacent), and on-off directional units. Thus, in spite of possessing no mutual ancestor since the pantotheres, the marsupial and placental infraclasses have 11 of 13 types of ganglion cell receptive field in common. This may not be surprising when it is noted that the pigeon, a species from a different taxonomic class, shares the majority of these receptive field types. The discovery that concentric and non-concentric classes project to the visual cortex in rabbit and cat has undermined the thesis that there is a phylogenetically older non-concentric system associated with tectal visual analysis and non-deterministic, concentric system associated with the visual neocortex. Can it be that this repertoire of vertebrate retinal unit types comprises a single mechanism handed-on from the early reptiles and embodying tricks of coding which have been universally retained ever since? Some doubt must be cast upon this idea by the apparent absence of conventional concentric units in the frog and of non-concentric types in the monkey retina. On the other hand, the problem of encounter rates is well known, especially when intraocular recording has not been employed in survey studies. The established presence of the entire range of unit types in hunter and hunted, land and aerial, placental, marsupial and avian forms at least argues strongly for a role in extracting universal and fundamental features of the retinal image.
A DIVERSION: THE PHILOSOPHY OF UNIT NOMENCLATURE
A Rose by Any Other Name...

On 'Naming of Neurones' by Rowe and Stone

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Key Words. Retinal ganglion cells • Classification of neurones • Naming of neurones • W, X, Y terminology undesirable

Abstract. Rowe and Stone dispose of descriptive terminologies for retinal units as 'essentialist' and argue for a 'neutral' multifactorial system redefined from previous W, X, Y nomenclatures. It is argued here that descriptive terminology need not be either essentialist or undesirable and that the W, X, Y terminology is too confused with false associations to be satisfactory. The synthetic power of the W, X, Y nomenclature was lost with the discovery of concentric W cell receptive fields, the projection of the W cells to the LGN and the suggestion of overlap between X and W conduction velocity groups. It was made methodologically unsound by its employment for the naming of Nissl stained soma classes before the identity of individual somas with the physiological entities can be demonstrated. Although now redefined, the identity of symbols in the several vintages of the W, X, Y system leads to inevitable confusion. The current tripartite division of a multidimensional quality space without cluster analysis is not neutral because it emphasises conduction velocity but represses the diversity of W receptive fields. The concise, apt and memorable descriptive receptive field terminologies are more desirable in that they are operationally based and approximate the unit's transfer function - the parameter most significant to the central nervous system in its information processing task; thus they are more suited for cross species comparison because of a probably bountiful source of functional hypotheses. The lack of taxonomic neutrality in the W, X, Y system has recently been emphasised by its failure to adequately classify rabbit retinal receptive field categories.

Introduction

In their survey of cat retinal ganglion cell classification Rowe and Stone [1977] have condemned the main alternative [Cleland and Levick, 1974a, b] to the W, X, Y nomenclature, and other descriptive terminolo-
W, X, Y Versus Descriptive Terminology

The Meaning of the W, X, Y Terminology Has Been Changed Too Often!

One of the earliest objections to the W, X, Y nomenclature was that it employed the X and Y symbols of *Enroth-Cugell and Robson* [1966] which were linked with the linear/nonlinear terminology. *Rowe and Stone* [1977] argue that *Enroth-Cugell and Robson* established the properties of their X and Y units by tests other than for linearity and nonlinearity and think it justifiable for a classification not employing tests for linearity to be based on the X, Y symbol pair. This is debatable, but what is clear, and rather ironic, is that *Stone and Hoffmann* [1972] and other workers [Cleland et al., 1969] have equated the X and Y of *Enroth-Cugell and Robson* with linear and nonlinear properties. This view is now inherent in the literature and means that the X, Y terminology is confusing for some workers. It is to be noted that, subsequent to the report of *Enroth-Cugell and Robson* [1966], many who did not employ the nonlinear/linear test avoided the use of the X and Y terminology even when convinced that it applied to their own categories; *Stone and Hoffmann* [1972] chose rather to imply the identity of their units with those of *Enroth-Cugell and Robson*. Indeed, it was the addition of W to the X, Y pair by *Stone and Hoffmann* [1972] which proscribed the use of the X and Y symbols for linear and nonlinear properties by expanding the dichotomous classification. It should be noted that because *Enroth-Cugell and Robson* [1966] did not measure conduction velocity, it remains possible that their sample of X and Y units included some which *Stone and Hoffmann* [1972] would have labelled W cells. *Stone and Fukuda* [1974a] argue against this on...
Hughes 54

the basis of electrode selectivity [Stone, 1973], but this has been questioned [Levick and Cleland, 1974].

Stone and Hoffmann's [1972] report on W cells differed from that of Stone and Fukuda [1974a] by describing a tripartite division of unit conduction velocity which was paralleled by a threefold grouping of receptive field classes. They distinguish the two concentric, X and Y, classes from the nonconcentric W cell class. In addition, Hoffmann [1973] reported that W cells project to the superior colliculus, and not to LGN and cortex, in contrast to the X cells which project to the LGN and not to the superior colliculus. Stone and Fukuda [1974b] saw the W cells, with their midbrain projections and nonconcentric fields, as functionally akin to the retinal units of the phylogenetically more 'primitive' rabbit and lower vertebrate retinas; the midbrain/cortical dichotomy of destinations also happily tied in with the current, in-vogue, concept of the 'two visual systems' by providing each with an independent retinal sampling array. No wonder the W, X, Y terminology became popular. But, as Huxley wrote, here we have: 'The great tragedy of Science – the slaying of a beautiful hypothesis by an ugly fact.'

Up to this point it had been possible to argue that the W, X, Y nomenclature represented some intrinsic feature of neural organisation which would be clumsy to discuss in terms of parallel descriptive terminologies dealing with conduction velocity, receptive field type, destination and so on. The midbrain projecting nonconcentric, W cell system could be regarded as a conceptual entity in its own right. The reports of Cleland and Levick [1974a, b] and Stone and Fukuda [1974a] which described the presence of a large population of concentric units amongst those with low conduction velocities struck at the roots of the W, X, Y terminology's usefulness; in the absence of three receptive field classes the tripartite W, X, Y terminology was brought solidly back to its basis in conduction velocity grouping. It thus effectively became redundant because the $T_1$, $T_2$, $T_3$ conduction velocity terminology of Bishop et al. [1969] could replace it and was, indeed, argued by Stone and Fukuda [1974a] to parallel their own nomenclature. A comment by Stone and Fukuda [1974a] which refers to the W, X, Y grouping of receptive fields obviously suggests some residual confusion with the earlier nomenclature; the W cells could no longer be grouped together on the basis of their receptive fields, of which there were at least five types including concentric forms, but only in terms of their lower conduction velocities than X and Y cells.

Alas, the W cell population is now understood to project not only to
the midbrain but also to the visual cortex via the LGN C laminae; X and Y cells project to both midbrain and cortex. Even the conduction velocity grouping has been called into question by Cleland and Levick [1974a,b] who describe an overlap in the conduction velocities of their slower brisk-sustained units and faster sluggish units thus implying an overlap between the W and X categories of Stone and Fukuda [1974a].

What now remains as a basis for the elegant tripartite division? According to Rowe and Stone [1977] simply a long list of properties, often made forcibly clear-cut in order to fit the three groups. With the loss of its unifying power over the descriptive categories the W, X, Y classification developed a striking disadvantage in that its now deceptively simple facade became coupled with a variety of abandoned associations, such as the nonconcentricity of W cells. Rowe and Stone [1977] are not troubled by these associations; they regard the W, X, Y terminology as possessing the asset that its meaning may be frequently revised, thus taking advantage of both its flexibility and the patience of its followers.

W, X, Y 1974 Confuses Morphology and Physiology

However, even further potential for confusion lurks in the W, X, Y terminology. It is disingenuous of Rowe and Stone [1977] to suggest that the W, X, Y system is physiologically based and that the \( \gamma, \beta, \alpha \) system is its morphological correlate. This may be true for the tabulature of their most recent publication but it is not applicable to the W, X, Y terminology, overtly identical, of Fukuda and Stone [1974]. These authors employed Boycott and Wässle’s subsequently published [1974] dimensional criteria to classify the cat Nissl-stained retinal ganglion cells W, X, Y rather than as, say, C, B, A cells to parallel the Golgi defined \( \gamma, \beta, \alpha \) categories. Their photographs show W, X and Y labels on cell soma [Fukuda and Stone, 1974, 1975b]. Dye marking experiments with results from 12 cells [Fukuda and Stone, 1974] justify this implied identity but these were from the peripheral retina where classification is most straightforward. No worker to date has claimed to reliably distinguish between individual members the three Golgi-defined morphological cell classes in all parts of the retina on the basis of Nissl-stained material; indeed, even the peripheral ganglion cell densities of Hughes [1975] and Wässle et al. [1975] are considerably higher than those of Stone [1965] or Rowe and Stone [1976] which indicates a divergence in criteria for the identification of
neurons let alone their subclassification; it is a further step to equate the identified neurone with the physiological entity. It was obviously premature to employ the same terminology for a classification based upon histological methods as was previously employed for electrophysiological data when it was beyond the capabilities of the techniques to unequivocally establish that identity. In fact, Rowe and Stone [1976], in a recent discussion of cells in the cat visual streak, appear to appreciate this point and have reverted to a *small, medium, large* classification for histological purposes which parallels the W, X, Y grouping. Even now, Rowe and Stone [1977] incorporate soma size into their table of otherwise physiological properties.

Although Rowe and Stone [1977] have now accepted the earlier position of many workers and inform us that it 'seems necessary to retain two terminologies', i.e. the morphological and physiological 'each in its appropriate context', we must regard the work of Fukuda and Stone [1974, 1975] as part of the current literature. It behooves us, therefore, to warn the unwary that these papers employ an W, X, Y terminology (W, X, Y1974?) which applies equally to morphologically and physiologically defined entities. Surely such a flexible nomenclature cannot be suited to 'those not working in this particular area but interested nevertheless in following it'. It is difficult enough for those working in the field to keep up! Indeed, the ready changes of the W, X, Y terminology without overt redefinition recalls Humpty Dumpty: "When I use a word" said Humpty Dumpty in rather a scornful tone, "it means just what I choose it to mean - neither more nor less'.

Other workers [Cleland and Levick, 1974a, b; Cleland et al., 1975] have retained separate morphological and physiological classifications without disadvantage relative to the pre-1977 W, X, Y terminologies and have thus avoided the pitfalls inherent in their premature fusion. Their terminologies are in this respect similar to that now espoused by Rowe and Stone [1977] but the nomenclature of Cleland et al., has been dubbed 'essentialist' and we are bid ignore it on that count alone.

Descriptive Terminology Is Not Essentialist

Rowe and Stone [1977] reject descriptive terminology on two counts; it implies that some limited aspect of the unit's performance is the essence of its properties and thus, perhaps falsely, more important than others,
and that the descriptive terminology is a source of confusion between the description of a neuron and its functional role. But are descriptive terminologies 'essentialist'? Well, this is an instance where the legal concept of mens rea applies; such a nomenclature is essentialist if its user believes that it identifies essence but purely descriptive if he does not! A descriptive terminology thus need imply neither essence nor neglect of other features of a unit's behavior.

Numerous descriptions of directionally selective units have been published in which the term 'directional' is employed as a descriptive name but there is no evidence that their authors have failed to examine other properties of the units by means of flashed light spots, broad stimuli which extend into the inhibitory surround and so on. The name does not restrict the activities of the investigator, it is descriptively apt, economical and memorable. The latter quality is important; extended neutral terminologies of the class I, II, III variety rarely pass into common usage because few people can remember what is what.

Close examination of papers dealing with the classification of retinal units in frog, rabbit and cat has not led to the identification of one in which it would be possible to identify the author's intentions with those of an 'essentialist' as defined by Rowe and Stone [1977]. Several papers have put forward the 'key-feature' and 'trigger-feature' approach to unit classification and naming [Barlow, 1961; Barlow et al., 1963; Barlow and Levick, 1965; Levick, 1967] but it is quite apparent that this is conceived as an operationally defined manipulation of the receptive field's light distribution which brings about the maximal response of the unit. As Levick [1967] says of the rabbit retinal 'edge-detector': 'One is implying no more than that the relevant observations of unit behaviour are thereby most economically described and most meaningfully interpreted.' – 'It is not claimed that edge-detection is the only good description or even the best. There is always some doubt that a sophisticated stimulus revealing subtle behaviour has been missed.'

It is immediately apparent that Levick [1967] is thinking in operational terms at the level of stimulus presentation and not in terms of subsequent processing. It is simplistic and tendentious of Rowe and Stone [1977] to ignore these clearly nonessentialist statements; the naming technique described by Levick [1967] is obviously similar to that in publications on the cat retina [Cleland and Levick, 1974a, b]. Rowe and Stone are thus able to dismiss other classifications by imputing to them what is clearly not the intention of their authors.
There have been attempts to identify in the early stages of neural processing what have been described as 'functional natural invariants' [Maturana et al., 1960], or 'password stimuli' [Barlow, 1961], which were regarded as the releasers of specific behavioral responses. A considerable literature has grown up around these concepts as applied to the frog [Ingle, 1968]. Although possibly premature [Hughes, 1971] in the absence of behavioral work, such hypotheses have been a great stimulus to investigation in the work which tested their predictions [Grüsser and Grüsser-Cornehls, 1968; Butenandt and Grüsser, 1968] and the field as a whole has probably benefitted more from their postulation than from a neutral, descriptive terminology. However, Barlow's [1953] 'fly detector' or the term 'bug detector' for a class of frog unit were never more than a hypothesis of a provocative nature, not a formal part of the nomenclature; Lettvin et al. [1959] simply remarked: 'We have been tempted, for example, to call the convexity detectors "bug-perceivers", but it is clear that they regarded the central interaction of all classes of unit as necessary before invariants such as prey and enemy could be recognized [Maturana et al., 1960]. Essentialism is thus not a necessary feature of a descriptive terminology.


Part of the task of Rowe and Stone [1977] has been to clear up some of the confusion arising from the earlier nomenclatures of Stone and his co-workers. This has been simply achieved by ignoring them and redefining the meaning of W, X, Y terms. The latest W, X, Y1977 terminology [Rowe and Stone, 1977] claims to provide a three class system, one class subdivided, which is allegedly neutral or nonessentialist. The three main groups of this classification are defined in a table listing several quality dimensions; a typical description of a unit might thus be W1/off centre/tonic/linear. Rowe and Stone suggest the absence of a one-to-one correspondence of the main categories with any of the dimensions of classification to be an advantage! But if the units require specification by cataloguing their properties along these quality dimensions then how does the tripartite division arise? It is quite apparent that an author with different interests could justifiably claim the presence of more, or fewer, main groups.
depending upon whether he were a 'splitter' or a 'lumper'. Moreover, remember that Cleland and Levick [1974a, b] even question the separation of the sluggish and brisk sustained conduction velocity groups.

A truly neutral terminology would have dropped the tripartite classification because it gives inordinate emphasis, in the fashion of an essentialist nomenclature, to the conduction velocity division and strongly suppresses the various W cell receptive field categories which Cleland and Levick [1974a, b] would rank equal in importance to the two classes of field with fast conducting axons. Instead, either quality cataloguing would be employed, thus retaining a separate terminology for each quality dimension in the manner of Cleland and Levick [1974a, b], or the data would have been subject to full-blown numerical taxonomic analysis. Thus, if there were N quality dimensions in use, the N-dimensional quantitative description of all members of the sample population would be subjected to analysis in an N-dimensional quality-hyperspace in order to objectively determine whether obvious clustering occurs and the dimensions along which it is most prominent. Unless clustering enables efficient subdivision of the quality space, it is not justifiable to employ a unified terminology to replace the straightforward property list. Rowe and Stone [1977] have imposed, probably for historical reasons related to the development of their terminology, an arbitrary tripartite division on this N-dimensional quality hyperspace without in any way justifying it.

It is obvious that the major problems do not arise in terminology relating to the X and Y cells; it is the W class which causes difficulty. Having made the major divisions essentially on the basis of conduction velocity, Rowe and Stone [1977] have subdivided the W group into those with tonic or phasic firing patterns and principally, etc., crossed or uncrossed projections from the temporal retina. Conduction velocity differences have been equated with the tonic/phasic division but this is not in agreement with the results of Kirk et al. [1975] and, in any case the conduction velocity must be measured for each unit and is not an independent criterion, as it is for X and Y classes, because there is overlap between classes of field. The differential distribution of the classes relative to the visual streak is presumably based on electrophysiological encounter rates which are too illunderstood and variable between workers to form a satisfactory basis for classification. Again we find soma size listed as a property distinguishing the W1/W2 classes in spite of Rowe and Stone's statement that a separate morphological classification is best retained. The curious effect of the W, X, Y classification in dividing up the receptive field
Hughes 60

classes almost suggests indifference to the information processing role of the retinal units.

**Descriptive Nomenclature Based on Unit Receptive Field and Firing Properties Offers Greatest Usefulness and Functional Relevance**

It is the task of the retinal neurophysiologist to give account of the processing whereby invariant features of the retinal image brightness distribution are analyzed and converted into patterns of impulses passing along numerous channels. The behavior of a single neuron in the normally functioning nervous system may be described under specified conditions, in terms of the transfer function which relates variation of the stimuli in its receptive field to the firing pattern of its axon. The information processing capability of the cell may thus be established and certain bounds are irrevocably placed upon the range of tasks in which it might be hypothesised to be employed. The information processing capabilities of the cell are unique to it, may be operationally defined and are very readily classified for units of the visual system by a descriptive terminology based upon quantitative or qualitative investigation of their transfer function. Evolutionary considerations strongly suggest that the categories of receptive field so defined may have functional significance but until such singular roles are verified, or after they have been disproven, the operational basis of the classification stands. Consideration of the literature suggests that, for a given species, there is more agreement between workers on operationally defined classes than for most other parameters of the neuron.

For given feedback conditions the information transfer capabilities of a visual cell are independent of its projection in the central nervous system, although to some degree related to its axonal conduction velocity; both of these factors determine the information processing capabilities of the next level of processing and partially establish the overall transfer function of subsequent cells. It seems almost self-evident, however, that the very feature, the receptive field and its selective properties, which is employed by the central nervous system in processing visual information should form the basis of a unit classification rather than secondary factors, such as destination and conduction velocity, or factors which underlie the mechanism of the receptive field, such as the form of the dendritic tree, cell soma size, and so on. Naturally enough such descriptive termi-
Tecnologies have been pragmatically established as the norm amongst those who have established single unit receptive field nomenclature for a variety of sensory systems and species.

Rowe and Stone [1977] suggest that receptive field nomenclature leads to a great number of small classes which form only a tiny proportion of the total number of retinal cells. But the cat retina can be completely covered by an array of neurons with dendritic trees some 2° diameter yet forming only about 1.3% of the total ganglion cell population. If these cells have small soma then the electrophysiological encounter rate will suggest them to be even rarer yet they can subject the entire unicellular retinal field to their own distinctive analysis.

The relative agreement between authors on the classification of receptive fields is important because it provides a common reference frame for discussion of the numerous factors upon where there is no agreement. In the current W, X, Y terminology the differential retinal distribution of the cell classes is incorporated into the descriptive table and is thus a parameter in their discussion. An author who disagrees with the results of Rowe and Stone [1977] must implicitly challenge their whole terminology rather than direct himself to a discussion of quality dimension of their results with which he disagrees. Revision subsequent on his success would of course be readily achieved in their system. The situation in regard to the W, X, Y [1974] terminology is worse in so far that reference to the receptive field classes automatically implies the corresponding histological distributions and identities reported by Fukuda and Stone [1974] and based on techniques questioned elsewhere [Hughes, 1975].

The W, X, Y [1977] Terminology Cannot Be Applied to the Rabbit Retina and Thus Falls as a Multispecies Terminology Which Might Embody Functional or Evolutionary Significance

Subsequent to the writing of the body of this text there have been two studies of retinal ganglion cell classification in the rabbit retina [Caldwell and Daw, 1978; Vaney et al., 1978] which agree that the brisk transient and sustained (Y and X) units cannot be distinguished upon the basis of conduction latency and that the conduction latency of directional units falls in the brisk rather than the sluggish (W) range. Caldwell and Daw [1978] have adopted what they described as a schizophrenic terminology in which sluggish is opposed to X and Y rather than to sustained and
transient. It is clear, if conduction latency is ignored, that the cat and rabbit have at least 9 common receptive field classes with similar temporal responses. Had the W, X, Y terminology been truly taxonomically-neutral then it could have incorporated these new findings. Its identity with conduction velocity grouping, already criticized in the cat, is its downfall in application to the rabbit. Again we see that cross-species uniformity is most obvious in the description of the spatial and temporal properties of the receptive fields and emphasise the advantages of terminology based on these features.

Conclusion

There can be no doubt of the extensive experimental contribution of Stone and his colleagues. The fact that their work was carried out under the umbrella of the W, X, Y1972, W, X, Y1974, and W, X, Y1977 terminologies without even subscripting the symbols suggests that terminology is no significant barrier to progress; language is much more 'transparent' than Rowe and Stone would have us believe: 'What's in a name? That which we call a rose by any other name would smell as sweet.'

Rowe and Stone [1977] have handled their discussion more in the manner of Baconian philosophers than practicing scientists. It is a petty but amusing irony that their emphasis on words and meaning has much in common with the position defined by Popper [1976] as essentialism! Perhaps I, as well as Rowe and Stone [1977] should note his antiesentialist exhortation against 'the path to intellectual perdition': 'Never let yourself be goaded into taking seriously problems about words and their meanings. What must be taken seriously are questions of fact, and assertions about facts: theories and hypotheses; the problems they solve; and the problems they raise.'

The real problems in the naming of cat retinal neurones have not arisen out of the subtle influences of ill-chosen classifications but from methodological inadequacies and nomenclatures pushed to imply what is not unequivocally established. Receptive field classification and property cataloguing keep our feet on the ground; why superpose a premature synthetic structure? With property cataloguing, the flexibility and revisability which Rowe and Stone [1977] so emphasise as assets to a terminology become less important. Although the W, X, Y terminology may continue to be employed for the cat by some workers it must by now be apparent
that its reality falls short of the claims made for it by Rowe and Stone [1977]. Its potential as a synthetic multispecies terminology is negligible in view of recent results from the rabbit unless it is to be yet again revised and become even more confusing.

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"IN PRAISE OF THE PAIR TREE"......A FINAL
COMMENT ON 'NAMING OF NEURONES'

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ABSTRACT

Rowe and Stone (1979) describe dichotomous
classifications as 'trivial' and incompatible with
a falsificationist methodology. It is pointed out
below that they are in error by distinguishing
between dichotomous and other classifications
because all classifications are reducible to a
dichotomous form. It is argued that the reiterated
charges of essentialism against the descriptive/
dichotomous terminology are groundless and that it
is compatible with a falsificationist methodology.
Rowe and Stone oversimplify falsificationism in its
practical application and place more emphasis on
accommodating failure of the terminology than on
the need for 'strong' predictive hypotheses which
survive for a significant length of time with a
constant terminology. The underlying structure of
the descriptive/dichotomous system is here
formalised for the first time and suggested to meet
Rowe and Stone's requirements as satisfactorily as
their own system. Its 'down-to-earth', descriptive
and modular terminology is equally compatible with
the needs of the practising scientist and
philosopher.
'A person cautious in argument will... constantly reaffirm the moderate and defensible position with which he started.... He has stated moderately and truly that 'some Xs are Y', but his opponent argues against the proposition that all Xs are Y. If he answers his opponent's arguments at all, he can only do so by defending the proposition 'All Xs are Y'. Then he has fallen in the trap.' (Thouless, 1953)

Rowe and Stone (1979) have rejected my defence (Hughes, 1979) of the dichotomous/descriptive terminology against their criticisms (Rowe and Stone, 1977). Neglecting Thouless's advice, I will not risk boring the reader by reaffirming my 'moderate and defensible position' but will rather take issue with two aspects of their reply.

Perhaps the most important is their description and dismissal of dichotomous classifications as trivial; this is misleading and requires countering. On a more philosophical note, I must protest at their continued reliance on stigmatising the position of Cleland, Levick and myself as essentialist and setting it in contrast to 'right-thinking?' falsificationism. I argue that they oversimplify the falsificationist position by underemphasising verification. A descriptive terminology which embodies lasting predictive theory rather than ad-hoc hypotheses is more compatible with the falsificationist approach. It is concluded that Cleland and Levick's (1974a,b) descriptive nomenclature has a powerful formal basis which is presented in outline below. It is a hypothetico-deductive system with an underlying structure so like that suggested by Rowe and Stone (1977; 1979) to be advantageous in their own system that their emphasis on essentialism seems to have blocked realisation of the similarities. The potential for formal expression in Cleland and Levick's system would suggest it to be a more convenient classification.
DICHOTOMOUS CLASSIFICATION IS FAR FROM TRIVIAL!

Rowe and Stone (1979) comment: 'Hughes seems to miss the central point of logic that the dichotomous classifications....although logically rigorous and easy to fit to new phenomena are, in the final analysis, trivial.' Unfortunately this 'presupposition' is logically flawed in drawing a distinction between dichotomous and other classifications. Ultimately, any classification may be reduced to a dichotomous form (Jevons, 1958). Even if some of the parameters of classification are finite numerical variables they may be regarded as implicitly a compacted dichotomous arrangement.

\[
\begin{array}{c|c|c}
\text{number} & \text{one} & \text{not one} \\
\hline
\text{two} & \text{not two} & \\
\end{array}
\]

and so on.

Thus, if Rowe and Stone's contention is true, all classifications, including the W,X,Y system must be trivial. A conclusion clearly at odds with their thesis. Why do they claim this triviality? Because, to a falsificationist, dichotomous classifications: 'cannot be tested, i.e. they cannot fail to fit the observations (either a receptive field is concentric or it is not). Because they can so easily fit new observations, the brisk/sustained etc. classification and terminology are unlikely to lead to any conceptual development in our understanding of the visual system... The ready applicability of the terms to a range of species results from the circumstance that the classification is trivial rather than from any heuristic power.'

But the data in a dichotomous classification can generate falsifiable theories; the presence or absence of concentric units in the retinas' of various vertebrate classes led Maturana (1964) to suggest that the visual systems of non-mammalian vertebrates were
'determinate', with much retinal pre-processing, and that mammals had 'indeterminate' systems which employed only concentric units at the retinal level. A single-level dichotomous classification leading to a non-trivial and testable hypothesis! Stone and Hoffman's (1972) own suggestion that the non-concentric units of the cat supply only the mid-brain while the concentric units project to the lateral geniculate nucleus was the same dichotomous hypothesis but applied to different regions of one animal rather than to the same region in different species.

As the number of qualities increases, such bifurcate classifications become the potential source of many more hypotheses. If n qualities are considered then we have $2^n$ combinations (ABCDEFG,...;ABCDEFG...). To find some combinations and not others may be significant if we are simply exploring single unit properties and have no reason to expect either their presence or absence; if we do have expectations then they may be confirmed or denied. It is possible to test or establish class divisions by means of multivariate analysis along a mixture of binary and continuously variable dimensions.

THE DESCRIPTIVE DICHOTOMOUS CLASSIFICATION EMBODIES A QUALITY LIST

Implicit within the descriptive/dichotomous classification is a procedure not yet presented as a formal classification or identification key. Misunderstanding arises in Rowe and Stone's (1979) comments because of this lack. I therefore present an illustrative model based on the battery of tests of Cleland and Levick (1974a).

Ten tests have been designated in binary form for application to the concentric units encountered at a given eccentricity. An identification letter is given next to each test's description in figure 1.
At each level of the tree a plus sign means the presence of the quality (A) and a minus sign its absence (a). A circle around the symbol indicates an empty set for the purpose of this classification. In practice there might be members for every possible set. With ten tests there are 1024 possible categories; such a tree incorporates all of the results of a given set of tests and places each unit in a specific category. The entire description of each unit is available at all times in its quality list ABCDefGhij, ABCDefGhij...etc. The system has the capability of representing an enormous range of individual or interspecies variation, contrary to Rowe and Stone's (1979) claim.

Classification may be achieved by downward division, optimally with theoretical foreknowledge, but failing that by pragmatic selective testing followed by upward regrouping of elements according to similarity, a formal polythetic synthesis. In practice both procedures are carried out repeatedly as hypotheses are concerning function generated and as the selective tests are optimised. The primary classification tree may thus be a data base with considerable redundancy but the results of this may be expressed in a compacted form for the purpose of formal discussion. The construction of such a formal tree requires hypothesising the degree of difference between elements which is to be regarded as insignificant and the extraction of the most efficient route for dichotomisation of the population to the required degree of compression.

Of the 1024 possible elements in the tree of figure 1, Cleland and Levick (1974a) identified only 12 as a major non-null sets. In the consideration of upward grouping by similarity, Taxa formation in Rowe and Stone's (1977; 1979) terminology, they
chose to identify as one class, the brisk-transient units, those elements with the descriptions ABCDEfGhij or ABCDEfghij; i.e. those identical in all factors other than maintained firing rate (G,g). The tests less cleanly dichotomise the class which they identified as brisk-sustained because of the spread in their optic tract latencies and three elements were designated acceptable as members of this class. Qualities which are invariably associated or found to be irrelevant to the classification may subsequently be compacted with other properties or dropped.

It will be clear that the complete binary definition of a class from the property list will have a form such as,

A and B and (c or C) and (f or G)...

Set theory, Boolean algebra and propositional calculus are specifically directed to the analysis of such propositions. By their aid the laws of correlation between the various properties may be systematically arrived at. Detailed discussion of these procedures may be found in Ledley (1960) and of classification in general in Jevons (1952) and Harvey (1969). The digital computer is peculiarly suited to handling such classificatory procedures and the overt usage of formal category descriptions based on suitable test batteries could offer considerable advantages.

The distillation (I will not say essence!) of the downward dichotomous test tree is shown at the bottom of figure 1 as the regrouping of the elements into their formal categories. It is this inductive conflation of the test-tree dichotomies which gives rise to the published dichotomous categories. It should be realised, however, that the test tree results represent the basic data and, contrary to Rowe and Stone's (1977) claim that it forces subdivision, may represent even a quantised continuum. Whether or
not this appears in the formal terminology depends on the investigator's proclivities when inductively generating the working categories. Either way the test data is not lost.

Cleland and Levick (1974a) have selected the terms concentric-nonconcentric, brisk-sluggish and transient-sustained as terminology for the formal categories derived from the segment of the tree shown in figure 1. Thus a reference to a brisk-transient unit, BT, is simply a shorthand description of the full test profile contained in the binary listing above.

ROWE AND STONE (1979) IGNORE MULTIDIMENSIONAL DICHOTOMISATION.

An alternative way of considering the bifurcate tree is presented to its right in figure 1. Each quality dimension is represented by a binary value (1 present; 0 absent) which is fed to an electronic gate. The description of the acceptable requirements for a class then, becomes the specification of the function of the logical gate. The order of bifurcation in the tree is immaterial to the location of a given unit in its class; however, if monothetic theoretical considerations suggest a specific order be given hierarchical significance then it may be adopted but equally readily changed at will for another purpose.

Failure to recognise this possibility for simultaneous dichotomisation of a population along numerous quality dimensions appears to lead Rowe and Stone (1979) astray in their assessment of dichotomy. Thus they say (Rowe and Stone, 1977): 'if it is insisted that X and Y cells have been.....all linear and nonlinear retinal ganglion cells, then arguably there can be only two categories of retinal ganglion cell, and the X/Y terminology cannot deal with the newly recognised cell groups except by regarding them
as varieties or subtypes of X and Y cells.' Of course, in a dichotomous system all retinal ganglion cells must be either 'linear' or 'non-linear' but it does not follow that new categories of cell can only be represented as subgroups of this division. As we have seen, to the right of figure 1, it is possible to divide the property space along other dimensions simultaneously without limit; brisk/sluggish, sustained/transient etc. This flexibility has been simply demonstrated by the addition of the brisk/sluggish category to the sustained/transient division established in an earlier paper (Cleland et al., 1971). With n parameters we have $2^n$ categories of retinal ganglion cell.

Rowe and Stone (1979) suggest that: 'the alternative non-essentialist approach is to regard the linear and non-linear summation properties of X and Y cells as only one of their properties rather than as a definition of their separate categories.' But this is a false distinction because it exactly defines the approach of the dichotomous classification.

It is important to realise that when the X and Y terminology is employed to define only linear and non-linear categories of cell then it is used, not in the sense of Rowe and Stone (1977, Table II) as representing the entities defined by their entire property list but, in what I understand to be the original sense of Enroth-Cugell and Robson (1966), before the ability to define dichotomy along one dimension was abolished by Stone and Hoffman's (1972) addition of the W category. Only subscripts can make this apparent.

**DESCRIPTIVE TERMINOLOGY IS NATURAL TO DICHOTOMOUS CLASSIFICATION.**

The dichotomous tree of figure 1 is a subset of an infinitely larger tree; a part of this is outlined in figure 2. With only a few classificatory steps
such trees become unwieldly and may be handled to advantage in the form of several small trees adapted to whatever aspect of the system we are discussing.

The organisation of an extensive tree can be relatively 'natural', in figure 2 one portion deals with the subset we are considering, another with their responses to stimuli and the final section with their projections. Now, if a unit projects to the accessory optic trace we describe it as such; if, with others, to the LGN then we class them together as LGN-projecting, rather than by a numeric designation which must be redundantly decoded in some way. The cells which we record have distinguishing and often descriptive anatomical names, such as 'displaced amacrine'. When dealing with the receptive field it appears sensible to continue with such a convenient nomenclature and employ the stimulus and response properties in naming. But not all descriptive names enjoy a strong functional hypothesis. Names like concentric-brisk-transient are as neutral as 'X' in predicting functions but convey more information. They can be a mouthfull! But, just as chemists face greater problems and solve them by abbreviation, so we may simply use such letter combinations as BS, BT, SS etc.

In laying out such a tree our attention is drawn to commonly neglected classes, such as non-encountered ganglion cells or displaced amacrines. It is often assumed that 'linearity' and 'sustained' properties are linked like 'transient' and 'non-linearity'; however we must retain logical space for 'non-linear sustained' units until a mechanistic explanation is available which excludes them. Consideration of the formal categories may thus be helpful in promotion of hypotheses. If a strong predictive theory is available then it may be possible to pre-allocate logical space for classes not yet encountered or tested for. This might be done when considering a
Fourier Analyser model of the retina which required multiple spatial frequency channels at one location. Thus, the full dichotomous/descriptive classification has the potential of doing the very opposite to Rowe and Stone's (1977; 1979) claim that is suppresses attention to factors other than those of the formalised name. A view which seems unrealistic because the investigator is always aware of the extensive test-tree on which his classification is based.

DICHOTOMOUS CLASSIFICATION POSSES MANY CLAIMED ADVANTAGES OF W,X,Y1977

Rowe and Stone (1979) contrast the large number of features upon which their classification is based with the small number employed by Cleland and Levick (1974a,b) and imply it to therefore be less arbitrary. It is apparent that they are comparing their property list with the formal and compressed dichotomous terminology rather than with its implicit dichotomous test-battery. But any practical system can deal only with an insignificant proportion of all possible parameters. Their suggestion that an ideal classification would be based upon all features of a cell is profoundly unrealistic. Even so few as 100 dichotomies give rise to $1.27 \times 10^{30}$ categories. Thus, in general, no classification can be organised to deal with all requirements including evolutionary relationships, homologous systems, naming and identification. It is essential to be guided by some hypothesis in selecting a few relevant parameters. Even Rowe and Stone's property list (1977, table II) is no exception; cat colour, the anaesthetic used, the weather, grating response and endless other parameters are judged irrelevant. Like the test-battery of Cleland and Levick (1974a,b) both sources of data are short, selective and theory laden.
Rowe and Stone (1979) thus ignore the processes underlying the dichotomous classification when comparing it with their own system. The test battery is equivalent to their property list; the stage of polythetic synthesis provides the opportunity of defining formal categories according to inductive generalisations and enables specification of economical procedures for the identification of the group to which a cell belongs. The flexibility of the dichotomous system and its possibility of continual revision have been indicated above and are entirely compatible with the description of a desirable system as outlined by Rowe and Stone.

It seems to me that Rowe and Stone's (1977) ideal progression would have been to the formalisation of their property list (their table II) in a dichotomous quality tree with some numerical parameters. Now they may have cut themselves off from the considerable representation benefits of a developed dichotomous classification by disparaging its use in the rival to their nomenclature. In principle, the dichotomous system has all the power claimed for their own classification and a more satisfactory potential formal structure such as is outlined above.

In spite of these underlying similarities Rowe and Stone would still argue that the selection of parameters from the test battery for use in the terminology depends upon asseveration, 'the issuing of a solemn statement that these properties are essential.' The suggestion that it seems almost self-evident that the properties of a unit's receptive field should be emphasised as a basis for classifying cells in the visual system (Hughes, 1979) is identified as such a dogmatic, essentialist asseveration and said to be without supporting argument. But the statement is included in the argument. It is, that the nature of a unit's response
to visual stimuli may be very relevant to its ultimate role in the visual system and thus provides a basis for predictive theory. It does not preclude an additional stage of classification based upon projection as an aid in tracing out function. The selection of these aspects of the test battery for naming purposes at the stage of polythetic synthesis is an hypothesis as to functional significance, is falsifiable and is not the enunciation of an essentialist and unarguable axion.

ESSENTIALISM HAS BEEN A RED-HERRING!

Rowe and Stone (1979) persist in dismissing Cleland and Levick's (1974a,b) terminology on the convenient charge of essentialism because of its use of descriptive terminology to identify key features; search for essence, they imply, is incompatible with the hypothetico-deductive falsificationist approach often considered de rigueur for the fashionable scientist.

Popper (1963) states that: 'it is false to view science as stating ultimate explanations, the essences of things.' The scientist: 'aims at finding a true theory or description of the world...which shall also be an explanation of the observable facts. Theories always remain conjectures as opposed to indubitable knowledge.' The existence of essence is not denied, Popper only declares the futility of claiming its identification. The scientist certainly aims at approximating ultimate explanation.

I have pointed out (Hughes, 1979) that Levick (1967) explicitly encompasses the possibility that his descriptive rabbit unit terminology has missed an important aspect of subtle unit behaviour or that the results have been misinterpreted; such a disclaimer is incompatible with belief in the identification of essence. Rowe and Stone (1979) reply that the
discussion is centred on cat retina and that Cleland, Dubin and Levick (1971) are explicitly essentialist because they use the phrase; 'does not convey the essential nature.' just as Levick (1967) mentions; 'capturing the essence of a rabbit unit's behaviour.' But this is trivial. These phrases are not philosophical jargon but employ the term 'essence' in its common meaning of the 'reality underlying phenomena' (O.U.D.) for which the scientist is justifiably hunting. A scientist ceases to be a falsificationist not by searching for underlying truth but by believing that he has found it with absolute certainty. Even in the cat papers (Cleland and Levick, 1974b), we find that the authors retain a 'nagging feeling of uncertainty' whether the unit descriptions are 'spuriously complex' or 'the physiological categorisations significant or incidental.'

Rowe and Stone (1979) comment that, in making these caveats, Levick and Cleland (1974b) are simply concerned that the 'true' essence of the unit's behaviour has been missed. This argument is untenable; if you think that the 'true essence' may not have been identified and that further work may falsify your hypothesis then you can only be described as working within a falsificationist framework.

The charge of essentialism is a red-herring and has practical significance only via the corollary that such an approach is incompatible with a falsificationist procedure. Indeed, it would not be of consequence if Cleland and Levick secretly believe that they have identified essence, as long as they and others continue testing the validity of their beliefs by experiment.

ROWE AND STONE OVERSIMPLIFY FALSIFICATIONISM.

W,X,Y1977 terminology has been presented as structured in accord with the falsificationist model
of scientific methodology and able to ride out inevitable error because of its flexible, non-feature linked structure. By contrast Rowe and Stone (1979) equate Cleland and Levick's 'nagging feeling of uncertainty' with evidence of a kind of weak-kneed essentialism rather than of adherence to falsificationism. Why? Perhaps because they assume that a falsificationist expects to be wrong and will, in accord with Popper's injunction, joyously welcome falsification of his theories rather than be worried about their failure. But Popper's thesis is more complex than this!

The starting point (Popper, 1958, 1963, 1972) is that falsification by one instance has a power equal to that of endless verifications. But, in practice: '...disproof of a hypothesis is contingent on the stability of the theories employed in interpreting matters of fact, so that the refutation of a supposed explanation may be no more definitive that its verification.' (Nagel, 1967). Waddington (1977) and others also argue that in practical scientific work the variability of experimental results and human error make falsification as unreliable as verification—'Technik ist alles'—and that it as often pays to set out to 'prove' as to disprove an hypothesis in the real world.

However, Popper (1963) has overtly stated that, although the logical power of scientific methodology ultimately stems from stringent attempts to falsify, there remain in practice several additional and important requirements which a new theory must fulfill in addition to agreeing with the facts better than its predecessor. For good theories: 'we need not only successful refutation, but also positive successes'. The theory should survive for a significant time: 'We must not see theories as nothing but stepping stones.' We seek discoveries: 'geniuine guesses about the
structure of the world.' We should not have the attitude: 'of merely producing theories so that they can be superseded.' Popper (1963). Positive successes mean verifications: 'science would stagnate, and lose its empirical character, if we should fail to obtain verifications of new predictions!' Popper (1963). The verification of prediction (Popper normally prefers 'corroboration') is thus an important factor even in the falsificationist approach.

Rowe and Stone (1979) rightly wish to incorporate modifiability into their terminology but are so concerned with theories which fail rapidly that they neglect to consider those which survive for a long time. Theories should not be expected to incorporate their predecessor's failure only to fail immediately themselves when put to the test. If that happens, Popper points out that we may be chaining 'weak' ad hoc theories which lack the power of explanatory insight into the world possessed by 'strong' predictive theory.

DESCRIPTIVE/DICHOTOMOUS TERMINOLOGY PREDICTIVE AND W,X,Y AD HOC.

Rowe and Stone (1977) describe the W,X,Y 1977 terminology as an hypothesis as to the functional niches of the cell categories and suggest (Rowe and Stone, 1979) that this grouping has had a far reaching impact on our understanding of the visual pathways. I, however, cannot identify impact which is not encompassed in other nomenclatures. The hypotheses associated with the W group have been almost exclusively anatomical. Originally the W cells were the specialised mid-brain input. Why? Because they were not concentric and not found to project to the lateral geniculate nucleus (L.G.N.). However, negative results are not a sound basis for anatomical and electrophysiological theories; concentric units turned up projecting to the mid-brain and W cells were
encountered in the L.G.N. 'C' laminae. In the updated theory, the projection via the C laminae is said to indicate that the W cells are a 'functionally separate group' (Rowe and Stone, 1976). But would the presence of X and Y in the same laminae necessarily suggest, therefore, that these classes are not functionally separate? Apparently not. In the rabbit we find all the receptive field classes encountered in the cat retina projecting to an un laminated LGN. Are these classes not to be regarded as functionally separate in this animal? Or should we opt, like Cleland and Levick (1974a,b) to place more emphasis on the information processing capability of the receptive fields in the initial classification.

I find the functional hypotheses offered by the new W,X,Y system elusive; even Rowe and Stone (1977) point out that the roles of Y and W cells are not satisfactorily defined. Only the X cell has been attributed with a specific function, a role in 'pattern vision' (Fukuda and Stone, 1974). But similar suggestions have been put forward independent of the W,X,Y terminology by Enroth-Cugell and Robson (1966), Ikeda and Wright (1972) and Cleland et al. (1971). Similarly, the vague conception of Y cells as involved in 'movement perception' is embodied in many places in the literature.

The theories which have been associated with the W,X,Y terminology for the generation of 'functional groupings' correspond to the 'weak' kind; their origin is in the month to month trends in the process of discovery. They are 'working tools' but have little predictive power in terms of function; and are frequently falsified; it is natural that Rowe and Stone should argue that terminology not be centered on them and opt for continual revision of the underlying meaning of their symbols (Hughes, 1979).
But Popper's falsificationism need not be associated with such an approach (Rowe and Stone, 1977; 1979); he is quite opposed to it. What is required is that nomenclature be based upon 'strong', long lasting, predictive theory. In the descriptive/dichotomous terminology a relatively 'strong' theory may be propounded by embodying certain aspects of the filtering operation of a receptive field class as key-features in the terminology, e.g. local edge detector, in order to suggest how the output of the units may be employed centrally. However, the constant change of meaning associated with the W,X,Y system is avoided because the neutral, fall-back, form of the nomenclature is simply descriptive; when theories fail it remains useful for designatory purposes because it embodies the outcome of the classificatory testing procedure unlike certain other descriptive terms such as 'bug-detector' (Hughes, 1979). It is unsatisfactory for Rowe and Stone (1979) to claim that it is a circular process to test whether a local edge detector is really a 'local edge detector' because the name is operationally descriptive whether the functional implication is correct or not.

The basis of descriptive terminology within the dichotomous system has varied. Concentric units could be described as 'contrast detectors' to be consistent with other parts of the nomenclature; instead they are described by relatively abstract features of their performance in a test-battery which designate no functional hypotheses. The terminology of non-concentric units is richer in functional implication; the embodied hypotheses are not powerful as theories go but are testable, long standing and distinctly not ad hoc. It is curious that Rowe and Stone (1970) insist on emphasising the advantages of classification by their nebulous 'functional grouping' in contrast to the 'physical properties' of the dichotomous
descriptive system which embodies much more obvious functional significance in its terminology; although I do not want to claim too much for it!

I thus advocate the employment of descriptive terminology for naming the receptive fields at a given anatomical level in terms of the stimuli which elicit their best response. A similar receptive field classification is present in the W,X,Y system and has remained, like a worm in the bud, relatively unchanged throughout several alterations in the W,X,Y terminology. The W,X,Y₁₉₇₇ system, however, fuses the data base of the receptive field nomenclature with projection and other parameters. In my opinion the outcome is a muddle; the terms are isolated from reality and associated with vague concepts; the different orders of theory become confused.

If Hubel and Wiesel (1962; 1965) had adopted a descriptive/dichotomous classification for their cat cortical receptive fields by employing such terms as 'edge', 'bar' and 'terminated bar'-selective then their hierarchical theory could have been more independent of the unit classification and many current complications avoided (Henry, 1977). In the manner of optimal programming, a nomenclature should be kept highly modular and the dichotomous/descriptive terminology is well suited to that end.

CONCLUSION

Philosophy is good at raising questions and science at answering them but the questions raised and answered seldom have much in common. The vast increase in our understanding of the universe which is directly attributable to the activities of scientists during the last millenium has come about in spite of the absence of any rigorous and generally accepted theory of scientific method. As Medawar (1969) has said: 'Ask a scientist what he conceives...
the scientific method to be, and he will adopt an expression that is at once solemn and shifty eyed: solemn, because he feels that he ought to declare an opinion; shifty eyed, because he is wondering how to conceal the fact that he has no opinion to declare.'

In practice a scientist is not committed to a consistent, watered-down, philosophical theory of science. He works with the rich reality and can justifiably employ a variety of approaches, even when they are mutually incompatible, as with the wave-particle duality in physics. If a theory works, then even a falsificationist should hardly be concerned that its author is essentialist. Philosophical justifications are of little significance.

So, with steady gaze and avoiding solemnity I might summarise my reply to Rowe and Stone (1979) in the phrase 'falsification-schmalsification!' They draw a 'false dichotomy' between the W,X,Y and the descriptive/dichotomous systems. Both approaches are compatible with the hypothetico-deductive method; whether falsificationism embodies the essence of scientific method is immaterial. I have suggested that, once the charges of essentialism and triviality are cast aside, Rowe and Stone's (1977; 1979) requirements are better met by the implicit qualities of the rival system. It is a benefit of this debate that the formal basis of the dichotomous/descriptive approach has been expounded. But the issue has probably been decided by practising scientists unconcerned with philosophical niceties. Caldwell and Daw (1976) entitled their first communication 'X,Y and W cells in the rabbit retina' but by 1978 their nomenclature was 'schizophrenic' and avoided 'W' (Caldwell and Daw, 1978). Recently Rodieck (1979) has argued that the diversity of 'W' cells is too great for the term to be profitably retained.
REFERENCES


Figure 1. Each letter on the left hand side of the figure symbolises an operational test to be applied to a recorded retinal unit. The set of ten tests (A - J) were chosen from those employed by Cleland and Levick (1974a,b) for the construction of a model test tree. Each test is scored 1 or 0 (yes or no) and the sequence of binary decisions thus defines the test tree forming the bulk of the figure. Only non-null sets are traced through the whole tree and the 'rarely encountered unit' tree is not developed.

The second stage of the classification is the formal polythetetic synthesis of classes from the non-null sets at the output of the tree. Cleland and Levick (1974a,b) group them as brisk transient (BT), brisk sustained (BS), sluggish transient (ST) and sluggish sustained (SS).

It is false to think that there is any necessary order or hierarchy in the test tree, although it can be introduced if desired, and this is shown to the right of the diagram. The result of each test is then fed into a logical gate whose specification is that of a class and which takes no account of test order.

The symbolic logical (Aa) and binary (1,0) representations of all sets making up each class of the polythetetic synthesis are shown. The dotted line traces the specification of sets accepted by Cleland and Levick (1974) as forming the brisk transient class. The diagram is discussed in detail in the text.
A
NEURONES OF RETINAL GANGLION CELL LAYER

B
SPIKE GENERATING NOT SPIKE GENERATING

C
ENCOUNTERED NOT ENCOUNTERED

D
ANTIDROMICALLY ACTIVATED NOT ANTIDROMICALLY ACTIVATED

E
CONCENTRIC NOT CONCENTRIC

F
LINEAR NON LINEAR LINEAR NON LINEAR

G
BRISK SLUGGISH BRISK SLUGGISH

H
TRANSIENT SUSTAINED TRANSIENT SUSTAINED TRANSIENT SUSTAINED

I
BIFURCATE NOT BIFURCATE

J
BRANCH A B BRANCH A

K
IPSI CONTRA IPSI CONTRA IPSI CONTRA

L
LGN NOT LGN LGN NOT LGN LGN NOT LGN

M
LAMINA A NOT A

N
LAMINA C NOT C

O
SC. NOT SC. SC. NOT SC.

P
ACC. OPT. T. NOT ACC. OPT. T.
Figure 2. The polythetic synthetic classification (BS, BT etc), and its implicit and formalisable test tree, are only a part of an infinite tree of which a segment is displayed here. The ease with which commonly neglected elements of the system are brought to mind in the rigid dichotomous description will be apparent after brief consideration. Underlining indicates a terminated set in the diagram. Such a tree is best delineated by descriptive terms rather than an abstract coding. It is equally arguable and its implicit components, like the test tree of Fig. 1 should also be presented in a descriptive terminology.
At given eccentricity

A: Concentric surround
   1 = YES
   0 = NO or NULL SET

B: < 3 ms O.T. latency

C: > 3 < 4 ms

D: > 4 < 5 ms

E: 120 spike/sec to TWIRL test

F: Clearly modulated by fine grating

G: Maintained rate > 20/sec

H: Very regular maintained firing in S.C.T.

I: Very slow grating for modulation

J: > 10 sec to standing contrast

BT

ABCD[fGhij] 111101000
ABCD[fghij] 111100000
AbCD[eFgHij] 1011011001
AbcDeFgHiJ 1001011001

BS

AbcDeFgHiJ 1000011001

Formal polythetic synthesis

ST

AbcDeFgHiJ 1001010110
AbcDeFgHiJ 1000010110
AbcDeFgHiJ 1000010111
AbcDeFgHiJ 1000010111

Descriptive / dichotomous operational classification
EYE MOVEMENTS
VERGENCE AND VERSION
VERGENCE IN THE CAT

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(Received 23 March 1972)

NOMENCLATURE

\[ P \] interpupillary distance
\[ M \] inter-marker distance
\[ S \] inter-reflex distance
\[ D \] suffix; indicates distant look
\[ N \] and \[ NC \] suffix; indicates near look or near look with vergence
\[ \alpha \] suffix; indicates measurement from photograph rather than absolute value
\[ \Delta P \] change in \( P \) during vergence
\[ \Delta M \] change in \( M \) during vergence
\[ \gamma \] change in \( S \) during vergence
\[ \theta \] vergence angle for one eye. Measured at the intersection of the visual axis with a parasagittal plane
\[ A \] distance of apparent pupil plane from corneal vertex in mm
\[ C \] distance of centre of rotation of eye from corneal vertex in mm
\[ R \] distance of centre of corneal curvature from corneal vertex in mm
\[ O \] distance between pupil and flash reflex for one eye in mm
\[ L \] distance between marker and flash reflex for one eye in mm
\[ \phi P \] change in pupil and reflex separation during version
\[ \phi M \] change in marker and reflex separation during version
\[ \theta_a \] version angle for one eye. Measured similarly to \( \theta \)
\[ s \] suffix; straight look
\[ a \] suffix; angle look
\[ r \] suffix; right
\[ l \] suffix; left
\[ aR, aL, L, D, h, \Delta D \] and \( \Delta L \) see Figs. 7 and 8
\[ X \] see Fig. 2.

INTRODUCTION

Do cats converge? The diversity of recorded opinions suggests that the answer cannot be obvious to direct inspection. The whole literature on eye vergence in mammals can be quoted, rather than summarised, within a page because it consists only of passing observation devoid of experimental data.

According to LINDSAY JOHNSON (1901),

"Man and the true monkeys, alone have the power of convergence. . . . No other mammal in the wild state can converge, but I have seen domestic cats and dogs which have been taught, by repeatedly holding food near their eyes, to converge to some extent."

This view is contested by HARRIS (1904) who records having seen attempts at convergence on the part of bears and various ungulates but such movements were,

"of course imperfect."

Elsewhere he states that,
"In man and a few of the higher mammals with binocular stereoscopic vision, the movements of the eyes are perfectly co-ordinated... in convergence."

It is clear from the text that he regards the cat and larger felidae as members of this group of vergers but he does not specifically record the observation of vergence movements in these animals.

Bartels (1929) similarly attributes vergence to a wider group of species than did Johnson. Man, the apes and cat are said to verge and the ability is claimed to be better developed amongst the carnivores than the herbivores although present in both groups. The references given to support these statements deal mainly with the horse.

The discussions of vergence in Vincent (1912); Warden, Jenkins and Warner (1936); Walls (1942 and 1961) and Walk (1962) all clearly derive from the sources mentioned above.

Examination of the recent literature reveals little more. Elul and Marchiafava (1964) refer to,

"the absence of true convergent, binocular vision in the cat."

And Zernicki and Dreher (1965) who have had the opportunity, like the previous authors, to observe restrained conscious cats comment

"However, the convergence of the eyes does not appear to play a major role in the mechanism of acute vision in the cat, as shown by the presence of rather poor convergence in the intact cat."

In contrast, Blakemore (1970) has claimed the presence of convergence in cats,

"But careful observation shows that most cats do in fact converge their eyes upon suddenly presented, close objects."

The rat and rabbit are the only other mammals to obtain mention in the literature on vergence. Many authors believed that the rat (Johnson, 1901; Harris, 1904; Vincent, 1912) and the rabbit (Brehé, 1936) were unable to move the eyes spontaneously and the question of vergence did not arise. In contrast, Lashley (1932) claims that such eye movements are common and that vergence movements are readily seen in the rat. Spontaneous eye movements have now been reported in the rabbit (Hughes, 1970) but vergence has not been observed (Collwein, 1970; Hughes, 1971).

The vague state of our knowledge of vergence in mammals in general is summarised by a footnote in a recent symposium on eye movements (Zuber in Bach-Y-Rita, Collins and Hyde, 1971).

"Vergence movements are probably only found among some mammals, and may be restricted to primates and higher animals."

That some tonic vergence is present in the cat is readily apparent. The visual axes of the paralysed animal diverge by about 4° (Nikara, Bishop and Pettigrew, 1968). If this were the case in the conscious animal it would not be possible for both areas centrales to be brought to bear simultaneously upon any real object. Tonic vergence must at least bring the visual axes to a parallel condition.

In the monkey, the presence of the neural substrate for the control of vergence movements is known since such movements may be induced by the stimulation of the frontal eye fields (Jampel, 1960). Passing comments of similar responses to the stimulation of the cat cortex (Jampel, 1960) have recently obtained some weak support from studies on the oculomotor cortex of this animal. Dreher, Zernicki and Santibanez (1970) report,
Vergence in the Cat

1963

"In the frontal area the movements in the vertical plane were occasionally accompanied by convergence movements."

and SCHLAG and SCHLAG-REY (1970) comment,

"Convergence movements were suspected more than once, but they were never easily repeatable."

THEORETICAL BACKGROUND

The basic purpose of this investigation was to determine whether the cat converges when looking from a distant to a near object. Qualitative investigation was begun with direct observation rapidly followed by videorecording and stop motion playback, but with neither method could vergence be detected with confidence. A potential source of error in the qualitative observation was noted during videorecording in that the pupils of the cat dilate, as part of its visual orientating reaction (ELUL and MARCHIFAVA, 1964), when an object is suddenly presented to it. It is difficult to observe simultaneously both the inner and outer margins of the pupils in both eyes so that a view of the inner margins of the pupils approaching one another can lead to the conclusion that vergence has occurred when it has not.

The work reported here thus consists only of the subsequent quantitative photographic observations whose interpretation requires extensive theoretical treatment.

If the distance between the centres of the pupils is \( P_D \) when the cat is looking at a distant object then the criterion for convergence in looking from it to a near object is a reduction in the interpupillary distance by an amount \( \Delta_P \) to \( P_{NC} \)

\[
P_{NC} = P_D - \Delta_P
\]

where \( P_{NC} \) is the interpupillary distance for the near look when convergence occurs; in the absence of convergence the interpupillary distance for the near set would be \( P_N = P_D \) and no change would be observed (Fig. 1).

Marks painted on the cornea may be employed with greater accuracy than the pupil in a similar technique in which the criterion for vergence is a reduction in the distance between the marks from \( M_D \) to \( M_{NC} \).

Photographic negatives were made which show the cat's eyes when,

(a) looking at a distant object (200 cm)
(b) looking at a near object (25 cm or 10 cm).

The cat was free to move a little back, forth and sideways so that his distance from the camera, and thus the photographic magnification, varied. It is clear that some standard is required for normalising the photographic magnification. A scale fastened to the head is not adequate as it is inevitably not in the same position as the eyes and may undergo translations with respect to the camera different from those of the eyes during rotation of the head about a bitemporal axis. Instead, the distance between the corneal reflections of the electronic flashgun used in taking the photographs was taken as a standard—although it will be seen that the use of this is not straightforward.

Thus, if the interpupillary distance of an animal involved in a distant look is \( P_D \) and at the same time the distance between the corneal reflections of the flash is \( S_D \) then the interpupillary distance on the negative, \( P_{DP} \), will be related to \( S \) on the photograph, \( S_{DP} \), in the following fashion,

\[
P_{DP} = \frac{P_D}{S_D} 
\]

\( P_{DP} \) is thus the slope of the regression line obtained by plotting \( P_{DP} v S_{DP} \).
Fig. 1. A diagrammatic representation of photographs of the cat's eyes during a distant look and near looks both with and without vergence. The black bar represents the pupil, the black circle is the reflection of the electronic flash and the black star is the corneal marker. The open symbols in the vergence diagram indicate the positions of the various features during the preceding distant look. The explanation of the symbols is to be found in the text and Nomenclature.

If the cat does not converge when looking from the distant to the near object then the near look data set \((P_{SN}, S_{SN})\) will lie on the same regression line as the distant look data set.

If the cat does converge when looking from the distant to the near object on the midline then each eye will rotate medially through an angle \(\theta_v\) which is the difference between the

Fig. 2. Diagram of a horizontal section through the right eye of the cat with the optic axis and cornea shown in two positions; D and d respectively for the distant look and N and n for the near look. The separation of the centre of curvature of the cornea, \(R\), from the centre of rotation of the eye, \(C\), means that, during vergence, the image of the distant electronic flash is displaced laterally from \(ld\) to \(ln\) by the amount \(\gamma/2\) which is smaller than the displacement, \(A_{p}/2\), of the apparent pupil, \(A\). Other symbols are to be found in the text or Nomenclature.
Vergence in the Cat 1965

Vergence associated with the distance look \( \theta_d \) and that associated with the near look \( \theta_n \) (Fig. 2). Such a rotation will bring the pupils nearer to one another by the amount \( \Delta_p \) which is a function of the angle \( \theta_n \) and the distance between the centre of rotation of the eye, \( C \), and the apparent plane of the pupil, \( A_D \) and \( A_N \), (the pupil, as seen through the cornea, appears in front of its actual position; the entrance pupil of Vakkur and Bishop, 1963), where \( C \) and \( A \) are given as the distance of the centre of rotation and apparent plane of the pupil from the corneal vertex in mm. \( \Delta_p \) is thus given by,

\[
\Delta_p = 2 \cdot \sin \theta_n (C - A_N) - 2 \cdot \sin \theta_n (C - A_D)
\]  

(Fig. 2). The regression line for the near-look-with-convergence data set \( (P_{NCp}, S_{NCp}) \) will thus drop below the line for the distant look data set.

Because the centre of curvature of the cornea, \( R \) mm from the corneal vertex, is displaced from the centre of rotation of the eye, \( C \), the distance between the reflection of the electronic flash in each eye will be reduced from the distant look value \( S_D \), if the animal converges, by an amount \( \gamma \) which is given by

\[
\gamma = 2 \cdot \sin \theta_n (C - R) - 2 \cdot \sin \theta_D (C - R)
\]  

(Fig. 2).

From equation (1) we may write by analogy a similar equation for photographs of a near look with convergence in which \( P_D \) and \( S_D \) have been reduced by \( \Delta_p \) and \( \gamma \) of equations (3) and (5) respectively. Thus we have

\[
P_{NCp} = \frac{P_D - \Delta_p}{S_D - \gamma} \cdot S_{NCp}
\]  

which now defines the regression line for the near look data set. The vertical distance between the distant and near look regression lines may now be derived to give an estimate of the magnitude of the change which the method must detect at any given photographic magnification. At any given value of \( S = S_D = S_{NCp} \) the distance between the lines is given by the difference between \( P_D \) and \( P_{NCp} \).

Thus,

\[
P_D - P_{NCp} = \frac{P_D}{S_D} \cdot S_{NCp} - \frac{P_D - \Delta_p}{S_D - \gamma} \cdot S_{NCp}
\]  

\[
= \left[ \frac{P_D}{S_D} - \frac{P_D - \Delta_p}{S_D - \gamma} \right] \cdot S_{NCp}
\]  

\[
= \left[ \frac{\Delta_p}{S_D - \gamma} \right] \cdot S_{NCp}
\]  

because \( P_D/S_D = 1 \)

\[
= (\Delta_p - \gamma) \cdot \frac{S_{NCp}}{S_D - \gamma}
\]  

The regression line for the near look data set may thus be written in an alternate form to (6) above,
\[ P_{NCP} = P_{DP} - \left[ \frac{\Delta P - (P_D/S_D)\gamma}{S_D - \gamma} \right] \cdot S_{NCp} \]  

The significance of this equation may be seen in the diagram of Fig. 3.

**Fig. 3.** An illustration of the meaning of equation (11). Interpupillary distance, measured from distant look, \( P_{DP} \), and near look, \( P_{SN} \), photographs, is plotted against the corresponding separation of the corneal light reflex, \( S_S \) or \( S_D \). The regression lines for \( P_D \) and \( P_{NCp} \) are shown. Inspection reveals that the absolute decrease in interpupillary separation, \( \Delta y \), upon vergence at unity magnification, \( S_N = S_D - \gamma \), is only detectable by this photographic technique as a smaller separation of the regression lines, \( \Delta_P - P_D/S_D \gamma \), because of the concomitant reduction of \( S_D \) by the amount \( \gamma \).

At a given value of \( S_{NCp} \) any actual reduction in the interpupillary distance during vergence, \( \Delta_P \), is thus represented by an apparent reduction on the photograph,

\[ \Delta_{PP} = P_{DP} - P_{NCp} \]  

From (10) above, when a photograph is at unit magnification and,

\[ S_{NCp} = S_D - \gamma \]  

\( \Delta_{PP} \) is given as,

\[ \Delta_{PP} = \Delta_P - \gamma. \]  

Now when the stimuli are presented near to the midline and \( \theta_N \) is not large we may substitute from (3) and (5) into (14) above and simplify considerably by letting \( A_D = A_N \) (the error is about 1 per cent at the distances used) if \( \theta_D \ll \theta_N \). We obtain

\[ \Delta_{PP} = \Delta - \gamma = 2. \sin (\theta_N - \theta_D). (R - A_N) \]  

so that at unity magnification the position of the centre of rotation of the eye, \( C \), has dropped out of the equation (15).

The apparent vergence, \( \Delta_{PP} \), given by \( \Delta_P - \gamma \) at unit magnification is thus smaller than \( \Delta_P \) by the amount \( \gamma \) and is consequently more difficult to detect; the direct result of using a
Vergence in the Cat

magnification reference standard \( S_p \) which changes upon vergence of the eyes. We have, however, avoided the problems of either restraining the cat or of attaching near to its eyes standards which untrained animals spend a great deal of time attempting to remove.

For photographs at magnification other than unity the apparent reduction in interpupillary distance may be written in full by combination of equations (5), (10) and (15).

\[
\Delta r_p = \frac{2 \cdot \sin (\theta_N - \theta_D) \cdot (R - A_N) \cdot S_{NCR}}{S_D - 2 \cdot (\sin (\theta_N - \theta_D) - (C - R)}
\]

if \( \gamma \ll S_p \) then,

\[
\approx \frac{2 \cdot \sin (\theta_N - \theta_D) \cdot (R - A_N) \cdot S_{NCR}}{S_D}
\]

Thus, if we have an estimate of the position of the centre of rotation of the eye we may use equation (17), if not then (18), to define the position of the theoretical near look regression line relative to the pragmatically determined distant look regression line by calculating the vertical separation between them at two well separated values of \( S \). The lines will not, of course, be parallel. The pragmatically determined set of near look results should lie on the appropriate near look theoretical regression line of convergence has occurred under the conditions of the experiment.

The accuracy of the technique will be discussed in the methods section but it should be noted that the use of the pupil for these measurements introduces several possible sources of error.

(1) The centre of the pupil is estimated for each eye by taking the mean of the distance between the inside and outside margins. Symmetry of expansion is thus assumed. If this does not occur then any systematic tendency for a larger or smaller pupil during the near or far look sets alone would lead to spurious indications of vergence.

(2) The distance of the pupil from the corneal apex is not fixed. If it dilates, then it recedes as its margins ride over the convex anterior lenticular surface away from the apex of the lens. This effect is small, however, in comparison to the movement of the pupil towards the corneal apex during the forward movement of the lens surface accompanying accommodation for near vision. Such changes may not relate to the degree of vergence.

The magnitude of the medial movement of the pupil during rotation of the eye is thus subject to factors other than the angular deviation of the eye. The justification for the use of the method is that it is easy and does not render the animals unco-operative. It is moreover adequate for the detection of vergence, our main aim, if not for the quantitative study of its magnitude because the worse combination of circumstances would not more than halve the separation of the lines.

The corneal marker is much more reliable (egg membrane or rapid drying paint on the anaesthetised cornea) and its use has the added advantage that the mark is further from the centre of rotation than is the pupil so that the reduction in the intermarker distance is greater than that in the interpupillary distance for a given vergence.

A set of equations similar to those for the pupil case may be derived for corneal markers separated by the distance \( M_p \). The use of the slightly simplified equations requires that
$M_p/S_D$ must be in the order of 1; $A_N$ is absent and the final equation for the apparent decrease in intermarker distance $\Delta_{M_p}$ is given by,

$$\Delta_{M_p} = \frac{R \cdot S_{NCP}}{S_D/2 \cdot \sin (\theta_N - \theta_D) - (C - R)}$$  \hspace{1cm} (19)

if $S_D \gg \gamma$ then,

$$\approx 2 \sin (\theta_N - \theta_D) \cdot \frac{S_{NCP}}{S_D}$$  \hspace{1cm} (20)

For the reasons mentioned above it is possible to attribute any reduction in the intermarker distance in photographs of the near look set to vergence movements alone and thus the magnitude of the vergence movements in terms of angle may be derived by solving equation (19) for $\theta_N$ when we obtain,

$$\theta_{N_M} = \arcsin \left[ \frac{S_D/2}{\Delta_{M_p}} \left( \frac{S_{NCP}}{\Delta_{M_p}} + (C - R) \sin \theta_D \right) \right]$$  \hspace{1cm} (21)

in simplified form from (20),

$$\theta_{N_M} = \arcsin \left[ \frac{\frac{\Delta_{M_p} \cdot S_D}{2 \cdot S_{NCP} \cdot R} + \sin \theta_D}{\sin \theta_D} \right]$$  \hspace{1cm} (22)

In the later experiments measurements have been made using pupils and markers in the same photographs. Equations (21) and (22) as derived for the pupil have the form,

$$\theta_{N_p} = \arcsin \left[ \frac{S_D/2}{(R - A_N) S_{NCP} + (C - R) \sin \theta_D} \right]$$  \hspace{1cm} (23)

in simplified form,

$$\theta_{N_p} = \arcsin \left[ \frac{\Delta_{N_p} \cdot S_D}{2 \cdot S_{NCP} \cdot (R - A_N)} \sin \theta_D \right]$$  \hspace{1cm} (24)

The vergence angle predicted by the marker and pupil methods may be compared but in the latter case they will only be valid if the correct assumptions are made about the state of accommodation when setting the theoretical value of $A_N$. Ideally the comparison of the results from the two methods should enable the state of accommodation of the animal to be assessed in each photograph as demonstrated in a later section but this is not very reliable with the present techniques.

**A CHECK OF THEORETICAL ASSUMPTIONS INDEPENDENT OF WHETHER VERGENCE OCCURS IN THE CAT**

The validity of the theoretical development and of the values assumed for the various parameters used in the calculation of the theoretical near look regression line from equations (18) and (20) may be tested without obtaining vergence movements. If no vergence movements are detected then, the following test having been made, it is known that the failure is due to their absence rather than that they are unexpectedly small as a result of a false theoretical prediction. The method depends upon inducing the cat to make version movements which may readily be obtained and controlled.
Two sets of photographic negatives are required with the animal's head restrained,

1. the cat looks straight ahead at an object \( x \) cm away
2. the cat looks at an object \( x \) cm distant but placed so as to subtend an angle \( \theta_a \) to the point of intersection of the line joining the anterior nodal points of the eyes with the midsagittal plane.

Assume the photographs to be of unit magnification. The distance \( O \) between the pupil centre and the flash reflex spot is measured in the straight look set, \( O_s \), and in the angle look set, \( O_{s,1} \) for the left, \( O_{a,1} \), and \( O_{a,1} \) for the right, \( O_{a,r} \) and \( O_{s,r} \), eyes. Reference to Fig. 4 will show that the difference \( \phi_{pp} \)

\[
\phi_{pp} = O_{s,1} - O_{s,1}
\]  (25)

is given by the difference between the pupillary movement resulting from the rotation through the angle \( \theta_a \),

\[
\sin \theta_a (C - A_x)
\]  (26)

and the corneal reflex movement,

\[
\sin \theta_a (C - R)
\]  (27)

so that

\[
\phi_{pp} = O_{s,1} - O_{s,1} = \sin \theta_a (R - A_x).
\]  (28)

At other magnifications the apparent version is given by the value at unity magnification multiplied by the ratio of the apparent photographic inter-reflex distance to the absolute value under the same conditions, thus (28) becomes

\[
\phi_{pp} = \sin \theta_a (R - A_x) \frac{S_{xc}}{S_{dp}}.
\]  (29)

For vergences up to 10°, \( \gamma \) is so small relative to \( S_a \) that its neglect introduces an error of only 2 per cent or less; \( \phi_{pp} \) is thus effectively independent of any change in standing vergence.
\[ \phi_{pp} = \sin \theta_a (R - A_x) \cdot \frac{S_{NCP}}{S_p}. \]  

(30)

The similarity of (30) to (18) above is obvious

\[ \Delta P_p = 2 \cdot \sin (\theta_N - \theta_D) (R - A_N) \cdot \frac{S_{NCP}}{S_p}. \]

Thus for any given vergence we may choose a version-photographed at similar magnification—such that,

\[ \sin \theta_a = 2 \cdot \sin (\theta_N - \theta_D) \]

(31)

and

\[ A_x = A_N. \]

(32)

with the result that the apparent reduction in the interpupillary distance upon vergence through the angle (\(\theta_N - \theta_D\)) is equal to the change in the separation of the corneal light reflex and pupil centre for one eye during a version of twice that angular magnitude if the stimuli are in both cases presented at the same distance so that accommodation is equal. In such a case

\[ \phi_{pp} = \Delta_{pp} \]

(33)

but the amplitude of \(\phi_{pp}\) during the readily obtained version is independent of whether the animal verges or not and thus enables testing of the theoretical prediction of the magnitude of \(\Delta_{pp}\) to be carried out. The results of the version, or angle look, test may alternatively be used—as they are in a later section—as a substitute for the theoretical line for \(\Delta_{pp}. \phi_{pp}\) provides a readily determined pragmatic expectation line for the vergence results which can be established individually for each cat without the need for assumptions about, or measurements of, the parameters of equation (18).

It should be noted that a similar set of observations may be made by the use of a corneal marker so that the angle look data set becomes \(L_{a,1} - L_{a,1}\) or \(L_{a,r} - L_{a,r}\) where \(L\) is the distance between the marker and the corneal flash reflex. In this case only equality (31) applies for a given value of \(S_{NCP}\); the plane of the entrance pupil is not relevant and the change in intermarker distance \(\Delta_{mp}\) and the reflex-marker distance \(\phi_{mp}\) for vergence of magnitude \(\theta_N - \theta_D\) and version of magnitude \(2 (\theta_N - \theta_D)\) respectively is identical even if the stimulus is presented at different distances in the two cases.

\[ \Delta_{M_p} = \Delta_M - \gamma = 2 \cdot \sin (\theta_N - \theta_D) R \cdot \frac{S_{NCP}}{S_p}. \]

(34)

\[ \phi_{M_p} = L_{a,1} - L_{a,1} = \sin \theta_a (R) \cdot \frac{S_{NCP}}{S_p}. \]

(35)

**METHODS**

The cat was seated in a shallow box placed 65 cm in front of a Zenith 35 mm camera fitted with a 55 mm lens and loaded with Pan F film (Fig. 5). The box was narrow and restricted lateral movement but the animal was free to turn its head, move back, forward and sideways as well as to reach for the stimuli with its paws. Attempts to leave the box were frustrated by an attendant but the cat was otherwise left completely unrestrained.
The Megablitz electronic flash used when taking the photographs was placed 22 cm sideways of the camera in order to prevent an afterimage from being formed on the area centralis. A minimum interval of 30 sec occurred between photographs. The animal showed no sign of distress or avoidance of the flash. Human subjects placed at the same position reported no discomfort.

When taking the distant look photographs the stimuli were presented to the cat behind and slightly above the camera at a distance of two metres from the animal. In this case the turning of the cat's eyes and head to the stimulus was easy to see and the photograph could readily be taken at the appropriate moment.

Two means of stimulus presentation were used for obtaining the near look photographs. Sometimes objects were brought up from a distance—usually with a wiggling motion—and, when the animal's attention had been caught, a lateral motion was induced so that the head and eye tracking could be seen to be occurring as the object came to the 10 cm marker. If attention was still clearly held at this point the photograph was taken. In other cases the object was popped up from behind a screen at the appropriate distance in front of the cat. Each session lasted from 5–10 min.

The final position of the stimulus varied but was always near to the midline and usually slightly to the side away from the flashgun so that the subsequent photograph shows that the eyes and head were directed towards the stimulus and not to the camera or to the experimenter who stood behind the flash. As a further test of the direction of gaze the stimuli were presented above or below the level of the eye so that the pupil could be seen to be appropriately elevated or depressed.

A great variety of stimuli were used to attract attention and these were frequently changed: squeaky rubber toys, bunches of keys, cotton wool on a string, kittens, guinea pigs, food, a rubber glove and most successful of all, a pencil, a stick with about 1 in. of string at the end and a feather duster with the feathers tied together to limit the visual angle it subtended. The feather duster and pencil were, in agreement with Marchalavafa and Elkur (1964), found to be more effective in holding attention than food and other animals.

The conditions and methods used in the production of the angle look photographs were very similar to those described for the near and distant look sets. The major difference was that the animal's head was restrained by an assistant and prevented from rotating so that the cat could only move its eyes to look at an object not on the visual axis. The stimuli were presented at a distance of 100 or 25 cm either straight ahead of the animal or displaced 20° sideways so that a conjugate version was required for their inspection. The stimuli were made to pop up from behind a screen at the appropriate distance from the cat. The photograph was taken when the version movement had been seen to occur.

For the marked eye set the cornea and conjunctiva were first anaesthetised with cocaine or proparacaine and then a tiny spot of either Dibon's mixture (1926; gelatin and barium sulphate) or rapid drying cellulose paint was applied to the cornea away from the visual axis. The remaining procedures were carried out in a similar fashion as for the animals with unmarked eyes.

### Measurements

Measurements of $P_e$, $S_e$, $M_e$, $\phi_e$, and $\phi_p$ were made on the photographic negatives with a travelling microscope which could be read to ±0.001 mm on a sharp border. The magnification of the cat's head on the 35 mm negatives was usually such that the distance between the pupil centres was about 30 mm so that the reading error amounted to 0.06 per cent of the distance under measurement. At unit magnification the distance between the anterior nodal points of the cat's eye is about 36 mm so that the error in measurement would be ±0.01 mm absolute. This, of course, represents optimum accuracy with sharply defined negatives.

The set of measurements for each photograph was repeated at least twice and consisted of a reading taken from each of the margins indicated by a letter in Fig. 6. $S_p$ was estimated from $(AH + BG) / 2$; $S_e$ from $(CJ + DI) / 2$ and $M_e$ from $(EL + FK) / 2$. 

---

**Fig. 5.** Arrangement of camera and subject when making photographs for the near look set.
This estimate of the interpupillary distance is satisfactory if the pupils are of constant size in each photograph of a set or if the medial and lateral pupillary margins move symmetrically. In this experimental series the pupil dimensions were not constant so that a check of symmetry of dilation was carried out. The average value of the pupil width at the widest point for the two eyes was plotted against the normalised interpupillary distance from the distant look data sets of three animals. No correlation was noted between the two over the range of 1-60 mm in pupil width. The small magnitude of the standard deviation of the points in the distant look sets from the mean regression line is further evidence that any asymmetry of pupillary expansion is small enough to give no spurious indications of vergence or divergence. No similar problem arises in the measurement of $S_D$ and $M_F$ as the reflex and mark dimensions are fixed.

For the straight and angle look sets:

$$A + B, \quad C + D, \quad E + F, \quad G + H, \quad I + S, \quad K + L$$

were recorded. From these readings $O_a, O_b, L_a$ and $L_b$ (p. 13) could be calculated for the determination of $\phi_a$ and $\phi_b$, in left and right eyes. The mean of the set was accepted as a final result.

The influence of movements of the animal upon the accuracy of the method

In all of the photographs used the cat deviates little from its standard position at the centre of the negative. The movement of the head was seldom more than 2-0 cm sideways, and not much more back and forth.

The graphical method for analysing the results automatically allows for the variations in the magnification of the photographs resulting from axial movements of the cat's head but this is based upon the assumption that $S_D$ and $S_A$ are not influenced by other head movements. This assumption must be justified because the flashgun is not infinitely distant. We must begin by deriving the value of $S_D$ at some fixed vergence; for convenience we assume the visual axes to be parallel.

Reference to Fig. 7 shows that the angle $\theta$, which is made between the parasagittal plane passing through the centre of rotation of each eye and the axis upon which the image of the flash lies, the line running from the electronic flash to the centre of the cornea $R_c$, will be larger for the right than for the left eye because the flashgun, $F$, is situated nearer than infinity and is to one side of the midsagittal plane, $M$.

The displacement of each image from the parasagittal plane will thus be different for the two eyes and the magnitude of $S$ will be less than the distance (36 mm on average) separating the two parasagittal planes containing the visual axes.

![Fig. 7. Plan view and dimensions of the experimental setup used during photography. $L$ is the distance between the camera on the midsagittal plane and the electronic flash, $F$. The origin of the difference in the size of $aR$ and $aL$ is displayed. See text for explanation.](image)
Vergence in the Cat

From Fig. 7 we have

\[ \alpha_R = \arctan \frac{L + b}{D} = 19.8^\circ \]  \hfill (36)

\[ \alpha_L = \arctan \frac{L - b}{D} = 17.0^\circ \]  \hfill (37)

From Fig. 2 it is readily seen that the displacement, \( X \), of each image, \( \Delta \), of the flash from the parasagittal plane containing the visual axis is given by,

\[ X = \frac{R}{2} \sin \alpha \]  \hfill (38)

where \( R \) (mm) = RV of Fig. 2, because the image of the flash is formed at \( R/2 \) when \( D > R \). From (36) and (37) above we have \( \alpha_R \) and \( \alpha_L \) so

\[ X_R = \frac{R}{2} \sin \alpha_R = 1.458 \]  \hfill (39)

\[ X_L = \frac{R}{2} \sin \alpha_L = 1.26 \]  \hfill (40)

\[ X_R - X_L = 0.2 \text{ mm.} \]  \hfill (41)

If the pupil centres, the corneal centre of curvature and the centre of rotation are assumed to fall on one line then the value of \( S_{0-0} \) would thus be given by

\[ S_{0-0} = \left( P_{0-0} - (X_R - X_L) \right) = [36.0 - (0.2)] = 35.8 \text{ mm} \]  \hfill (42)

If \( P_{0-0} = 36.0 \text{ mm} \). The smaller value for \( S_p \) actually obtained from the slope of distant look regression lines may result from the presence of a degree of vergence and the pupils being placed lateral of the visual axis.

Now when the cat moves his head back, forth and sideways it is the difference between \( \alpha_R \) and \( \alpha_L \) and thus between \( X_R \) and \( X_L \) that will change so that \( S_p \) itself will undergo absolute variations quite independent of the photographic magnification changes in \( S_{0-0} \).

For the estimation of the magnitude of this error we have allowed a theoretical movement of the cat which considerably exceeds that to be found in the photographs used. The range is assumed to be 6 cm forward, backward, left and right of the standard position. Within this range the largest difference in the value of \( S_p \) would occur between case 1 in which the head is 6 cm in front and 6 cm to the left of the standard position and case 2 in which the head is 6 cm behind and 6 cm to the right of the standard position. This worst case for the variation in \( S_p \) subsequent to head movement is given by the difference,

\[ E = S_{0-1} - S_{0-2} = (X_R - X_L)_1 - (X_R - X_L)_2 \]  \hfill (43)

The situation is shown in Fig. 8.

![Fig. 8. Geometry upon which is based the estimate of the changes in \( S_p \) resulting from movements of the cat's head. The diagram is explained in the text.](image_url)
From Figs. 7 and 8 it is seen that the following equalities apply

\[ X_{R} - X_{L} = \frac{R}{2} \sin \left[ \arctan \frac{L + \Delta L}{D} \right] - \frac{R}{2} \sin \left[ \arctan \frac{L - \Delta L}{D} \right] \]

\[ = 0.232 \text{ mm} \]

\[ X_{S} - X_{C} = \frac{R}{2} \sin \left[ \arctan \frac{L + \Delta L}{D + \Delta D} \right] - \frac{R}{2} \sin \left[ \arctan \frac{L + \Delta L}{D + \Delta D} \right] \]

\[ = 0.172 \text{ mm} \]

from equation (43)

\[ E = (X_{R} - X_{L})_{1} - (X_{S} - X_{C})_{2} = 0.232 - 0.172 = 0.06 \text{ mm.} \]

The variation in \( S_{0} \) as a result of even these excessive assumed head movements amounts to only 0.06 mm, about 0.002 per cent of its value, and the method is seen to be exceedingly insensitive to movements of the head as was reported by Cowey (1963).

Further consideration of the situation will also reveal that if \( S_{0} \) or \( S_{CP} \) is measured along the line joining the images of the flash in the left and right eyes then the method is uninfluenced by the rotation of the head about its long axis. This assumes that the cornea is not astigmatic.

**Test of the method with a human subject**

The accuracy and sensitivity of the technique was examined by the use of a human subject who was photographed in the same fashion as the cat while looking at a small marker placed on the midline at various distances from the eyes.

The results were plotted in the usual fashion as the interpupillary distance, \( P_{p} \), against the inter-corneal reflex distance, \( S_{c} \); the data for each degree of vergence was fitted with a regression line.

The pragmatically determined regression line for the distant look, 200 cm, was used as a reference and the drop to each of a set of theoretically determined regression lines, corresponding to each of the vergences in the experimental series, was calculated by means of equation (11)

\[ P_{D} - P_{CP} = \frac{\Delta P - (P_{D}/S_{D}) \cdot \gamma}{S_{CP} - \gamma} S_{CP} \]

The experimental and theoretical lines are shown in Fig. 9.

The values of the various parameters were obtained from Gullstrand's schematic eye (Helmholtz, 1925).

\( P_{D} \) for the subject was 6.5 cm, \( P_{D}/S_{D} = 1.01 \), \( R = 7.7 \text{ mm} \) and the centre of rotation of the eye, \( C \), was taken as 13.5 mm (Park and Park, 1933). The pupil was assumed to lie on the front surface of the lens. According to Fincham (1937) the lens was adjusted forward by 0.5 mm during a change of accommodation from 1 to 8 D. The position of the lens anterior surface was estimated for each viewing distance by linear interpolation from this value. The apparent plane of the pupil, \( A \), was then calculated by means of the schedule of Bennett and Francis (in Davson, 1969, Vol. 4). These data enabled \( \Delta P \) and \( \gamma \) to be calculated from equations (3) and (5). It is to be noted that the regression lines were calculated for values around \( S_{CP} \) of 4.7 mm which corresponds to a magnification of 0.012—approximate value for the head in the photographic negatives. The theoretical results, plotted in Fig. 9 with the experimental findings, are shown below in Table 1; the angles of vergence are given as changes relative to the vergence required for viewing at 200 cm (56° vergence for one eye's visual axis from the sagittal plane).

**Table 1**

<table>
<thead>
<tr>
<th>Distance</th>
<th>200</th>
<th>45</th>
<th>35</th>
<th>25</th>
<th>15</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vergence</td>
<td>0°</td>
<td>3°12</td>
<td>4°23</td>
<td>6°28</td>
<td>11°15'</td>
<td>17°11'</td>
</tr>
<tr>
<td>( P_{D} - P_{CP} )</td>
<td>0.0264</td>
<td>0.046</td>
<td>0.071</td>
<td>0.134</td>
<td>0.19</td>
<td></td>
</tr>
</tbody>
</table>

\( (S_{CP} = 4.7 \text{ mm}) \)

The agreement between the theoretical predictions and the regression lines obtained is very good; the method even picked up 1-1° of vergence between the 45 and 35 cm lines. The 0.134 mm vertical separation of the distant and 15 cm regression lines in the human, is similar in magnitude to the theoretical separation of the distant and 10 cm regression lines the cat at a similar magnification. The separation is readily seen in the human results of Fig. 9 and should be equally readily detected if present in the cat.
RESULTS

Eighteen cats were studied with this photographic technique. The first twelve were examined without a corneal marker; of these, five showed little or no sign of vergence. The remaining seven gave unequivocal evidence of vergence.

Unfortunately a group of animals which did not clearly demonstrate vergence came at the beginning of the series and it was initially concluded, because the vergences were not appropriate to the stimulus presentation distance, either that the movements observed were artifacts, perhaps resulting from displacement of the pupil during accommodation, or that the cat was capable of vergence to a fixation distance little nearer than about 40 cm. The animal described later as cat "C" was then discovered to reveal vergence of the theoretical magnitude.

It was shortly after this that the corneal marker method was introduced in order to provide a more accurate means of examining those animals apparently not verging to the expected extent.

The results still remained variable. On the one hand there were animals like cat "A" who showed the expected vergence fairly consistently—but not invariably—while on the other were animals like cat "B" which showed no sign of vergence even in the results from the corneal marker data. Of the six cats with marked eyes two revealed vergence of the expected magnitude, two rather less and two revealed no vergence at all. These individual differences will be discussed later but in the results section are included examples from each group. The population of animals with unmarked corneas is also represented below because the technique, although subject to great limitation, can still demonstrate the presence of
vergence and, more importantly, does so without the use of local anaesthetics or interference with the eyes which is an asset in the use of unrestrained cats as voluntarily cooperating subjects.

**CAT "A"**

![Graph](image)

**Fig. 10.** Results of various investigations on cat "A", a good "verger". In this and subsequent graphs the intermarker and interpupillary distances, measured on photographic negatives, are plotted against the corresponding inter-reflex distances for distant and near look data sets. The open circles and the accompanying regression line form the distant look set for the inter-marker distance, $M_{25}$, and the interpupillary distances, $P_{25}$. The capital letters indicate the 25 cm near look measurements for the marker set when below the marker distant look line and for the pupil set when below the pupil distant look line. Each letter thus represents both measurements from one photograph. The same is true for small letters except that they represent the 10 cm near look set. The black discs indicate 10 cm near look measurements for which no corresponding marker reading was available. The distant look lines are accompanied by regression lines for expected vergence, at 25 and 10 cm viewing distances, which were based upon measurements of the marker set, $M_{25}$ and $M_{10}$, and pupil set, $P_{25}$ and $P_{10}$, during versions. These lines indicate the position of the near look points from photographs in which the cat is viewing at the corresponding nominal presentation distance. This cat often achieved vergences close to the magnitude expected for fixation of the nominal fixation plane. The variability of the results is taken up in the discussion.

**Distance look set**

The results of the measurements on the distant look photographs of cat "A" are plotted as the points in Fig. 10 to which a regression line has been fitted. The various parameters for the general equation of the line,
Vergence in the Cat

\[ y = c + bx \]

are shown in Table 2 below where \( \overline{S_{D}} \) and \( P_{D} \) are given in mm.

| Pupil set 36 | \( \overline{S_{D}} \) | 2.822 | 0.9751 | 0.2043 | 2.956 \( \pm 0.00175 \) |
| Mark set 8   | 2.7979 | 0.91813 | 0.6692 | 3.238 \( \pm 0.001097 \) |

**Quantitative prediction of near look regression lines**

The expected drop in Fig. 10 from the distant look regression line to the near look pupil set is given by the simplified equation (18),

\[ \Delta P_{p} = 2 (\sin \theta_{N} - \theta_{D}) (R - A) \frac{S_{NC}}{S_{D}} \]

and that for the drop from the distant to the near look mark set is

\[ \Delta M_{p} = (\sin \theta_{D}) R \frac{S_{NC}}{S_{D}} \]

or equation (20).

We thus require numerical values for the above parameters. \( P_{D} \) for this cat was 36 mm and is assumed to be of this value for the subsequent animals. The various vergence angles required for each fixation distance are thus,

- 200 cm \( \theta_{p} = 31' \)
- 25 cm \( \theta_{p} = 4^\circ 7' \theta_{D} = 7^\circ 12' \)
- 10 cm \( \theta_{p} = 10^\circ 12' \theta_{D} = 19^\circ 22' \)

According to Vakkur and Bishop (1963) the corneal radius of curvature \( R \) has an average value of 8.5 mm. For this cat the curvature was measured with a keratometer as 8.4 mm and the result is used in the calculation.

From the distant set we have \( P_{D} / S_{D} = 1.045 \); because \( P_{D} \) is 36 mm \( S_{D} \) must be 34.4 mm. This leaves only the specification of the apparent pupil plane to be carried out.

The results of Fisher (1971) show that Young's modulus of polar elasticity for the cat lens is too high for accommodation to be effected by change in the shape of the lens. This finding confirms the hypothesis of Vakkur and Bishop (1963) that accommodation in the cat is carried out by forward movement of the lens as a whole. According to the schematic eye of these workers, a forward movement of 1 mm of the lens produces a 3.5 \( D \) change in the power of the eye.

The anterior vertex of the lens is situated, at a point 1, 5.2 mm behind the corneal vertex in the cat schematic eye at rest and moves forward appropriately during accommodation (Vakkur and Bishop, 1963); if not more than about 6 mm wide the pupil may be considered to lie in the same plane.
The apparent plane of the pupil is, however, determined by the refraction of the cornea (corneal power, 39 D; refractive index of the aqueous humour, 1:336) and is calculated from 1 by the following equation,

\[ A = \left( \frac{1}{1 - \frac{1}{1.336}} \right)^{-1} \]

(SOUTHALL, 1964).

In carrying out the theoretical calculations it is necessary to conform to the requirements of the schematic eye. The results of VAKKUR and BISHOP indicate the eye to be 1·5 D hypermetropic. It is thus necessary to assume a forward movement of the lens to bring the animal to emmetropia whether this seems to be likely in the real animal or not. We thus determine the following values for A at the various distances required.

<table>
<thead>
<tr>
<th>Distance (cm)</th>
<th>( A ) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>4·0</td>
</tr>
<tr>
<td>25</td>
<td>3·04</td>
</tr>
<tr>
<td>10</td>
<td>1·5</td>
</tr>
</tbody>
</table>

When calculating the theoretical lines we must be confined to the known range of accommodatory power in the cat. VAKKUR, BISHOP and KOZAK (1963) have considered the literature on this subject and conclude that the amplitude of accommodation in the cat is about 4 D. ELUL and MARCHHAFAVA (1964) report a maximum change of refraction for near objects of about 4–5 D using an infra-red technique. Most reports suggest ranges of accommodation less than this and only MORGAN, MAHONEY and OLMSHEAD (1943) suggest that accommodation of up to 11·5 D is to be found in the cat. It thus appears possible that the cat cannot accommodate more than is appropriate to a viewing distance of about 25 cm. The calculation of \( \Delta_{p} \) for 10 cm was thus carried out using the same value for A as for 25 cm. The results appear to bear out this assumption. It will be remembered from equation (31) that when

\[ \sin \theta_{p} = 2 \sin (\theta_{s} - \theta_{d}) \]

then \( \Delta_{p} = \phi_{p} \) and \( \Delta_{m} = \phi_{m} \) at a given value of \( S_{w} / S_{c} \). Substitution of the above values for the parameters in equations (18) and (20) thus determines the position of the version or angle look lines as well as that of the near look lines. At unit magnification calculation gives \( \Delta_{p} = 1·7 \) mm and \( \Delta_{m} = 2·7 \) mm for fixation at 10 cm. Theoretical values of \( \phi_{p} \) and \( \phi_{m} \) for the corresponding \( \theta_{p} \) are identical and only these are given in the tables below.

**Angle look results**

The cat was caused to look 20° sideways with the head restrained. Version data from the pupil and marker positions were obtained for a viewing distance of 25 cm. Another set of pupil results was obtained with a viewing distance of 100 cm in order to change the state of accommodation. The reasons for this will become apparent later. The results were consistent and averaged values are presented below in Table 3 computed as described in the Methods section.

The actual and theoretical apparent displacements of pupil and corneal markers for a 20° version are thus seen to differ by about 16–20 per cent in cat "A". Considering the assumptions made this is not an excessive deviation, but no specific source has been assigned.
Clearly, when considering the near look data, the results should be compared with the pragmatic expectation of the vergence magnitude, in the form of the results from the appropriate version,

$$\theta_a = 2 (\theta_n - \theta_p)$$

rather than with the theoretical predictions (see page 1968). The results above require conversion to values of version appropriate to the specific vergence angles of interest i.e.

for vergence to 25 cm \( \theta_a = 7^\circ 12' \)

for vergence to 10 cm \( \theta_a = 19^\circ 22' \).

The apparent vergence movements of the pupils and marker obtained for 20° of version thus require reduction in the ratio of the sines of \( \theta_a \) and 20°. We thus obtain,

Near look results

The measurements made on the near look set of photographs of cat “A” are presented in Fig. 10 accompanied by the marker and pupil distant look regression lines and the expected vergence lines which are based upon the angle look data set for the same animal. It is thus easy to see how near the value of each point representing \( P_{N_p} \) or \( M_{N_p} \) approaches that predicted for the 25 cm or 10 cm viewing distances.
The variation in the apparent vergence achieved at any given viewing distance is considerable and an average value did not appear to be appropriate. Variation of such a degree is not found in the distant look data sets so that the method is apparently free of any gross random error; the fluctuations in the near look data set are thus assumed to be significant and to require individual investigation. This is taken up in the next section.

It is clear that cat “A” is verging to some extent in most of the near look photographs and in certain cases the vergence appropriate to the viewing distance is achieved. The magnitude at the vergence movements is generally much greater for the 10 cm than for the 25 cm viewing distance.

### The vergence accommodation relationship

From each near look photograph we obtain an observed value for \( \Delta_{MP} \). For that stimulus presentation distance we also have an expected value, \( \Delta_{MP} \), which is equal in magnitude to the corresponding angle look prediction, \( \phi_{MP} \), when \( S_{NCp1} = S_{NCp2} \) and \( 2(\sin \theta_{N2} - \theta_D) = \sin \theta_D \). The ratio of the observed and expected values is given by,

\[
\frac{\Delta_{MP1}}{\phi_{MP}} = \frac{\Delta_{MP2}}{\phi_{MP}} = \frac{2(\sin \theta_{N1} - \theta_D)}{2(\sin \theta_{N2} - \theta_D)} \times \frac{S_{NCp1}}{S_D} \times \frac{S_{NCp2}}{S_D}.
\]  

Solving equation (47) for the angle of vergence, \( \theta_{N1} \), associated with the observed value of \( \Delta_{MP1} \), we have,

\[
\theta_{N1} = \arcsin \left[ \frac{\Delta_{MP1}}{\phi_{MP}} \times \sin \theta_{N2} + \sin \theta_D \right].
\]

From \( \theta_{N1} \) we may calculate the fixation distance, \( F \), by elementary trigonometry. No assumptions about the pupil plane are involved and any constant under or over estimation of the results by the theoretical equations is irrelevant.

\[
F = \frac{1.8}{\tan \theta_{N1}} \text{ cm.}
\]

For one photograph the ratio between \( \Delta_{p} \) and \( \Delta_{MP} \) may be written as

\[
\frac{\Delta_{p}}{\Delta_{MP}} = \frac{2(\sin \theta_{N} - \theta_D)}{2(\sin \theta_{N} - \theta_D)} \times \frac{(R - A)}{R} \times \frac{S_{NCp}}{S_D},
\]

\[
= \frac{R - A}{R}
\]

\[
\therefore A = R - R \left( \frac{\Delta_{p}}{\Delta_{MP}} \right)
\]

from equations (18) and (20). In equation (50) \( A \) is the only unknown so that after re-arrangement equation (51) provides a means for its determination when \( \Delta_{p} \) and \( \Delta_{MP} \) are available from one photograph.

An equation similar to (51) may be obtained for the angle look set (53). In the original data for the 20° angle look set (Table 3) we have a value for \( \phi_{p} \) when the viewing distance was 100 cm. In this instance the lens will be displaced from its position in emmetropia by an
amount necessary to generate only 1D of power. Correcting the values in Table 3 to uniform magnification we have for the position of the lens anterior surface with 1D of accommodation,

$$ A = R - R \cdot \frac{(\phi_{20}^* F200 \text{ cm})}{(\phi_{20}^* M_P)} $$

$$ = 8 \cdot 1 - 8 \cdot 1 \frac{(0.09)}{(0.1845)} = 4.1 \text{ mm behind corneal vertex} $$

We are now in a position to determine the state of accommodation of cat “A” in each near look photograph. Equation (51) enables the apparent position of the pupil to be calculated. Subtraction of this value from the effective resting position, 4.1 mm, gives the forward movement of the lens; the effect of this on the power of the eye is obtained from Vakkur and Bishop’s figure of 3.5D per mm of lens forward displacement (1963).

The refractive state of the eyes of the cat in each near look photograph may thus be related to the fixation distance calculated from equations (48) and (49). The two factors are plotted against one another in Fig. 11 for each of the alphabetically indexed points of Fig. 10. It is obvious that the fixation distances fall into two groups which correlate well with the 25 cm and 10 cm stimulus presentation distances. The maximum accommodation...
achieved for 10 cm fixation is only 5·5 D, rather than the expected 10 D, which is in keeping with the limited accommodatory power believed to be available to the cat (p. 1978). That which is available is exploited more in the viewing of objects at 10 cm than at 25 cm because 50 per cent of the 10 cm, compared with 25 per cent of the 25 cm, photographs revealed accommodation of more than 2·5 D. The lack of correlation of the accommodation evinced in the remaining photographs with the animal's fixation distance is in keeping with the variability of the accommodatory response of the cat as described by ELUL and MARCHIAFAVA (1964). The technique is clearly being pushed to its limits but it is worth noting that the pupil plane for 1 D, p. 1981, is very close to that predicted by the cat schematic eye, p. 1978.

**CAT "B"**

![Diagram](image)

**Fig. 12.** Cat "B". The near look points, black discs, fall on the corresponding distant look regression lines for both marker and pupil sets, open circles. There is no suggestion of vergence in the results.

The results are shown in Fig. 12. Regression lines are plotted for the marker and pupil distant look data and for the prediction of the 10 cm vergence based upon the 19°22' version data for the same animal. The parameters of the distant look regression lines are as follows,
Vergence in the Cat

Table 5

<table>
<thead>
<tr>
<th>No.</th>
<th>$\bar{S}_{dp}$ (mm)</th>
<th>$b$</th>
<th>$c$</th>
<th>$\bar{P}_{dp}$ (mm)</th>
<th>S.E. (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marker</td>
<td>18</td>
<td>2.362</td>
<td>1.008</td>
<td>0.305</td>
<td>2.686</td>
</tr>
<tr>
<td>Pupil</td>
<td>18</td>
<td>2.362</td>
<td>1.008</td>
<td>0.076</td>
<td>2.455</td>
</tr>
</tbody>
</table>

The mean drop from the distant look regression line to the angle look line is shown below (Table 6).

Inspection shows that the points of the near look result set fall on the distant look regression line; indeed, a statistical investigation gives no indication that the two sets of results might be from different populations. The largest deviation amongst the points of the near look set from the marker distant look line corresponds to a fixation point 47 cm from the eye. It is clear that cat “B” did not carry out any eye movements that could be accepted as significant fixation vergences.

Table 6

| $\theta$ | $S_{NCp}$ (mm) | $\phi_{dp}$ (actual) (mm) | $\phi_{dp}$ (theory) (mm) | Actual $\phi_{dp}$
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>19°22'</td>
<td>2.340</td>
<td>0.148</td>
<td>0.18</td>
<td>0.82</td>
</tr>
<tr>
<td>19°22'</td>
<td>2.340</td>
<td>0.076</td>
<td>0.095</td>
<td>0.80</td>
</tr>
</tbody>
</table>

CATS “C” AND “D” (NO MARKER)

The parameters of the pupillary distant look regression lines of Fig. 13 for cats “C” and “D” are as follows (Table 7).

Table 7

<table>
<thead>
<tr>
<th>No.</th>
<th>$\bar{S}_{dp}$</th>
<th>$b$</th>
<th>$c$</th>
<th>$\bar{P}_{dp}$</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat “C”</td>
<td>13</td>
<td>2.8</td>
<td>1.02</td>
<td>0.02</td>
<td>2.88</td>
</tr>
<tr>
<td>Cat “D”</td>
<td>21</td>
<td>3.01</td>
<td>1.056</td>
<td>-0.08</td>
<td>3.096</td>
</tr>
</tbody>
</table>
Fig. 13. Measurements on photographs of cats "C" and "D" plotted in the same fashion as for Fig. 10. For these animals only pupil data sets are available. The open circles represent measurements in the distant look set and the black discs form the 10 cm near look set. The black triangles indicate readings from photographs for which cat "C" was presented with stimuli 25 cm distant. Clearly cat "C" can fixate with appropriate vergence both 25 and 10 cm distant stimuli. The range of vergence of cat "D" appears to be more limited; its greatest vergence corresponds to a fixation point about 22 cm distant.

The mean drop from the distant look to the angle look line is,

<table>
<thead>
<tr>
<th>Cat</th>
<th>( \theta_v )</th>
<th>( s_{NC} ) (mm)</th>
<th>( \phi_{Petit.} ) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;C&quot;</td>
<td>19°22'</td>
<td>2.9</td>
<td>0.085</td>
</tr>
<tr>
<td>&quot;D&quot;</td>
<td>19°22'</td>
<td>3.075</td>
<td>0.08</td>
</tr>
</tbody>
</table>

The angle look regression line is for a viewing distance of 100 cm in both cases and thus indicates the expected position of the near look points corresponding to a vergence of 10 cm with only 2.5D of accommodation (p. 1978). A larger decrease in interpupillary distance would be expected in the case of greater accommodation.
Inspection of the near look points for cat "C" in Fig. 13 reveals that some actually fall on the $\phi_{f_n}$ angle look regression line and thus indicate a fixation point at 10 cm if the cat is accommodating to the same extent as during the angle look determinations. The angle look set was, however, obtained for a stimulus presentation distance of 100 cm so that more accommodation is to be expected when viewing objects 10 cm distance in the near look set. In those near look samples for which this is true the comparison of the result with the corresponding angle look line will indicate a fixation point nearer than is the case and consequently, an excessive angle of vergence. It is readily established, however, if the schematic eye has no more than 5-5D of accommodation available (of which 1-5D is used to eliminate its hypermetropia), that this overestimate cannot be greater than about 20 per cent when accommodation is maximal and proportionally less when it is not.

The largest vergence indicated in the results from cat "C" thus lies between 9-6° and 8-5°, depending upon the state of accommodation, which corresponds to a fixation distance of between 10 cm and 12 cm.

In contrast, however, the largest vergence in the results from cat "D" is between 4-75° and 3-9° which corresponds to a fixation distance between 22 cm and 26 cm.

A limited number of 25 cm near look results were available for cat "C" and are also plotted in Fig. 13 as black triangles along with the corresponding version regression line. The cat clearly achieved the expected vergence.

**CAT "E" (NO MARKER)**

![Graph of vergence data for cat E](image)

Fig. 14. Cat "E". These results are plotted in the same fashion as in Fig. 10 but contain only the pupil data sets. The distant look points are plotted as open circles and the near look points as black discs. There is no sign of vergence. Possible explanations for these findings are offered in the Discussion; more quantitative details are given in the results section.

The parameters of the distant look regression line for cat "E" (Fig. 14) are as follows (Table 9).
The mean drop from the distant look regression line to the angle look line is,

\[ \frac{\Delta \theta}{\Delta \phi} \]

In this case the near look set falls on the same regression line as the distant set and statistical investigation shows that there is no significant difference between the two sets of data. There was no indication of vergence under the conditions of this experiment in spite of the presence of all the usual signs of interest in the stimulus.

DISCUSSION

Variability of the results

The results from 11 of the 18 investigated cats unequivocally reveal the presence of vergence movements. In four of the animals instances of fixation close to the 10 cm stimulus presentation plane were recorded; seven cats showed apparently inappropriate vergences to 17 cm or further and seven showed little or no sign of vergence change. All the four cats specifically tested revealed vergences appropriate to the 25 cm stimulus presentation distance. The main aim of the experiments was thus achieved in that the ability of the cat to

Fig. 15. A set of photographs of cats carrying out some of the acts of visual fixation described in this paper. Photographs A and B show distant and near looks respectively for one cat. The presence of greater vergence in the near look photograph is readily detectable by observing the relationship between the pupils and the corneal reflection of the electronic flash. In photograph A the reflection and pupil coincide in the animal's left eye but are well separated in the right eye, the reflex being medial of the pupil. In the near look photograph, B, the light reflexes are almost equidistant from the centre of each pupil. The pupils have thus moved toward one another. A similar change in the relationship of the pupils and the corneal reflex can easily be seen between the distant, E, and near look, F and G, photographs of the second animal. Examination of distant, C, and near look, D, photographs of a non-verging cat reveals no change in the relative distance between the corneal reflexes and the pupil centres. Photograph H shows a cat looking straight ahead at an object one meter distant. Photograph I shows the version resulting when the stimulus is suddenly presented at the same distance but displaced 20º sideways along the horizontal. For one eye the change in the separation of the corneal reflex and pupil between the straight and angle looks is roughly twice the magnitude to be expected for a change in midline vergence from infinity to 10 cm. A point worthy of note, which is to be taken up elsewhere, is that the laterally placed flash produces shadows on the pupil which bring into relief the subadjacent lens. This may be seen most readily in the right eye of the cat in photographs A and B. The ring of shadowed pupil on photograph A is confined to the apex of the lens but during accommodation for near vision, in photograph B, the lens protrudes much more and the shadow circle is doubled in diameter. A similar change can be seen when comparing photograph E with F and G.
Vergence was demonstrated in 11 animals and in at least four animals it was shown that the magnitude of the vergence varied appropriately with the stimulus presentation distance over the range from 200 to 10 cm.

Apart from the considerable individual differences in the maximal vergence achieved it is obvious from inspection of figures 10-14 that in the results from cats revealing vergence there is great variation in the magnitude of the vergence apparently achieved upon successive presentations of the stimulus at the same distance. Calculation shows that in these animals the standard deviation of the marker and pupil near look sets is from three to ten times greater than that of the corresponding distant look set. There is no reason to assume that any error of measurement is greater for the near set so that the variation must be taken to indicate real differences in the intermarker or interpupillary distances.

It is also apparent that the majority of near look photographs of some cats, and all photographs of others, indicate a vergence smaller than that to be expected if the animals were fixating the stimulus in the nominal presentation plane.

There is little room to doubt that the results from the cats with marked corneas represent the true vergences. Failure to reach the expected magnitude might be regarded as resulting from an excessive predicted value. The expected vergence is, however, based upon the amplitude of \( \phi_m \) or \( \phi_p \) in a suitably selected angle look carried out by the same cat and is thus pragmatically founded and not subject to assumptions of parameter values.

Reasons have been given in the methods section for assuming that the cat is actually looking at the stimulus but the use of the predicted vergence line is based upon the assumption that the animal is fixating the stimulus in the nominal presentation plane. For some narrow stimuli this may be a valid assumption but, with the exception of a vertical pencil and a key ring, most of these stimuli were not successful in arousing the subject's interest. The most effective stimuli were larger and extended for at least several centimetres back from the presentation plane, beyond which they did not protrude. This ambiguity of the stimulus plane means that there is a range of distances at which the cat may be carrying out satisfactory fixation of the stimulus. This explanation provides a basis for the unimodal distribution of results in Fig. 10 in which nearly all the near look observations are less than the expected value whether for the 10 or 25 cm presentation distances.

The work of Westheimer and Mitchell (1956) and Yarbus (1957) on the human shows that, during fixation, version and vergence are carried out simultaneously but that in the latter stages vergence continues alone. Too precipitate photography may thus produce an incomplete record of the vergence achieved if the completion of fixation version is used as an index of the attainment of maximum vergence. This possibility is also compatible with the persistent apparent underachievement of the expected vergence.

According to Zernicki and Dreyer (1965) and Dreyer and Zernicki (1969) the fixation reflex can be rather variable in the cat. The nature of the stimulus influences its duration and the reflex habituates rapidly to repeated presentation of stimuli until only abortive fixations occur. Similarly, the studies of Elul and Marchiafava (1964) shows that the accommodatory response is also very labile and not in continual 1:1 correspondence with the stimulus presentation distance. The amplitude of accommodation is reduced upon repeated stimulus presentation, eventually ceasing, and this habituated state may be transferable from one stimulus to another.

Although experimental sessions were kept short in the above series it was clear towards their end that, in spite of frequent changes of stimuli, habituation of the fixation reflex had set in. If sessions were carried out on successive days the state of unresponsiveness in the cat
appeared to become chronic. That habituation of the vergence reflex also occurred was obvious when the results from a good verger were considered in temporal order. One good verger was kept as a demonstration animal but became useless for this purpose within a very short period. It is clear that habituation of vergence is almost certainly a source of considerable variation in the results.

The variability of the accommodatory response described by Elul and Marchiafava (1964) will, of course, lead to variations in the apparent vergence demonstrated in the pupillary sets quite independent of the magnitude of vergence. The expected apparent vergence is thus subject to the unpredictable influence of up to 4 D of accommodation on the apparent pupil plane.

In passing, it is worth noting that the lack of correspondence between the fixation plane and the accommodatory state of the eye to be seen in the results from cat “A”, Fig. 11, is in keeping with the findings of Elul and Marchiafava (1964).

Some cats gave no evidence of an ability to verge more than about 6° (cat “D” Fig. 13). The 10-cm presentation distance, which corresponds to about 10° of vergence, was selected to produce an easily detectable movement for qualitative investigation. It now appears that this may be too close for most cats and that 15–20 cm might be more appropriate. It should be noted that the ability of the animal to attain a given mean change in the value of φM or φp during an angle look confirms only the values of any parameters used in theoretical prediction and indicates the size of the apparent movement which will occur if the cat verges. It does not establish the ability of the animal to verge by that amount. In the human the maximal vergence may only be about 1/3 of the largest version that the same eye can carry out.

The results from the cats showing vergence movements are not unlike those reported for human beings. Surprisingly large errors, up to 2°, have been described in the position of the human eyes after vergence (Alpern, 1957; Westheimer and Mitchell, 1956). The maximum vergence angle for individual humans varies widely, as in the cat, and in the results of Duane (1933) ranges from about 15° to 38° but, of course, there is no physiological equivalent of the apparent absence of vergence in some cats which is reported here.

Duane’s results are particularly interesting because he concludes that maximum vergence in humans is uninfluenced by age but is subject to improvement by practice. This brings to mind the observation of Lindsay Johnson (1901), quoted in the introduction, to the effect that cats and dogs may be trained to verge. Now large vergences which could be directly observed without measurement are to be seen in only about one-fifth of the laboratory bred cats used in this study. In marked contrast were the family of Danish farm cats, four adults and four kittens, which I had the opportunity to examine after the completion of this work. All of the adults, which are known to be very good hunters, and three of the kittens could readily be seen to verge. It appears probable that practice may play a part in accounting for the difference in the readiness of the two groups of animals to verge to very close objects.

So far no explanation has been put forward for the complete absence of vergence movements from all of the photographs of one animal, e.g. cats “B” and “E”. For the cats with marked corneas, e.g. “B”, it might be argued that, in spite of the use of a local anaesthetic, some residual irritation suppressed the movements. Magnus (1924, p. 154) reports the reduction of a reflex eye movement from 99° to 26° as a result of irritation of the cornea in the rabbit. Such an explanation cannot apply to cat “E” whose eyes were unmarked and in any case version movements of the eyes were readily apparent in cat “B”.

It is unlikely that habituation accounts for the failure to record vergence in seven animals because the measurements were made only on the results of the first session of
Vergence in the Cat

1989

filming when at least the initial presentations would be expected to produce some indication of vergence.

The apparent absence of vergence movements in the five cats for which only pupil data sets were obtained might be suggested to result from the coincidence of the pupil apparent plane and the centre of rotation of the eye. It was, however, pointed out earlier that the method is independent of the position of the centre of rotation and such an explanation could not be applied to the animals bearing a corneal marker (e.g. cat “B”) who also failed to reveal vergence movements.

The absolute magnitude of the decrease in inter pupillary distance which may be directly observed during a vergence to 25 cm or 10 cm has not so far been discussed because it requires an estimate of the position of the centre of rotation of the eye. The results of Richardson and Davis (1960) suggest a centre of rotation 11.7 mm behind the corneal vertex while Zernicki and Drehner (1965) find it to be 10 mm behind the cornea in the pretrigeminal cat. The absolute changes in the intermarker or interpupillary distance may be calculated from equations (20) and (18) respectively if the centre of rotation of the eye is assumed to be at the average of the two positions reported in the literature. 5.5D of accommodation is assumed and \( S_D = S_{NCP} \), i.e. unity magnification. We thus have,

\[
25 \text{ cm } \Delta_{P_{25 \text{ cm}}} = 2 \sin 3°36'. (10 - 3.04) = 1.0 \text{ mm.} \tag{55}
\]

Pupil
\[
10 \text{ cm } \Delta_{P_{10 \text{ cm}}} = 2 \sin 9°51'. (10 - 3.04) = 2.6 \text{ mm.} \tag{56}
\]

Marker
\[
25 \text{ cm } \Delta_{M_{25 \text{ cm}}} = 2 \sin 3°36'. (10 - 9) = 3.6 \text{ mm.} \tag{57}
\]
\[
25 \text{ cm } \Delta_{M_{25 \text{ cm}}} = 2 \sin 3°36'. (10 - 9) = 3.6 \text{ mm.} \tag{55}
\]

Stryker and Blakemore (personal communication and in preparation) have recently carried out a study of saccadic and disjunctive movements of the cat eye. They obtain an absolute value for the horizontal movement of one pupil during a 20° angle look of 2.2 mm when 2.5D of accommodation would be required by the schematic eye (p. 1978). Under these conditions the theoretical magnitude is given by (26),

\[
(sin 20°). (10 - 3.9) = 2.4 \text{ mm} \tag{59}
\]

and the result is in good agreement with their finding. All three of their cats verged, up to 7° for one eye, but partial and complete failure of vergence during apparent fixation as well as rapid habituation were also noted. Their findings are in good agreement with those reported here and have the advantage that recording of the eye movements was continuous.

Is vergence less necessary for the cat than for man

It is hard to believe that the cats which showed no vergence are in fact incapable of it but there are several indications that this animal demonstrates a greater degree of lability in its vergence movements than does man or the monkey. Even a “good verger” like cat “A” was not converging during fixation in about 20 per cent of the near look photographs. That this is simply a sampling error is made unlikely by the occurrence of fixation without vergence on ciné film records, and in the results of Stryker and Blakemore (in preparation). The rapidity of vergence habituation suggests a considerable degree of tolerance of the resulting
fixation disparities on the part of the cat who may continue to show fixation when vergence is chronically habituated.

The results suggest the invalidity of the assumption (Noda, Creuzfeldt and Freeman, 1971) that conscious cats, restrained in a stereotaxic apparatus during unit investigation, are verging simply because they show fixation movements.

"The animal followed it with its eyes, indicating that objects on the screen attracted the cat's visual attention and that they were perceived and that they could be fixated. It may be assumed therefore that the eyes were converged on the level of the screen."

These observations naturally lead to the question as to whether there is any physiological indication of a reason why the cat might need to verge less frequently than the human subject.

Lindsay Johnson clearly associated the presence of convergence with that of a macula; the vergence movements being necessitated for near binocular acute vision because of the limited angle for detailed vision subtended by the macula.

"From the Lemur downwards in all other Mammals, except Simiae, habitual convergence ceases, and the macula is absent but in its stead we find a larger sensitive area."

The larger sensitive area led him (1901, p. 64) to conclude,

"This leads to the consideration of whether binocular vision is possible without convergence, even in animals with a great divergence of the optic axes."

The idea that a larger region of more acute vision occurs in the cat has been put forward more recently by Dreher and Zernicki (1969) as an explanation of why it may demonstrate abortive fixations more frequently than man,

"in man the difference in resolving power between the peripheral retina and the area centralis is much stronger."

In fact, comparison of the retinal ganglion cell density nasal and temporal of the cat area centralis (Stone, 1965) with the plot of acuity in the human eye for the same angular displacements (Wertheim, 1894) shows that both factors have fallen off to about 33 per cent at 5° and 20 per cent at 10°. The horizontal arm of higher ganglion cell density which is found in the cat retina deals with the temporal field and is well away from the region involved in central binocular vision. There is consequently little support for the idea of a more extended area centralis in the cat. The limited size of the area centralis of the cat has been known since Chiervitz's description in 1891.

The eyes of the cat are, however, set closer together than those of the human being—36 mm as against 62 mm respectively; a displacement of 1° nasal or temporal of the area centralis or fovea will consequently be to retina dealing with a region of visual space on the midline which is, for a given fixation distance, less displaced from the fixation point in the human than in the cat. A greater depth of the cat's midline visual space is thus dealt with by retina with a ganglion cell density near to the peak value of the area centralis. The benefit of this in eliminating the need of vergence for reason of acuity would be very great for a distant fixation point and small for a near one. The resting fixation distance in the cat has not, however, been measured.

Although bringing up the problem of acuity, Lindsay Johnson did not discuss that of single vision. The separation of the two eyes leads to the generation of visual parallax which means, in the absence of vergence movements, that the image of objects not in the fixation plane will fall on non-corresponding regions of retina and that they will, in the absence of any special provision to the contrary, be seen double.
Vergence in the Cat

The recent discovery of cortical cells in area 17 of the cat which require binocular stimulation with stimulus disparities which differ from cell to cell was originally interpreted as providing a "plausible basis for binocular depth discrimination and stereopsis" (Barlow, Blakemore and Pettigrew, 1967); the same authors, implying the absence of vergence in the cat, point out that,

"Possibly the cat, a hunting animal, surveys a wide range of depth at low accuracy, whereas man, a sophisticated tool maker, surveys a narrow band at high accuracy, varying the position of the band with his vergence movements."

More recently, the fact that the cat cortical units are for the most part sensitive to vertical as well as to horizontal disparity has made it difficult to be satisfied with the idea of the majority of them playing a role in depth discrimination. Instead, Joshua and Bishop (1970) have suggested that they are employed in the generation of single vision for which purpose vertical disparity is as important as horizontal. The system may be regarded as substituting a means of neural fixation for vergence over a range depth. The magnitude of this range and the acuity change occurring within it would determine the relative need for vergence movements in different species.

The presence of residual eye movements appears to have led to reports of an excessive range of stimulus disparities for cells in the cat area 17; the most recent work of Joshua and Bishop (1970) indicates a standard deviation of only 0.5° for the horizontal disparity of units within 4° of the area centralis.

If the largest divergent disparity is taken as appropriate for an object at infinity then it is readily shown—when the population is assumed to have a range of three S.D. of convergent, and three S.D. of divergent disparity—that units would be available for the provision of single vision for depths from 34 cm to infinity. The fixation point, assuming this to be the resting state, would be 69 cm in front of the line joining the anterior nodal points of the eyes.

Under these circumstances all objects on the midline from infinity to 34 cm from the cat would form images on the retina within 1.5° of the area centralis. The results of Stone (1965) show that the ganglion cell density of the retinal region dealing with the most extreme distances—34 cm and infinity—would have only dropped to 66 percent of that dealing with the fixation point. Acuity would thus be fairly uniform within the retinal area giving rise to single vision.

The region of retina in which an image gives rise to single vision is equivalent to Panum's fusional area in the human eye. The problem is, as Rodieck (1971) has pointed out, that the arbitrary range of disparity chosen to represent the area is not necessarily equivalent to the range used by the cat. Joshua and Bishop (1970) have constructed a theoretical horopter for the cat but assume single vision to obtain for only one standard deviation of disparity. Panum's area in the cat would then be only 1° in dia. compared to the 3° assumed above. Even in this case a fixation distance of 200 cm would give single vision without vergence from infinity to 100 cm from the cat compared with only 170–260 cm in the human under similar circumstances.

It is thus arguable that the cat may be relatively lazy about verging for stimuli beyond 30 or 40 cm distant especially because accommodation appears not to be tightly linked with the degree of vergence (Elul and Marchiafava, 1964). The most generous estimate of the extent of Panum's area does not, however, explain the absence of vergence in animals looking at stimuli nearer than 34 cm if the resting fixation distance is 70 cm. Bringing the assumed resting fixation distance nearer results in large losses in the distant range of single
vision with only small gains in the near point for single vision. It thus seems likely that cats which do not verge to near objects, whether chronically (cats "B" and "E") or acutely, are tolerating diplopia or are perhaps suppressing the image from one eye and using monocular fixation.

It is worth noting that apart from the total range of disparities of cortical units there are two other hints that the cat may hold a resting fixation distance of about 70 cm.

(a) Elul and Marchiafava (1964) have found the resting accommodation of the cat to be about $-0.5$ to $-1.0D$ when it is awake. According to Glickstein and Millodot (1970) a standing error of refraction would make this correspond to a range from $-1.0$ to $-1.5D$, suggesting that in the resting state the eye is focussed for a plane between 100 and 69 cm.

(b) Karl Smith (1936) made the surprising, and as yet unconfirmed, observation that the visual acuity of the two cats which he investigated was maximal for a stimulus placed at a viewing distance of 75 cm and that it decreased for stimuli at either 50 or 125 cm. He discarded variation in illumination as an explanation. Such results would be consistent with presbyopic myopia but an animal with a resting fixation point at 75 cm who did not bother to converge or diverge might give similar results if he directed his midsagittal plane towards the stimulus and depended upon Panum's area and the acuity of the pericentralis retinal region for carrying out the discrimination.

**Do other mammals verge?**

A general discussion of the literature on this point has already been given in the introduction but a few experiments similar to those described above were carried out on dogs and rats. In both of the examined dogs the eyes appeared to verge when directly observed because the sclera become visible at the margins but this was found to result from a change in the position of the head during near viewing and the results of the photographic analysis gave no indication of vergence in animals clearly investigating the stimuli presented.

Several rats were photographed when examining a shelf below their jumping stand or objects held out to them at various distances. It was not possible to confirm the observation of Lashley (1932) mentioned in the introduction that vergence is readily seen in the rat. In fact spontaneous eye movements also did not appear to be as plentiful as he records.

Obviously, however, these negative findings cannot be given much weight in view of the limited sample used and the indications of individual variability observed in the results from the cat.

It is clear that further work along these lines must be carried out with animals trained to fixate at known distances during some visually directed task. If a photographic method is employed it should involve the use of cine film in order that the disadvantages of the sampling method described above are eliminated.

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Vergence in the Cat

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Abstract—A photographic technique has demonstrated that 60 per cent of unrestrained cats are capable of vergence movements during the examination of interesting near objects. The remaining cats showed no sign of vergence under the conditions of the experiment. Vergence movements habituate rapidly in those cats that demonstrate them and they do not invariably appear during fixation. Possible reasons for the apparent liability of vergence in the cat are discussed.

Some information on the accommodatory state of the eye during vergence and its relation to the fixation distance was also obtained as a by-product of the techniques used.

Résumé—On démontre par une technique photographique que 60 pour cent des chats libres de leurs mouvements sont capables de mouvements de vergence durant l'examen d'objets rapprochés intéressants. Les autres ne montrent pas de signe de vergence dans les conditions de l'expérience. Chez les cats capables de mouvements de vergence, ceux-ci deviennent rapidement habituels et ils n'apparaissent pas invariablement durant la fixation. On discute les raisons possibles de cette labilité apparente de la vergence chez le chat.

Comme sous-produit de la technique employée, on obtient aussi des données sur l'état d'accommodation de l'œil pendant la vergence et sur sa relation avec la distance de fixation.


Резюме—С помощью фотографической техники показано, что 60½% кошек в свободном состоянии способны совершать вергенионные движения при исследовании интересующих их близко расположенных объектов. Остальные кошки в условиях эксперимента признаков вергениии не обнаруживали. Вергенионные движения быстро становятся привычными у тех кошек, которые их обнаруживают, однако они не всегда появляются при фиксации. Обсуждаются возможные причины выраженной лабильности вергениии у кошек.

В качестве побочного результата при использовании этой техники была получена некоторая информация о состоянии аккомодации глаза во время вергениии и его связи с расстоянием до фиксируемого объекта.
THE ANATOMY AND PHYSIOLOGY
PRIMARY VISUAL CORTEX
**The Organization of Binocular Cortex in the Primary Visual Area of the Rabbit**

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**ABSTRACT**

A conscious rabbit which crouches in the 'freeze' position has an unequivocal 24° wide binocular field formed by the overlap of the two 12° sectors of unocular optical field which extend nasal of its midline. Although this investigation reveals that the horizontal representation of the unocular visual field in cortical visual area I extends more nasal than in earlier reports, it is found to terminate at the midline when the eyes are set in the standard freeze position. The 12° sectors of unocular field nasal of the midline were not represented in spite of being served by retina. The binocular field of the rabbit in the freeze posture thus appears to have no binocular representation in visual area I.

Nevertheless, the presence of a binocular region was confirmed in rabbit visual area I but the projections to it, via the contralateral and ipsilateral eyes, deal with the nonoverlapping sectors of monocular field when the eyes are in the freeze position. The maps of the visual field obtained in one hemisphere via the contralateral and ipsilateral projections were subject to a horizontal divergent disparity of some 18°. The presence of binocular single units in these regions was also confirmed but their two receptive fields were necessarily located in different visual hemispheres and again subject to an 18° mean horizontal divergent disparity. They could not therefore simultaneously be well fused by any localized feature of an object and are thus precluded from involvement in binocular single vision during the freeze position.

The systematic, rather than reportedly random, topography of the ipsilateral projection to visual area I ensured that it could be well fused with the similarly organized contralateral projection by means of an 18° vergence of the eyes from the freeze position. The horizontal receptive field disparity of binocular single units is then brought to a zero mean value which enables their receptive fields through each eye to be simultaneously stimulated by the same part of an image. Tested units thus evinced response summation; a minimum requirement for binocular vision. In this equivalent primary position of the eyes, the entire binocular visual field is completely represented in visual area one of both hemispheres.

The rabbit thus appears to employ a two-state cortical system for forward vision. In the freeze position the visual field attains a coulpean extent of 360° but the receptive fields of cortical binocular units are widely divergent. The classic binocular optical field may then project to some other region of brain which subserves binocular vision. Upon examination of a frontal object the animal must verge its eyes in order to obtain binocular single vision in visual area I; it then temporarily surrenders part of its rear visual field. An explanation in terms of ocular image quality is suggested.

This study has been reported in preliminary form by Vaney and Hughes (79).

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The lateral eyes and narrow binocular field of the rabbit have often led to the conclusion that it has poor frontal vision and lacks stereopsis (Harris, '04). In spite of this, the rabbit preferentially employs its binocular field for visual discrimination tasks (Van Hof and Lagers-van Hassel, '73), the corresponding region of far temporal retina is differentiated by a specialized ganglion cell distribution (Hughes, '71; Provis, '79), and the optics serving this area are corrected so as to be free of oblique astigmatism (Hughes and Vaney, '78). The rabbit visual cortex contains a population of single units with binocular receptive fields (Van Sluyters and Stewart, '74), and it has also been shown that the rabbit is capable of ocular vergence movements (Zuidam and Collewijn, '79). These results suggest a potential for binocular vision similar to that of cat and monkey.

Nevertheless, descriptions of the rabbit's binocular system indicate some curious features which require explanation, if a satisfactory understanding of its functional organization is to be achieved. For each position in an array of cortical recording sites, Thompson et al. (50) located the point in the visual field at which a flashing light spot elicited a maximal evoked response. The eyes were set with the optic disc centered in the coronal plane which included the anterior nodal point of the eye and whose projection into the visual field defines the 0° meridian. From these results they constructed maps of the visual field representation in cortical areas Visual One (V1) and Visual Two (V2). Their maps indicated that the nasal limit of cortically represented visual field lies on the 70° meridian. The far nasal sector of each visual hemifield, from 70° to 90°, was thus not represented in the contralateral cortex; a vergence of 40° would be required to close this "gap" and ensure complete cortical representation of each visual hemifield.

The "gap" may, of course, be an artefact. The rabbit retina does not possess an ophthalmoscopically definable fixation area which can be used to align the eyes; experiments may thus be carried out with them in an abnormal position. However, a behaviorally defined eye position is available. When a rabbit is frightened it may attempt to avoid detection by crouching low to the ground in the "freeze" posture (Brecher, '36; Hughes, '71). In this condition the eyes remain stationary after taking up a position with the retinal visual streak horizontal and the optic nerve head projecting into the visual field some 9° forward of the coronal plane (Hughes, '71). However, even after the results of Thompson et al. (50) have been converted to this standard state, a gap remains in the mapped cortical representation of the frontal field, although it is reduced to some 11° for each hemifield.

Van Sluyters and Stewart ('74) have examined the organization of binocular visual cortex by locating the receptive field position of single units. After conversion to the freeze position, their results show that receptive fields of binocular units, as mapped through the eye contralateral to the recording sites, were encountered from 60° to 90° nasal of the lateral 0° meridian. The visual field of the contralateral eye was thus represented in V1 by single units right up to the 90° nasal meridian; the two eyes gave complete coverage of the frontal field. The findings of Swallow ('77) are similar. The results of both reports indicate that there is no gap in the cortical single unit coverage of the binocular field.

This curious difference between the evoked potential and single unit maps has not been investigated. Several other puzzles remain. Van Sluyters and Stewart ('74) described the receptive field of binocular single units in primary visual cortex as possessing divergent disparities of more than 20° when converted to the freeze position. The contralateral and ipsilateral receptive field of binocular single units thus projected into different visual hemifields and could not be stimulated simultaneously by one object whatever its distance from the animal; under these conditions, such cells could not provide a substrate for binocular single vision. Van Sluyters and Stewart ('74) also emphasized unusual receptive fields and a seemingly random topographic organization to support the view that the rabbit binocular cortex is markedly different from that of the cat. By contrast, Choudhury and Ghent ('73) describe their cortical map of the 25° wide ipsilateral field of the rabbit as topographically organized, although its angular position differs from that of Van Sluyters and Stewart ('74).

The present study was undertaken to resolve the above problems and to determine whether the rabbit might be capable of binocular vision. The difference between the evoked potential and single unit maps was verified and explained by a very compressed cortical representation of the extreme nasal field. The topography of the maps was reinvestigated and the divergent disparities of binocular receptive fields confirmed. A hypothesis is presented to explain the unusual features of rabbit binocular organization and interpret its function in conventional terms. The results have been presented in preliminary form (Vaney and Hughes, '79). Choudhury ('80) has recently proposed an unconventional binocular apparatus for the rabbit but does not address the difficulties inherent in previous observations.

**METHODS**

**Preparation**

Recordings were made from the visual cortex of 14 rabbits (2.6-3.3 kg) with agouti fur and brown eyes. Such animals would be expected to have normal decussation patterns (Sanderson, '75). Anesthesia was induced with 2.5% thiopental sodium and maintained for surgery with 0.7-1.5% halothane in 70:30 nitrous oxide:enflurane. Paralysis was subsequently induced with 20 mg of gallamine triethiodide and maintained by intravenous infusion at 5 mg/kg/hour in a 5% glucose solution (4 ml/hour). The ventilation rate was 65 strokes/minute with a minute volume V = 1.3 (37 + 393W) ml, where W is the weight in kg. A setting 30% greater than for the unanesthetized rabbit (Edwards et al., '59) precluded distress. Temperature was monitored subcutaneously and maintained at 37.5°C by a homeothermic blanket. Penicillin in doses of 250,000 units was given on each of the 2 or 3 days of the experiment.

The electroencephalogram and occasionally the electroencephalogram were monitored but no acute changes were noted and heart rate remained constant when nocuous stimuli were applied. Local anesthetic ('Xylocain') was infused at intervals around pressure points and the subcutaneous probe.
RABBIT BINOCULAR CORTEX

Topographic mapping

A craniotomy was made over each visual cortex and a ring was sutured to the scalp to form a well. Dura mater was reflected and the brain was covered with liquid paraffin. A 1-mF electrode was set vertical; the resulting maps are thus Horsley-Clarke projections uncorrected for curvilinear curvature. Penetrations were attempted every 0.5 mm but large blood vessels were avoided. The region of visual field corresponding to each penetration was located by audio monitoring of the electrical activity evoked by a neon flash or light spot. The margins of this receptive field were mapped by card stimuli in the same manner as for single unit receptive fields.

Single unit recording

The procedures employed are described elsewhere (Vaney and Hughes, '82). Stable units possessed wave forms typical of cell bodies (Bishop et al., '62) and injury discharges occurred upon electrode advance.

Visual stimulation

Receptive fields were examined on a tangent screen 132 cm from the anterior nodal points of the eyes and plotted with spots and bars projected from a Keeler "Pantoscope" (60 candelas cd/m²) on a dimly lit background (7 cd/m²). Cardboard stimuli on a contrasting white (20 cd/m²) or black (0.6 cd/m²) background were used to determine the minimum discharge borders for single unit receptive fields. The disparity of binocular receptive fields was calculated from the separation of their geometric centers after correction for the interocular distance.

A computer drove the visual stimulator (Hughes and Snow, '73), analyzed the single unit responses to moving stimuli, and presented the results in various forms of histogram (Vaney and Hughes, '82).

Eye positioning

The headholder provided an unobstructed 120° horizontal field of view and ± 60° in the vertical. A Baush & Lomb indirect ophthalmoscope was mounted on a ring concentric with the animal table which permitted it to be rotated through 360° of azimuth and ± 30° of elevation; the anterior nodal point of each eye could be centered on the rotation axis of the table, the ophthalmoscope level brought into coincidence, and the angular projection of centered retinal landmarks read directly.

A ring sutured behind the limbus was connected to a manipulator which permitted each eye to be set in a standard position by independent X, Y, and Z translations as well as roll, pitch, and yaw adjustments about its anterior nodal point. Zero-power contact lenses of 7.75-mm radius and 13-mm base diameter were fitted; the natural pupil was employed. Hughes and Vaney ('79) found the binocular field of the rabbit eye to be within 1° (1 diopter) of emmetropia so that no spectacle correction was employed and the screen distance was neglected. The corners were bathed daily with normal saline to prevent clouding and treated with "Neosporin" ophthalmic solution (Wellcome).

Eye alignment and stability

The rabbit fundus contains no viable fixation area so that the optic nerve head, myelinated band, and overlying blood vessels must be used as landmarks for setting the eye in a standard position. Their projection has been determined for the conscious rabbit in the freeze posture by Hughes ('71). Because of their importance, these observations were repeated for the horizontal plane. A camera was positioned above the rabbit's head and two observers photographed while simultaneously fixing the left and right hyaloid artery remnants by means of hand-held ophthalmoscopes. Measurements on the photographs confirmed that the center of the optic nerve head projects a mean 9.2°–9.5° nasal of the coronal plane.

The adopted coordinate system is thus based on the freeze position of Hughes ('71). Its origin is defined by the intersection of the horizontal and coronal planes passing through the anterior nodal point. The center of the optic nerve head then projects 9° nasal and 18° inferior in the visual field. The major blood vessels overlying the nasal and temporal myelinated bands are horizontal and the visual streak projects close to the horizon. If considered as projecting onto a sphere large enough to make the interocular distance negligible, then the unioocular optical fields, each 192° wide, overlap in front of the animal to define a binocular optical field which is 24° wide (Hughes, '71, '72).

The eyes were set in the freeze position at the beginning of an experiment. The projections of three landmarks defined by blood vessel crossings some 20° nasal, adjacent, and 20° temporal to the optic disc were monitored at intervals. It was found that a given landmark remained within a 1° circle for up to 5 days: This range of movement was judged acceptable.

Effect of contact lenses on cortical projections

The application of contact lenses has been demonstrated to change the projection of retinal landmarks into the rabbit visual field (Hughes and Vaney, '81). This increase in eccentricity is maximal near the nasal 90° meridian and decreases toward the 0° meridian. The mean shift, ΔX°, as a function of temporal eccentricity from the 90° meridian, X°, is,

\[ ΔX° = 0.0627X° − 2.42° \]

for a range of 25°, beyond which it may be neglected (Hughes and Vaney, '81).

The azimuth coordinate of all single and multunit receptive fields has thus been corrected to compensate for the mean lateral shift in projection caused by the application of a spherical contact lens. The corrected azimuth, Xc, was calculated from the plotted azimuth, Xp, thus:

\[ (90° − Xc) = (90° − Xp) − 2.42/(1 − 0.0627) \]

where (90° − Xp) is the eccentricity of the plotted field from the midline and (90° − Xc) is the eccentricity of the corrected coordinate.

Note that the magnitude of a vergence is subsequently expressed as the combined angular rotation of both eyes.

RESULTS

Representation of the visual field in V.I

Rabbit visual areas I (V.I) and II (V.II) are defined electrophysiologically. Within V.I, encountered receptive field positions shift systematically toward the midline field as...
the recording electrode is moved laterally across the cortical surface. A reversal in the direction of this shift and an increase in receptive field size denotes entry into area V.II, whose map of the visual field is a mirror image of that in V.I. Both areas have previously been mapped (Thompson et al., '50; Choudhury and Whitteridge, '65; Hughes, '71; Woolsey et al., '74). The border between V.I and V.II, the "decussation" line of Thompson et al. ('50), has been equated with the boundary between the historically defined areas 17 and 18 (Hughes and Wilson, '69; Bousfield, '78).

Low-impedance electrodes were used to map the projection of the visual field via the ipsilateral and contralateral eyes to sites in V.I of both hemispheres. This permitted confirmation of earlier maps of the nasal visual field, topographical comparison of the contralateral and ipsilateral retinal projections, assurance of symmetry in the two hemispheres, and investigation of eye positions which might permit fusion of the contralateral and ipsilateral maps on each hemisphere as a basis for binocular single vision.

The topography of the projection from contralateral retina to V.I as defined by multunit responses. Recording sites are indicated in Figure 1A for both hemispheres. Some restrictions were imposed on the placement of electrodes by the blood vessel pattern, which is illustrated in Figure 1B for the right cortex. Dipping indicates unexposed cortex. The projection of the nasal visual field via the retina of the contralateral (left) eye onto the right cortex is mapped in Figure 1C as determined from the responses at electrode locations in Figure 1A. Figure 1D shows the corresponding projection from the central visual field (right) eye onto the lateral margin of V.I in the left hemisphere.

The map confirms the earlier finding that the cortical magnification factor progressively increases in passing horizontally across the visual field from the temporal representation to the border between V.I and V.II (Thompson et al., '50; Choudhury and Whitteridge, '65; Hughes, '71). The position and orientation of the 0° vertical meridian resembles that of previous maps (Thompson et al., '50; Hughes, '71; Woolsey et al., '74); the central extent of the horizontal meridian bends more anteriorly than in the map of Hughes ('71) but less than that of Woolsey et al. ('74). In this breed of rabbit, the border between V.I and V.II lay at an angle of 45° to the midline (Woolsey et al., '74), rather than parallel to it (Hughes and Wilson, '69; Choudhury and Ghent, '73).

The topography of binocular V.I as defined by multunit responses. Electrode positions for which multunit responses to binocular visual stimulation could be recorded in V.I are indicated by discs in the inset boxes to the left of Figure 2; squares represent penetrations in V.II. These cortical positions correspond to those in Figure 1C. To the right of Figure 2 are maps of the frontal visual field which show the 90° nasal meridian and the 0° horizontal. The points in these plots represent the geometric center of the area of visual field from which a light spot could elicit a detectable multunit response through both eyes at the cortical recording site with the corresponding number and symbol; these areas are referred to as multunit receptive fields. The points appear as they do when plotted on a tangent screen in front of the animal but their coordinates have been corrected for the finite separation of the eyes and the influence of contact lenses (Hughes and Vaney, '81). The projections to right cortex are shown separately from those to left cortex; each plot of the visual field displays projections via the contralateral and ipsilateral eyes which are differentiated by connection with bold and light lines, respectively. It is apparent that the receptive fields of the projection via the contralateral retina are confined to the contralateral visual hemifield and those of the projection via the ipsilateral retina to the ipsilateral visual hemifield. The two projections to one cortical hemisphere thus deal with different visual hemifields when the eyes are in the freeze position.

These results are typical of several rabbits. At sites in binocular cortex, the multunit receptive fields through the contralateral eye ranged from about 18° eccentricity of the 90° meridian to the "decussation" line, some 7° eccentric. The "decussation" lines defined by multunit responses thus project to the 85° meridian. The "decussation" lines of left and right cortical hemispheres must therefore diverge by some 14° when projected into the visual field.

In agreement with previous studies, a substantial sector of the frontal visual field was not represented in the cortical multunit responses mapped via the contralateral eye.

Comparison of the upper and lower maps of the visual field in Figure 2 clearly shows, for each eye, an overlap of at least 7° between the sectors of visual field which are served by retina projecting to ipsilateral and to contralateral cortex, respectively. This arrangement precludes a sharp retinal decussation. It also follows that the region of frontal field not represented in the "gap" in the multunit map of contralateral cortex is nevertheless represented in the projection of the same eye to the ipsilateral cortical hemisphere.

Given that the freeze position occurs naturally in the conscious rabbit, it is surprising to observe from Figure 2 that the projections via the ipsilateral and contralateral eyes to a recording site in binocular cortex do not arise from corresponding retinal points. The results from 31 sites in binocular cortex showed a mean divergent horizontal disparity of 17.7° ± 3.5° between the multunit receptive fields of the contralateral and ipsilateral eyes. The mean vertical disparity was zero. The outcome is that the projections to ipsilateral and contralateral cortex from one eye deal with the same hemifield and do not overlap the median sagittal plane. The mapped projections from different eyes arriving at one cortical binocular area thus have no part of the visual field in common and could not be simultaneously stimulated by an edge at any distance from the animal.

The topography of binocular V.I as defined by single unit recordings. The extent of the cortical representations defined by single unit encounters differed from that mapped by multunit responses. All the studied receptive
Figure 1
Fig 2. The inset boxes display the stereotactic coordinates and topography of the more lateral electrode insertions in the left and right binocular visual cortices of the brain in Figure 1; discs represent penetrations of VI and squares of VII. The eyes were set in the freeze position. The location in the visual field at which a visual stimulus evoked a binocular multiunit response for each electrode site is marked by a corresponding number in separate field maps for the left and right hemispheres; cortical sites receiving only monocular input are not represented in the visual field maps. The ipsilateral and contralateral projections to one cortical hemisphere arise from separate visual hemispheres, are some 18° divergent, and cannot be simultaneously stimulated by a real object. Receptive fields 7° and 34° were those of units deeper than 7 and 34 but in the same oblique penetrations.

Fig 3A. The distribution of receptive fields encountered in the binocular region of visual area 1 is shown as a function of eccentricity for monocular and for binocular units in the contralateral and ipsilateral projections to the right cortex. It is apparent that the absolute encounter with monocular and binocular units peaks at 5° to 10° eccentricity. B. The percentage division of the encountered single units between those possessing monocular or binocular receptive field is plotted as a function of eccentricity for the contralateral projection. The proportion of binocular encounters again peaks at 5° to 10° eccentricity.
fields lay within ± 5° of the horizontal and therefore originated from cells of the visual streak. No binocular units were encountered with the receptive field of the contralateral eye more than 20° temporal of the 90° meridian. Figure 3 shows the number of single units encountered in the binocular area of primary visual cortex for each 5° step of horizontal eccentricity from the 65° to the 120° meridian.

Contralateral receptive fields of binocular units were encountered from 15° temporal of the 90° meridian to 2° nasal. The largest number of encounters with monocular and binocular units, Figure 3A, and the largest percentage encounter with binocular units, Figure 3B, were found from 5°-10° temporal of the midline.

Ipsilateral receptive fields of binocular units were also encountered from 20° temporal of the 90° meridian to 1° nasal. The ganglion cells projecting to ipsilateral and contralateral cortex from one eye are again concluded to be intermingled. However, single unit recording shows that this occurs over some 18° of retina rather than the 7° suggested by multunit responses. Single unit responses show the nasal visual field to be represented in contralateral primary cortex from the 0° to the 90° meridian and in the ipsilateral cortex from the 70° to the 90° meridian. Why, therefore, does a "gap" exist in the representation of the frontal visual field when mapped by multunit responses?

An explanation of the presence of a "gap" in the map of contralateral visual field as mapped by multunit responses in V.I. Examination of the multunit mapping data indicated that the last penetration of V.I and the first in V.II were separated by only 0.5 mm in the Horsley-Clarke horizontal plane. This suggested the possibility of a compressed representation of the extreme nasal visual field. Detailed exploration of this region by electrodes inserted perpendicular to the cortical surface revealed the 81° meridian of V.I to be separated by about 0.9 mm from the 82° meridian of V.II. Even if the representation of nasal visual field in V.II is neglected, this would permit a magnification in V.I of less than 0.1 mm/degree for the field from the 80° to the 90° meridian in contrast to that of more than 0.2 mm/degree close to the 80° meridian. In fact, the magnification factor of the nasal field representation in V.II appeared to be similar to that of the same area in V.I so that the mean magnification factor for the 0.9-mm strip separating the 81° meridians in the two areas might be no more than 0.5 mm/degree. The obvious trend for an increase in the horizontal cortical magnification factor when passing from the representation of temporal to nasal field thus appears to reverse nasal of the 80° meridian. More detailed exploration was impeded by the presence of large blood vessels in this region, Figure 3B. During relatively coarse mapping experiments the area is presumably overlooked.

The optical binocular field in the freeze position

Geometric studies on the pupil retinoscopic reflex (Hughes, 71) show that the rabbit possesses an unisocular binocular field in the freeze position; the unisocular optical fields extend 10°-12° beyond the 90° meridian and overlap to create a binocular optical field some 24° wide. In spite of this, the above results show that cortical multunit or single unit responses were not detected significantly beyond the representation of the 90° meridian in V.I.

It might be that this region of visual field from which light could enter both eyes (binocular optical field; Hughes, 76, 77) is not served by retina so that its neural representation would be lacking in V.I. The extent of the field served by retina (binocular retinal field; Hughes, 77) was therefore determined. With the eyes in the freeze position, the indirect ophthalmoscope was used to measure the extent of the binocular optical field in the absence of contact lenses, it was then set directly ahead of each eye in turn. A needle was inserted into the rear of the eye so as to protrude at the projection of the 90° meridian defined by the ophthalmoscope cross hairs. The position of the eye was confirmed to be unchanged and it was then enucleated. One retina was prepared as a flat mount and examined microscopically; the needle hole was found to be separated by normal retina from the ora terminalis. The other eye was frozen with the myelinated band horizontal and sectioned until the needle hole was reached. It was separated from the ora terminalis by 1.3 mm of retina. This would subtend some 8° of visual field beyond the 90° meridian in the freeze position retinal magnification factor for this region is 0.16 mm/degree; Hughes and Vaney, 51). The binocular retinal field in the freeze position must therefore be at least 16° wide and serve a major part of the binocular optical field. The absence of a representation of the latter region in V.I thus does not arise from a lack of corresponding retina. It may be that a few single units represent this region of field but that its topography is even more compressed than that of the 80°-90° sector.

Our understanding of the projections to area V.I which deal with rabbit nasal field are summarized in Figure 4 for the left eye in the freeze position with the optic nerve head projecting 9° nasal of the corneal plane. Oblique hatching indicates the extent of visual field defined by multunit and single unit responses as projecting to cortical area V.I; the extent of field defined by single unit responses alone is indicated by dots. The multunit and single unit components of the field projecting to ipsilateral cortex are defined by similar conventions but with the orientation of the hatching reversed.

The contralateral representation of nasal field in area V.I ranges from the 0° meridian, which is close to the optic axis in this position, to about 1° nasal of the 50° meridian. The ipsilateral projection ranges from about the 70° meridian to the 90° meridian. Binocular units with a contralateral field through this eye were encountered only in the sector designated by an arc corresponding to the region of bilaterally projecting retina.

The broken line indicates the position of the "decussation line" previously defined by multunit responses and thought to designate the most nasal region of the visual field represented in the contralateral projection. According to single unit criteria this limit now corresponds to the 90° meridian. The contralateral and ipsilateral projections arise from a common region of temporal retina and thus no decussation line can be defined in the classic sense. It will be apparent that the contralateral projection to area V.I from one eye and the ipsilateral from the other deal with separate hemispheres of visual field in this eye position and could not provide a substrate for stereoscopic binocular vision.

The binocular optical field of the animal in the freeze position arises from the overlap of the two sectors of optical field from the 90° meridian to the limit of the optical field some 12° nasal of it. The majority of this optical sector is served by retina which extends for up to 8° nasal of the 90° meridian. However, no representation of this retinal
Field was encountered in V.I and it is unlikely that it is involved in cortical stereoscopic vision.

Receptive field disparity of binocular units

The receptive field properties of 55 binocular units recorded in rabbit primary visual cortex have been reported elsewhere (Vane and Hughes, '82). The horizontal and vertical disparities between the contralateral and ipsilateral fields of 21 of these units from four rabbits are plotted in Figure 5A. The horizontal disparity range of 17° for all units is about twice that for binocular units of individual animals. Monocular units with either contralateral or ipsilateral receptive fields projected to similar points in visual space as the contralateral and ipsilateral receptive fields of previously, or subsequently, recorded binocular units at the same site. This is in keeping with the systematic topographic representation of the ipsilateral and contralateral projections which is clear in Figure 2 and emphasized subsequently.
The mean horizontal disparity of the 21 well-defined binocular receptive fields from four rabbits is 17.7° ± 2.5° and the mean vertical disparity of the same set is 0.13° ± 3.4°. This value is identical to the mean multunit receptive field disparity analyzed above for 31 sites in V.I.

Although the range of horizontal disparity for pooled binocular units is broad, it is more restricted for binocular units with contralateral fields at a given eccentricity from the midline. This is because the magnitude of the horizontal disparity between the contralateral and ipsilateral fields of binocular units decreases progressively as the contralateral field approaches the midline (Fig. 5B). The mean horizontal disparity for units whose contralateral receptive field is 15° from the midline exceeds that of units only 5° from the midline by some 5°. The contact lenses did introduce a differential shift in receptive field projections which would change disparities by about 0.5° over this range of eccentricity. However, this small effect has been corrected for in the results (see Methods) and it is concluded that the observed variation of disparity with contralateral receptive field eccentricity is not an artefact.

It is emphasized that the disparities of multunit and single unit binocular receptive fields remained divergent after correction for the prismatic effect of the contact lenses (Hughes and Vaney, '82). Eye positions were monitored and shown to be stable for the duration of the experiments (see Methods) so that the divergent disparities do not arise because of movements from the freeze position. However, the simultaneous stimulation of both receptive fields of binocular cortical cells by the same region of an object requires zero or convergent disparities. The substantial divergent disparities of the single and multunit binocular receptive fields thus indicate that the binocular area of V.I. cannot be employed for binocular single vision in the freeze position.

Requirements for functional binocular vision. If the multunit receptive fields of cortical sites in one hemisphere as mapped via the contralateral and ipsilateral eyes, Figure 2, are brought together by a symmetrical vergence equal in magnitude to their mean 17.7° horizontal disparity, then a fused field plot, results (Fig. 6). The similar topography of the ipsilateral and contralateral projections ensures that they are generally well fused. It is apparent from the magnitude of the necessary vergence that the mean horizontal disparity of binocular single unit receptive fields would also be reduced to zero in this eye position.

Under these conditions of 18° total vergence beyond the freeze position, the anterior binocular visual field projects through the right eye and onto the cortex as shown schematically in Figure 6. The extents of binocular field served by a projection through the right eye to the contralateral and ipsilateral hemispheres are separately indicated in Figure 7A. The horizontal extent of field known from single unit mapping to be represented in binocular cortex is indicated by hatching, vertical to the left of the midline and horizontal to the right. A bold line boxes the component of the projections which is also mapped by multunit responses. The scheme for the left eye would be coextensive but inverted.

A horizontal cross-section shows the right eye, Figure 7B, as appropriately rotated 9° beyond the freeze position. The sectors of retina which are then served by visual field to the left or right of the midline are indicated by vertical and horizontal hatching. Short and long arcs respectively define the extent of retina which projects to ipsilateral or to contralateral cortex. Solid lines indicate the projection of retinal sectors defined by both multunit and single unit responses; broken lines indicate the projection defined by single unit encounters alone.

The horizontal extent of the binocular field represented on left and right visual cortex, as mapped by single unit responses through the right eye, is shown in Figure 7C. With the eyes verged 18° from the freeze position, binocular single units were encountered in contralateral cortex with receptive fields ranging from at least 8° right, to 6° left of the midline; the angular range in ipsilateral cortex was of the same extent. The mirror symmetry of the two
Fig. 6. This figure presents the same information as the visual field map of Figure 2 but after a total vergence of the eyes by some 18° from the freeze position, an amount equal to the mean single or multiunit receptive field horizontal disparity. The multiunit receptive fields of ipsilateral and contralateral projections to the binocular area of each hemisphere fuse quite well. The arrays for both cortical hemispheres are symmetrical and a 7° wide strip centered on the visual field midline obtains a binocular multiunit representation in both cortical hemispheres. The diagram thus presents the relationship between the various projections when the eyes are in the equivalent primary position.

Projections from one eye indicate that the coordinates of the representations projecting via the left eye would be topographically fused in each hemisphere with those illustrated for the right eye.

For the eye position of Figure 7 it is apparent that the whole binocular field is represented on each hemisphere; it is bilaterally mapped when defined by single unit studies. Multiunit responses reveal only part of the binocular projection so that the bilateral representation appears to be a strip only 6° wide, Figure 6.

The confluence of vertical and horizontal hatching in the schematic diagram of Figure 7C defines the position of the midline in relation to the boundaries of the cortical map of binocular field. The representation of the contralateral binocular hemifield is wider than the ipsilateral even though the binocular field is divided symmetrically
Fig. 7. The regions of anterior field dealt with by the ipsilateral and contralateral projections of the right eye are diagrammed for the visual field, A, the retina, B, and visual cortex, C, with the eye verged from the freeze position by 9° to the equivalent primary position. The extent of binocular field served by the contralateral and ipsilateral projections from the right eye are separately diagrammed, 7A. Hatching designates the horizontal extent of field known from binocular single unit recording, vertical to the left of the midline and horizontal to the right. A bold line haves the component of the projections also mapped by multiunit responses. The scheme for the left eye would be coextensive but of opposite symmetry. Some 36° of visual field thus projects to binocular cortex and symmetrically bridges the midline. B. A horizontal cross section shows the right eye appropriately rotated 9° beyond the freeze position. The sectors of retina served by field to the left or right of the midline are indicated by appropriate hatching. Short and long arcs define the extent of retina projecting ipsilaterally or contralaterally. Solid lines indicate definition by multiunit response and broken lines by single unit responses. C. The extent of binocular field represented in the projections of the right eye to left and right visual cortex are shown along the horizontal meridian, HM, as defined by single unit responses. As in Figure 7A, binocular single units were encountered in contralateral, left cortex with their fields ranging from 8° right to 8° left of the midline. The ipsilateral range was the same. The mirror symmetry of the two projections from the right eye shows that the projection via the left eye would represent field coordinates topographically fused in each hemisphere with those illustrated. The striking point is that the binocular field would be represented in this eye position on both cortical hemispheres in its entirety. The lateral limit of area VI then does not coincide with the 8° meridian indicated in Figure 7C by the confines of the vertical and horizontal hatching.

The “equivalent primary position” of the eyes

The eye position in which the rabbit optic nerve head projects 18° forward of the coronal plane containing the anterior nodal point forms a useful reference state which we define to be the equivalent primary position for a species whose retinal fixation area has not been identified. The fused ipsilateral and contralateral projections in the cortical maps of the visual field and the zero receptive-field mean disparity so obtained resembles the situation in the cortex of a species having defined fixation areas which have been so aligned that the eyes are in the primary

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position. The equivalent primary position is also remarkable in the rabbit because of the unique symmetry by which the cortical representations of binocular field in both halves of the brain have their left and right boundaries at corresponding eccentricities in the visual field.

DISCUSSION

Topographical representation in visual area I

Thompson et al.'s (50) report of a "gap" in the cortical representation of the rabbit anterior visual field was confirmed when multiunit responses were used for mapping with the eyes in the freeze position. However, the above results equally support the implication of reports by Van Sluyters and Stewart (74), Swadlow (77), and Bousfield (78) that contralateral single units are encountered in binocular cortex with receptive fields located in the "gap." The compression of the cortical representation of the field from the 80° to the 90° meridians and its obscuration by major blood vessels may cause this sector to be overlooked during exploration by multiunit responses.

According to Thompson et al. (50) the region of retina which projects to contralateral visual cortex is sharply bounded from that projecting to ipsilateral cortex; the boundary between the projections forms the "decussation" line in their maps. If the eyes were set so that the projections of the decussation lines into the visual field were parallel, then the ipsilateral and contralateral projections from the left half of the visual field would arrive only at the right cortex. Their cortical representation is thus entirely contralateral in terms of the visual field. The decussation line for the eyes vorged some 11° from the freeze position would then define the nasal limit of the field in each cortical map, the visual field midline, and the boundary between areas V.I and V.II.

Although Van Sluyters and Stewart (74) do not comment specifically on this matter, their results and those above indicate that the ipsilateral and contralateral projections from one eye to binocular cortex deal with the same region of visual field and arise from a common area of retina (Figs. 6, 7). Retina nasal of the 12° meridian projects contralaterally to V.I, but temporal of that meridian it projects bilaterally. This description is based on single unit recording. However, even when multiunit recording is employed a bilateral projection may be defined from temporal retina. A decussation line as understood by Thompson et al. (74) was not confirmed.

The current findings differ from those of Van Sluyters and Stewart (74) in that the width of field served by binocular units appeared greater in their results; however, they make no correction for the prismatic influence of the contact lenses and it is possible that the eye positions may not have been standardized and stabilized to the extent they were in this series. More importantly, Van Sluyters and Stewart (74) described the ipsilateral projection to V.I as lacking topographic organization. In sharp contrast, it is shown above to be ordered in identical fashion to the contralateral projection and to contain a topographic representation of the binocular visual field. This result introduces the possibility of achieving binocular single vision by the systematic fusion of the entire contralateral and ipsilateral projections in each hemisphere when an appropriate vergence of the eyes is made.

If the eyes are vorged to the extent represented by Thompson et al. (50) as assuring a cortical representation devoted to contralateral field, it is instead found that the entire binocular field is represented in both hemispheres. The boundary between V.I and V.II then occurs at the limit of the cortical representation of ipsilateral binocular visual field and is not, as Thompson et al. (50) suggest, coincident with the representation of the visual field midline.

Is binocular single vision possible in the freeze position?

The encountered ipsilateral and contralateral projections to points in binocular cortex, and to binocular single units, arise in the freeze position of the eyes from retina dealing with different hemifields and subtending divergent axes in visual space. Such receptive fields cannot provide for binocular single vision under these conditions. Although it does not appear to be served by the binocular area of V.I, the rabbit in the freeze condition does possess a 24° binocular optical field which is generated by the 12° of each uniconal optical field which extends beyond the midline (Hughes, 71, 72). It has been previously assumed (Hughes, 72, 77) that this binocular optical field is imaged on retina which projects to contralateral binocular cortex and provides a basis for cortical binocular vision but this region of field does not appear to be served by binocular V.I. It is confirmed that at least 8° of the uniconal optical field which extends beyond the midline in the freeze position is served by retina which contains ganglion cells (Fig. 4). However, no representation of this region has been found in V.I at the multiunit or single unit level, although it does supply the superior colliculus (Vaney et al., 81). Only such a pathway from extreme temporal retina could provide for binocular single vision or stereopsis when the eyes are in the freeze position. No other region of retina serves a binocular field of view. However, if a very compressed or nontopographic binocular representation of this region of retina does exist in V.I or some other region of the brain, it follows that any associated binocular units would require zero mean disparity in the freeze position and would thus differ from those encountered in the identified binocular cortex.

Can cortical binocular single vision be achieved in rabbit?

The mean horizontal disparity of encountered binocular units was 17.7° ± 2.5° (n = 21) in the freeze position. Van Sluyters and Stewart (74), who obtained the similar value of 17°, point out that this mean divergent horizontal disparity precludes the same region of an object at any distance from simultaneously stimulating both receptive fields. Although accepting that a 10° vergence for each eye would fuse, or bring to convergent disparity, some 75% of their binocular unit population, Van Sluyters and Stewart (74) are reluctant to accept the possibility of simultaneous stimulation of binocular receptive fields They emphasize the "abnormalities" which they encountered: random topography of the ipsilateral projection, "split" monocular receptive fields, binocular units with different contralateral and ipsilateral receptive fields, and a wide range of relative vertical and horizontal disparities. The wide range of disparities and random ipsilateral topography suggest that uncontrolled eye movements and contact lens effects may underlie some of their findings.

By contrast, it has been shown above that the ipsilateral and contralateral maps have a similar topography and are
RABBIT BINOCULAR CORTEX

capable of fusion when the eyes are in the equivalent primary position. The 17.7° vergence required to achieve this from the freeze position of the eyes is equal to the mean disparity for a sample of binocular single unit receptive fields in the freeze position. The achievement of zero mean horizontal disparity would thus introduce the possibility of real objects simultaneously stimulating the ipsilateral and contralateral fields of binocular single units.

In contrast to Van Sluyters and Stewart (74), Vaney and Hughes ('82) point out that rabbit binocular units appear to be organized to take advantage of this possibility. Thirty-four out of 38 such units possessed similar ipsilateral and contralateral receptive fields. Of equal importance, the simultaneous stimulation of contralateral and ipsilateral fields causes obvious summation of the response of investigated units (Vaney and Hughes, '82). Such a property would be functionally significant under natural conditions only if the receptive fields actually attain the zero or convergent disparities which topographic fusion produces in the equivalent primary position.

When Van Sluyters and Stewart published there was no evidence for vergence in the rabbit (Hughes, '71; Collewijn, '77) and it is understandable that they decided against the possibility of binocular fusion. However, more recently Zuidam and Collewijn ('79) have demonstrated convergent eye movements in freely moving rabbits. Their maximum reported convergence of 18° would suffice to ensure that a majority of the cortical units recorded in this study possessed horizontal disparities appropriate to some position in real space.

The identified single units of rabbit primary binocular cortex are thus organized so as to permit binocular single vision but it cannot be achieved when the eyes are in the freeze position. It could occur if the eyes verge a minimum 18° to the equivalent primary position; this implies possible two-state cortical organization.

Why should the rabbit have two states for dealing with its frontal binocular optical field?

It is strange that the binocular optical field of a rabbit in the freeze position is served by retina but apparently not represented in visual area I. Although the anterior opties of the rabbit eye are free of oblique astigmatism and the retinotopic slice of the binocular retina is close to emmetropia (Hughes and Vaney, '78; but see De Graauw and Van Hof, '77, '79), it may be that image quality in this extreme peripheral region remains inadequate for binocular fixation tasks (Van Hof and Lagers-Van Haselen, '73; Van Hof and Steele Russell, '77). The quality of the choroidal image does appear to deteriorate in the far periphery as judged by opthalmoscopy (Hughes and Vaney, '78).

The need for a two-state cortical system to deal with the binocular field is thus arguable. The rabbit has the most lateral eyes of any mammalian species and it obtains an image over some 360° of visual horizon. A tonic vergence with the eyes swung forward so that the binocular field is imaged by less oblique, better-quality optics would necessarily reduce the extent of the rear visual field and generate a blind area dangerous for an animal relying on early warning for escape. Increasing the field-of-view of the eye to eliminate the blind area under such circumstances is probably not possible for conventional dioptrics, which already provide the rabbit eye with a 182° horizontal field (Hughes, '72). However, a two-state system might accept some 18° of divergence between the receptive field of binocular cortical units and the temporary loss of binocular vision when the animal is in the freeze condition. This would permit surveillance to the rear while the animal crouches motionless (Hughes, '71). The projection from extreme nasal retina to the superior colliculus might provide some form of binocular vision under these conditions. If the rabbit moves or binocularly fixes, the eyes could then converge on approaching targets (Zuidam and Collewijn, '79) and gain the advantage of superior optics with only temporary loss of field to the rear of the head.

The absolute disparity of binocular receptive fields and the horopter

In the equivalent primary position the mean horizontal disparity of single units is 0° ± 2.5° S.D. and their mean vertical disparity is 0° ± 3.4° S.D. Although these are pooled values, the range of any given eccentricity approaches a magnitude suitable for binocular single vision or stereopsis (Barlow et al., '67; Nikara et al., '85; Joshua and Bishop, '70), but neither capability has yet been demonstrated in the rabbit.

Joshua and Bishop ('70) report a slight negative gradient of binocular single unit disparity with increasing eccentricity in the cat cortex and this is suggested to define a horopter concave toward the animal. By contrast, in passing from the 90° to the 70° meridian in rabbit we find a comparatively gross increase of about 10° in the mean divergent disparities.

If a vergence of magnitude adequate to make all single unit disparities convergent should occur in rabbit then an oblique horopter lacking bilateral symmetry would be implied. The two cortical hemispheres would each map the entire binocular field but their horopters would be mirror images which would intersect on the midline to form an X shape in horizontal cross section. An apparently similar suggestion has been made by Choudhury ('60). This interesting possibility requires confirmation.

Bilateral cortical representation of the midline field has been reported for mouse (20°, Drager, '75), sheep (30°, Clarke and Whitteridge, '76), and golden hamster (20°, Tiao and Blakemore, '76). Although substantially wider than the 1.5° reported for the cat (Leicester, '68; Blakemore, '69), these regions of binocular overlap do not represent the entire extent of the binocular field as in the rabbit.

Does the rabbit possess a functional retinal fixation area?

In cat and monkey the retinal decussation passes close to the fixation area. By analogy, Hughes ('77) suggests that the projection of the cortical or retinal decussation line into visual space might enable the definition of the behavioral fixation axis as its intersection with the horizon's representation in a species lacking an obvious fixation area. Clearly, however, the complexities associated with the concept of decussation in the rabbit indicate that this suggestion is unlikely to have general applicability.

The results from primary visual cortex indicate a region of total overlap of the ipsilateral and contralateral projections rather than a narrow band or clear-cut decussation. With the eyes in the freeze position the cortical representation of the midline visual field is very compressed and quite unlike what might be expected in the projection of a fixation area. In the equivalent primary position of the eyes, it is retina some 10° nasal of the optic nerve head.
which projects to the midline. Indeed, the largest number of contralateral units encountered in the binocular cortex were in this region (Fig. 3), and the horizontal cortical magnification factor reaches a maximum value here. It is possible that it contains a functional fixation area whose centralis. This is the only possible that the large cell node identified by Provis (79). Alternatively, the rabbit and other species may retain relatively uniform horizontal acuity over some 6–10° of frontal visual field and thus dispense with the frequent horizontal reorientizations of head and eyes required by species with a delimited area centrals.

LITERATURE CITED


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Single Unit Receptive Fields in Rabbit Primary Binocular Cortex

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Summary. The receptive fields of 125 single units recorded from the binocular region of rabbit primary visual cortex have been analysed. The population of 43% radially symmetric, 23% directional, and 23% orientation selective units is similar to that of rabbit monocular visual cortex. The relative scarcity of orientation selective units and the absence of orientation columns differentiates rabbit from cat primary visual cortex. However, the majority of binocular units had similar receptive fields in each eye and very unconventional receptive fields were not encountered. Tested binocular units demonstrated summation upon simultaneous stimulation of both receptive fields. In conjunction with findings reported elsewhere, these results suggest that rabbit and cat possess a similar provision for binocular vision in spite of some differences in their cortical organisation.

Key words: Rabbit – Binocular cortex – Visual receptive fields – Single units

The binocular area of rabbit primary visual cortex has been described in detail only by Van Sluyters and Stewart (1974). They concluded that a single object would stimulate few, if any, pairs of binocular receptive fields because of divergent disparities, the lack of evidence for vergence, the dissimilar receptive fields of 50% of binocular units and lack of topography suggested by the presence of unusual “split” fields. Their opinion that the rabbit binocular system is imprecise and must involve a different kind of processing to that of the cat was supported by descriptions of random topography in the cortical representation of the ipsilateral visual field, the absence of columns and a large proportion of indefinite receptive fields.

By contrast, Hughes and Vaney (1982) find the visual field to be systematically represented in ipsilateral and contralateral rabbit primary visual cortex and conclude that binocular cortex in rabbit and cat has many features in common. The following is a brief account of the single unit receptive fields encountered during the study and is not intended to be a definitive classification.

Methods

Techniques have been described elsewhere (Hughes and Vaney 1982). Surgery was carried out under 0.5% – 1.5 Halothane in 70 : 30 nitrous oxide : carbogen. Binocular primary visual cortex was exposed up to its boundary with visual area two (Hughes 1971; Hughes and Vaney 1982) at the representation of the horizontal meridian of the visual field.

Paralysis was induced and maintained by i.v. infusion of gallamine triethiodide. The ventilatory minute volume was set 30% greater than recommended for the anaesthetised animal in order to preclude distress from CO₂ accumulation over the 1.5% level in the gas supplied. Body temperature was controlled by a homeothermic blanket system; the ECG, and occasionally the EEG were monitored. Xylocain was infused at intervals around pressure points. No signs of arousal were noted during the 2–3 days of the experiments.

Electrodes were pulled from filament-lined glass capillary tubing. After filling with 4M NaCl their impedance was 8 MΩ at 50 Hz. An electrode was arranged perpendicular to, and just above, the brain surface. A perspex well which surrounded the craniotomy was then almost filled with 3% “Ionagar” (Oxoid) in normal saline and covered by silicon fluid (DC200, Dow Corning) to prevent drying. Conventional recording facilities were employed. Topographic mapping was used to ensure data collection from electrophysiologically defined area V1. The majority of stable binocular and monocular units had waveforms and subsequent injury discharges typical of cell bodies rather than axons (Bishop et al. 1962).

A tangent screen was positioned 132 cm in front of the anterior nodal points of the eyes. The eyes were sutured to rings

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Table 1. Projection and types of the receptive field classes encountered in rabbit primary binocular cortex and subjected to detailed analysis

<table>
<thead>
<tr>
<th>Receptive field category</th>
<th>Contralateral</th>
<th>Ipsilateral</th>
<th>Binocular</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radially symmetric</td>
<td>17</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>52</td>
</tr>
<tr>
<td>Suppressed</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Direction selective</td>
<td>8</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>Axial* orientation</td>
<td>13</td>
<td>3</td>
<td>1</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>Directional* orientation</td>
<td>8</td>
<td>1</td>
<td>3</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Indefinite*</td>
<td>11</td>
<td>1</td>
<td>2</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>+ unclassified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>77</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>13</td>
<td>35</td>
<td></td>
<td>125</td>
</tr>
</tbody>
</table>

* These classes were not segregated into ON/OFF types
Preferred direction or axis of directional and orientation selective classes taken together:
0° axis: 38%  90° axis: 36%
45° axis: 17%  135° axis: 9%

and set in the "freeze" position (Hughes 1971). Contact lenses of zero power were fitted to protect the corneas from drying and did not change the +2D refraction in the anterior field. This is believed to represent emmetropia at the outer limiting membrane (Hughes and Vaney 1978). The 0.76D working distance was not corrected because of the uncertainties associated with anterior field refraction in the rabbit. Receptive fields were sought and plotted using spots and bars projected by a handheld Keeler "Pantoscope" (60 cd/m²) on a dimly lit background (7 cd/m²) and they were qualitatively analysed by handheld cardboard stimuli on a contrasting white (20 cd/m²) or black (0.6 cd/m²) background.

After a receptive field had been located and defined it was centred on a 60 cm television monitor situated 115 cm in front of the anterior nodal points. The background luminance of the screen was 4.0 cd/m²; superimposed black and white stimuli were respectively 1 log unit above and below this level. Raising prisms enabled fusion of the left and right receptive fields of binocular units. Quantitative receptive field analysis employed a stimulus-pattern generator (Hughes and Snow 1973). An HP2114B computer processed both spike data and stimulus position information in order to generate response profiles for the single units.

Results

Rabbit binocular cortex deals with visual field from the 72° to the 90° meridian when the eyes are in the "freeze" position (Hughes and Vaney 1982). In this area, thirty tracks in six rabbits encountered a mean of 9 ± 6 units per track. Ninety-five percent of the investigated fields were situated within ±5° of the horizontal meridian so that they lay within the projection of the visual streak. Two hundred eighty-two units were encountered but the receptive fields of 28 of these were not located within 20 min. The receptive fields of 129 units were located but they were lost before analysis was complete. Hundred twenty-five units were held for between 1 and 12 h which provided adequate time for detailed investigation and classification. Of these, 35 possessed binocular receptive fields. Some tracks passed through monocular cortex and the opportunity was taken to examine the receptive fields.

Receptive Field Classification

Receptive fields were grouped into six categories (Table 1). Fifty-two of the 125 units were radially symmetric because of the similarity of their response to a stimulus entering along any radius. Their ON, OFF or ON/OFF centre receptive fields could often be mapped with a flashed light spot; some revealed an antagonistic, others only an inhibitory, surround organization (Fig. 1A). These fields were not divided into stationary and motion selective groups.

The maintained firing of one unit was inhibited by a light spot anywhere in its large receptive field. No stimulus was found to increase the firing rate of this suppressed-only unit. A similar unit in monocular cortex was inhibited by a dark spot. These units
are reminiscent of the uniformity detectors in the rabbit retina (Levick 1967).

Fifty-eight units possessed receptive fields which were not radially symmetric. Twenty-nine direction selective units (Fig. 1B) showed a maximum of response for stimuli moving in one, the preferred, direction and little or no response to movement in the opposite, null, direction. Movement in the null direction sometimes inhibited their spontaneous discharge. The stimulus entry angles producing consistent excitation ranged from ±20° to ±135° of the preferred direction, mean ±70°. Directional selectivity did not arise from the interaction of mapped excitatory and inhibitory elements of the receptive fields and was equally apparent to light spots, disc stimuli and bars or extended edges. Extended stimuli sometimes indicated the presence of an inhibitory surround.
The 29 receptive fields without radial symmetry were selective for linear stimuli oriented over a limited angular range. The stimulus selectivity of eleven such orientation selective units was explicable in terms of their separate excitatory and inhibitory regions. Two units had orientation specificity defined by adjacent, mutually inhibitory, dark and light discharge regions (Fig. 1C). The orientation selectivity of five edge selective units could not be accounted for. Five orientation selective units responded better to a bar than to an edge; their central discharge regions were bordered by inhibitory areas.

At least five of the orientation selective units responded best to line stimuli of limited length. Such end-zone inhibition beyond one or both of the lateral borders of the discharge region is characteristic of hypercomplex units (Hubel and Wiesel 1965; Henry 1977).

Of 29 orientation selective units, 17 responded to movement in either direction along the preferred axis of motion. The remaining 12 were directional orientation selective units and responded to movement along only one direction of the response axis.

Fourteen cells with heterogeneous properties comprise the indefinite (Van Sluyters and Stewart 1974) and unclassified units. Indefinite units gave localised but variable responses and failed to respond to repeated stimulation. Unclassified fields were located but could not be grouped with other categories for various reasons.

**Receptive Field Size**

Receptive field size was expressed as the geometric mean of the major and minor axes of the hand plot. The mean field diameter for units with different classes of receptive field was similar for tracks within the monocular or binocular representation of the streak. The mean diameter of 47 radially symmetric fields was 4.0° ± 5.9° and 2.8° ± 1.9° for 52 directional and orientation selective fields. The contralateral and ipsilateral fields of binocular units were not significantly different in size and, in spite of the great obliquity of the optics, they could be as small as 1° in diameter.

**Encounter Proportions**

Of 125 units whose receptive fields were localized within 20° of the midline and analysed, 77 (62%) had a contralateral field alone, 13 (10%) had an ipsilateral field alone, and 35 (28%) had binocular receptive fields. Non-orientation selective units formed 77% of the total sample and comprised 43% radially symmetric, 23% directional, and 11% indefinite units (Table 1).

No evidence was obtained for columnar organization with respect to receptive field type, or preferred orientation, in either monocular or binocular cortex; signs of clustering were insignificant. However, the pooled preferred directions, or axes, of directional and orientation selective units do indicate a preponderance of vertical and horizontal (74%) over oblique orientations (26%) (Table 1) as has previously been reported in reuna (Levick 1967; Oyster 1968) and cortex (Ogawa et al. 1968).

**Binocular Receptive-Fields**

Of 125 units recorded from binocular cortex, 62% were stimulated via the contralateral eye alone, 5% were contralaterally dominated, 18% equally responsive through each eye, 5% ipsilaterally dominated, and 10% stimulated via the ipsilateral eye alone.

The properties of the units stimulated through the ipsilateral eye alone resembled those of the ipsilateral fields of binocular units. This suggested that a corresponding contralateral field had been overlooked. However, exhaustive search did not even reveal indefinite or inhibitory "gating" fields (Bishop 1973; Henry et al. 1969).

Two of 35 binocular units possessed one indefinite field; no double indefinite fields were encountered. Only one binocular unit had dissimilar fields; both were radially symmetric ON fields but the contralateral field revealed a slight OFF response as shown in Fig. 1A. The difference is minor. Preferred directions and orientations were always the same for both receptive fields of binocular units.

The binocular unit population (Table 1) comprised 54% radially symmetric, 29% directional, 11% orientation selective and 6% indefinite units. Binocular summation upon simultaneous stimulation of both receptive fields was revealed in all six of these units which were examined in detail (Fig. 1D). Such interaction is strong support for the involvement of these units in binocular vision.

**Discussion**

**Binocular Organization**

The population of units described above differs from that of Van Sluyters and Stewart (1974). Indefinite fields were rare, the receptive fields of both eyes were similar for all binocular units, no split fields...
were encountered, contralateral dominance was less obvious, some units possessed only an ipsilateral field and a smaller proportion of binocular units, 28% compared to 79%, were encountered. The lack of anesthesia during Van Sluyters and Stewart’s (1974) experiments could underlie these differences. However, Choudhury (1980) reports only 47% of binocular units to possess similar fields in anaesthetised preparations.

It is not our intention to emphasise or explain the differences between the two samples. We rather assume that, like the binocular units above, some 50% of Van Sluyters’ and Stewart’s (1974) and 47% of Choudhury’s (1980) units did require similar inputs from each eye. The divergent disparities of these receptive field pairs led Van Sluyters and Stewart (1974) to argue against their possible role in binocular vision. However, the recent demonstration that rabbits can verge up to 18° (Zuidam and Collewijn 1979) now provides a means for the conscious animal to fuse on a single object the binocular receptive fields found to be divergent in the experimental situation.

Obviously some unusual features of rabbit binocular cortex, such as indefinite fields and binocular units with different types of ipsi- and contralateral receptive fields, still require explanation. However, Hughes and Vaney (1982) differ from Van Sluyters and Stewart (1974) in that they describe a potentially functional range of horizontal disparities and a systematic representation of the ipsilateral visual field in rabbit binocular cortex. In addition, it is reported above that some rabbit binocular units demonstrated summation when both of their receptive fields were simultaneously stimulated. This would be a minimum requirement for the employment of such units in a binocular apparatus for single or depth vision. Taken together, these results suggest that the organization of rabbit binocular cortex has much in common with that of the cat. Stereopsis (Fox and Blake 1971; Packwood and Gordon 1975), and possibly single vision (Packwood and Gordon 1975), have now been demonstrated in cat and it is commonly argued that the binocular apparatus forms their substrate. It thus seems likely that the rabbit will be revealed to possess these capabilities.

Absence of Columns

The lack of orientation columns in rabbit visual cortex appears to be a genuine difference from the cat. The above results confirm earlier reports (Arden et al. 1967; Hughes 1968; Stewart et al. 1971). Specific search for orientation columns (Bousfield 1977; Murphy and Berman 1979) has only revealed slight clustering.

Single Unit Population and Encounter Rates

The above population of 43% radially symmetric, 23% directional, and 23% orientation selective units in rabbit primary binocular cortex resembles the 39% symmetric, stationary, and motion selective units, 9% directional and 15% orientation units of Van Sluyters and Stewart (1974). Both samples are like those encountered in monocular cortex (Hughes 1968; Ogawa et al. 1968; Chow et al. 1971)

The proportion of orientation selective units in rabbit cortex was substantially lower than the 99% (Murphy and Berman 1979) to 100% (Hubel and Wiesel 1962) usually encountered in cat primary visual cortex. Nevertheless, the orientation selective units of both species have very similar edge, bar and contrast selectivity, which may be associated with hypercomplex and directional properties. Murphy and Berman (1979) came to a similar conclusion when comparing rabbit monocular cortex receptive fields with those of cat.

It is, therefore, the non-orientation selective receptive fields which have no commonly encountered equivalent in cat primary visual cortex and appear to set the rabbit apart. However, there have been reports of these receptive fields in cat primary visual cortex (Baumgartner et al. 1965; Denny et al. 1968). Similar rare encounters with non-concentric units (Stone and Fabian 1966; Rodieck 1967) suggested the description of a large population in cat retina (Stone and Hoffmann 1972). Although commonly neglected, non-orientation selective units may form up to 30% of the encountered population of some regions of cat primary visual cortex (Joshua and Bishop 1970). The single unit populations of cat and rabbit cortex may thus turn out to be less disparate than is currently accepted.

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TECHNICAL NOTE

A VISUAL STIMULATOR EMPLOYING A T.V. RASTER DISPLAY

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A GREAT variety of visual stimulators for the exploration of the receptive field properties of single units has been described in the literature. Commonly these consist of slide projectors coupled with a rotating mirror, or of a mechanical system for moving card stimuli. By these means reliable repetitive movements at controlled stimulus velocities are obtained. For qualitative investigation the experimenter falls back on the much more flexible “wand and card”.

The cathode ray tube has been little exploited as a means of presenting stimuli during experiments on animals. The following device generates a great variety of visual stimuli on the screen of a standard commercial 625 line TV raster and enables the experimenter to carry out hand controlled exploration of receptive fields with as great a variety of stimuli as card can provide, while enjoying the added advantages of continuously variable size, instant contrast reversal, brightness and contrast control, and immediate transfer to automatically controlled movement of the stimulus at a pre-set velocity, along a selected path, at a variable repetition rate.

The disadvantages of the method are the possibility of spurious responses arising from the presence of the raster lines, responses to flicker resulting from too low a frame repetition rate (although this should be limited by the use of 2:1 interlaced scan), and the limitation on the upper movement velocity set by the appearance of discontinuous movement, which may be eliminated only by increasing the frame repetition rate.

The circuit consists of two main parts; a stimulus pattern generator and a system for controlling the position of the pattern on the screen. A block diagram is given in Fig. 1.

The position of the stimulus is controlled by the output of the two voltage controlled monostables which precede the pattern generator (Fig. 1). One of these is triggered at the beginning of each line, and when resetting after its determined delay, gives a voltage change which is used to establish the horizontal position of the stimulus pattern. The other voltage controlled monostable is triggered by each frame pulse, and thus determines the vertical position of the stimulus.

The adders which precede the voltage controlled monostables have various inputs. The simplest of these is a potentiometer determined voltage, one feeding the horizontal and the other the vertical monostable, which determines the monostable period and thus the stimulus position. The two potentiometers are mechanically linked to a joy-stick assembly of the kind used for model aircraft radio control, which provides a means of moving the stimulus about by hand, with the same degree of convenience normally associated with the traditional wand and card stimulus.
Provision is made for automatic movement of the stimulus along the horizontal lines of the raster. Movement at other orientations is provided by rotating the C.R.T. about its long axis. The movement generator also provides a voltage which is fed into the voltage controlled monostable to vary its delay and thus provide displacement of stimuli across the screen. This voltage is obtained from a ramp generating integrator whose output is also fed to two triggers; one of these is set to give a pulse at zero volts—the threshold of the other is variable. When the threshold of either of these triggers is exceeded, the output pulse changes the state of a bistable which provides the input to the integrator. The consequent inversion of the input voltage to the integrator reverses the direction of the ramp, thus producing alternate positive and negative slopes, which act on the voltage controlled monostable so as to move the stimulus back and forth on the screen at a rate determined by the integrator time constant, and for a distance determined by the setting of the adjustable trigger threshold voltage; both of the trigger outputs are synchronized to the frame pulses to stop unwanted flyback and changes of movement direction during a frame scan. A press switch is provided for resetting the relationship between the bistable and the integrator output which may be disturbed during switching.

Movement in one direction is obtained by switching the input of the integrator to the d.c. supply, and the reset to the synchronised adjustable trigger output. In both the single and double sweeps the length and rate of the movement are controlled independently.
A hold facility is provided in order to give an adjustable delay between the sweeps. This consists of a reed relay which open-circuits the integrator input for the delay period, which is determined by the period of a monostable triggered from the variable threshold trigger.

Provision has been made for up to a ±45° change in the orientation of vertical edges and bars generated by the pattern unit by increasing or decreasing the width of the horizontal monostable position pulse from line to line during one frame and resetting it between frames. This is achieved by feeding another, faster ramp into the adder which precedes the monostable. The amplitude of the ramp is directly related to the orientation of a vertical edge on the screen. A second ganged potentiometer is required to provide correction to ensure rotation of the edge about the centre of the screen. Positive and negative slopes may be selected.

In the simplest case, the output of the vertical or horizontal monostable may be fed directly through the pattern generator to provide a single vertical or horizontal edge with provision for phase change, so that it may be set to be black on the left or right of the screen or at top or bottom. Horizontal and vertical edges may be simultaneously selected in various phases to provide a corner which occupies one of four positions on the screen.

A second edge, which is variable in position, may be added to form a vertical or horizontal bar. All of these stimuli may be combined in various ways; horizontal bar with vertical edge to make a tongue; horizontal bar with vertical bar to make a vertical rectangle or square according to the width settings. Finally a third and fourth edge may be added to make another bar which can be superposed on the first to provide a contrasting band of variable width and position for the investigation of laterally inhibitory borders of bar shaped fields. The various permutations of edges provide a great range of stimuli (Fig. 2). The logic incorporated ensures that at crossing points, the edges are suppressed in such a manner as to enable terminated bars to be set up. Once established the stimuli may be centred at any point on the screen.

Fig. 2. A selection of the stimuli obtainable from the unit by combining edges is shown above. It must be remembered that movement, contrast and shuttering are also under the experimenter's control.
An alternative source of stimuli is provided by the annulus generator—for simplicity these annuli are square! The outer and inner boundaries of the annulus are defined by a large and small square respectively. The vertical and horizontal separation of the edges of each of these squares is determined by a pair of monostables whose duration is set by a single twin ganged potentiometer which thus provides a single size control. Each of the edge generating monostables is triggered from the appropriate voltage controlled monostable by an intermediate delay monostable. This arrangement is necessary to ensure that the squares remain in a fixed position on the screen as they change in size; the delay monostable and the corresponding edge separation monostable are so connected to the same potentiometer that as the duration of one increases, that of the other decreases. For the square to remain stationary the delay monostable duration must change by half the amount of the edge monostable. The arrangement of the monostable connections is clear in Fig. 3g.

A final source of stimuli is provided by a square/sine wave generator consisting of an oscillator, triggered by the resetting of the voltage-controlled monostable during the first line of each frame; on subsequent lines the output of the monostable provides synchronizing pulses, and the system is reset at the end of the frame. The oscillator is continuously variable and has a series of frequencies which give a stable array. The output provides
Technical Note

3a. Integrator input

3b. Movement generator

3c. Movement and hold

3d. Voltage controlled monostable

492
Technical Note

Shuttering

From pulse movement generator

Small square pattern generator

The above section is duplicated; one unit supplies horizontal, and the other vertical, lines.

Horizontal C_v=165KpF; C_y=330pF
Vertical C_v=330pF; C_y=330pF

Fig. 3 (a-h). The following working drawings are included to minimize design work on the part of anyone building the circuit. They are not referred to in the text but are self-explanatory when examined in the context of the block diagram of Fig. 1. No plan for the triggered oscillator of the grating generator has been provided as the design is straightforward if required.
an array of bars modulated in intensity in a sine or square fashion which is moveable by the joystick or by automatic control. The pattern logic system enables the bar raster to be trimmed above and below and mixed with other patterns in a useful way, e.g. an annulus may be generated with a bar pattern superposed in its illuminated area to test for edge selective surrounds.

The output of the pattern generators passes through an inhibit gate, operated by the shuttering oscillator, or by a push button which allows flashing of the stimuli. A synchronizing system is provided to ensure that the flashes are appropriately related to the frame pulses and movement generator. The video output signal passes directly, or through an inverter which enables instantaneous reversal of contrast, to a contrast control. The direct signal gives a white stimulus on a grey background and the inverted a black stimulus on a grey background. When the stimulus has been inhibited the whole screen remains grey.

The unit contains its own line and frame pulse generating system.

The system is effective with cortical cells but has not been tried out with retinal units. It is possible that use of the normal frame presentation rates may produce sufficient flicker to elicit spurious responses from units of the transient class.

In this preliminary form the T.V. tube was mounted in a ball bearing race, with its screen vertical, thus enabling rotation through 360°. With appropriate earthing no extensive screening was found necessary to prevent interference pick up by the microelectrode FET input system. The animal looks at the image of the screen in a front silvered mirror which is gimbal mounted. This arrangement enables a unit to be centred fairly rapidly but is only suited to recording from the cortex and other locations where small shifts of visual projection occur between successive units.

Some care must be taken to ensure that the raster line spacing—determined by the choice of T.V. tube screen size—subtends a visual angle at the selected working distance which is below the behaviourally determined visual acuity of the viewing animal. This will not, however, guarantee protection from unit responses to the lines; it is well known that optokinetic acuity is greater than behavioural acuity in most lower mammalian species.

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CALLOSAL TERMINATIONS ALONG THE BOUNDARY BETWEEN VISUAL AREAS I AND II IN THE RABBIT

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INTRODUCTION

In the cat the right and left halves of the visual field are separately represented in topographical fashion on the respective contralateral cerebral hemispheres. Furthermore, each half field is represented three times within a hemisphere upon adjacent areas of cortex. From medial to lateral these areas are named visual I, visual II and visual III. The boundary between visual I and visual II is formed by the cortical representation of the medial edge of the half visual field. Because the eyes are forward looking in the cat this boundary corresponds to the vertical meridian which passes through the area for acute vision (area centralis) of each eye.

There is anatomical and electrophysiological evidence in the cat that the visual areas of the two hemispheres are interconnected along the boundary between visual I and visual II by fibres running in the corpus callosum. Points more medial or more lateral to this boundary, with receptive fields away from the vertical meridian, have only sparse connections with the other hemisphere.

In contrast to the cat, the rabbit’s eyes are laterally placed and it is therefore of interest to know whether the callosal fibres are again confined to the boundary between the right and left halves of the visual field. We have consequently determined the relationship in the rabbit between the cortical region receiving callosal terminals, as shown by anatomical degeneration methods, and the electrophysiologically constructed map of the projection of the visual field onto the cortex. This comparison is made easier by the absence of the deep sulci which are found in the cat.

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EXPERIMENTAL

Anatomy

Three rabbits (weights 4.5, 2.6 and 3.0 kg resp.) were anaesthetized with Nembutal* and the caudal 18 mm of occipital cortex of one hemisphere was removed lateral to the splenial sulcus. This included the visual areas I and II. 5–10 days later the animals were again anaesthetized with Nembutal and perfused with 10% neutral formol–saline. The caudal 15 mm of brain was cut in the coronal plane using a freezing microtome. Sections were cut nominally at 0.03 mm and were stained with cresyl violet and the Nauta method at approximately 0.6 mm intervals. The position of the Nauta degeneration on the undamaged cortex was marked directly on the slides using a fine felt tipped pen. The slides were then projected with a magnification of 3 times onto paper and the outlines of the sections and the position of the degeneration drawn. Before the sections were cut the intact hemisphere was penetrated by 2 needles, approximately 1 cm apart, parallel to each other and to the long axis of the brain. Later, when the brain was being reconstructed, sections could be orientated and related to the midsagittal plane by the position of the holes. The maps were reconstructed by measuring the distance of the degeneration from the midline, perpendicular to the midsagittal plane, i.e. an orthographic projection. Linear shrinkage of less than 10% occurred during the Nauta procedure but this has not been taken into account as it would introduce an error of less than 1 mm in the position of the degeneration.

Fig. 1. The caudal part of a rabbit's left cerebral hemisphere viewed from above. The limits of Nauta degeneration, after removal of the contralateral visual area, in each of the 3 animals is marked.

* Abbott Laboratories Ltd., Queenborough, Kent, Great Britain.
The pattern of Nauta degeneration seen in the 3 brains was similar; the maps are reproduced in Fig. 1. The reconstructed dorsal views of the brain show a dense strip of moderately coarse degenerated fibres running rostrocaudally across the visual cortex. The degenerated fibres could be seen to extend from the white matter in a predominantly radial fashion. Only a few fibres could be seen penetrating as far as the bottom of layer III. However, after a similar operation in the rat and opossum, degeneration has been seen to extend as far as layer I with the Fink and Heimer stain. Rostral to the region plotted in Fig. 1 the degeneration becomes more extensive and within another millimetre it was found throughout the whole dorsal surface of the hemisphere.

Electrophysiology

A map showing the dual projection of the visual field onto the rabbit cortex was used to determine the topographical relationship between the region receiving the callosal projections and the visual areas I and II. The map, made for other purposes, was obtained electrophysiologically by determining the co-ordinates of the point in the visual field from which a small light spot flashed on and off, provoked the maximum response for each of some 80 electrode positions. Tungsten electrodes were used and the rabbit was anaesthetized with urethane.

Unlike the cat, the rabbit does not have a circular area centralis which can be used as the centre of a system of visual field co-ordinates. Instead, for acute vision, the rabbit has a visual streak which stretches horizontally across the retina. A horizontal band of myelinated fibres emanating from this region can be seen with the ophthalmoscope and is used as the basis of the parallels of the visual field co-ordinates. The zero vertical meridian of the system of co-ordinates is arbitrarily chosen to run through the optic nerve head. The parallels of the co-ordinate system were arranged to run parallel to the myelinated band, and thus to the visual streak, because these are normally held horizontal in the conscious rabbit.

The orientation and co-ordinates of the optic nerve head and the myelinated band of the eye were determined ophthalmoscopically at the beginning and termination of the experiment, during which the eye remained sewn to a brass ring. The data were later transformed nomographically to a system of parallels and meridians arranged so that the projection of the centre of the optic nerve head (which is 20° above the streak) lay at the intersection of the —20° parallel and the central vertical meridian. All results were reduced to this standard eye position. The visual field co-ordinates associated with each electrode insertion on the cortex were marked on a plot of the stabs and then the arrangement of the meridians and parallels onto the cortex viewed in dorsal projection was drawn. The skull was not removed up to the posterior pole so that the upper visual field must be more extensively represented than appears in the results. The map of visual II will be distorted at its lateral margin because of the greater obliquity of the brain surface in that region.

Three complete maps of visual I and visual II were obtained. One typical result is shown in Fig. 2. The map will be treated in detail in another publication. The

Brain Research, 12 (1969) 19-25
Fig. 2. The same view of the cerebral hemisphere showing the representation of the visual fields. VI, visual area I; VII, visual area II. T, temporal and N, nasal to the vertical meridian through the optic nerve head. D.L., decussation line. The dotted region is the maximum extent of the callosal terminations in the 3 animals of Fig. 1.

Contralateral half visual field is projected upon the rabbit cortex in a topographical fashion with the vertical meridians running roughly rostrocaudally, parallel to the midline. The medial boundary of the map, the temporal limit of the visual field, is always located at the splenial sulcus. The visual field representation in the lateral part of the cortex is a mirror image of the map on the medial part of the cortex. The medial area is visual I and the lateral area visual II. The transition between visual I and visual II is marked with a bold line in Fig. 2 and is the 'decussation line' of Thompson et al. The decussation line corresponds to a vertical meridian 70–80° nasal to the projection of the optic nerve head in space. Because on the retina it corresponds to the line of division between ganglion cells giving rise to the crossed and uncrossed optic nerve fibres, it is the meridian at which the two halves of the visual field, which are projected to the different hemispheres, meet (Fig. 3). The dotted region in Fig. 2 shows the position of the previously described degenerated callosal fibres from the contralateral visual area. It is clear that the callosal fibres project to a strip running astride the boundary between visual I and visual II in the rabbit. It should also be noted that the greater part of the dorsal projection of the cortical map is involved in the representation of the visual streak (+10° to −10° parallels).

The callosal fibres are thus less extensive in the rabbit than in the rat but occupy a similar position. The relation of the callosal fibres to visual I and visual II is similar to that in cat.1,2,4,8,16.

It is to be noted that the transition between visual I and visual II, the decussation line, runs more parallel to the midline on Fig. 2 than in the map of Thompson.
et al. Our electrophysiological map, however, correlates well with the arrangement of the callosal degeneration.

In one mapping experiment, a cut was placed somewhat medial to the electrophysiologically determined decussation line. In toluidine blue-stained sections of the brain it was found that the cut lay just medial to the ill-defined transition region between area 17 and the area occipitalis. It would thus appear that area 17–occipitalis border corresponds to the visual I–visual II junction defined electrophysiologically. In support of this is the finding that the position of the transition zones between area 17 and occipitalis in the diagrams of the smaller rabbit brains studied by O'Leary and Bishop corresponds with the position of the decussation line in the

Brain Research, 12 (1969) 19-25
map of Fig. 2. It would thus appear that in the rabbit, as in the cat, the electrophysiologically defined visual regions correspond to the cytoarchitectonically determined cortical fields. The visual cortex of the rabbit as determined by Rose and Malis\textsuperscript{12} on the basis of lateral geniculate nucleus projections is much more extensive than visual I and must embrace the area occipitalis as well. This would suggest that the rabbit primary and secondary visual areas receive separate innervation from the L.G.N. such as has been demonstrated in the cat\textsuperscript{15}.

DISCUSSION

In conclusion, our results show that only a narrow band within the visual cortex is linked through the corpus callosum to the opposite hemisphere. This band lies along the junction of visual I with visual II and, in terms of visual space, corresponds to the junction between right and left halves of the visual field. This would suggest that these fibres may be concerned with linking together the two halves of the visual field which are represented in different hemispheres. The correspondence of the region bearing the callosal projections with that representing the binocular portion of the visual field indicates that the callosal projection may correlate range finding processes in the two hemispheres.

It is alternatively possible that these fibres may play a role in transferring information for pattern discrimination from one hemisphere to another as in split chiasm cats\textsuperscript{9} and monkeys\textsuperscript{5}. The fibres described above would, however, serve such a function for only a limited portion of the nasal visual field. Behavioural evidence\textsuperscript{14} suggests that the rabbit uses its lateral visual field rather than the nasal for detail vision so that this role for the fibres appears unlikely. The interhemispherical transfer of information for the lateral field may occur by means of more anterior callosal fibres arising from some region of the cortex which carries out further processing of visual information.

SUMMARY

The distribution of the terminals of the corpus callosum within the visual areas of the rabbit were studied by removing the occipital cortex on one side and looking for Nauta degeneration in the other hemisphere. The degeneration was found as a narrow band running rostrocaudally over the occipital cortex.

In other rabbits an electrophysiological map of the visual field was produced by recording, with tungsten electrodes, the response to a small flash of light. The map consisted of 2 visual areas which are mirror-images of each other, lying side by side with visual area I medial to visual area II.

When these maps of the visual field were compared with the distribution of the callosal degeneration, it was found that the band of degeneration coincided with the junction between the 2 visual areas.

The significance of these results is discussed.

\textit{Brain Research}, 12 (1969) 19-25
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Single Unit Observations in Conflict with Van Hof's Theory of Orientation Discrimination in the Rabbit

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Single unit observations are presented which cast doubt on Van Hof's theory of orientation discrimination in the rabbit. An alternative interpretation of his behavioral findings is put forward.

Introduction

Van Hof (8) has investigated whether the orientation selective units of the rabbit retina may be considered to be the exclusive intermediaries of orientation discrimination. He interprets the results of his behavioral experiments as suggesting that this cannot be the case and that separate mechanisms are used in the determination of the orientation of continuous bars and rows of dots. This interpretation of the results appears to follow from an assumption stated early in the paper: “Since the output of the orientation detectors will only give relevant information if a straight boundary is projected across the receptive field, it seems a plausible inference that these detectors do only participate in the discrimination of bars.” And in the summary: “Since straight boundaries are not relevant to the ‘dot’ mechanism, it seems unlikely that orientation detection is exclusively performed via the orientation detectors.”

Previous experience of rabbit retinal and cortical orientation selective units (4) suggested that orientation selectivity would be displayed, contrary to Van Hof's assumption, to both continuous and discontinuous bands centered on the receptive field. During recording from the rat visual cortex, the opportunity was taken to present the stimuli used by Van Hof (8) to an orientation selective unit similar to those of the rabbit cortex and to determine whether orientation selectivity could be demonstrated in the case of a row of dots.

Methods

Cells were recorded by means of 2–3 megohm glass micropipettes filled with sodium chloride in area VI of rats' anesthetized with urethane. The
dura mater was left intact and a layer of agar (3%) covered the exposed surface and limited pulsation.

The animals were either fitted with contact lenses or the cornea was covered with a thin layer of silicon fluid (1 cs) to preserve its optical quality. The animals were refracted and assumed to be emmetropic when about 6d of hypermetropia was indicated (2). Stimuli were made up in various sizes on slides (2×2 inch) and projected onto a tangent screen at a distance of 0.5 m from the eye of the rat. The refractive error for the animal would thus be about 2d which is perfectly compatible with the normal functioning of the orientation selective units (5).

Results

The majority of the orientation selective units to be encountered in the rat visual cortex have been similar to the orientated edge selective units of the cat (3); flanking regions are not usually present on both sides of the receptive field. The field consists of an “on” area and an “off” area connected in reciprocally inhibitory fashion. In some cases stimulation of the “on” area or the “off” area alone failed to elicit an excitatory response so that the opposing region dominated and a clear preference for black or white stimuli respectively would be noted.

Once a unit was recorded its identification as orientation selective was rapidly achieved by the movement of extended black or white bars through the appropriate region of visual field against a background of the reverse contrast. When such an extended stimulus is moved perpendicular to its long axis along various radii through the receptive field then firing is found to be maximal for one specific axis of movement, the preferred axis, and absent for movement along the axis perpendicular to it, the null axis. The selectivity of the response depends entirely upon whether the two regions of the field, the “on” and the “off” area, are stimulated simultaneously or sequentially. In the former case there is no response, in the latter there is a response. Firing may thus be elicited even for movement in the null axis as long as the stimulus does not overlap into the opposing part of the receptive field. It is thus possible to map out the extent of the two regions by moving a small stimulus back and forth along the null axis while slowly approaching the excitatory/inhibitory boundary from the periphery with a simultaneous movement along the preferred axis. Firing will be elicited until the border is overlapped. The same movement repeated from the opposite side of the border will map out the opposing region of field if it also gives an excitatory response. The border between the two regions of the field may be precisely defined by projecting a bar of light parallel to its long axis, (i.e.) parallel to the null movement axis, and moving it slowly towards the border along the preferred axis while flashing it.
on and off. Firing will be elicited until it just straddles the border when it will cease although sometimes returning in the opposite phase relationship to the flash if the movement is continued into the opposite part of the field. The results obtained with such an analysis for a horizontally selective unit are shown in Fig. 1.

Figure 2 provides an example of the application of Van Hof's stimuli to...
an orientation selective unit which responded best to white stimuli—the reverse contrast to those used by Van Hof but otherwise similar. The behavioral stimuli were bars 10° wide and rows of dots 10° in diameter spaced with their centers 15° apart. The stimuli presented to the unit shown consisted of a bar 11° wide and dots 11° in diameter spaced 20° apart.

When the row of dots or the bar was projected with the long axis horizontal and located in the excitatory region of the receptive field without overlapping into the inhibitory area, then a good response could be obtained as the stimulus was flashed on and off. In this case the bar or the row of dots is orientated parallel to the excitatory/inhibitory border.

Flashing of the bar parallel to the preferred movement axis or perpendicular to the excitatory/inhibitory border elicited no response from the unit (Fig. 2A) but if the stimulus was cut off at the border and confined to the "on" area then a pronounced response to its flashing was obtained (Fig. 2B).

The differential response to the two orientations of the bar clearly results from the simultaneous activation of the inhibitory and excitatory regions when the bar is perpendicular to the border between them. A similar orientation selectivity will be manifested, contrary to Van Hof's assumption, if the row of dots simultaneously activates the two regions. The receptive field of this unit was about 15° along its preferred axis so that an extreme test of the unit's ability to discriminate simultaneous stimulation of its two regions was provided when two dots, whose centers subtended an angle of 20°, were disposed symmetrically about the excitatory/inhibitory border with the axis joining their centers parallel to the preferred axis and perpendicular to the border. When the two dots were flashed simultaneously there was no response (Fig. 2C); when the inhibitory area was stimulated by flashing one of the spots alone then no "off" response was obtained (Fig. 2D) but when the "on" area was stimulated alone by flashing the spot which partially overlapped its field then a powerful response was obtained (Fig. 2E). It is thus clear that this unit would respond with orientation selectivity to rows of dots whose centers were separated by up to 20° at least.

Discussion

It is apparent that rows of dots such as were provided by Van Hof as behavioral stimuli for "rabbits" are adequate to elicit the orientation selec-

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such as the vertical bars to the left, do not give rise to a response as is indicated by the two small arrows and the — sign. Shorter tongues confined to the "on" or the "off" area elicit a response if of the appropriate contrast as long as they do not overlap the border between the regions.
Fig. 2. The insets A to E show the response of a horizontally orientation selective unit of the rat visual cortex to the flashing of the visual stimuli indicated by the interrupted lines. The border of the receptive field is indicated by a continuous line and the boundary between the excitatory and inhibitory regions is shown as a dotted line. The upper trace of each insert shows the output of the microelectrode; the next down indicates the flashing of the stimulus—down is on; the bottom trace is a 100 msec time marker. See the text for an explanation.

itivity which “rat” cortical orientation selective units normally demonstrate to continuous edges or bars.

Similar orientation selective units have been described in the rabbit retina (5) but, as Van Hof (8) has pointed out, these are too small to account for the behavioral response to dots whose centers subtend an angle of 15°. These units project to the LGN (7) and to the visual cortex (4) of the rabbit. Both Hughes (4) and Ogawa, Karita and Tsuchiya (6) found the orientation selective fields to be usually relatively small (~5°) but larger versions were encountered and Arden, Ikeda, and Hill illustrate one (1, Fig. 1B) which has a preferred axis length of 15°. There is no reason why the results obtained from the rat units should not be taken as applying equally to similar units in the rabbit cortex.

It is therefore the case that we may no longer accept Van Hof’s inference that the orientation selective units can be excluded from participation
in the discrimination of the orientation of rows of dots. The results of Van Hof (8) thus remain unexplained. Before attempting an alternative interpretation it is to be emphasized that the involvement of the orientation selective units in the behavioral process of orientation discrimination is being tacitly assumed for the continuation of the discussion but there is no clear evidence supporting the assumption.

The most important points established by Van Hof (8) which remain to be explained are therefore: (a) why rabbits trained to discriminate dotted lines (97.2 ± 1.2% score) transfer well to bars (98.9 ± 0.4% score) but animals trained to discriminate the orientation of bars (96.6 ± 1.6% score) transfer poorly to dots (62.1 ± 2.5% score); and (b) why the accuracy of orientation discrimination for dotted lines (10°) is lower than for bars (between $5 + 10^\circ$) for horizontal, 45° and vertical orientations. This second point will not be dealt with below; the differences are not very large and explanations are too dependent upon the nature of the mechanism underlying the transfer asymmetry (1).

The following explanation of the transfer asymmetry is put forward in spite of its oversimplification because it covers the facts at present available. We limit the discussion to the vertical/horizontal bar discrimination. It is assumed that the ability of the rabbit to carry out the task is based upon a comparison of the separately summed outputs of the vertically and horizontally selective unit populations. For each region of the visual field in which the illumination level is uniform there will be no response from the corresponding units in either population and no contribution to the summed output. Along each border of the vertical bar, however, all those vertically selective units whose two regions are not overlapped by the border will contribute to the summed vertical output pool, $V'$, but no such contribution will be made by the horizontally selective units to their summing pool, $H$, because the vertical border must intersect both regions of each of the constituent unit's receptive fields. During training to select the vertical bar the reward will come to be associated with a signal of magnitude $X$ from the vertical summing pool in the absence of an output from the horizontal summing pool.

During the training of the corresponding discrimination between a vertical and horizontal row of dots there will be a different population response. In the case of the vertical row of dots the gaps between the dots will reduce the output of the summing pool $V'$ from $X$, as for a bar, to some smaller value $Y$ either by lowering the number of contributory units or by reducing the output of each contributory unit if the receptive fields are larger in relation to the gaps between the dots. It is also apparent that the introduction of borders perpendicular to the axis of the dot row may, depending on the gap to field diameter ratio, introduce some firing into the horizontal
summing pool but in the absence of the necessary information we must ignore this possibility.

Thus, because the animal trained to choose the vertical bar associates a firing level in the vertical pool of magnitude $X$ with the reward it will fail to transfer the association to a vertical row of dots which give rise to a smaller pool output, $Y$.

In contrast, if the training is initially to the vertical row of dots then $Y$, the output of the vertical pool, will become the criterion for the reward and upon the presentation of the continuous vertical bar the output of the vertical pool, $X$, will exceed the criterion and transfer of the reward association from the row of dots to the continuous bar will readily occur.

The individual units at retinal and cortical level will fire well to the flashing of an extended narrow bar projected into the receptive field as long as it does not overlap the excitatory/inhibitory border. The ready transfer from continuous to outline bars would thus be explained because the output of the summing pools would be similar in both cases.

Van Hof (8) has pointed out that his conclusions are speculative but may provide a starting point for electrophysiological experiments. It appears appropriate to conclude by "putting the ball back into the behavioral court." If the above model is valid, then the transfer asymmetry should be demonstrable in the absence of straight lines or bars. Transfer from a row dots with wide spacing to a row of dots with relatively narrower spacing should be readily achieved but the reverse transfer should fail. If Van Hof's concept of a dot extrapolating analyzer was valid then asymmetry would not be expected in the case of transfer between rows of dots of different spacing.

References
The neural representation of visual space

DRASDO\(^1\) and Lennie\(^2\), attribute Rolls and Cowey\(^3\) with the demonstration that monkey cortical magnification factor, \(M\), varies as the square root of the ganglion cell density which deals with the corresponding region of visual field. Lennie's discussion is based on the development of this relationship for monkey and man. In fact, Rolls and Cowey report \(M\) to be proportional to ganglion cell density \(D\), not to its square root, both within and outside the foveal area.

Such proportionality between retinal ganglion cell density \(D\) and \(M\) has also been described at several stations of the visual pathways in a variety of species.\(^4\) It has been a long-standing puzzle as to why a linear measurement, \(M\), should be proportional to a retinal cell density.

The outcome of Drasdo's data manipulations, that human cortical magnification factor is proportional to the square root of the ganglion cells serving the corresponding region of visual field (that is, of his receptive field density, \(D\)), is thus not at all in agreement with the findings of Rolls and Cowey. If valid, it is the first instance of such a relationship and implies that the human visual cortex is organised in a quite different fashion from that of monkey or macaque, or indeed from that of cat and rabbit.

However, the data on which Drasdo's conclusions are based are not sound enough to warrant a belief that the human cortex deviates so markedly from the organisation apparently common to a variety of species. The ganglion cell distributions which he pooled\(^5\) were all obtained by the unreliable technique of counting on radial sections not subject to an Abercrombie correction.\(^6\) Also, there is no evidence of common criteria for ganglion cells, shrinkages were not measured and the distributions plotted separately can support quite different interpretations from those of Drasdo. It perhaps suffices to point out that the results of Van Buren indicate densities of from one to more than two orders of magnitude greater than those of Oppel at the equivalent eccentricity. Correction for optical magnification is a nicety in such circumstances.

Of course, it would be pleasing if Drasdo's results were valid, because the recent demonstration\(^7\) of proportionality between human acuity and cortical magnification factor could then relate acuity directly to ganglion cell angular separation. In my opinion the evidence is against such a relationship in man\(^8\) or cat\(^9\) but, as Lennie points out, knowledge of the distribution of the specific ganglion cell class involved in resolution tasks may elucidate the matter.

Note added in proof: Lennie's editorial remains explicitly misleading. This might have been avoided had Drasdo emphasised that he claimed differences between man and monkey which necessitated analysis in terms of \(D\), rather than \(D\), and \(M\). However, I remain unconvinced; the densities of Van Buren and Oppel differ more than 100-fold at the same eccentricity in the original data quite independent of errors introduced by reploting; similar large discrepancies occur throughout their data and preclude pooling.

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PROLEGOMENA
AN ATTEMPT AT SYNTHESIS FOR ONE SPECIES
TOPOGRAPHICAL RELATIONSHIPS BETWEEN THE
ANATOMY AND PHYSIOLOGY OF THE RABBIT
VISUAL SYSTEM

by

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(Oxford)

INTRODUCTION

Even a casual glance at the rabbit head suggests that the visual field of the animal is unusually large. This point was clearly recognised by the eighteenth century poet who described the rabbit as possessing:

'Unsleeping eyes, by Nature raised
To take the horizon in ........'

In fact this description is more appropriate than the author can have imagined because the major feature of the rabbit retina is now known to be the 'visual streak', an extended area centralis which receives the image of the animal's visual horizon.

In the subsequent pages I have brought together a collection of observations which reveal the extent of the interdependence between the specialised eye of the rabbit and the organisation of its central nervous system.

The work presented here is thus unified by the theme of the topographical relationship between the visual field and its representation in the central nervous system, both in the overt mapping sense and in more subtle ways. My use of this theme stems from the influence of Professor D. Whitteridge who has emphasised the importance of mapping studies (Whitteridge, 1965); some of the material is a continuation of work begun by Whitteridge and his co-workers (Choudhury & Whitteridge, 1964; 1965; Kerr & Seneviratne, 1963). Other components, including most of the single unit studies, were carried out some years ago under the supervision of Professor Whitteridge at Edinburgh University.

One initial problem faced in carrying out such a topographical study is that
of comparing observations made on different individuals or in different regions of the same animal. It is thus necessary to adopt some standard state for relating the observations. The main points to be established are the orientation of the head, the absolute position and orientation of the eyes in space and their degree of convergence. The majority of the observations made to establish these facts have thus been carried out on rabbits in the freeze state in which they crouch motionless with the nasal bone about 35° away from the vertical. It is quite possible that changes occurring in normal behaviour will thus make some of the results stated below misleading.

**Visual Field**

*Visual Field 'In Vivo'*

**Total Visual Field** The ophthalmoscopically observed limits of the retinal light reflex were used to determine the total extent of the monocular and binocular fields of view of an intact rabbit in the freeze condition with its eyelids retracted. The results are similar to those of Pisa (1939) and are shown in Fig. 1 as determined in one characteristic animal. The binocular field begins some 30–40° below the horizontal and extends to 70° beyond the vertical in the midsaggital plane. The medial intrusion of the monocular field into the opposite half field is at a maximum of 15° for each eye in a region directly above the head. The total width of the binocular field is thus not in excess of 30° but varies with the action of the retractor bulbi and the position of the eyelids.

The visual field of the rabbit was thus found to be almost a complete sphere. The only totally blind areas are the optic nerve head and the narrow blind sector under and behind the head. The extent of this region is variable and depends upon the body and eyelid positions and upon the length of the animal's fur. The topography of the area is therefore indicated only approximately in Fig. 1 in which the ears are neglected.

**Limit of Field to Rear of Head** The rabbit has been described as possessing a useful binocular field to the rear of the head on the horizontal. There seems to be no possibility of this being true; statements to the contrary appear to stem from misinterpretation of Pisa's results (Pisa, 1939). His theoretical constructs of the rabbit binocular field show a narrow binocular field within 5°
RELATION BETWEEN ANATOMY AND PHYSIOLOGY

Fig. 1
The visual field of the rabbit divided into its respective binocular, monocular and blind regions.

of the horizontal but the above findings are in agreement with his observations on the intact animal which suggest that the rear limit of the binocular field is some 30–40° above the horizontal. As we shall see that the obstruction of view by extraocular structures forms the main limit to the field of vision at the rear then convergence alone would be inadequate to generate a binocular area at the horizontal level. Even when the head and ears are raised above the level of the body it is not possible to find evidence for the rearward directed binocular field. In Fig. 2 the camera axis is centred on the middle of the rabbit’s head and parallel to its long axis. It is possible to see only the corneal vertex of one eye and pupil of neither. As the camera was situated at a distance of three feet

Binocular reflex
Blind area
Optic nerve head & fibres
Photograph taken at a distance of 3 feet showing the absence of a view of the pupils when the camera is centred at the middle of the marked black spot. A binocular field of more than 2° could thus not exist.

from the animal it is possible to show that both pupils should be visible if there is a binocular field of more than 2–3° overall. It would have been necessary to move the camera sideways in order to obtain views of either pupil so that it appears that the rear limit to the monocular field runs back almost parallel to the long axis of the body. Ophthalmoscopic observation suggests that it terminates a degree or two lateral to a line parallel to the long axis. The figures presented on page (37) bear out this view. If the monocular overlap into the opposite half field is 12° on average then it is clear that with an overall monocular field of 192° the rear limit of the field must extend back parallel to the long axis of the head as long as there is no change in convergence.
There is, of course, no doubt as to the existence of a binocular field above the level of the horizontal to the rear of an animal in the freeze position.

'Blind Spot' The true blind spot of the rabbit is the region of optic nerve entry through the retina but the area may be effectively elongated along the horizontal. The optic nerve fibres fan out nasally and temporally to form a narrow band which obstructs the access of light to a region of visual field some 140° long and 8° wide projecting to the lower half of lateral field some 13–15° below the horizontal. Although this region is shown in Fig. 1 as being blind like the optic nerve head projection there are receptors and ganglion cells subadjacent to it for almost all of its length but the concentration of ganglion cells appears to be low. The position of the relatively blind area is such as to offer little impediment to predator detection; an important feature in an animal with lateral eyes as there is no complementary coverage of the blind area by the other eye.

The arrangement of the band of nerve fibres passing from the visual streak to the optic nerve head is clearly visible in the accompanying photograph of an eye opened under saline, Fig. 4f. In the hare (Fig. 4h) the arrangement is similar but the fibres arch upwards in the middle of their course towards the optic nerve head.

**Potential Visual Field**

*Unobscured Monocular Field* The monocular field, free of obstruction by eyelids or body margin, was determined by the technique previously described for excised eyes inflated to a pressure of 25 cm H₂O. The projections of the optic nerve head and optic axis were used to nomographically transform the results so that they represented the potential field of an eye in its normal position in the animal.

The monocular field of the isolated eye was found to extend for 192° along the horizontal and 180° along the vertical. (Average of 6). This difference of field extent is reflected in the corneal dimensions, quoted by Davis (1925), of 15.6 mm along the horizontal and 13.8 mm in the vertical.

*Unobscured Whole Field* The unobstructed potential binocular field was determined by reconstruction from the measured monocular field whose limits were plotted as though viewed on the front and rear surfaces of a sphere centred on the animal's head. The viewpoint lies on an axis passing from the centre of
the sphere through the point of intersection of the midsaggital plane with the nasal region of the equator. The sphere was assumed to be large enough to

![Diagram](image)

**Observed**

- Front
- Rear
- Front
- Rear

**Construct**

- Binocular field
- Blind area

Fig. 3

Measured and constructed potential binocular fields projected onto the surface of a sphere. See text.
make the interocular distance negligible. The region of overlap between the right and left monocular fields defines the potential binocular field and blind area. The results of this procedure are shown in Fig. 3b. The observed results from the intact animal are displayed in similar fashion in Fig. 3a as obtained at a distance of 1 m, so that the interocular distance may again be neglected. The only major difference between the constructed and observed fields is that the reconstructed binocular field is much more extensive to the rear and extends almost to the horizontal; the blind area is thus correspondingly less extensive. This is to be expected as it is in this region that the greatest obscuration of the field by the body occurs. The correspondence between the potential and observed visual fields is marked and indicates that almost all of the field of the eye has been exploited in this much preyed upon animal. This correspondence between the visual field defined by the organisation of the eye and that defined by the marginalia of the orbit may be an important factor in the interpretation of the eye movements of the rabbit and will be taken up again.

The plane of the iris and of the corneo-scleral junction may be seen to be inclined medially at the upper margin by an angle of about 15° when observed in an eye ‘in situ’. This 15° rotation of the 180° vertical monocular field generates the overhead binocular and lower blind areas. The subsequently demonstrated 13° inclination of the optic axis above the horizontal confirms this effective rotation of the field about an antero-posterior axis running through the anterior nodal point of the eye.

The photographs of the excised rabbit eye shown in Fig. 4 reveal the anatomical basis for the form of the binocular field in an animal with dilated pupils. Given that the two monocular fields are inclined towards one another by some 15° in the vertical, manifested by the large area of iris in the dorsal view (Fig. 4a) compared with the ventral view (Fig. 4c), then the lower nasal binocular field is obtained from the medial extension of the cornea in its lower nasal quadrant. This extension can be seen in the anterior view of the eye (Fig. 4d) as a medial retraction of the corneo-scleral boundary below the equator of the globe. The width of the binocular field in the upper nasal and temporal regions is kept constant by extensions of the cornea in the upper nasal and temporal quadrants. These are indicated by arrows in the dorsal view (Fig. 4a). No extension of the cornea is to be seen in the lower temporal region (Fig. b & c) where the blind area coincides with the body.

Fig. 4g shows a frontal view of a hare eye which is very similar in organisation to that of the rabbit.
The excised rabbit eye in (a) dorsal; (b) posterior; (c) ventral; (d) anterior; and (e) lateral, views. In (f) the has been opened to reveal the optic nerve head and myelinated band. Similar views of the hare: (g) anterior view; (h) eye opened.
RELATION BETWEEN ANATOMY AND PHYSIOLOGY

Relationship of Retinal Landmarks to the Visual Field

If a neurophysiological basis is to be provided for the interpretation of the visual behaviour of the rabbit it is essential to be able to relate area of retinal specialisation to given regions of the animal centred visual space. The area centralis, or 'visual streak' in the rabbit, is the most important region in this respect. It is unfortunately not usually possible to determine its position ophthalmoscopically, though differences in pigmentation may indicate its whereabouts on occasion, and recourse to indirect location must be made. Previous to reading Oyser's 1968 paper I had assumed that the streak was orientated parallel to the long axis of the myelinated fibre band and to the horizontal so that the band and its blood vessels provided an indication of the orientation of the streak.

Oyser (1968) reports that in conscious rabbits the main blood vessels have their axis inclined to the horizontal at an angle of 10.7° in the region neighbouring the optic nerve head. The preferred axis of one class of directional units and the direction of pull of the medial rectus muscle were found to deviate by a similar extent. It is thus possible that the visual streak is rotated, instead of being maintained horizontal, so that its long axis corresponds to the direction of medial and lateral rectus pull. It is alternatively possible that the blood vessels are tilted but that the streak is held horizontal. If this were the case then the axis of the visual streak and that of the blood vessels should diverge by some 10° with the greatest separation occurring on the temporal retina. In fact numerous observations show that the peak ganglion cell count and the mean line of the blood vessels are separated to the same extent at a distance of 5 mm to each side of the optic nerve head and that their long axes are parallel (Fig. 5b & c). The implication is thus that if Oyster's observations on the blood vessels apply to this population, the streak should be inclined on average 10.7° to the horizontal.

This arrangement would appear teleologically unsatisfactory. If the streak is horizontal, then the narrow region of peak count is directed close to the animal's horizon for almost its whole extent. This region of the visual field is of considerable interest and significance to a hunted species. If the streak is tilted, then the high resolution regions of the streak would intersect apparently arbitrary parts of the visual field except on the lateral axis. It is thus important to attempt to determine the orientation of the streak in animals sitting in the freeze condition.
A. Fundus photographs showing the blood vessels and optic nerve head of a rabbit in the freeze position. Note variation in orientation. B & C: Blood vessels and peak ganglion cell count of visual streak appear parallel in unfixed flat mount preparations of the rabbit retina.

**Orientation of The Visual Streak.** A fundus camera was used to obtain photographs of the myelinated band and blood vessels in unrestrained rabbits. The camera was arranged vertical with a spirit level. In four animals there was no consistent deviation of the blood vessels from the horizontal. In any one animal the orientation varied in successive photographs, Fig. 5A, but it is possible that the obscuration of the field by the camera upset the natural position.
In an alternate investigation the optic nerve fibres which pass down to the visual streak from the region adjacent to the hyaloid artery remnant were observed ophthalmoscopically in a lighted room. These fibres pass down the retina perpendicular to the visual streak and thus reveal its orientation (Fig. 5). By means of a slit ophthalmoscope their orientation was found to be 0.6° in a clockwise direction. (Average of 12 animals) The results ranged from 4° to —5°. The conclusion is thus that the streak, since it is parallel to the blood vessels and perpendicular to the fibres, must be nearly horizontal under these conditions.

In almost all cases of animals sitting on a table in the freeze condition it has been possible to run the ophthalmoscope along the table edge at constant level and keep the myelinated band and blood vessels centred in the field of view. On occasion restless animals have been encountered which show variations in the blood vessel position but these were not in the freeze position and evinced search behaviour. The results shown later suggest that variability in the streak position during activity is to be expected for a variety of reasons.

![Fig. 6](image-url)

Views of the excised rabbit eye showing the positions of the medial and lateral rectus insertions and their relationship to the myelinated fibre band and the visual streak.
The photographs of Fig. 6 show an excised eye from anterior, medial and posterior viewpoints. The arrowed black lines are the remnants of the posterior ciliary arteries which penetrate the sclera and roughly overlay the visual streak. These vessels pass around the globe parallel to the myelinated band of fibres leaving the optic nerve head. In Fig. 6D the underlying band of fibres has been marked by cautery after identification of its position by transcleral illumination. The fibre band and line joining the blood vessels are clearly parallel. The vessels at front and rear of the eye may be seen through the conjunctiva in conscious animals. Their position usually suggests that they are running parallel to the horizontal. This impression was confirmed in one case in which the cornea was anaesthetised, marked and then subsequently photographed when the animal was in the freeze position. Comparison of the photographs with others of the excised eye indicated that the myelinated fibres, posterior ciliary arteries and, consequently, visual streak were parallel to the horizontal.

The insertions of the medial and lateral rectus muscles may be seen to be quite broad but, when stretched, the line of pull is parallel to the myelinated fibre band and hence to the streak. In keeping with the findings of Rothfield (1912), Wessely (1916) and Prince (1964) I find the medial rectus to have a mean insertion slightly above the horizontal and the lateral rectus slightly below.

It is thus clear that because Oyster finds the muscle line of pull and the blood vessels to be parallel, which is confirmed above, then the whole eye must be rotated by some 10.7° under the conditions of observation used by him. Such rotation of the eye has been observed in free moving animals (page 89). The rabbits used by Oyster were not in the freeze condition (personal communication) and may have been using their binocular field rather than the streak.

Projection of Retinal Landmarks in Visual Field The horizontal extent of the monocular visual field has already been presented as 192° on average with the limit of the binocular field being 102° nasal of a line perpendicular to the long axis of the head and passing through the anterior nodal point of the eye. The optic nerve head projects 9° nasal of this line and the optic axis, determined by the Purkinje images, is 2.0° nasal of it. In the horizontal plane the optic nerve head is at the centre of the eye but the optic axis is somewhat temporal of the centre. These relationships are indicated in the diagram of Fig. 7B.

The monocular field extends 180° in the vertical (Fig. 7A). The optic axis was used as a reference when relating the results from different animals and projects on average 13° above the horizontal. Given the 15° medial tilt of the
field in the vertical plane it is clear that it is nearly bisected by the optic axis projection line. The projection of the other retinal landmarks was obtained by

![Diagram](image)

**Fig. 7**
Projection of retinal landmarks in the visual field of the rabbit in the freeze condition:
A in the vertical plane, B in the horizontal plane. BL, binocular limit; V, vertical; OA, optic axis; SP, streak peak ganglion cell count; H, horizontal parallel; ONH & BV, optic nerve head and blood vessels; DL, presumed projection of the decussation line; P, perpendicular to long axis of head.
measurements made on photographs of the investigator and an unrestrained sitting rabbit at the time when the required feature was under ophthalmoscopic investigation. The upper border of the band of myelinated fibres projects to \(-16\)\(^\circ\); the blood vessels leaving the optic nerve head and centre of the nerve head project to \(-13\)\(^\circ\); the hyaloid artery remnant projects to \(-0.5\)\(^\circ\) and the lower border of the myelinated fibre band projects very slightly above the horizontal (\(+1.5\)\(^\circ\)).

The projection of the invisible peak of the visual streak ganglion cell count was obtained by conversion of the linear perpendicular distance between it and the main blood vessels in 6 flat mounted, unfixed, hydrated and stained retinæ, to an angular separation in the visual field. The average distance was 2.8 mm (range of 6: 2.7–2.9 mm). Measurement of the distance between the transclerally viewed images of two distant light sources subtending a known angle to an inflated, excised, eye was carried out with a travelling microscope and gave a value of 0.17 mm/\(^\circ\) in the region under the optic nerve head (average of 12; range 0.15–0.19). The peak count of the visual streak must therefore project about 3\(^\circ\) above the horizontal.

No schematic eye is available for the rabbit in the literature. The necessary calculations and observations were carried out but will be published separately as they are not immediately relevant to this paper. The above observations on the scale of representation of the visual field image under the optic nerve head are in agreement with the finding of the schematic eye. The figure of 0.17 mm/\(^\circ\) leads to a posterior nodal distance of 9.75 mm which is in good agreement with the schematic value of 9.85 mm. It is worth noting that the schematic eye indicates the state of refraction of the animal to be 1D of hypermetropia which is in agreement with the figures in the literature. CHOU (1954) and STONE & LEARY (1957) report emmetropia and 2D of hypermetropia respectively.

**Retinal Organisation**

*Topographic Distribution of Retinal Cells*

The retinal image of the visual field is of fairly uniform magnification in the rabbit. In enucleated but inflated eyes the retinal image is compressed by less than 15\% at the temporal and nasal periphery compared with the region under the optic nerve head.

The various stages in the visual pathways are connected in strict topographi-
cal relationship to the retinal receptor mosaic so that the neural activity generated by the retinal image of the visual field is organised in such a fashion that the visual field may be said to have a projection within the CNS. The differential transformation of the central visual field projection may offer clues as to the fashion in which the animal uses its visual input. For the moment we are concerned with the transformation of the image within the retina. The differential transformation of the central visual field projection may offer clues as to how the animal uses its visual input. The overall transformation carried out by the retina is best looked at in the output stage – the ganglion cells – and we may return to the other layers later.

Ganglion Cell Distribution  The area centralis or visual streak of the rabbit was first described by CIEVITZ (1891). A limited count of the ganglion cell distribution was made by SENEVERATNE (1963) from radial sections. The more extensive map of retinal ganglion cell density in Fig. 8 has been made from a flat mounted retina which was arranged to be securely adherent to a gelatinised slide, before staining with cresyl violet, dehydration and mounting, thus ensuring a minimum of shrinkage. This was not corrected for in making the counts.

The isocount lines define successive areas in which the ganglion cell count increases in steps of 1,000 g.cell/mm² from 1,000 g.cell/mm² to 7,000 g.cell/mm². The origin of the term visual streak is apparent from the band of high ganglion cell count running under the retinal blood vessels for most of the length of the retina. The peak count of 7,000 g.cells/mm² is centred on the optic axis in the region under the optic nerve head. Along the streak the count drops off slowly to the 5,000 g.cells/mm² termination at each end but then declines more rapidly – a little less rapidly in nasal than in temporal retina. Thus, although the decline in density is most immediately noticeable in running from the streak peak count to the retina above or below, such a change also occurs along the length of the streak itself.

The upward bulges in the 1,000 g.cell/mm² – 4,000 g.cell/mm² isocount lines in the temporal retina occur in the region which normally receives the image of the nasal field from the horizontal to some 30° below. This region is also, of course, that which deals with the frontal binocular field. The nasal retina which deals with the temporal field at the same level bears no such inflections in the isocount lines.
A flat mount of the retina of the right eye of a rabbit. The blood vessels which overlay the myelinated band spread out from the optic nerve head. Ganglion cell isocount density lines are drawn in indicating the position of the visual streak. Numbers represent the ganglion cell count in thousands per mm². Note the area of high count under the optic nerve head and the upward bulge of the 3,2 and $1 \times 10^4$ isocount lines in the region of nasal retina which deals with the ground under the animal's nose.

It is of great interest to attempt to relate the line of decussation to the isocount contours. The necessary optic tract section has not been performed so that indirect, and not unequivocal, means must be used to determine the location of the line. If it is assumed that the retina has been removed along its full length then the 30 mm of the preparation represent some 192° of visual field along the horizontal. 1° in the visual field thus occupies 0.156 mm on this retina. The average binocular field is 12° so that the decussation line would fall as a vertical line tangent to the end of the 4,000 g.cell/mm² isocount. The optic nerve head would project 15° forward of the perpendicular to the head long axis, which is within the observed range. The above assumptions give an angular distance from the optic nerve head to the binocular field limit of 87°. On average this distance is 92° when observed in the living animal (range 87° – 95° in 12 cases).
so that the calculated value falls within the observed range and justifies the assumptions involved. Such a prediction is however based upon the assumption that the projection of the decussation line is parallel to that of the other eye and parallel to the long axis of the head and that the binocular field is not changed by convergence from the magnitude determined in the freeze position.

It is noticeable that the isocount lines are more widely spaced in progressing from the streak to the inferior retina, which deals with the sky above the horizon than in passing towards the myelinated fibre band.

**Ganglion Cell Diameter Distribution** The ganglion cell density map of Fig. 8 shows the arrangement of the specialised area centralis of the rabbit. Lévick (1967) has reported that the cells of this region possess rather specialised properties when compared with those of the more peripheral areas. The visual streak appears equipotential along its length in that the density of cells varies little. It was, however, decided to seek for evidence of differentiation by the examination of the distribution of ganglion cell diameter in various regions of streak and peripheral retina.

The diameter of the ganglion cells was measured at a magnification of \( \times 1,000 \) on photographs of 0.36 × 0.24 mm areas spaced at regular intervals on lines perpendicular to the visual streak and at various distances from the optic nerve head in both nasal and temporal directions. The cells were grouped into three diameter classes: 6-10 \( \mu \), 10-14 \( \mu \) and 14-18 \( \mu \), without correction for shrinkage. No ganglion cells were smaller than 6 \( \mu \) and the population larger than 18 \( \mu \) was significant only at the limits of the retina and was left off in order to make the diagrams clearer. The sample populations vary from 40 to over 400 cells.

The results are shown in Fig. 9 for the same retina as that in Fig. 8. The distance indicated in the top right hand corner of each set of results records the distance of the sample line from the optic nerve head. The abscissa is a mm scale indicating distance along the retina and the ordinate indicates the percentage of the sample at each point contributed by each of the three continuous lines which represent the divisions of the population. The ordinate to the right of the figure relates to the dotted line which represents the total ganglion cell density determined from the sample photograph. The arrow under the abscissa of each section indicates the peak count of the streak as determined from this curve. In passing from this arrow to the right the results come from progressively more inferior retina.
Ganglion cell diameter populations for points on sampling lines perpendicular to the visual streak at various distances nasal, N, and temporal, T, of the optic nerve head. The distance indicated by the figure in the upper right hand corner of each section. An arrow indicates the peak count of the streak on the abscissa which is marked out in mm. to indicate the distance of sampling points up or down the retina. The left hand ordinate gives the percentage of the sample at each point which is contributed by each of the populations coded by the symbols indicated in the top left hand corner of the diagram. The ordinate to the right gives the ganglion cell density in thousands/mm² for each sampling point on the interrupted lines.
The pattern of the change in population composition is very similar in cross sections of the streak running from 11.7 mm temporal to 10 mm nasal. The 6–10 μ count peaks on the streak while the 10–14 μ + 14–18 μ counts are symmetrically decreased. Thus in running 4 mm up the retina the population changes from about 50%, 10–14 μ, to 80–90%, 6–10 μ, on the streak peak. The central run, 2 mm temporal of the optic nerve head, is continued for some distance towards the periphery to show the relative uniformity of the population once the visual streak has been left behind. In the peripheral region the population is about 15%, 14–18 μ, 60%, 10–14 μ, and 25%, 6–10 μ.

The counts along strips running parallel to and on each side of the assumed decussation line show little difference from the standard pattern (10.7 & 11.5 mm temporal lines).

The anomalous form of the most nasal and temporal counts, in that the 10–14 μ count predominates and the 6–10 μ is considerably reduced, is partly

![Fig. 10](image-url)

Plot of the relationship between ganglion cell density and the percentage of each ganglion cell diameter class present in the population at that density. Points marked by the symbols shown in the upper left hand corner show the average percentage of each class of all points sampled at a given density. The line through each point indicates the range of the samples, which is large in some cases but the clumping was good.
accounted for by the absolute count of these areas being low like that of the regions below the streak nearer to the optic nerve head.

The diameter distribution thus confirms the uniformity of organisation of the streak that is suggested by the ganglion cell density counts. Apart from the most temporal and nasal limits of the retina, the population appears to be a relatively simple function of the total cell density at a given point and not a function of the absolute position on the retina.

To test this hypothesis the values of each component of the population were averaged over all the readings available from different regions of retina at a given ganglion cell density. The average value was then plotted for each component as the ordinate of a graph whose abscissa was a scale of ganglion cell density. The range of the values was indicated by a line drawn to the extreme values from the average point. The results form Fig. 10. The range is rather large for some of the values at low ganglion cell densities but the samples clump well and

![Graph showing distribution of receptor, bipolar, and ganglion cells](image)

Fig. 11

Distribution of the absolute count of receptor nuclei inner nuclear layer nuclei (coded as 'bipolars') and ganglion cells, along a line perpendicular to the visual streak. The counts start at a point just below the optic nerve head and pass down the retina. Distance along the abscissa and density as an ordinate. Note that the visual streak is represented in all layers.
only a few points fall at the extreme limits. The average line is quite indicative of the general validity of the above hypothesis. The population clearly alters from a predominant 6–10 μ group, (95% in the region of 5,000 g. cell/mm²), to a 60%, 10–14 μ, population in the 500 g.cell/mm² region. A model of the diameter distribution based on the simple relation shown in Fig. 10 would reproduce the basic form of the curves in Fig. 8 satisfactorily. It would be of interest to know if the diameter uniformity is correlated with functional uniformity along the streak and whether the constitution of the single unit population is a function of the ganglion cell density and not of absolute position.

Inner and Outer Nuclear Layers Cell density in the inner and outer nuclear layers must be investigated in radial sections rather than in flat mounts. The region under the optic nerve head was taken as representative of the majority of the retina. Fig. 11 shows the results of counts of nuclei in the outer and inner nuclear and ganglion cell layers at various distances below the optic nerve head in a 6 μ thick vertical radial section of the eye. The counts were subjected to an abercrombie correction (ABERCROMBIE, 1946).

The peaks in the plots of nuclear concentration in the three layers coincide on the retina and indicate that the visual streak is represented, albeit not so markedly, in the receptor and inner nuclear layers as well as in the ganglion cell lamina.

The ratio of the nuclear counts in different layers is illustrated in Fig. 12 in which the ordinate is a logarithmic scale. The marked parallelism of the receptor and inner nuclear layer counts is brought out in the ratio plot where the term 'bipolar' is used to stand for the nuclei of the inner layer including those of amacrine and horizontal cells but excluding MÜLLER cells. The receptor/inner nuclear layer count remains constant along most of the retina at a value of about 7 although it decreases to 5 on the peak of the streak. The potential increment in resolution offered by the increased receptor density at the visual streak may thus not be lost at the bipolar level since the nuclei of the inner layer show a parallel increase in density. The slight excess of inner layer nuclei over receptors in the area of the streak may indicate a lesser degree of convergence in the connections of the receptors. It is equally likely that the amacrine and/or horizontal cell population is increased in this region.

DOWLING (1968) has described the rabbit retina as displaying many more instances of bipolar cells which connect to amacrines alone than are to be found in cat or monkey retinae. If this reflects the greater sophistication of the
retinal units in this animal then these connections might be expected to be most common in the visual streak where Levick (1967) has demonstrated a greater variety of complex units than are present in the periphery (Barlow, Hill & Levick, 1964). High density of such contacts may be accompanied by an increase in the number of amacrine cells which would lead to the lowering of the receptor/inner nuclear layer nuclei ratio such as is found in this region (Fig. 12).

Fig. 12
The ratios of the number of cells in the outer nuclear (receptors), inner nuclear layer ("bipolars"), and ganglion cell layers plotted on a logarithmic ordinate scale for sampled areas on a line perpendicular to the visual streak and passing through the optic nerve head. Note the relative constancy of the receptor/inner nuclear layer cell ratio compared with the inner nuclear layer/ganglion cell ratio.
The relative uniformity of the ratio of receptors/inner nuclear layer cells suggests that they are organised into subunits of uniform composition, or of different composition but uniform distribution, and function throughout the retina. An analysis of the results of CHEVITZ (1891) reveals that a similar uniformity of the ratio of outer to inner nuclear layer counts exists in the cat retina.

The contrast between the receptor/inner nuclear count ratio and that of the inner nuclear layer cells/ganglion cells is marked. The latter changes from a value of 5 at the streak to one of about 95 in the peripheral retina and thus clearly reflects a different organisation at the area centralis. The overall receptor/ganglion cell ratio shows a similar form because of the constancy of the inner nuclear/receptor ratio and changes from about 35:1 at the streak to 700:1 at the periphery. It is clear that much less gross, quantitative EM data is required to reveal the organisation of the streak in any detail.

Cone Distribution There has been some controversy as to whether the rabbit possesses cones. In Prince’s book (Prince, 1964) an apology is made because the presence of cones is denied in one chapter and claimed in another. SJOSTRAND (Prince, 1964) describes two types of receptor: one is a typical rod while the other – the type II receptor – is characterised by a short outer segment which is surrounded by dense processes emanating from the pigment cell in which the end of the receptor is always embedded. The inner segment of the type II receptor extends half as far again as the rod inner segments towards the pigment cells. SJOSTRAND assumes these type II outer segments to be associated with the irregularly conical pedicles to be found adjacent to the outer plexiform layer. The unique form of the type II receptor caused SJOSTRAND to question the existence of cones in the rabbit retina.

There are various reasons for assuming that this receptor is functionally a cone. Apart from the form of the outer segment, its morphology is consistent with that of a cone. In Fig. 13B we present an illustration of the appearance of these receptors in a Golgi preparation. The outer segments seldom impregnate but the inner segments are broad. The nucleus is situated in the most scleral layer of receptor nuclei and is always connected to a conical pedicle which gives off fine tendrils from its base. All of these features are associated with cones in other species.

Fig. 13C shows a most fortunate E.M. section which ran through such a type II receptor from its insertion in the pigment cell to its pedicle and tendrils.
Cones in the rabbit retina.  

A Arrows indicate cone nuclei with patchy chromatin lying at the scleral boundary of the outer nuclear layer.  

os: outer segments; is: inner segments; rn: rod and cone nuclei; bn: bipolar, amacrine and horizontal cell nuclei.  

B Cone from a Golgi preparation of rabbit retina with distorted inner segment. The outer segments usually fail to impregnate.  

n: nucleus; p: conical pedicle; t: terminal filament.  

C A fortunate E. M. section along the whole length of a type II receptor showing the pigment cell lamellae enveloping the outer segment and its connection to a pedicle of the cone type with a terminal filament (× 6,000).  

D The pedicle at higher magnification showing the thin filament of the teledendron linking the nucleus to a pedicle with complex synaptic apparatus.
nucleus is situated in the outer layer of receptor nuclei. The cone foot with characteristically complex synaptic apparatus is shown in enlargement in the insert of Fig. 13D which clearly indicates the fine filament which connects the pedicle to the 'teledendron'. A crude count of Type II receptor density and of conical pedicles showed them to appear in similar quantity. The above Figs. 13C and D give unequivocal evidence for their association.

The nuclei of the type II receptors are located in a single layer of the receptor nuclei, the most sclerad, and are mixed with the common rod nuclei. Their chromatin is, as is the case in the cones of other species, fragmented in contrast with the two masses characteristic of the rod nuclei. The cone nuclei are indicated by arrows in Fig. 13A.

The evidence that there are functional cones in the rabbit retina is most convincing.

a. The eye has separate photopic and scotopic mechanisms (Dodd & Elenius, 1960) and the latter shows features of a cone system (Elenius, 1958).

b. Although the Purkinje shift is small in the rabbit (Elenius, 1956) there is electrocorticogram (Monnier, Vatter & Koller, 1964) and single unit evidence (Hill, 1962) for differential spectral sensitivity in the later stages in the visual pathway.

c. Nubler (1965) has demonstrated effective colour vision in the rabbit.

The correspondence of the morphology of the type II receptor with much of

![Fig. 14](image)

Cone density isocount lines for the region of central retina. Note the upward sweep of the $7.5 \times 10^3$/mm$^2$ isocount in the temporal retina dealing with the lower nasal field. The peak count coincides with the position of the visual streak in the ganglion cell layer (Fig. 5).
that of a characteristic cone, along with the evidence for receptors with functional characteristics of cones, appears to be an adequate basis for ceasing to withhold the term cone because this is no longer a term descriptive of the outer segment shape alone but is possessed of many functional connotations.

The single layer of cone nuclei makes it very simple to carry out a count of cone density on a flat mounted retina which has been arranged with the scleral surface upwards. The cone nuclei may readily be distinguished under oil immersion from those of the rods. A set of cone isocount lines are shown in Fig. 14. Comparison of these with ganglion cell isocount lines in Fig. 8 reveals that the cone count is maximal on the visual streak. The increase – from a peripheral value of about 7,000/mm² to a count on the streak of about 13,000/mm² – follows the overall change in receptor density and no marked change occurs in the percentage of the total receptor population that is made up by cones (8% of total) in descending the retina although in passing up towards the myelinated band the percentage drops more precipitously.

**Histology of the Rabbit Retina**

In spite of the differences between the single units of the rabbit and those of cat and monkey retinas there are no major distinctions to be drawn between the cellular elements of the different species as observed in methylene blue and GOLGI preparations, although differences are apparent in electron microscope studies (Dowling, 1968). Some drawings of impregnated cells from GOLGI preparations of the rabbit retina are shown in a montage in Fig. 15.

![Fig. 15](image_url)

Montage of retinal cells observed in Golgi preparations. See text of page (59) for key.
a. Receptors form two classes only; the rods (c, c' and c'') and the atypical cones (d).

b. The horizontal cells have their nuclei either within the outer plexiform layer (e) or within the inner nuclear layer (f).

c. Bipolars appear to be the characteristic classic forms, the mop bipolar with the limited teledendronic spread (g) and the flat bipolar with more diffuse teledendron (h).

d. The amacrine cells observed in GOLGI preparations have been few in number but either spread deeply into the plexiform layer (j) or limit their terminations to one layer (i). The extent of their terminal tree is quite limited compared with the cells described in the cat (LEICESTER & STONE, 1967).

e. Ganglion cells (k) have their dendrites confined to a limited region of the inner plexiform layers in the majority of cases but have not been well preserved in the GOLGI preparations.

Amacrine and ganglion cell architecture was more satisfactorily observed in methylene blue stained, flat mounted retinas. Two major classes of amacrine cell are apparent in the drawings of such preparations, Fig. 16. One set of cells has a relatively compact dendritic tree whose terminals are fine but arise from thickened processes near to the perikaryon. The nuclei of these cells are located in the inner part of the inner nuclear layer. Some send out several processes which branch at a fairly uniform depth while others have a single process which gives off branches as it penetrates deeper into the inner plexiform layer. The other class of amacrine cells is more rare but its cells possess larger dendritic trees (300 μ) than the previous group (100 μ) although the processes are much more sparse and the field oval in form. These amacrine cells are situated in the inner plexiform layer while the processes are distributed in a single plane just adjacent to the inner nuclear layer. The two major classes of amacrine cells appear to correspond roughly with those described by LEICESTER & STONE (1967) in the cat.

The ganglion cells stain readily with methylene blue and show an enormous variety of forms. One to seven branches may come from the perikaryon and spread deeply or shallowly in the inner plexiform layer. The dendritic tree of a cell may be up to 2 mm across its greatest diameter. No detailed study of these ganglion cell fields has been carried out. None of the so-called ‘multi-stratified’ ganglion cells has been observed in this animal.
Amacrine cells seen from the vitread surface of an 'in vivo' methylene blue stained flat mount of the rabbit retina. Two classes have been noted; bipolar type with extensive but sparse dendritic tree lying within one plane of the inner plexiform layer and a class with a dense circular or oval tree which branches at various depths in the inner plexiform layer. Figures indicate depth of the adjacent dendrite in μ.

Fig. 17 shows a specially selected group of ganglion cells from the region under the optic nerve head. Examination of the shape of their dendritic trees indicates them to be oval, a feature common to those in this region. The field is extended along an axis parallel to the streak and at right angles to the line of approach of the axon. Further along the retina it is to be noted that where the axon leaves the cell at some angle to the streak then the dendritic tree is elongated in a direction perpendicular to the axon, the arrangement is indicated in the insert B. The ovality of the receptive fields of the cells in the rabbit retina to be seen in the results of Barlow, Hill & Levick (1964) and which is commented upon by Levick (1967) thus has its morphological counterpart in the arrangement of the ganglion cell dendritic trees. Oval fields are also found in the visual cortex, again with the long axis horizontal (Ogawa et al. 1968). A retouched photograph of a large ganglion cell on the visual streak is shown
Fig. 17
Ganglion cells in the region under the optic nerve head of an 'in vivo' methylene blue preparation showing the tendency to ovality in the dendritic tree distribution. Inset A shows an amacrine cell to the same scale. Inset B shows that the orientation of the major axis of the field remains perpendicular to the incoming axon.

In Fig. 18 to illustrate the relation between the rays of myelinated axons, which ascend the retina to the band leading to the optic nerve head, and the axis of elongation of the cell's dendritic tree.

As in the cat (Leicester & Stone, 1967), it has not proved possible to stain the dendritic trees of the smaller cells of the area centralis.

**CENTRAL REPRESENTATION OF THE VISUAL FIELD**

The representation of the visual field in the central nervous system is of particular interest at three sites, the superior colliculus, the lateral geniculate nucleus and the visual cortex. Preliminary maps of the superior colliculus have been described by Kerr & Seneviratne (1963), of the L.G.N. by Choudhury & Whitteridge (1965) and an extensive map of the visual cortex has been presented by Thompson, Woolsey & Talbot (1950) and confirmed by Choudhury.
& Whitteridge (1964). The maps are in agreement in demonstrating the great extent of the representation of the visual streak in each of these regions. Quantitative comparison of the maps in the different areas has not been possible because the upper and lower field representations have not been reported and standard eye positions were not used.

The maps presented below are selected from the most extensive mapping experiments and are characteristic for their respective regions. Recording was carried out with tungsten electrodes of medium impedance which picked up several units at one time in rabbits anaesthetised with nitrous oxide or urethane. The region of visual field corresponding to a given recording site was located on the surface of an 'Airmark' perimeter, in which the animal's eye was centred, by means of a neon flash, small light spot and card stimuli. The co-ordinates of the points in the field were then transposed by a nomographic procedure to the case in which the eye is in the standard position described earlier. The electrode positions were determined histologically. The map of the L.G.N.
was defined with 250 points in 60 stabs, that of the visual cortex with 100 and that of the superior colliculus with 80 points in 50 stabs. The raw data and transposed forms are not included because of limitations of space. The map of the superior colliculus has been corrected for curvature of the medial and lateral surfaces by plotting measurements made in a medio-lateral direction, along the surface of the sectioned organ, in a plane projection so that the area shown is about twice that to be observed in dorsal view. The cortical map is a projection onto a horizontal plane with the head rotated 30° contralaterally about its long axis in order to minimise the compression of the VII map on the more curved lateral surface.

Fig. 19
A Schematic diagram showing the projection of the visual field co-ordinates onto the flattened retinal strip which includes the visual streak. B A theoretically constructed map of the visual field at some central region of the nervous system based upon the assumption of uniform convergence of the projection from the retina over the representation of the whole visual field and a uniform packing density of cells receiving the projections (page 72).

The conventions in the maps are similar. The 0° vertical meridian intersects the perpendicular to the long axis of the head which passes through the corneal vertex. The 0° parallel lies in the horizontal plane which intersects the corneal vertex. The optic nerve head thus projects 10° nasal in the field (see Fig. 19A) and the line drawn through the optic nerve head and parallel to the myelinated
fibre band projects 13° below the horizontal. The nasal meridia which segment the visual field into 30° wide lunes are marked N and the temporal, T. The limit of the representation in the nasal field is marked D and corresponds to the decussation line. The upper field parallels are given positive elevations and lower field negative elevations. The coordinate system is the equatorial orthographic projection used by THOMPSON, WOOLSEY & TALBOT (1950) which is more appropriate than the usual polar projection for the representation of the map in an animal with a visual streak.

**Superior Colliculus**

The most striking feature of this map, Fig. 20, is the great extent of the representation of the area of visual field between the −10° and +20° parallels which encompass the projection of the visual streak. The representation is markedly uniform along the length of the parallels although along the meridia it is clear that the upper and lower fields receive a reduced representation. In the most anterior part of the colliculus it was occasionally possible to record a hiss response from the visual field beyond the decussation line of the contralateral eye. A similar multiunit response was also obtained at the extreme nasal end of the map when stimuli were presented in the binocular field of the ipsilateral eye but no single units were recorded.

The correlation with the anatomical findings of GIOLLI & GUTHRIE (1969) is thus good for they find a very small ipsilateral projection to the anterolateral region of the organ. No responses were obtained from the field above the 80° parallel and consequently no representation of the region giving a binocular light reflex above the head was observed. No representation of this area in the projection of the ipsilateral eye to the superior colliculus has been noted in degeneration experiments (GIOLLI & GUTHRIE, 1969).

**The Lateral Geniculate Nucleus**

The L.G.N. of the rabbit is small; its dorsal projection is about 3 mm mediolateral and 3 mm anteroposterior. Electrodes were inserted along the tracks indicated in the coronal maps of Fig. 21 on a matrix of 0.5 mm steps. The field representation was determined at 300 μ steps during a stab. The results confirm and extend the observation of CHOUDHURY & WHITTERIDGE (1965). Fig. 22 shows a dorsal view of the L.G.N. with the projections of the meridia at its
surface marked in. The black dots adjacent to the scale of anteroposterior distances indicate the plane of the mediolateral runs of electrode insertions upon which the maps of the four coronal sections in Fig. 21 are based. In spite of the reported absence of retinal projections to the x sector (Giolli & Guthrie, 1969), it has not been possible to distinguish the region when mapping. The meridia and parallels appear to run through it without alteration. The first of the coronal sections shows a map (A.P. 11.5) which, in its lower part is within

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Fig. 20

The projection of the visual field onto the flattened surface of the left superior colliculus of the rabbit. The upper visual field is represented along the medial margin and the nasal field at the anterior margin. The meridia run from top to bottom of the map and the parallels across. In this and later maps the upper field parallels have positive and the lower field negative co-ordinates. The orientation of the eye during mapping is described on page (63).
the sector. These results are in agreement with those of Rose & Malis (1964) who find a projection line to be continuous through the α and β sectors when determined anatomically.

The series of sections runs in a rostro-caudal direction. The anterior map has

Fig. 21

The projection of the visual field into the lateral geniculate nucleus pars dorsalis and ventralis shown in coronal sections running rostral (A.P. 10.5) to caudal (A.P. 12.25). The vertical lines indicate the histologically identified electrode tracks. The decussation line is shown boldly and separates the medial and incomplete mirror image representation of the visual field from the main representation in the body of the dorsal L.G.N. In the diagram A.P. 11 the map has been extended to include some of the visual representation in the pars ventralis which appears to deal mainly with the nasal field.
a good representation of the upper visual field which does not appear in the subsequent maps, whose greatest recorded parallel elevation is no more than about 30°, but otherwise the maps differ little from one another.

A narrow area running along the medial boundary of the L.G.N. contains a second representation of the nasal half of the visual field, on a smaller scale than that in the major representation.

The representation along the meridia is similar to that in the superior colliculus with the region of the streak obtaining the greatest area. Along the horizontal, however, the temporal field obtains a markedly lesser representation than the extreme nasal field.

Binocular responses have been recorded from the appropriate medial region of the most caudal half of the L.G.N. It is in this region that the most nasal lune from N60° to the decussation line, D, obtains its greatest representation. In one experiment, binocular responses were obtained from the second representation as well as from the first and one section of the map from this case is shown in Fig. 22 where the region of the binocular projection is shown bridging the decussation line as a hatched area.

The ipsilateral degeneration in the L.G.N. observed by Giolli & Guthrie (1969) is most extensive in the rear half of the L.G.N. and its position correlates with that of the binocular representation in the coronal projection of Fig. 22.

In the coronal section A.P. 11.5 in Fig. 21 a part of the visual field representation in the ventral nucleus of the L.G.N. was mapped. It is predominantly concerned with the nasal field. The asterisk marks the position of the ipsilateral terminal degeneration observed by Giolli & Guthrie (1969) and this corresponds well with the electrophysiologically determined location of the binocular field representation.

A consideration of the relationship of the maps to one another indicates that in the rabbit, as in the rat (Montero, Brugge & Bettel, 1968) and the cat (Bishop, Kozak, Levick & Vakkur, 1962), a given point in the visual field receives a representation along a line running through the L.G.N. in a rostral-caudal direction. The orientation of these projection lines is similar to the columns of lateral geniculate degeneration described by Rose & Malis (1964) subsequent to limited cortical lesions. The presence or absence of functional differences along the length of one projection line remains to be determined.

The second visual area is puzzling. It is possible, since the representation is very narrow, that the region recorded is extrageniculate. If the projection of the rabbit L.G.N. is similar to that of the cat then the cortical areas VI and VII
would both be supplied from the primary representation in the L.G.N. (GLICKSTEIN, MILLER & KING, 1965; WILSON & CARR, 1967). The second representation in the cat, located in the medial interlaminar nucleus, projects to area VIII (GLICKSTEIN et al, 1965) which has not yet been observed in the rabbit.

Fig. 22

(Top) Dorsal view of the L.G.N. showing the projection of the vertical meridia on its surface. The black circles adjacent to the left hand scale represent the positions of each of the preceding coronal sections. The extent of the β sector in dorsal projection is shown and approximately indicated for the coronal sections in the accompanying sketches. The map of the visual field in the coronal sections appears to be continuous through the α and β sectors.

(Bottom) A coronal map of the visual field projection into the dorsal L.G.N. of another animal in which the notched region indicates the position of the electrophysiologically determined binocular area.
The map, Fig. 23, corresponds well with that of Thompson, Woolsey & Talbot (1950) and Choudhury & Whitteridge (1964), but has been extended to include the representation of the upper visual field in visual area I. This part of the map lies on the tentorial aspect of the posterior pole of the cortex and in order to reach it electrodes were driven down through the upper surface.

The representation of the field is similar in areas VI and VII although in the latter case the area of cortex is smaller. The streak obtains a considerable part

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Visual Cortex

Map of the projection of the visual field onto the surface of the rabbit cortex. The projection is made on a viewing axis 30° lateral of the vertical in the coronal plane. The decussation line, which is drawn bold, separates the areas VI and VII. The hatched region represents the area from which responses have been recorded from both eyes. The map has been unfolded along the dotted line; the upper field lies beyond the posterior pole on the tentorial surface of the brain. The black spots indicate the margin of visually excitable cortex.
of the map in both VI and VII. Along the meridia, the representation is similar to that in the superior colliculus and L.G.N. Along the parallels, however, the difference in the representation of the nasal and temporal limits of the field, already noted in the L.G.N., is more marked. In both VI and VII the most nasal $30^\circ$ of the map is considerably expanded relative to the representation of the lateral field. The representation of the temporal field beyond $60^\circ$ has not been obtained in this map as it must have been so compressed as to occur between subsequent electrode stabs. It is worth noting that the region from N $60^\circ$-D at $0^\circ$($10^\circ$) which deals with regions to the front of and below the nose, is greatly expanded and corresponds to the bulge in the ganglion cell isocount lines in the temporal part of the visual streak.

The map shows a binocular representation in the anterior region of the streak in both VI and VII. Although the field was mapped to an elevation of $100^\circ$ it was not possible to obtain a binocular response from the area over the animals head from which a binocular light reflex may be obtained.

**Magnification Factors in Superior Colliculus, L.G.N. and Visual Cortex**

The great variation in density to be observed in the distribution of retinal ganglion cells is surrendered at all subsequent stages in the visual pathway where the packing density of the elements is more uniform. The extent of the representation of any given region of the visual field at some central station would thus be expected to be determined, at least in part, by density of ganglion cells in the corresponding region of the retina.

A quantitative investigation of this hypothesis can be carried out with maps such as those shown in the previous section by comparing the magnification factor \( (\text{daniel} \& \text{whitteridge, 1961}) \), expressed as mm of tissue per degree in the visual field, with the ganglion cell density in the corresponding regions of retina. In many species the relationship has been shown to be good. (Monkey cortex, \text{daniel} \& \text{whitteridge, 1961}; \text{rolls} \& \text{cowey, 1970}; \text{frog tectum, jacobsen, 1962}; \text{baboon colliculus, vejbaesya, 1967}).

In Fig. 24 are presented normalised plots of the vertical magnification factor \( (M_v) \) along the $0^\circ$ meridian in maps of the superior colliculus, L.G.N. and visual cortex. The peak values were determined from the original data not from the maps themselves. Each of the curves is based on the results from two experiments. The correspondence between the set of normalised vertical magnification factor curves and the normalised ganglion cell count is clear.
Fig. 24

(Top) Normalised plots of the vertical magnification factor $M_v$ along the O° meridian of maps of the superior colliculus, L.G.N and visual cortex. The peak values were obtained from the original data. The normalised ganglion cell count along the corresponding region of retina is provided for comparison.

(Bottom) Normalised plots of the horizontal magnification factor $M_h$ along the O° parallel of superior colliculus, L.G.N. and visual cortex maps compared with the mean of the ganglion cell count along each sector of the visual streak as marked off below the abscissa.

The correspondence between the superior colliculus horizontal magnification factor plot along the O° parallel, $M_h$ of Fig. 24, and the normalised peak ganglion cell count is also quite good. The main difference between the curves lies in the reduction of the cell count in the temporal retinal region receiving the projection of the N 60°-D sector, while $M_h$ remains constant in the corresponding region.
The large reduction in the horizontal magnification factor along the 0° parallel of the visual cortex and, to a lesser extent, of the L.G.N. in passing from nasal to temporal along the map finds no equivalent in the peak ganglion cell count line and is a deviation far greater than that of the superior colliculus M\(h\) plot from the normalised peak ganglion cell plot.

All three of the regions considered possess projections from the ipsilateral retina in the sector containing the binocular area, N 60°-D, which means that this region may be expected to contain a correspondingly increased representation in the maps. In effect, the peak ganglion cell count projecting to a point close to the decussation line in, say, visual cortex may be regarded as being twice that given by the retinal count in the contralateral projecting area since the count from the corresponding ipsilateral projecting region of the other eye must be included in the model. Points in the field further away from the decussation line, but within the binocular field, would be represented at the cortical level by an effective peak count of lower magnitude since the count on the ipsilaterally projecting retina falls off rapidly in passing temporally.

The construction of the normalised effective peak count plot including the ipsilateral retinal counts requires a precise knowledge of the position of the decussation line which is not available. It cannot, however, lie nearer to the optic nerve head than at the limit of the 5,000 ganglion cells/mm² region so that adjacent to this the effective peak count would be about 10,000 g.cell/mm² in the uncorrected shrunken preparation, but the value falls off rapidly and is less than 7,000 g.cell/mm² within 12°. The normalised version of this curve thus declines more steeply at the nasal end than does the usual peak count curve but the temporal count does not decrease as rapidly as is the case in either L.G.N. or visual cortex and the correspondence with their M\(h\) curves is poor.

The difference in the extent of the correlation of the horizontal and vertical magnification factors with the peak ganglion cell density in the L.G.N. and visual cortex is not the only disparity. The normalisation of the scales masks the fact that the vertical magnification factors are some 8-10 times greater in magnitude than the horizontal in the region of the visual streak, even for ganglion cell counts equivalent in both directions. This is immediately apparent upon looking at the map if it is remembered that the elements of the field are 30° \(\times\) 30°, rather than 10° \(\times\) 10°.

It is not known whether there is any functional correlate of the difference between the vertical and horizontal magnification factor disparities. In the case
of the superior colliculus it is possible that the difference results simply from a generalised reduction of the scale along one axis and of its extension along another without any differential convergence of incoming retinal fibres. The construct, B, of Fig. 19 shows a pragmatically constructed model map of the visual field representation at some central area based upon the rearrangement of the retinal ganglion cells into a sheet of uniform packing density. Vertical stretching and corresponding horizontal compression would produce a map similar to that of the superior colliculus. The visual cortex and L.G.N. maps cannot be explained this way because in these maps the temporal representation along the horizontal is reduced relative to the lateral field while the vertical scale is increased very little. The consequent reduction of area in this sector indicates that actual convergence must have played some part.

If the visual streak is assumed to be represented by the regions bounded by the 2,000 g.cell/mm² isocount then it is found that the percentage of the total representation of upper streak and lower retina in terms of area in superior colliculus and cortex and volume in the L.G.N. corresponds much more closely to the ganglion cell count for the corresponding regions than to the corresponding percentage of the visual field. Thus,

<table>
<thead>
<tr>
<th></th>
<th>% Superior colliculus</th>
<th>% L.G.N.</th>
<th>% Visual cortex</th>
<th>% Visual field</th>
<th>% Ganglion cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper retina</td>
<td>32</td>
<td>37</td>
<td>30</td>
<td>50</td>
<td>31</td>
</tr>
<tr>
<td>Streak retina</td>
<td>41</td>
<td>42</td>
<td>48</td>
<td>17</td>
<td>47</td>
</tr>
<tr>
<td>Lower retina</td>
<td>27</td>
<td>21</td>
<td>22</td>
<td>33</td>
<td>22</td>
</tr>
</tbody>
</table>

17% of the visual field thus obtains 40-50% of the central representations and has its image processed by 47% of the retinal ganglion cells.

Along the horizontal the distinction is not clear cut in this way since the ganglion cell percentage in each lune is almost equal to the percentage of visual field corresponding.

The rabbit L.G.N. and visual cortex thus appear to deviate from the widely accepted hypothesis of Woolsey, Marshall & Boyd (1942) which states that the extent of the cortical representation in a primary projection area is related to the peripheral innervation density.
BRECHER (1936) has pointed out that the lack of aggressive capability in the rabbit makes it the subject of attacks by various predators against which its only protection is ‘freezing’ (Niederdrucken) or flight. The eyes thus play a very important role as ‘Warnorgane’ which is aided by their lateral position and by the powerful optokinetic and labyrinthine reflexes which stabilise the retinal image and facilitate the detection of movement within the visual field. He concludes, on the basis of his own work and the earlier literature, that the eye movements of the rabbit are of the most simple reflex kind and are devoid of a voluntary component.

BRECHER’S views gain some support from consideration of the role of the rabbit visual streak (CHIEVITZ, 1891; KERR & SENEVIRATNE, 1963). In the freeze condition, the animal obtains a great deal of information from the visual horizon, and some from nearly every part of the remaining visual field, in spite of the fact that all body and eye movements which predators are so well adapted to detect are inhibited in this situation. Even optokinetic nystagmus is inhibited (BRECHER, 1936, p. 382).

That the visual streak is used under conditions other than the freeze situation can hardly be doubted. DE KLEIN (1921 & 1922) has demonstrated that the neck and both static and kinetic labyrinthine reflexes are unusually effective for the maintenance of the orientation of the eyes in space during change of head orientation. In the context of our current knowledge of the organisation of the visual streak, his results (DE KLEIN, 1921) show that the visual streak remains parallel to the horizon, and receiving the image of that part of the visual field during rotations of the head about its long axis by 21° to right or left, rotation in the horizontal plane of 17° each way and rotation about a bitemporal axis of 55° up and 45° down from its normal position.

The rabbit appears to be peculiarly dependent upon the vestibular reflexes for its orientation in space. Thus, in contrast to similarly operated dogs and cats, bilaterally labyrinthectomised rabbits lack a visual righting reflex. The eye and head position of blind or sighted, normal or thalamic rabbits is indistinguishable when they are placed in similar positions (MAGNUS, 1924) but destruction of the labyrinths results in permanent loss of eye and head stabilisation although a small effect of vision on foot symmetry in unilaterally laby-
RELATION BETWEEN ANATOMY AND PHYSIOLOGY

Relation between Anatomy and Physiology

75

Rintheomised rabbits may be demonstrated (Magnus, 1924: p. 321, Figs. 152b & 152c).

Prior to the demonstration by Ter Braak (1936) and Brecher (1936), that more than half of the visual field must move before optokinetic nystagmus may be demonstrated in the rabbit, it had been concluded by Urbanitsch, 1910, and Bartels, 1920, that this animal displayed neither 'look' nystagmus nor fixation nystagmus. The 'Wilkurliche Fixation' described by Fleisch (1922) was shown to be a special case of optokinetic nystagmus by Brecher (1936). Although able to elicit optokinetic nystagmus, Brecher, (1936), Ter Braak, (1936) and Rademaker & Ter Braak (1948) have all been unable to demonstrate fixation nystagmus in the rabbit but it could be induced in dog, cat and monkey.

This failure has been claimed to be a consequence of the absence of an area centralis in the rabbit retina (Brecher, 1936; Ter Braak, 1936, 1948; Walls, 1942, 1962). The retina was assumed to be equipotential so that fixation would be of no value in tracking moving objects. The previous sections show that this is not the case even along the length of the visual streak. At the retinal level the potential resolution at ganglion cell level drops off markedly above and below the visual streak, the retinal image is of maximum magnification in the region under the optic nerve head where the visual streak ganglion cell count is maximal, and although the optical magnification is lower, the binocular region of the streak may be distinguished by its specialised central connections.

In the consideration of the behaviour of the whole animal it is also necessary to be concerned with the extent to which the central, as well as peripheral regions, show equality of representation and as we have seen there is clear evidence of greater magnification in the cortical representation of nasal and to a lesser extent of lateral visual streak which might be expected to have a behavioural correlate involving redirection of the eyes by voluntary control under conditions not dominated by the freeze reaction.

Behavioural evidence for the selective direction of these potential fixation areas is rare but available. Rabbits involved in Van Hor's discrimination testing choose to view the test stimuli with their binocular nasal field, and similar use of this part of the field may be observed in free moving animals.

On the other hand, Washburn & Abbott (1913) have observed that free moving rabbits view test cards monocularly, often with alternate eyes, so that the image of the card will fall on the region of specialised retina adjacent to the optic nerve head. They found that, on the majority of occasions, the rabbits elected to eat food presented to their lateral field rather than that simultaneously
provided in their nasal field so that the middle of the visual streak may be regarded as a secondary fixation area analogous to that often found in birds.

The erroneously claimed uniformity of the retinal organisation has often been used to explain the absence of voluntary or spontaneous eye fixation movements, as distinct from fixation nystagmus or tracking, in the rabbit. In fact such movements are present in this animal, as will be shown, but are not so common as in some other species. It must be made clear, however, that a voluntary component of eye movement is also involved in those redirections of the eyes achieved by head movements that are described above. In these cases it is at least necessary that the vestibular and neck reflexes, which would maintain the eye orientation fixed in space, should be opposed in order that the eyes may rotate with the head.

The subsequent observations of rabbit eye and head movements were carried out by either filming with a 16 mm cine camera or by videorecording and subsequent playback when 35 mm photographs of single frames of interest could be taken. The movements of the eye could be observed either by the relation of the pupil margin, or of marks on the corneal surface, to the corneal reflection of a light source at some distance from the animal. The quality of the photographs obtained from the television system was adequate for the purpose but unsuitable for reproduction and where necessary in the subsequent sections tracings have been presented instead.

There has always been a degree of confusion in the naming of the direction of eye movements and the number of terms available, especially in the German literature, is considerable. In the subsequent sections we shall refer to head or eye movements as being rotations from the normal position in the horizontal, vertical or parasagittal plane.

**Compensatory Eye Movements in Response to Passive Head Rotation**

In order to establish the range of positions that the eye can reach in the head of the rabbit, and for contrast with the positions attained during voluntary head rotations, the results of De Kleijn (1921) and Fleisch (1922) have been reinvestigated, extended and confirmed.

**Horizontal Rotation** The neck reflexes were found by De Kleijn & Magnus (Magnus, 1924) to provide static compensation for eye position when the head is rotated in the horizontal plane over an angle of about 20° to left or right. Such
Eye stabilisation during the passive rotation of the whole rabbit about its long axis in a direction medial or lateral of the examined eye. The deviation of the eye from its normal orientation in the vertical plane is plotted as ordinate. $O'$ is the normal eye and head position at the start of the experiment. In this, and the next figure, filled symbols are used for rotations away from the starting point and open symbols for rotations proceeding towards the starting point; a line of 45° slope passing through the starting point indicates the eye and head positions which would be assumed if the eye maintained fixed relationship to the head during its rotation. Each reading was made under static conditions. L, results of Fleisch (1922) for a blind animal. L + S₁, sighted rabbit rotated within range of good compensation. L + S₂, sighted rabbit rotated outside of range of good compensation. L + S + N, head twisted relative to the stationary body in a sighted animal thus involving the neck reflexes and extending the range of good compensation. Note hysteresis in the curves in which the range of good compensation is exceeded.
A range was observed but no quantitative examination of movements of passive nature in this plane were made.

**Longitudinal Rotation** VAN DE HOEVE & DE KLEIN (1917) show that when sighted rabbits are rotated about their long axis there is counterolling of the eyes in the vertical which partially overcomes the applied rotation. FLEISCH (1922) found that in blind rabbits the compensation was not perfect and disappeared entirely outside a range of about 15° rotation in each direction from the normal position so that the eye rotated along with the head. The results of FLEISCH (1922) are shown as Fig. 25 L in which the deviation of the eye from the normal position is plotted against the rotation of the head. The results represent the pure labyrinthine, reflex stabilisation of the eye.

FLEISCH (1922) claimed that perfect stabilisation under these circumstances involved visual fixation of a sighted animal (OKN). Rotations were thus carried out rapidly enough to eliminate this possibility (FLEISCH, 1922) as it was not desired to separate the kinetic and static labyrinthine reflexes. In Fig. 25 L+S a sighted rabbit was rotated about its long axis and compensation was found to be nearly perfect over a range of 20° either side of the normal head position for several cycles of rotation but outside of this range the eyes rotated with the head (Fig. 25 L+S). Upon return of the head from an excursion in either direction outside of the nearly compensated range it was found that stabilisation began before the normal position of the eye had been reached so that the curve shows hysteresis. The stabilisation of these abnormal positions is effective over a range nearly as great as in the normal position of the eye. If the animal is startled while the eye is held in an abnormal position then the eye resets to the normal orientation with a nystagmic flick.

In Fig. 25 L+S+N is an example of the curves obtained when the head is twisted relative to the body in a sighted animal in order to involve the neck reflex upon the eyes. The range of stabilisation may be up to 45° in each direction, which is nearly double that reported by DE KLEIN (1922), but is variable between successive runs and animals. The range is clearly greater than that in the case dependent upon the labyrinthine reflex alone. Hysteresis could also be induced in this case by rotation of the head outside the range of good compensation.

**Rotation About A Bitemporal Axis** In the case of passive rotation of the rabbit about a bitemporal axis, VAN DE HOEVE & DE KLEIN (1917) and DE KLEIN & MAGNUS (MAGNUS, 1924) found that the counterolling dependent upon the
labyrinthine reflex was inadequate to provide complete stabilisation of the eye. In Fig. 26 L+S a sighted rabbit has been rotated over a range of some 160° between points 2 and 3 without complete stabilisation being apparent. This part of the curve is like that of the above authors. It is noticeable, however, that in setting out from or returning to the original position there is good stabilisation over a range of some 30° or more. In Fig. 26 L+S+N₁ the neck reflexes were introduced by rotation of the head with respect to the trunk within the

Eye stabilisation during passive rotation of the head and body about the bitemporal axis. The nasal bone is in the normal position, 35° before the vertical, at 0° on the abscissa and the nose rotates up or down. The deviation of the visual streak from horizontality upon failure of stabilisation is indicated on the appropriate ordinate scale. L+S, eye stabilisation is poor in the absence of a neck reflex component. L+S+N₁, the neck reflexes were introduced by rotating the head up and down relative to the body within the range of good compensation. L+S+N₂, the range of good compensation is exceeded and the eye rotates with the head. Note hysteresis.
range of complete stabilisation so that many reversals of direction have occurred without deviation of the eye from its normal position. If this range, which corresponds with that described by De Kleijn (1922), is exceeded then the eye begins to rotate with the head and hysteresis is introduced to the curve upon reversal of the direction of rotation (Fig. 26 L + S + N).

The line, tilted at an angle of 45°, to be seen in all of the above plots is that which would be obtained if the eye rotated passively with head. It passes through the point representing the normal resting condition of the eye in the head which is with the myelinated band horizontal and the nasal bone 35° away from the vertical (0°, 0°). Outside the range of compensation the plots tend to follow a line nearly parallel to that of passive rotation but displaced by an amount proportional to the range of stabilisation.

Voluntary Horizontal Eye Movements

Rotation of Eye and Head  Cine film and video-recording reveal that readjustment of the relationship between the visual world and the retina is usually effected in the horizontal plane by a combined eye and head movement. Fig. 27A. Video-recordings indicate that the eye and head movements begin almost simultaneously but that the movement of the eye is effected more rapidly than that of the head, whereupon the eye remains locked in its new orientation and the head rotates around it. The eye movement is complete within one frame of a 16 frame/sec. film and rarely lasts for more than two 25 m. sec. 312 line rasters on the video tape. The duration is thus about 50 m. sec. for small rotations not greater than about 30°. At the end of the movement the eyes bear same relationship to each other as at the start and are recentred in the head when simultaneously observed from the front. With this system it would be possible to detect convergence movements in the order of 4-5° but they have not been observed.

More satisfactory results have been obtained by the use of two cameras to view both eyes simultaneously. Half of each picture was blocked off and the resultant images superimposed before video-recording. The results of such observations confirm those above. Collewijn (this symposium) has also failed to record evidence for convergence or divergence of the eyes during electronystagmography.

Rotation of Eyes Alone  On occasion it is possible to record eye movements
Spontaneous eye movements in the horizontal plane. Drawings were made from prints of a 16mm film; no frames omitted. The rotation of the eye may be identified by observing the position of the image of a distant light source on the cornea relative to the pupil margin. Between frames 1 + 2 in the first column, the eye rotates as the head begins a simultaneous movement. The head movement carries on for three more frames after the eye has ceased to rotate before the eye is centred.

In the second column is an example of a similar eye movement which was unaccompanied by a head movement, but in this case the eye rotates temporally.
in the horizontal plane which are not accompanied by head movements. Fig. 27b shows a unilateral view of such an eye movement which had been preceded by an ear rotation to the rear. It was some 15 seconds before the eye returned to its original position, again without head movement. Animals sitting in cages with opaque rear and side walls show such eye movements without head rotation when their nose is directed to the front of the cage so that they must be redirecting their binocular field.

Fig. 28
Right and left view of the head of the same animal obtained with two television cameras and a mixer unit. It may readily be seen that the right eye rotates towards the nasal field simultaneous with a rotation of the left eye to the temporal field. The movements are of similar magnitude and are thus not predominantly for the purpose of convergence. No head movement was associated.

Unilateral observations of isolated eye movements are subject to the charge
that they may be convergence movements seen from one side. The two camera system provides evidence that this is not the case (Fig. 28). Observation of the amount of sclera visible or the relation between corneal reflex and the pupil margin shows that both eyes move simultaneously and by the same amount. If convergence occurred it must have been of very small extent.

Voluntary Rotation of the Eyes in the Vertical

Rotation of Eyes and Head Special arrangements have to be made to induce a rabbit to display interest in anything well above or below its visual horizon.

Voluntary rotation of the eyes and head about the long axis when the rabbit examines something on the lateral fixation axis, which is perpendicular to the long axis of the head. Note that the relationship between the eyes and head as judged from the plane of the iris remains unchanged between A, the normal, and B the rotated position indicating that the eyes rotate as much as the head.
Some success has been obtained with mothers looking at their young or with a 'voyeur' buck looking at a doe. The rabbits were placed on a platform so that they could look down on another animal on the floor below. If jumping was not attempted the observer rabbit occasionally sat down and brought his lateral field to bear on the rabbit below by rotation of the head and eyes about the long axis. Photographs were taken of the animal from the front so that the relationship between the eye position and the head position could be determined by observation of the plane of the iris. In the normal head position the iris appears to be medially inclined by an angle of about 10-15°. In the case of animals looking down with the lateral field it was observed that the relationship between the iris and the head remained unchanged but that eyes and head were together rotated through an angle of up to 45°. Pictures are shown of an animal in the normal position and with the head and eyes rotated (Fig. 29 A, B). The rotation of the head and eyes is carried out quickly and the dynamics of the

Voluntary rotation of the eyes and head about the long axis of the head showing that the eyes remain in fixed relationship to the head during rotation in this plane (see Fig. 29). Note the correspondence of the points with the line of 45° slope which shows the relationship between the eye and head orientation assuming that the eye remains in fixed relationship to the head. Eye rotation away from the normal orientation in space in the vertical plane, as judged from the inclination of the iris in frontal photographs, is plotted as downward or upward when the head rotates medially or laterally respectively. The filled circles relate to head positions which lower the eye and open circles to those which raise it. The dotted horizontal line shows the relationship between the eye and head orientation when the labyrinthine and neck reflexes stabilise the eye during passive rotation of the head about the long axis (Fig. 25).
process have not been observed. It is not known whether the eyes precede the head, as in the case of rotations in the horizontal plane. In Fig. 30 the results so far obtained are plotted as eye and head rotations from the normal position. It is clear that the eye positions fall on a 45° line during voluntary observation with the lateral field and that the eyes must at least end up in unchanged orientation relative to the head. In view of the preceding demonstration of the magnitude of the range of perfect compensation in animals subjected to passive rotations when visual, labyrinthine and neck reflexes are operative, it is clear that these rotations of the head and eyes contain a voluntary component in that the stabilising reflexes have been prevented from manifesting their effect. In Fig. 25 L+S+W the stabilisation is perfect over a range of rotation at least 40° in each direction and is totally absent in Fig. 30 for rotations of 30° in each direction.

Rotation of the Eyes Alone There has been no sign during many hours of recording of any voluntary eye movement carried out in the vertical plane independently of the head. Such movements might justifiably be expected since the peak ganglion cell count on the visual streak is very sharply localised in the vertical and might be expected to be raised or lowered for the examination of objects producing images just above or below the peak.

Certain behaviour has been observed which suggests a possible substitute

![Fig. 31](image)

Successive stages of head bobbing in the vertical plane; drawn from film of a videorecording (see page 86).
for small vertical eye movements. If a rabbit is placed in a new environment then it immediately 'freezes'. Its first subsequent movement is usually a bobbing of the head up and down over a distance of 6–8 inches with the visual streak held horizontal. This movement permits the vertical scanning of the image of a cylinder of visual space at the level of the animal to be carried out simultaneously by the visual streak of both eyes. Fig. 31 presents successive frames from a video-recording of such a movement.

**Voluntary Rolling Movements of Eye and Head**

**Rolling of Eyes and Head** In order to study eye rotation in the parasagittal

![Voluntary rolling of the head and eyes in the parasagittal plane. Deviations of the eye from its normal orientation which accompany head rotation in the parasagittal plane are plotted as the ordinate. Upward rotation means that the temporal end of the visual streak is lowered by a rotation about the optic axis in the parasagittal plane, so that the binocular streak receives the image of a region above the horizon. The head positions are again derived from the nasal bone orientation and plotted along the abscissa. The 45° line indicates the relationship between eye and head orientation in space if the eye retains its normal orientation with respect to the head during their rotation. The horizontal dotted line indicates the range of good stabilisation of the eye when the head is passively rotated (see Fig. 26, L + S + N). See text (page 87) for symbols and explanation of results.**

Fig. 32
RELATION BETWEEN ANATOMY AND PHYSIOLOGY

plane during voluntary head rotation, video-recordings were made of buck rabbits with marked eyes when the animal was using his binocular field to look over the edge of a platform of variable height prior to jumping down to join a doe below.

If the eye were simply stabilised during voluntary examination of the floor then the horizontal dotted line of Fig. 32 should be obtained when eye deviation is plotted against head rotation within the fully compensated range. If, on the other hand, the eye rotates with the head during the voluntary movements then the points obtained should lie on the broken line with 45° slope in Fig. 32. The actual results are presented in this illustration. It is clear that the eye is not stabilised. Some points relating to a head position of 60° up to 90° down fall near to the 45° line indicating that in this range the eyes can rotate with the head. Below a voluntary downward head rotation of 30°, although some points fall on or near the line, it is clear that the head usually rotates more than the eye. It is possible that this minimises the obscuration of the lower nasal field by the nose.

A further point arises when the magnitude of the drop facing the animal is taken into account. The large open squares are from occasions when the animal is on a platform of height about 15-20 cm; the small open squares relate to a height of 50 cm; the large black circles and small black circles relate to a height of 100-130 cm. It is clear that the maximum deviation attained by the eye tends to increase with the drop facing the animal. The large rotations indicated by the open circles were, however, obtained at a height of only about 1.5 m but from a very adventurous rabbit. It is not possible to assume any inevitable correlation of height and extent of rotation since the animal may look at regions of floor other than his predicted point of impact. It was impossible to obtain any voluntary rotation in animals sitting free on a platform above about 1.5 m in height because they ‘froze’ and would not look over the edge. Only some of the points in Fig. 32 represent the occasion just before a jump. The cluster around that representing zero eye deviation in the normal head position were obtained from records made when the animal was well back from the edge of the platform.

It is important to note that a given head position can be associated with different eye positions and thus, when the head is voluntarily rotated 60° down, the eye may be from 30-60° deviated from the normal position whereas if the head is forced to this position the eye would remain undeviated as a result of the operation of the neck and labyrinthine reflexes. The deviation is not,
however, the inevitable concomitant of the voluntary movement since the eye remains undeviated in an animal which is washing its feet with its head rotated some 60° down (indicated by the star in Fig. 32). A voluntary component to the eye rotation is consequently revealed. The main point of this section is thus that the eye rotates with the head during a voluntary downward or upward fixation with the binocular field, but that this is not an inevitable result of head movement and that the extent of the rotation may be a function of the magnitude of the drop facing the rabbit.

The dynamics of the process remain unexplored. The movement of the head during the time in which the eye is rotating blurs the iconoscope image and the relationship between the eye and head positions cannot be determined.

Rolling of the Eyes Alone  During these experiments it has been observed that animals moving off from the freeze position, in which the mark on the eye and the myelinated band were horizontal, occasionally rotate their eyes to raise the binocular end of the visual streak by 10–20° so that the position described by Öyster is taken up (Öyster, 1968). Similar small deviations from the normal eye orientation may be observed after return to the normal head position subsequent to forced rotations outside the range of complete compensation. The voluntary rolling may be eliminated, like the hysteresis error, by startling the animal with a loud noise or shaking.

If a rabbit with a marked eye in which the visual streak is horizontal is slowly lifted from the floor by means of a firm grasp of the neck and rump fur then the eyes may be seen to rotate in steps so as to direct the binocular area to the floor below. When the rabbit is raised to about 2 m the eye may rotate by about 40° and sometimes returns to normal by steps as the animal is lowered again to the ground. The phenomenon is variable between rabbits and a quiet alert animal is required for its demonstration which may be aided by a slight shake at some stage during the lifting.

This phenomenon might be regarded as a reflex part of the 'Liftreaktionen' (Magnus, 1924) resulting from labyrinthine or retinal stimuli. The conditions may, however, be changed in the following way. If the rabbit is seated on a raised platform and lifted until he is just above the surface then the eye will remain with the streak horizontal. If the animal is then swung, either forward or sideways, out over the floor about 120 cm below then the eyes usually show a rotation of rather variable magnitude but not involving a head rotation. When the animal is swung back over the table then they may flick back to the
horizontal. In this case streaming of the retinal image or labyrinthine stimulation must be very different to that in the original situation. It appears clear that some visual cue, probably stereopsis, is being used to detect the drop. The ability to detect such drops is clearly present from the progressive reluctance of adult animals to jump from platforms of increasing height. This ability appears within about 10 days of visual experience (Walk, 1966) and it has been shown that the rabbit is unique amongst small animals in that visual cliff avoidance is visually and not contact dominated (Schiffman, 1970).

The Mechanism of Voluntary Eye Movements

Observed Eye and Head Positions Requiring Explanation. It is clear from the previous sections that there is a voluntary component available for eye movements in all three planes. In the case of horizontal and rolling eye movements the eye may voluntarily move independently of the head; in the case of the vertical movements this has not yet been observed. In both planes, the eye movements manifest without head movements are similar to saccades observed in cats and primates although the rotatory eye movements isolated from head rotation require special circumstances to be elicited.

Some insight into the central organisation for the production of voluntary eye and head movements may be gained from an examination of the extensive data on rotation movements. Thus in Fig. 33A the rabbit is sitting with its head in the normal position and the visual streak is horizontal. When the head is rotated downwards to avoid a preferred piece of lettuce, B, or to wash the feet, C, or when it is forcibly rotated by the experimenter, D, then the streak remains horizontal. Stabilisation for upward rotation of the head is effective over only a limited range, E.

When the rabbit is sitting, the eye may be in its normal position, A, have the slight rotation described earlier, F, or it may show a pronounced rotation accompanying a downward rotation of the head, H. The eye position is neither inevitably linked to that of the head, because the head may be considerably but the eye only slightly rotated, G, nor is the eye position determined by the neck extension because similar eye deviations are to be seen in Fig. H and I although the neck is extended in one case and not in the other; moreover, different eye positions are apparent in Fig. G and I in both of which the neck is extended. During a voluntary upward look, however, the eye appears to invariably rotate as much as the head, J, just as is the case during voluntary
rotation about the long axis.

Although there are quite apparent differences in the characteristics of eye, or eye with head, movements in the three planes considered it is clear that, whether the eyes move with the head or precede it, at least four sets of reflexes operate in a sense which would oppose the voluntary movement. These are the static and stato-kinetic reflexes of the labyrinth, the neck reflexes and the visual reflexes which give rise to OKN. As has been previously stated, their effect must in some way be offset for the voluntary rotation of the eyes to be established.

Optokinetic Nystagmus May Be Ignored In This Context

Optokinetic nystagmus has been shown (Collewijn, 1969) to have a very limited acceleratory capacity in the rabbit so that 1° per second is the maximum rate of rotation that may be fully compensated for within one second. Voluntary head rotations produce accelerations greatly in excess of this with which only the kinetic labyrinthine reflexes can cope. The failure of Magnus (1924) to observe temporary optic compensation of eye or head position during rotation of a bilaterally labyrinthectomised animal (Fig. 80 and Fig. 109) must be related to the rate with which the animals were brought to the observed positions. Fleisch rotated his rabbits very slowly when demonstrating the involvement of OKN in the compensatory positions of the eyes. We are thus not required to consider the means by which OKN might be overcome during voluntary rotations since its frequency response is too low for it to be effective in this context.

Possible Mechanisms for Voluntary Eye and Head Rotations

In view of some of the unusual features of the rabbit eye movements, and in an attempt to come to some understanding of the nature of the central mechanisms involved, it appears incumbent upon us to examine some rather unconventional possibilities for the generation of the voluntary deviations of eye and head position.

Voluntary Eye Rotations

1. Rotation of the eye with the head is not achieved by the inevitable suppression of the stabilisation mechanism during the voluntary rotation of the head. The stabilisation of the eyes normally observed during passive rotation of the head can be equally effective during the voluntary rotation of the head in all of the three planes considered earlier e.g. Fig. 33 b and c and 5 below.

2. Rotation of the eye with the head is not achieved by the voluntary rotation
RELATION BETWEEN ANATOMY AND PHYSIOLOGY

Fig. 33
Drawing made from photographs of rabbits with corneal markers parallel to the visual streak. The variable relationship between the eye and head positions in the parasagittal plane is discussed on page (89).

of the head into the range of incomplete compensation of eye orientation. In Fig. 30 and 32 it can be seen that voluntary eye and head rotations may be of equal magnitude although within the range accompanied by perfect eye stabilisation during passive movements.

3. Voluntary rotation of the eyes with the head is not a passive rotation subsequent to the suppression of the stabilising system. The eye rotation may precede that of the head in at least one plane and, because the eyes may move independently of the head in two planes, it must be concluded that a specific motor command for eye displacement enters the oculomotor pool.

4. The conjunction of 1 and 3 indicates that the eye and head movement commands may be activated independently, although the observation of combined rotations in all three planes indicates that this is not always the case.

5. The eye stabilisation system is operative in at least two planes at the moment of cessation of a voluntary rotation. During a voluntary rotation of the eye and head in the horizontal plane, the eye may achieve its final position before the head and is held in a fixed spatial orientation by the action of the stabilising reflexes as the head continues to rotate.

In the case of voluntary rotations of the head and eyes in the parasagittal plane it is possible to forcibly rotate the head of a rabbit which has just voluntarily attained a given eye rotation without disturbing the maintained eye
Eye rotation in the parasagittal plane plotted against the head orientation in the same plane during forced cycles of raising and lowering of the nose when, A, the eye is in the normal position and B, C and D when the eye has undergone a voluntary rotation with the head and is in that position at the initiation of the forced movement. In all cases the eye is stabilised during the forced rotations. See page 92 for the significance of this observation. The numbers indicate the successive sampled positions observed in a video-recording.

deviation. The eye orientation during such forced head rotation in animals with the eyes voluntarily deviated to different extents are shown in Fig. 34. The eyes clearly remain stabilised during several cycles of head rotation, which was carried out rapidly enough to occlude the manifestation of OKN.

6. Labyrinthine and neck reflexes, rather than either visual fixation or OKN, are responsible for the stabilisation of eye position during voluntary maintained deviation of the head and eye from the normal position. Although the presence of smooth visual tracking has not been demonstrated in the rabbit, it was decided to eliminate the possibility of its involvement by performing the rotations of the above experiment, 5, in a room darkened immediately after the animal had voluntarily rotated its head and eyes. When the lights were switched on after the forced head rotations had been carried out, the animal was usually found to have its eye orientation unaltered.
7. The eye motor command appears to enter a feed-forward motor system. The feed-forward nature of the vestibulo-ocular reflex arc and the apparent absence of a stretch reflex in the eye muscles (Mcanie & Adler, 1932; Whitteridge, 1960) mean that the superposed O.K.N., joint receptor, vestibular and command inputs to the eye may be regarded as feeding the motor pool directly without entering a position servo-loop.

A simple superposition model is thus completely adequate to account for these observations on eye movement control. Eye movement is obtained by a direct command signal. If the head moves passively then the vestibular input to the summing region fluctuates accordingly and provides compensation in the same fashion as it does when the eye is in the normal position. If the head moves during rotation of the eye then the time course of the two displacements may be different but the eventual net rotation of the eye relative to the head will be a function of the difference between the command and vestibular inputs. During head rotation unaccompanied by eye rotation, the vestigial and neck joint receptor input provides stabilisation of the eye.

In such a system the vestibular and neck reflexes acting upon the eyes would be operative during the kinetic phase of both head and eye movements. There is no need for 'a powerful and complete blocking of the vestibular ocular connections' (Robinson, 1968) during combined head and eye rotation. In fact a specific addition to the scheme would have to be made to prevent the operation of the reflexes during the kinetic phase because stabilisation is automatically provided by the same labyrinthine and neck reflexes which have been shown to be operative immediately upon the cessation of the kinetic phase, when the eye has reached its new position.

The presence of active vestibular stabilisation of the eyes during voluntary head rotation has not been demonstrated in the rabbit but Graham Brown has shown that such stabilisation is operational during head and eye movements elicited in monkeys by stimulation of their frontal eye fields (1922).

Head Movement Control

Less information is available about the interaction of the head motor and stabilisation systems. The neck muscles receive the normal x/y motor input which provides a feed-forward pathway for head movement.

The head of the rabbit is equally well stabilised against passive displacements whether it is in the resting, or voluntarily displaced, position. The reflex systems which stabilise the head must be thus reset in some way so as to accept the new
orientation as the norm and are not simply suppressed. The mixing of command and vestibular inputs to the neck muscles is not, however, another case of superposition such as has been suggested in the case of the eye motor system. The physical link between the head and the vestibular apparatus means that a servo-loop is present which is made up of the motor path to the head and the labyrinthine reflexes on the neck muscles. The head movement command signal must inevitably enter this loop, whose gain is unknown. An error signal resulting from the combination of the command and vestibular inputs will thus establish maintained rotation of the head while leaving the vestibular loop active during and after the kinetic phase of the movement. The central organisation of the system is obscure, as has been pointed out by Whitteridge (1960), and doubtless complex since it involves the reciprocal innervation of antagonist motor pools with command and stabilisation impulses which differ in organisation for movement in each of the three planes considered.

The predicted head stabilisation against displacements occurring during the kinetic phase of rotation has not, to my knowledge, been demonstrated although there is every reason to believe that it would be found.

Fig. 35

Block diagram embodying the findings relating to the control of eye and head movement in the rabbit. See page (95) and argument beginning on page (90).
The Overall System for Eye and Head Movement in the Rabbit

The results of the previous considerations are summarised in the Fig. 35 which is consistent with all the findings and can account for:

a. Stabilisation of the head and eye during passive rotations of the animal.
b. Stabilisation of the eye during passive rotation of the head.
c. Voluntary rotation of the eye accompanied by stabilisation of the eye and head during static and kinetic phases of movement.
d. Voluntary rotation of the head accompanied by stabilisation of the eye and during the static and kinetic phases of movement.
e. Combined voluntary rotation of the eye and head accompanied by their stabilisation during static and kinetic phases of movement.
f. Interaction of vestibular and optokinetic reflexes on head and eyes.

The diagram is grossly simplified. The separate eye and neck muscles have not been considered at all and the differences in the relative contributions of labyrinthine and neck reflexes in stabilisation against rotation in different planes has been left out. All the interaction of the various pathways has been shown diagrammatically as established for the rabbit but no implication is made about their specific circuitry or location in the central nervous system.

General Discussion about Rabbit Eye Movements

The command signals discussed in the above sections are themselves part of complex servo-loops involving the visual cortex and other regions. In man these give rise to predictive tracking, smooth tracking and fixation of stationary objects which are scanned with the fovea by a rapid succession of saccades. In monkey and lower animals predictive tracking appears to be absent (Fuchs, 1967) but in dogs, monkeys and man smooth tracking can be demonstrated as the slow phase of cortical fixation nystagmus. Even this response has not been observed in rabbits (Rade-maker & ter Braak, 1948). Smooth following of a striped sinusoidally oscillating drum moving at low frequencies may be carried out by the sub-cortical OKN system (Collewijn, 1969) but this author points out that the system is a velocity servo and fixation tracking never reveals itself in the experimental situation. The fast phase of OKN is not related to the position of the stripes on the drum.

Even in the case of saccadic movements the rabbit differs from the cat, monkey and man in that the rabbit almost never shows the rapid series of
saccadic fixations evidenced by these animals. The relatively rare eye movements reported earlier are all that is observed. In view of the findings of Robinson & Fuchs (1969) it is tempting to relate the poor development of saccadic movements in the rabbit to the apparent absence of frontal eye fields in the animal.

The earlier pages have shown that the potentiality of the rabbit visuomotor system is, contrary to previous belief, similar to that of other mammalian species. The infrequency of voluntary saccadic movement in the rabbit is to some extent compensated for by the extensive use of combined movements of the head and eyes. Examination of the information presented on retinal and visual field organisation in the earlier sections suggests that the low frequency of voluntary eye movements and the predominance of eye and head movement is accounted for in two ways:

a. The large size of the visual field and extent of the visual streak.
b. Relative extents of the potential and actual visual field.

In the first case the visual field is so large that many of the orientation movements and saccades of the less well endowed animals are not necessary. During active exploration the second factor comes into play because even quite small reorientations of eye position unaccompanied by head rotation would inevitably convert part of the binocular field to monocular field and introduce blind areas to other regions because the unobscured field of the eyes is so similar in extent to the actual field measured in the animal. The finding, reported in the mapping section, that the binocular area of the LGN and cortical maps only corresponds to the nasal binocular field suggests that, although it may have been missed, the upper field binocular reflex to be seen in Fig. 2 may not possess a bilateral central projection but is simply a reserve to minimise the development of blind areas during rotation of the head. It must be remembered that even in human beings voluntary eye movements of more than about 30° are usually accompanied by head movement.

There is, however, a different quality in the voluntary fixations of the rabbit eye or eye and head to those observed in the cat. They last longer. The impression is that the eye is brought to a new orientation in space but that no specific region is closely fixated. The voluntary movements of the eye alone which have been observed occur in the two planes in which the binocular area is shifted usefully, either up and down or horizontally, as do the majority of combined eye and head movements. The binocular region of retina is, like other regions, much more uniform in its organisation along the horizontal than is the case in
many other species but it is also, in view of the upward bulges in the ganglion cell isocount lines of temporal retina (Fig. 8), more uniform in the vertical as well. This may mean that there is not such a powerful stimulus for the precise recentering or scanning of objects of interest when the binocular field is in use as there is in animals with a more limited circular area centralis.

I feel obliged in terminating this discussion to point out that in one circumstance it has been possible to elicit what may be a rapid succession of saccades of fixation in a rabbit. This has been done by swinging a piece of cabbage in front of the animal in a horizontal arc. The head will follow the cabbage with a smooth tracking motion and clear nystagmic flicks can be observed. It has not yet proved possible to obtain a record of this behaviour so that it is not known whether the nystagmus is a secondary vestibular form dependent upon the head movement or whether the eyes are preceding the head in the previously recorded fashion (Fig. 27) but in a series of rapid fixations.

SINGLE UNITS OF THE RABBIT VISUAL SYSTEM

In the previous sections the rabbit has been shown to possess a specialised eye whose features are reflected in the topographic organisation of the central visual stations and in the oculomotor behaviour of the animal. It thus seems appropriate to round up with a comparison of the single units the rabbit retina and visual system with those of the other species commonly investigated.

The visual stimuli used in the following experiments were provided either by the hand movement of card figures mounted on thin wires against a contrasting background or by the hand movement of a projector forming an image of a selected slide on a sheet of paper in the stimulus plane of the animal. In some experiments the paper sheet was mounted on the arm of a perimeter and in others it was fixed to a screen 1 m away from the animal. In later experiments the visual stimuli could be moved automatically at selected velocities and directions. The receptive fields were mapped out on the paper and filed. In all cases the eye was sutured to a brass ring and a contact lens fitted to bring the image of objects in the stimulus plane into focus.

Single Units of the Retina

The single units of the rabbit retina have been investigated in detail by Barlow, Hill & Levick (1964) and those of the visual streak by Levick (1967). These
A. HUGHES

workers used direct retinal recording of the ganglion cells but in my work I have recorded from the optic nerve of decerebrate rabbits with platinum black tipped Woods' metal electrodes. The demonstration of a surround in units of concentric organisation has been greatly facilitated by projecting an image of an iris diaphragm onto the screen; during expansion the increasing area and moving edge elicit a response from ON areas, during contraction OFF areas are stimulated and ON-OFF areas are easily delineated. The majority of the 230 units investigated fell into classes similar to those of Barlow et al. (1964) and Levick (1967) but not all those of the latter worker were observed. It was felt necessary to introduce a little further subdivision but the units so divided were not novel types. The following is a brief description of the classes found. The percentage of each class will be observed in Table II.

1. Small Field Concentric with Transient Centre ON or OFF Response

The field centres, mapped by a card disc, are about 0.5-3.0° in diameter. A small light spot centred on the field elicits a transient ON or OFF response but the surround can only rarely be induced to generate an excitatory response. The surrounds are antagonistic to the centre but do not necessarily overcome its response. The expanding spot test clearly reveals the excitatory response from the surround to be of the opposite form to that from the field centre. The test has the further advantage that it permits the extent of the surround to be estimated without threshold tests and it was discovered to be as large as 10-20° in many cases.

The surround demonstrated by this test cannot immediately be equated with that of other tests for the stimulus is of complex form. It is possible that the expanding circumference of the spot elicits the McIlwain 'Peripheral Effect' (1964), which is a potentiation of the surround and centre excitatory response. The moving boundary of a constricting spot is not enough, however, to elicit a response until the central region of an OFF centre unit is reached. Shadows or rapidly (50-100/sec) moving stimuli do not elicit a response from this class.

2. Rapidly Dark Adapting Units

A few OFF units with small receptive fields, 0.5-1.0° centre diameter in bright light (3 ft. L.), showed a rapid increase in field centre diameter as the overall illumination was decreased. The response was almost immediate and did not involve the lengthy periods involved in dark adaptation of cat units. The field
diameter increased by some 5 to, on occasion 10, times the original magnitude. The change could readily be revealed if a neutral density wedge were inserted in the beam during the expanding spot test.

3. Concentric ON or OFF Centre With Maintained Firing

The most characteristic of these units, which have receptive fields about 3° in diameter, will show firing at a maintained rate for as long as 15 minutes after a spot illuminating the field centre has been turned on or off depending on the unit type. Others show a degree of adaptation in the firing. Introduction of illumination into the surround region reduced the firing rate markedly. The rate of firing saturated rapidly as the light intensity of a centred spot was increased. Under suitable lighting conditions the maintained units follow, by grouped firing, the fastest movement that could be generated by hand (300°/sec). The firing could be modulated from the centre or surround. If a unit is responding by maintained firing to the presence of a white disc in the centre of its field then a second white disc (5°) in diameter will exercise a considerable maintained inhibitory effect on the firing if placed in the surround up to 10° from the centre. The surround inhibition could be modulated by rapid movements. If stimuli were moved in the surround then inhibition of the centre could be obtained up to 15° away in some cases.

If the intensity of illumination of the field centre is changed incrementally or decrementally then firing is momentarily inhibited or the units fire in bursts depending on the nature of the unit and the direction of the illumination change.

It is my opinion that these units can be placed in a separate group to the following for descriptive purposes but they may well be part of one continuum including group 4.

4. Large field, dimming or shadow sensitive units

These units correspond to the large field OFF units of Barlow et al. (1964) except in that a small number of ON units with similar properties have been recorded. If a light spot is confined to the centre of an OFF unit then a fairly smooth decay in the firing frequency occurs when it is turned off but as the spot is enlarged to take in the surround then the response becomes more bursty, giving a characteristic squeaky quality to the unit firing if this is monitored auditorily.

Centre and surround can both be mapped out with a small light spot when
the centre region is found to be about 4\(^\circ\) in diameter and the surround about 8\(^\circ\) in diameter. The expansion test generates vigorous response from centre and surround. The centre size determined by this test is the same as that determined by the exploring spot but the surround can be activated within a 20\(^\circ\) of the centre. Shadows or small objects are more effective than card stimuli in the stimulus plane for eliciting firing. The firing is not very maintained and will give a burst for the passage of very rapidly moving stimuli (300\(^\circ\)/sec). Records have, however, been obtained of these units firing to the movement of an edge through the field at speeds as low as 0.1\(^\circ\) per second.

The units respond to very small decrements or increments in light intensity (depending on centre type) of either whole field or centre illumination. The surround does not powerfully inhibit the centre response which no doubt accounts for the response to large shadows. 1–2 % changes in illumination level will each generate a small burst of firing during either dimming or brightening depending upon the field centre response.

5. Directional Unit of the ON-OFF Centre Type

These units were identical to those described by Barlow et al. (1964) and Barlow & Levick (1965). The field centres respond on and off of a small spot of light. If the spot is enlarged to encompass the surround then the response is reduced in magnitude. The inhibitory surround so demonstrated was not, however, powerful enough to eliminate the response to the passage of a projected grating of 2" bar period and 50° wide swept through the 3° diameter receptive field centre in the preferred direction. One important point is that firing was obtained over radii of entry into the field in a sector of some 270°. No response was obtained over the remaining 90° unless the velocity was extremely low. Firing in the preferred direction was present up to 150°/sec and down to less than 0.5°/sec. Firing in the null direction did not appear until the rate of less than 0.5°/sec was reached.

6. Adjacent ON and OFF Area Unit

This unit class is equivalent to the orientation detector of Levick (1967). These units were very rare and are the only members of one of the novel classes described by Levick to be found in this series. Responses could be obtained to the movement of a black disc or bar along either a horizontal axis or vertical
RELATION BETWEEN ANATOMY AND PHYSIOLOGY

axis. Firing was not obtained for movement along an axis perpendicular to that from which the response was obtained unless the card was displaced to one side or the other of the null axis. In the latter case a centripetal response might be obtained from one side and a centrifugal from the other. The fields thus consisted of an ON and an OFF area exercising mutual inhibition. Wide or properly centred stimuli moving along the null axis stimulate both areas of receptive field so that no response is obtained; in the perpendicular direction the two components of the field are stimulated sequentially and a response is elicited. Appropriate use of card stimuli centred on each region may demonstrate the maintained nature of the inhibition reciprocally exerted between them. After inhibition, the response of an area is potentiated. The fields were about $2^\circ \times 4^\circ$ along their minor and major axis respectively. This feature of selective response to motion in either direction along a certain axis and not to motion along another axis perpendicular to it appears more likely to be representative of the natural behaviour of the unit than is the orientation selectivity to static flashed or moving stimuli illustrated by Levick's choice of terminology (Levick, 1967).

**Single Units of the Superior Colliculus**

These observations were carried out in animals under ether or light urethane anaesthesia. The records were obtained with tungsten microelectrodes which were usually inserted into the superior colliculus in the region representing the central portion of the upper part of the visual streak representation. This arrangement ensured that the electrode encountered the laminae of the organ in perpendicular fashion and at equivalent depths in successive insertions thus enabling comparison of the depths at which unit types were obtained in different preparations. A diagramatic representation of characteristic collicular receptive fields is presented in Fig. 36.

**Multi-unit Evoked Response** The upper 0.6 mm of the superior colliculus gives a marked multiunit response to the flashing of a small light spot in the appropriate part of the visual field. The off response is particularly noticeable because of its oscillatory form and long decay time (6 sec). The movement of small, $1^\circ$, card stimuli produces an equally marked response but larger stimuli, $10^\circ$, were much less effective. Large shadows were often effective stimuli although large card forms with well defined edges would not elicit a response. Detailed
Fig. 36
Single unit receptive fields of the superior colliculus. 1, very small field off centre unit from surface. 2, unit similar to large field off unit of the retina. 3, uniform on-off field. 4, directional unit similar to retinal form. On-off centre and firing over 270° sector. Null direction indicated by white arrow. 5, large field on-off unit with a non-homogeneous receptive field. From the stratum opticum downwards are found 6, long field unit which responds to on and off of flashed light spot but movement is the best stimulus. 7, the deeper units have varied properties; most do not respond to a light flash but will fire to movement, some show habituation to repeated stimuli and others have preferred directions but not true directionality of the retinal kind. 8, in the deepest regions are the quadrant A, and hemifield, B, units which fire best to small moving objects. The border of the fields are very sharply defined.
examination of the multiunit evoked response revealed a well developed lateral inhibition. Flashes of a 1° spot of light gives rise to a well developed oscillatory response and a more abruptly terminating on response. When the light spot is 4° in diameter then the off response is reduced and the on response increased.

The multiunit response shows a well developed decremental dimming response and in some cases a less well developed incremental brightening response. The gross response will synchronise with the rapid movement of small card stimuli or with flickering shadows in a restricted region of the visual field without habituation. Under unstimulated conditions the region from which the multiunit response may be recorded remains silent.

The multiunit response is recorded from the stratum griseum superficiale of the superior colliculus in which there are a variety of cell types as well as the bushy terminals of the incoming optic tract fibres. The responses of the population recorded in this region were thought to be very similar to those of the retinal large field off units and an investigation of the units was attempted. Such large field off units, and directional units, had been observed in the fibres of the brachium of the superior colliculus in earlier experiments.

*Units in the Upper 0.8 mm of the Colliculus*

*OFF Units.* Within about 0.2 mm of the surface of the colliculus it has only been possible to record off units with very small fields, in the order of 1° with a well developed inhibitory surround, and the spike shape characteristic of a fibre. They were difficult to isolate and examine.

Below, and sometimes amongst the above units, it was possible to isolate some large amplitude spike potentials, a few of which showed prepotentials or gave an injury discharge which identified them as cells. The fields possessed a predominantly off centre about 4° in diameter but even a 1/2° light spot elicited a slight on response when flashed. In the transition from a 1° to 10° spot and then to whole field illumination there is a marked decrease in the duration of the transient off response and an increase in the amplitude of the on response. The units gave a good decremental dimming response to the 1° spot which was reduced but not eliminated under whole field illumination. The firing fell into synchrony, without habituation, with the rapid movement of small stimuli or of shadows in the receptive field. The properties of the units are similar to the much more readily recorded multiunit population and to the large field off units of the optic nerve and retina.
ON-OFF Units. In the deeper parts of the stratum griseum superficiale the units are more readily recorded and from about 0.5 to 0.8 mm down they are predominantly classed as ON-OFF units. The receptive fields varied in size from about 0.5° to 20° in diameter. Lateral inhibition was noticeable in many of the units and some 50%—mainly those with fields of less than 5° in diameter—were noted as being especially sensitive to rapid movements.

A number of the ON-OFF units possessed large, 10-20° diameter, fields in which the distribution of ON and OFF areas was rather patchy. These units were clearly identified as cells by their spike shape. The large field ON-OFF units did not follow rapid movement well and in more than half the cases the fields showed a marked inhibitory surround.

Directional Units. A number of directional units were recorded to the lower margin of this region. The fields were 2-3° in diameter and their characteristics were similar to those of the optic nerve. None were clearly identifiable as cells.

The multunit evoked response is much reduced as the electrode descends below about 0.5 mm and is replaced by a fibre hiss at a depth of about 0.8 mm, when it is concluded that the stratum opticum has been reached. The response is sharply localised. Units are difficult to isolate in this region but large field OFF, concentric and directional types have been recorded with fibre characteristics. As described by Hamdi & Whitteridge (1953), it is usually found that slowly moving stimuli in the visual field temporal and nasal of that giving rise to the main evoked response will elicit fibre activity. This region extends no more than 5° in the vertical but can be up to 20° long. The response is not simply of incoming fibres for there is a response from nasal field whose fibres should have terminated before the central colliculus. It is most likely that the hiss ‘wings’ represent the input fibres for the units located in the subadjacent region.

Units Recorded from 0.8-1.7 mm

Long Field Units. When mapped with a neon flash these units were found to possess a receptive field in the form of a bar 30°-100° long by 5°-20° high giving a predominantly off response and separating the two halves of a large region, up to 100° in diameter, from which a weak on or off response could be elicited. The larger bars were found in the deeper layers and all the fields were orientated with the band parallel to the visual streak. Responses are obtained from the band alone when the field is searched with moving card stimuli. The
response to flashing of a light at different points along the band is variable in magnitude or patchy and is of much shorter latency (40-50 m. sec) than that of the surround (100 m. sec).

The response of the band (which gives rise to the description of long field units) is similar to whole field or 2° flash and consists of a massive off and more restricted on component. The maintained activity is usually inhibited after the transient on response. The flashing of a spot of light 5° in diameter at points ranging from the band to 30° below shows that the off response decreases with distance from the band. The response to movement of a card disc falls off much more rapidly. The response to movement along or across the band is equally readily elicited. A good decremental dimming response is obtained from the band and firing falls readily into synchrony with fast movement. The units vary in their readiness to respond to slow movement. The majority of the units show pre-potentials or injury discharge and thus are identified as cells. The properties of the band region are in many ways reminiscent of those of the large field off units of the retina.

Units Found Below 1.3 mm
The previously described units fall into this class but have been grouped with the more superficial of their kind in the above description. Apart from the long field units it becomes increasingly difficult to study and describe the properties of the deeper units. The action potentials of the majority were characteristic of cells. Classification was not readily carried out. More than half of the units respond to the flashing of a light spot within the receptive field but the response to moving card stimuli was much better. A few units in the deeper layers would respond to fast movement but the majority fire only to slowly moving figures (less than 10°/sec). When mapped by a light spot, or by their movement response, the majority of the fields extend 8°-40° along their major axis. Circular and oval fields were recorded.

Single units from the lower parts of this region (1.8-2.6 mm) are in general much more difficult to investigate. The majority will not respond to light spot, whole field or decremental dimming stimuli. Slow and, more rarely, fast movement will elicit a response but this habituates very rapidly in the majority of cells. Some units show greater response to movement in one direction, which is usually parallel to the horizontal. Detailed examination of the receptive fields is difficult because regions of the field disappear and return during stimulation.

Between 2.0 and 4.0 mm below the collicular surface some unusual units
were encountered but histological controls were not available and their exact location is not known. Their action potentials are like those of cells; the receptive field consisted of exact quadrants, hemispheres or the whole of the visual field. The margins of the fields run along the horizontal or the central vertical meridian and they are bounded by the limits of the visual field. The responses do not habituate to movement. The response to large stimuli is

Fig. 37
Lamination of the unit classes in the superior colliculus. The ends of the black bars indicate the depth at which the most superficial and the deepest example of each unit class was recorded. An approximate scale relates the depth measurements to the laminae of the organ. Only electrode insertions perpendicular to the laminae have been included.
inferior to that generated by small objects as in the case of cortical hyper-complex units. A few of the cells show spontaneous activity but they do not respond to the flashing of a light spot or to rapid movement.

Lamination. The units of the superior colliculus evidence a marked degree of functional lamination. This arrangement is revealed by the plot of Fig. 37 where the most superficial and the deepest points at which each class was recorded set the limits of a line representing the extent of their distribution.

Single Units of the Visual Cortex Area (VI)

Single unit recording from units in the rabbit visual cortex is not easy. Two phenomena, burst discharge and ‘switching off’ of cortical activity play havoc with attempts at systematic analysis.

Ether, and later nitrous oxide, has been found to be the most satisfactory means of holding the animal at the right anaesthetic level. The recording was carried out either with Woods’ metal filled micropipettes tipped with platinum black or with tungsten electrodes varnished with bakelite. The cortex was exposed through a small hole in the skull and covered with a 3% solution of warm ‘Ionagar’ to prevent pulsation without obscuring sight of the cortical blood vessels. In a good preparation the upper layers of cortex are active but if the animal is too deeply anaesthetised only layers 5 and 6 appear to be functional. A diagrammatic representation of the various classes of receptive field encountered in area VI is shown in Fig. 38.

All units with receptive fields characteristic of class 6 possessed action potentials of a form suggesting that the units were cells; long duration, complex or multiphasic waveform, large amplitude and in some cases a prolonged injury discharge at the termination of recording. Similar features indicated that some units at least, of each of the remaining classes, other than class 1, were cells.

1. Small Receptive Fields These units were most commonly recorded from the upper half of the cortex and possess fields from 1–3’ in diameter when mapped with the 10’ spot of an Airmark perimeter. The fields appear to be homogeneous in their response, which is most commonly of the ON-OFF variety although a few ON or OFF types are encountered. The response to wide card stimuli is less than that to narrow and thus an inhibitory surround
Single units of the visual cortex. 1, small field on-off. 2, medium field on-off. 3, cortical directional unit showing restricted range of entry radii along which stimulus movements elicit firing. 4, on-off adjacent unit. 5, directionally selective orientated bar sensitive unit. Spot indicates field location. Black arrow indicates movement and stimulus required to elicit firing. 6, very large field on-off unit in which movement produces best response. Some prefer stimuli smaller than the receptive field.

is assumed to be present although it could not be revealed by spot stimuli. The response to a flashed light spot is transient and that to rapidly moving stimuli is not very good. Spike waveforms characteristic of cells were not obtained.

2. Medium Field Units (5-10°) These units possess receptive fields of homogeneous ON-OFF type about 5-10° in diameter. The units respond to the
movement of small objects or shadows within the receptive field and fire in response to step like changes in illumination. Their properties are to some extent reminiscent of the retinal large field type but they respond to both incremental brightening and decremental dimming. The response to such stimuli is markedly decreased when the surround is included which suggests the presence of lateral inhibition. The units will follow movements at rates up to 50–100/sec.

3. **Directional Units**  Only one half of the recorded cortical population of directional units would respond to the firing of a spot of light flashed in their receptive field and all of these were of the ON-OFF type possessing fields from 3–7° in diameter. The response to card stimuli was good and the preferred direction remained unchanged after contrast reversal of the stimulus and background. Firing could be elicited by the introduction of a small object into the field along radii forming a sector from 90° to no more than 180° in extent and thus the cortical fields differed from those of the retinal units which respond to stimuli entering over a 270° sector. The preferred direction was almost always at right angles, or parallel, to the visual streak but 20% of the units evinced other preferred directions. Lateral inhibition is revealed only if very wide edges are used (20°–30°) when the response is reduced but not eliminated.

4. **Adjacent ON and OFF area unit**  The properties of these units are very similar to those of the retinal variety and it was occasionally possible to map adjacent ON and OFF areas, but included in this class are units which behave as 'axial motion' or 'Orientation' detectors without possessing fields which can be mapped with a light spot. In the case of units with maintained firing it was found that a black disc placed in the ON area against a white background will eliminate firing, just as in the case of the retinal units. A flashed bar twisted by more than 25° each way from the optimum orientation elicits little response. Edges entering the field twisted a similar amount away from the optimum orientation were similarly ineffective. The major axis of the fields is from 6–12° long and the minor about 4–6°. Five fields with a preference for oblique stimuli were recorded.

5. **Oriented Slit or Bar**  Four units with more sophisticated requirements were obtained. They fired spontaneously and were difficult to locate. The adequate stimulus was finally discovered to be a narrow bar or thin wire (1 mm at 30 cm)
passed across the field at a certain orientation (in two cases this was 45° to the vertical). A single edge would not cause firing so that a lateral inhibitory region must be present on each side of the excitatory area. A slight change in the orientation of the wire eliminated the response. One of the fields responded to the motion of the bar in one direction only, the others responded to back and forth motion. The rarity of these units in the rabbit contrasts markedly with their abundance in cat and monkey.

6. Very Large Field Units (20-40) It is possible to record single units with very large receptive fields in the region from 0.75 mm to 1.5 mm below the cortical surface. These units are often present, firing spontaneously, in preparations whose upper cortex is not at all responsive. The action potentials are usually of large amplitude and complex waveform. Lesions have been made when recording from these units which show the electrode tip to have been in layers V and VI. The majority of the units show a homogeneous ON-OFF field but a few pure ON types have been recorded. The fields were exceedingly sensitive to small movements of card stimuli or shadows. Many more spikes were obtained in the response to moving stimuli than to a light flash. The units are quite unlike anything recorded in the optic nerve. A few of the units would not respond to stimuli half the diameter of the receptive field although response to small stimuli could be elicited all over the field. In this respect they resemble the hypercomplex units of the cat visual cortex.

7. Unidentified Fields Seventeen units have been held for an adequate length of time for routine analysis without classification having been made satisfactorily. Response to visual stimuli could be elicited without a field being located so that units with more sophisticated stimulus requirements are probably present.

8. Lamination of Visual Cortex Units Most of the unit classes were similar to one another in the pattern of their depth distribution and were predominantly located in the upper half of the visual cortex. In Fig. 39 the unhatched columns indicate the total number of units recorded within each cortical lamina 0.25 mm thick. (Total of 203 units). The hatched columns indicate the distribution in depth of the very large field units which were concentrated in the lower half of the cortex.
Depth histogram showing the number of single units recorded within 0.25 mm thick laminae of the rabbit visual area I. The hatched columns indicate the very large field on-off units which are found only in the lower layers of the cortex. Other classes of units have been lumped together because they show similar distributions.

9. No Columns Observed in the Rabbit Area VI  
Electrode insertions perpendicular to the surface of the cortex encountered single units whose receptive fields were centred about one point in visual space. During a single electrode insertion it was always found that successive units differed in receptive field properties and did not possess common features like preferred direction or orientation sensitivity. In view of this observation and the rarity of the orientated slit sensitive fields it is clear that the rabbit visual area I is organised in different fashion to that of the cat. Eye dominance columns were not looked for and may be present.

Single Units of the Visual Cortex Area VII

It has proved very difficult to record the units of this region and only two have been investigated. They had large fields, in the order of 60° in diameter, which were directionally selective to the movement of quite large objects (10° edge) in a naso-temporal direction along lines parallel to the horizontal. Other angles of entry produced no firing.
% of units of various categories at stations on the central visual pathways

**Optic Nerve Units (230)**

- Concentric, small field, transient: 16%
- Maintained: 13% ON 33%
- Large field shadow sensitive: 4%
- Concentric small field transient: 21%
- Maintained: 13%
- Dark adapting: 4% OFF 56%
- Large field shadow sensitive: 18%
- Directional: 9% ON + OFF 9%
- On/Off adjacent: 2% Other 2%

**Superior Colliculus (97)**

- Very Small field off: 16%
- Large field off: 8%
- On-off, homogeneous, I surround: 10%
- Directional on-off: 5%
- Long field: 23%
- On-off habituating or not: 18%
- Movement + habituating + directional: 13%
- Quadrant and hemisphere: 7%

**Visual Cortex (I area, 202)**

- Small field on off: 23%
- Medium field on off: 15%
- Directional, simple and complex: 18%
- On/off adjacent, simple and complex: 15%
- Very large field: 19%
- Bar not edge: 2%
- Unclassified: 8%

The multiunit response recorded from VII may be elicited from a much more extensive region of the visual field than is the case of that in area VI. It appears possible, therefore, that many of the units will eventually be found to possess large receptive fields.
Comparison of Single Unit Observations with the Literature on Rabbit Visual Units

The optic nerve of the rabbit has only been studied by Thompson (1953) who classifies the units in terms of response to the flashing of light spots. The three groups of units, ON, OFF and ON-OFF were recorded directly from the retina by Granit (1947) but detailed analysis of the retinal units has only been presented recently (Barlow, Hill & Levick, 1964; Barlow & Levick, 1965; Levick, 1965 & 1967; Barlow & Oyster, 1967 and Oyster, 1968). None of the results reported here are in conflict with their more extensive findings.

It is important to note from the table of percentages that, the results obtained from the optic nerve correspond more closely to the peripheral units of the retina (Barlow et al. 1964) than to the population obtained by Levick (1967) from the visual streak, in spite of the fact that 50% of the optic nerve fibres come from the visual streak (defined as a ganglion cell count of more than 2,000 mm²).

An examination of the optic nerve by means of the electron microscope reveals that of a total count of 276,000 fibres (based on a distributed sample of 6,110 fibres), all myelinated, the range of diameters is 0.5 µ to 6.5 µ with 85% of the fibres less than 3.0 µ and more than 90% greater than 1.0 µ in diameter. The distribution was unimodal with the modal diameter 1.5 µ.

Thus in recording from the optic nerve there is a considerable disadvantage relative to retinal recording in that the largest fibres are only about 6.5 µ in diameter, which is equivalent to the smallest ganglion cell to be encountered during retinal recording. Most of the fibres from the visual streak region, which contains the smaller cells, will be in the order of 1.0 to 2.0 µ in diameter and are probably difficult to isolate. The fact that the ganglion cells with the largest receptive fields, the large field off units, and presumably the largest axons form 4.7% of the streak, and 11% of the peripheral populations (Barlow et al. 1964; Levick, 1967) of ganglion cells but make up 18% of the recorded optic nerve population supports this interpretation of the results. The absence of the local edge detectors and uniformity detectors (Levick, 1967) from the optic nerve population may thus be accounted for although other technical factors may be involved.

The units of the superior colliculus have been neglected in the literature until recently. Hamd & Whitteridge (1953) were the first to describe the change in shape of the multiunit evoked response field as the electrode passes down
to the stratum opticum. An early account of the collicular units by Horn & Hill (1966) has emphasised the multimodality of many of the cells but this has not been explored in the previous section. A later report by Hill (1966) contains a more extensive classification which reports the long field units, directional units and habituating types. The large field off units of the S.G.S. do not appear to have been observed. A most detailed account is contained in Schaeffer's (1966) paper on units recorded from freely moving animals. The laminar organisation of collicular units outlined in crude form above is shown in great detail by Schaeffer by means of electrolytic lesions. Small fields do not appear to have been observed by him and, because he does not use the contrast reversal test of directionality, it appears possible that many of his units that appear to be directionally selective along the horizontal are in fact long field units.

An early paper by Lomo & Mollica (1962) has reported on the sensory interaction in cortical units of the rabbit but stimulation was by means of flashed diffuse light. Similar methods were used by Velyka (1965). Arden, Ikeda & Hill (1967) have published an account of cortical units in the rabbit which is in essential agreement with the above account (preliminary form, Hughes, 1968). Directional and on-off adjacent units are described. The great proportion of the units reported by Arden et al. appear to have possessed very large receptive fields unlike those described above. It is possible that the majority of these units were obtained in the lower regions of the cortex in animals more deeply anaesthetised than those used in my own series of experiments. My findings with respect to cortical units are most in accord with the description of Ogawa, Karita & Tsuchiya (1968); although their nomenclature is different, it is apparent that they recorded small, discrete, receptive fields whose properties are very similar to those described in the earlier sections.

Comparative Aspects of Retinal Units

Some years ago, when it was believed that the mammalian retina has an output consisting only of concentric units, Maturana (1964) suggested that two types of visual system, related to the presence or absence of a neocortex, are to be found amongst the vertebrates:

1. A deterministic system present in the amphibians reptiles and birds, in which the function of the ganglion cells is highly specialised and unambiguous. The fundamental elements of form, edges, corners, points, colour, movement etc.
are discerned at the level of the retina and are as such projected to the tectum.

2. An indeterministic system, found in mammals where the output of each ganglion cell is unspecific and unambiguous the fundamental elements of form and colour being transferred to the geniculate and cortex as the combination of the output of a whole neighbourhood of ganglion cells.

The finding of nonconcentric units in the retina of the rabbit (Barlow et al., 1964; Levick, 1967), cat (Rodić & Stone, 1965; Stone & Fabian, 1966; Spinelli, 1966; Spinelli, 1967) and ground squirrel (Michael, 1968) necessitated the abandonment of the hypothesis in its original form. Concentric units alone have, however, been reported from the rat (Brown & Rojas, 1965) and monkey (Hubel & Wiesel, 1960). Conversely, reinvestigation of the frog retina (Keating & Gaze, 1970) reveals that the earlier reports (Maturana, McCulloch, Lettvin & Pitts, 1960), although occasionally misleading, are supported in their failure to describe units with the concentric on and off excitatory receptive fields (Grüsser, Grüsser-Cornehls & Bullock, 1964) originally recorded in the cat retina by Kuffler (1953).

Hubel & Wiesel (1961) suggested that their failure to record nonconcentric units in either optic nerve or L.G.N. of the rabbit might be accounted for if such units passed to the superior colliculus as small axons. The geniculo-cortical projection would thus contain only concentric units. This hypothesis provides a means of explaining the presence of concentric and nonconcentric units within one species and the variations in their proportion between species. The superior colliculus is regarded as a phylogenetically ancient deterministic system in which each class of nonconcentric unit might be a 'Key Feature' detector (Barlow, 1961) while the cortical concentric unit projection represents the more recent indeterministic system.

The presence of such an arrangement might, although not necessarily, be expected to reveal itself by an increase in the percentage of retinal concentric units in a series of animals ranked in ascending order of 'encephalisation'. If we exclude the rat, such is in fact found to be the case when the percentage of concentric units near the area centralis, averaged from reports in the literature, is plotted against the ratio of visual cortex/superior colliculus area determined from published material;

<table>
<thead>
<tr>
<th>frog</th>
<th>rabbit</th>
<th>cat</th>
<th>monkey</th>
<th>rat</th>
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<tr>
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<td>42</td>
<td>68</td>
<td>76</td>
<td>100</td>
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<td>0</td>
<td>1.5</td>
<td>9.0</td>
<td>30</td>
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% concentric | Area VC/SC
The results from the rat may be erroneous but in fact the information on rabbit cortical units and the results of Takahashi, Levick & Oyster (1969) are in total contradiction with the attractive hypothesis above.

All six classes of rabbit retinal unit have been described as represented in the L.G.N. by Takahashi et al. (1969); my own observations confirm them with respect to the large field off, concentric, and directional units. It is also clear that at least two of the retinal classes, the directional and orientation sensitive units, reach the cortical level relatively unchanged. The rabbit visual cortex, at least, thus does not receive the projection of concentric units alone. A similar situation may exist in the cat because Kozak, Rodieck & Bishop (1964) report that 4% of the cat L.G.N. units possess directional sensitivity.

As a corollary, it must be noted that not all of the six retinal unit classes have been described in the superior colliculus of the rabbit. At present only the large field off, and directional units have been observed – apart from the ordinary concentric units. The evidence thus indicates that, in the rabbit, the nonconcentric units project along the geniculo-striate pathway, and to some extent to the superior colliculus, whereas concentric units project to both regions.

**Phylogenetic and Ecological factors** It is a simplification to say that the concentric units decrease in percentage as we ascend the vertebrate series shown above. In fact the quality of the retinal units changes markedly in passing even from frog to rabbit. Thus the frog units are never concentric and are universally 'complex' or 'hypercomplex' in their properties. According to Dowling (1968) the difference between frog and monkey retinal units can be accounted for by the interposition of at least one extra synapse in the pathway between the receptor and ganglion cell. He finds very few direct bipolar to ganglion cell contacts in the frog retina whereas in the cat and monkey nearly all ganglion cells receive direct bipolar inputs. The rabbit and ground squirrel have retinas intermediate in organisation. Dowling concludes: 'the trend towards complexity of retinal structure correlates with less encephalisation of neural function.' Thus, like many workers, he places the emphasis upon phylogenetic status as the determinant of retinal unit population. It is well, however, to remember the sophisticated adaptations to specific environments (Walls, 1942) that are to be found in the visual system. Ecological factors appear to be concerned in the determination of the topographical distribution of retinal ganglion cells in the ground and arboreal squirrels (Hughes & Whitteridge, in prepara-
tion). Changes in the form of this distribution may well be accompanied by modifications in the properties of the single unit population such that little evidence of the original phylogenetic ground plan remains.

Sufficient species have not been examined for the relative importance of the phylogenetic and ecological influences on the retinal unit populations to have been determined. The results available from the ground squirrel and rat are consequently important but because the accounts have not been confirmed their discussion has been delayed until now.

The ground squirrel is often quoted as possessing a retinal unit population equivalent in complexity to that of the rabbit. An examination of the results of Michael (1968a, b, c) reveals that, if colour sensitive units are classed as basically concentric in organisation, 77% of the retinal units are concentric and 23% are directional on-off directional units. This set is more akin to that of the cat than to the six classes of the rabbit. Michael comments that some units with more sophisticated requirements are encountered in the optic nerve (1968b) but they do not feature in his 100% count. Recording was carried out in the optic nerve and this may result in biased sampling (page 113); sophisticated units may thus be present in large quantity. The equivalence of structure between ground squirrel and rabbit retina (Dowling, 1968) indicates that a greater variety of units might be expected but until they are confirmed the position of the ground squirrel in the series of mammals shown above is not clear.

If a greater variety of unit classes were to be recorded, then the fact that the ground squirrel, a rodent, is unrelated to the rabbit (McKenna, 1969; Van Valen, 1964) could be thought to lend weight to the hypothesis of correlation between phylogenetic status and retinal unit population. Work carried out with Professor D. Whitteridge (Hughes & Whitteridge, in preparation) has shown that this is not the case. The ganglion cell distribution in the antelope ground squirrel (Citellus leucurus), which is a relative of the Mexican ground squirrel used by Michael, possesses a visual streak. The environmental factors common to the way of life of the rabbit and ground squirrel, or secondarily, the common visual streak may have been as important as phylogenetic status in establishing any functional similarities between the units of the species.

The rat, however, does not possess a well developed visual streak (Hughes & Whitteridge, in preparation); in retinal topography and life style it contrasts with the related ground squirrel and the much more distantly related rabbit (McKenna, 1969) while all three species enjoy similar phylogenetic status. The report by Brown & Rojas (1965) that the rat possesses concentric units alone
A. Hughes

at the retinal level is thus crucial. Although unconfirmed, it stands as an obstacle to the acceptance of the phyllogenetic serial concept of retinal organisation. Work is in progress to settle the matter. It is worth noting that directional cells have been recorded in the L.G.N. of the rat by Montero, Brugge & Beitel (1968).

The question of the significance of the different proportions of concentric and nonconcentric units projecting in the retino-cortical pathway of different species and an implicit problem, as to whether the concepts of determinism and indeterminism may be usefully applied to the understanding of unit function within the cortex of one animal, must be deferred. As Hubel & Wiesel (1965) have stated: 'a proper understanding of the part played by any cell in a sensory system must depend . . . . . on a knowledge of how the information it conveys is made use of at higher levels.'

Roles of the Superior Colliculus and Visual Cortex

Until recently the superior colliculus has been regarded as little more than a centre through which the cortical visuomotor regions operated the eye muscles (Holmes, 1938: Crosby & Henderson, 1948). The findings of Schneider (1967) have summarised the indications of many earlier, debatable ablation studies of localisation of function in the visual system. The total ablation of the superior colliculi eliminated the ability of the golden hamster to orientate towards a visual stimulus but left pattern discrimination unaffected. Conversely, ablation of the striate cortex almost totally eliminated pattern discrimination but left the visual orientation reflex intact. The complimentary nature of the results suggests a remarkable division of function between the two regions.

More recent work shows that the residual visual capacity after striate cortex ablation is attributable in part to the involvement of a tecto-thalamo-cortical pathway (Diamond & Hall, 1969) but that the role of this path varies considerably between species. In the tree shrew (Snyder & Diamond, 1968) pattern vision is possible in its absence and must be attributed to collicular function. In the hedgehog (Hall & Diamond, 1968) the residuum is more extensively reduced after removal of the ascending path. Results like those from the hamster, which at least indicate collicular involvement in orientation to visual stimuli after the ablation of visual cortex, are available from rat (Lashley, 1939), cat (Sprague, 1966: Fischmann & Meikle, 1965) and monkey (Humphrey & Weiskrantz, 1967). Extensive visual cortex lesions in the rabbit leave orientation
and object vision relatively intact (Ten Cate & Van Herk, 1933; Ten Cate, 1935).

Failure of visual orientation after collicular ablation has been observed in animals other than the hamster e.g. cat (Sprague & Meikle, 1965) and monkey (Denny-Brown, 1962). Such ablations are associated with deficits in orientation towards acoustic, tactile and nociceptive stimuli so that the organ appears to be involved in more general orientation responses.

The neural substrate of the orientation response, or 'visual grasp reflex' (Hess, Bürger & Bucher, 1946), has been revealed as the co-existence of an eye motor map which corresponds topographically with the retinal projection within the superior colliculus (Apter, 1949, 1950). A similar organisation of the rabbit superior colliculus has been established by Schaeffer (1968) which reveals appropriate eye, head and ear motor maps which direct these organs to the region of visual field represented in the corresponding retinotopic projection.

That the superior colliculus plays a role in the detection of visual stimuli is clear from its contribution to orientation in the destriate animal. In the rabbit, this role may be reflected by the uniform magnification of the visual field representation on the organ which contrasts with the cortical emphasis on the representation of the nasal and, to a lesser extent, lateral fixation areas; the retinal regions redirected by the orientation response. In the absence of the orientation response, the visual cortex of a hamster lacking the superior colliculi reveals its own capacity for stimulus detection by an increased number of 'freeze' responses (Schneider, 1967). Whatever the potentiality of the superior colliculus in the absence of the visual cortex it is essential to remember their normal co-operation and the presence of extensive neocortical projections (Garey, Jones & Powell, 1968), whose function is obscure and influence on the collicular units varies from species to species.

**Comparative Aspects of Superior Colliculus Units**

*The Role of Rabbit Superior Colliculus Single Units* In an earlier section it has been pointed out that the upper part of the stratum griseum superficiale in the rabbit has a conspicuous multunit response and contains single units which are very similar in properties to the large field off units of the retina. The multiunit response and the large field off units both:

1. Show evidence of an inhibitory surround.
Fig. 40

Similarities in the responses of an optic nerve large field off unit, collicular large field off cell, massed discharge of the superficial superior colliculus and a collicular long field unit to: A; off then on of a small spot of light (2-3°) centred in the receptive field; B; decremental dimming and incremental brightening of a small light spot; C; rapid movement of a shadow stimulus back and forth through the receptive field of the unit. In the case of the multiunit discharge, an extra response has been included; B shows the reduced off and increased on response relative to the prominent off response in A when a larger spot of light is flashed (10° in diameter), thus demonstrating the presence of lateral inhibition in the population response. The illustrations C & D thus correspond to B & C in the other cases. Downward movement of the monitor trace indicates a reduction in the light intensity falling on the receptive field and upward movement the reverse. Time 100 msec. For discussion of responses see page (119).
2. Evince prolonged firing at 'off' of a small centred light spot but a rather brief 'on' response.
3. Give a good response to shadows or to very small movements of an object in the receptive field.
4. Respond to fast movement and repeated movement without habituation.
5. Give a dimming response to step changes in light intensity but not a brightening response.

In Fig. 40 are off, on, dimming and rapid movement responses for an optic nerve large field off unit, a single unit from the upper layer of the superior colliculus and for the multiunit evoked response of the stratum griseum superficiale.

The above evidence suggests that the large field off units pass to the upper layers of the superior colliculus in such large numbers that they predominate in the population response. There is normally very little spontaneous activity in this layer so that a burst of firing is indicative of 'activity' of the kind detected by the large field off units e.g. rapid movement, shadows + small moving objects, in the corresponding region of the visual field.

Levick (1965) has described the large field off units as 'alarm units'. In view of the preceding demonstration of the involvement of the superior colliculus in orientation to stimuli it appears appropriate that the organ should possess in its upper layers an 'early warning system' – the large field units constitute the fast fibre population of the optic tract (Lederman & Noell, 1968) – fed by 'alarm' units situated all over the retina (Hughes, 1968). It is suggested that, when 'activity' occurs at some point in the visual field, this superficial sheet of cells activates the subadjacent topographically related column of cells by means of its descending connections (Cajal, 1911) and elicits physiologically the same orientation response that is obtained by electrical stimulation or strychnine application under experimental conditions. The absence of cortical projections to this upper half of the stratum griseum superficiale in the rat (Lund, 1966, 1968) suggests that the layer may function normally in the absence of the striate cortex. The class of stimuli which elicits orientation responses in animals lacking the visual cortex is very similar to those which excite the large field off units (Sprague, 1966; Schneider, 1967).

The unit properties tend to be laminated within the radial column of deeper collicular cells. The long field cells have properties reminiscent of the large field off type (Fig. 40) and may be synthesised from them providing generalisa-
tion of ‘activity’ parallel to the long axis of the visual streak.

Oyster (1968) has presented evidence for the involvement of on-off directional units in the generation of OKN which is a response mediated by the superior colliculus. It would thus appear that another of the collicular unit classes, the directional, may reasonably be attributed with a specific, but not necessarily sole, function. There is no evidence, however, which would unequivocally establish the deep collicular directional units involved in the generation of OKN.

In a qualitative sense the responsiveness of the deeper units of the superior colliculus bears some resemblance to that of the visually guided behaviour evidenced in destriate animals. In particular, the responsiveness to moving rather than to stationary stimuli, and habituation to repeated stimuli have behavioural counterparts. The latter feature would not be provided by the upper sheet of ‘alarm’ units – their failure to habituate is one of their most distinguishing features.

The deepest region of the superior colliculus contains the very large quadrant and hemifield units whose properties are suggestive of involvement in motor output but there is no apparent correspondence of the visual field division with eye muscle direction of pull and again we observe extensive spatial generalisation for an unknown function.

Tectal Units in other Species In spite of differing investigative techniques there appears to be a considerable similarity between the units of the superior colliculus in the different species of mammal. In all the vertebrates the superficial tectal receptive fields are small and the deep fields very large.

The multiunit response of the upper part of the stratum griseum superficiale appears to lack directional specificity and to respond without habituation to rapid oscillatory movement of shadow or card stimuli in the rat (Humphrey, 1968), cat (Sprague, Marchiavava & Rizzolatti, 1968), and rabbit (Hughes, 1968). The superficial small units of the monkey tectum do not appear to be similar to those of the above species (Humphrey, 1968).

Long fields similar to those of the rabbit have been observed in the rat by Humphrey (1968) but they are not so extensively elongated. They have not been described in any other species. On the other hand large fields with straight margins corresponding to the vertical or horizontal have been observed deep in the tectum of monkey (Humphrey, 1968), cat (McIlwain & Buser, 1968), rabbit and pigeon (unpublished observations) but not in the rat (Humphrey, 1968).

It is a common feature of rat, ground squirrel, rabbit, cat and monkey tectal
RELATION BETWEEN ANATOMY AND PHYSIOLOGY

units that the response to stationary light spot stimuli becomes progressively poorer, so that the units respond to moving stimuli alone, and that the incidence of habituation to repeated stimuli becomes greater as the deeper layers of the superior colliculus are penetrated.

Perhaps the major species difference at the tectal level, if confirmed, is indicated by the failure of Humphrey (1968) and Siminoff, Schwassmann & Kruger (1967) to find directional units in the rat tectum and of Humphrey (1968) to find such units in the monkey tectum. In the rabbit I find two classes of directional unit, both of which are directional under reversed contrast, an upper superficial group which have the properties of a retinal directional on-off type and a deeper group which may be described as possessing a preferred direction rather than possessing a preferred null axis. The results differ from those of Schaefer (1966) who finds 70% of the rabbit tectal units to be directional and more in keeping with those of Hill (1966) who finds the class to form 20% of the total. In both rabbit (Schaefer, 1966) and cat (McIwain & Buser, 1968: Sprague, Marchiafava & Rizzolati, 1968) it appears that the proportion of directional units increases as the deeper layers are approached.

The species differences can be correlated to some extent with the apparent differences described earlier in retinal unit populations. The rat and monkey, reported to be without retinal directional units, are claimed to possess no such tectal units. The rabbit and ground squirrel, which possess a large retinal population of directional units also possess similar units in the superficial layers of the tectum. The directional units in their deeper layers appear to be similar to those deep in the cat superior colliculus as described by Marchiafava & Pepeu (1966) and Sprague et al. (1968). The superficial directional units of the cat colliculus do not appear to be either optic tract fibres or cells connected to such fibres because, although their percentage is high (50%), within the colliculus, there is no evidence (Sterling & Wickelgren, 1969) that the brachium of the organ is richly supplied with retinal directional units such as are to be found in the rabbit and ground squirrel.

The observation of Wickelgren & Sterling (1969) that the directional units of the cat upper collicular layers disappear after cortical cooling or ablation suggests an organisation different to that of rabbit and ground squirrel, in which the retinal afferents alone prevent such an event. The directional units of the deeper layers of the cat tectum do not disappear upon cortical ablation (Marchiafava & Pepeu, 1966). The units of the rabbit (Hughes, unpublished observations) and rat (Humphrey, 1968) appear little changed after cortical
ablation. These differences suggest the possibility of correlation between cortical dependence of the collicular units and the retinal unit populations but the evidence is too sparse for any generalisations to be made.

The tectal units of all species are thus mainly concerned with the detection of moving stimuli, many of them requiring movement in a specific direction. Habituation to repeated stimulus presentation is common and there is some lamination of function correlated with a topographic organisation of the retinal projection. Many of the fields are large and respond to activity in different regions equally well. Such fields often respond better to small rather than large objects and show an inhibitory surround. These have been described as analogous to the hypercomplex units of the visual cortex by Sterling & Weckelgren (1969). The requirements of the units are in general not so precise as those of cortical units, are more likely to involve large stimulus movements and to be less specific about stimulus size and orientation. These similarities of tectal organisation between species may reflect the similarity of the residual visual capacity which exists after ablation of the striate cortex in animals as different as the hamster and monkey (Schneider, 1967; Humphrey & Weiskrantz, 1967).

Comparative Aspects of Visual Cortex Units

The major contribution to our knowledge of visual cortex single unit organisation has been provided by the work of Hubel & Wiesel on cat and monkey, two animals which appear to have many features of their cortical unit populations in common. The results of Hubel & Wiesel inevitably provide a standard for the comparative consideration of the work on the rabbit cortex. No detailed single unit studies have been carried out on the visual cortex of any mammals other than cat, rabbit and monkey. At present, the results from cat and monkey appear to be very different to those from the rabbit. Although generally confirming the work of Hubel & Wiesel on the cat cortex, certain features revealed by other workers somewhat reduce the apparent disparity between the descriptions of cortical organisation in that animal and the rabbit so that consideration is given to their findings below.

Cat Visual Area I According to Hubel & Wiesel The cat visual cortex units are not organised in concentric fashion like those of the retina (Hubel & Wiesel, 1959: 1962) but are all, whether in areas I, II or III (Hubel & Wiesel, 1965), especially responsive to the movement of straight line stimuli such as an edge,
slit or bar at some specific orientation. This is true even if other, more sophisticated, requirements are superimposed. Most of the cat cortical units respond best to moving stimuli but amongst those responding to flashed light spots are two classes. The ‘simple’ units have elongated receptive fields which may be mapped out in terms of excitatory and inhibitory regions so that the optimum bar stimulus may be determined from an examination of the plot; ‘complex’ units may respond to the light but cannot be mapped in such a fashion. The complex and simple units alike are unaffected by the length of bar stimuli as long as their orientation is optimal but the complex units require less precise location and appear to generalise the requirements of the simple unit in space. Lower and higher order hypercomplex units, now found in area VI in the cat (Hubel & Wiesel, 1968), have more restricted requirements for stimulation and it is necessary that a stimulus bar or slit be terminated at one or both ends before a response may be obtained.

The specific orientation requirements of the edge, bar and slit receptive fields soon revealed that all of the cells encountered during one electrode insertion perpendicular to the cortical surface were selective for the same orientation. Adjacent insertions were discovered to record units with similar orientation requirements so that a region could be defined, on the surface of the cortex and extending through all its layers, in which all the units possessed the same orientation requirements. This volume of tissue was called a column (1963).

Eye dominance columns have also been described in the cat but these are not so discreetly organised as orientation columns (Hubel & Wiesel, 1962). Such columns are functionally defined. The presence of eye dominance columns has, however, been demonstrated independently by an ingenious anatomical technique (Hubel & Wiesel, 1969).

Some indication of lamination within the depth of the visual cortex has been obtained in the cat (Hubel & Wiesel, 1962) and monkey (Hubel & Wiesel, 1968) which suggests that complex units are not located in the region of layer 4 but occur above and below. Since the complex and hypercomplex units of a given column possess the same orientation selectivity it has been suggested by Hubel & Wiesel that the column provides the means for the economical ‘wiring up’ of the complex and hypercomplex units from simple units according to the hierarchical concept of cortical unit organisation (Hubel & Wiesel, 1962, 1965). The arrangement in the monkey appears to be similar but organised in a more discreet and sophisticated fashion (Hubel & Wiesel, 1968).
The orientation sensitivity of many cortical cells has been confirmed by various investigators (Baumgartner, Brown & Schulz, 1964; Blakemore, 1970; Campbell, Cleland, Cooper, Enroth-Cugell, 1968; Campbell, Cooper, Robson & Sachs, 1969; Creutzfeldt & Ito, 1968; Pettigrew, Nikara, Bishop, 1968; and Spinelli & Barrett, 1968).

The presence of orientation columns has also been confirmed by Blakemore (1970) who divides them into two categories. Constant depth columns contain orientation specific binocular units whose disparity is similar so that stimuli within one plane of the visual field, at a given convergence, will activate them. In a direction column, the disparities vary considerably so that the units are activated by stimuli at different depths in the field but located along a single projection axis running from the eye.

Circular and concentric receptive fields associated with cortical units have been classified as arising from L.G.N. afferents by Hubel & Wiesel (1959; 1962). Baumgartner et al. (1964), Spinelli & Barrett (1968), and Joshua & Bishop (1970) have, however, described many such units with binocular inputs. The fields recorded by Spinelli & Barrett (1968) and Joshua & Bishop (1970) were influenced neither by the shape of a stimulus nor by its orientation. Circular fields formed 44% of a sample of 165 cortical cells (Spinelli & Barrett, 1968).

16% of the circular and 17% of the edge and bar selective fields were described as directionally selective by Spinelli & Barrett (1968). A similar emphasis on the presence of units with directional properties has been made by Pettigrew, Nikara & Bishop (1968) who find 35% of simple and 48% of complex units to have responses so asymmetrical as to form a separate directional class. Directionality of response has not been emphasized by Hubel & Wiesel as a property of cortical units, it figures in none of their tables, and when reported is regarded as possibly arising from the symmetry of flanking areas of the receptive field (Hubel & Wiesel, 1959).

The single unit populations obtained by the different investigators may not be comparable as a result of variations in the anaesthetic used. It is noticeable that Hubel & Wiesel work with light nembutal anaesthesia while the other investigators use either light nitrous oxide anaesthesia or work with conscious animals. According to Burns (1968), the cortical units of the unanaesthetised preparation are somewhat 'less fussy' about the stimulus required to drive them. Orientation selectivity of the cells is claimed to be invariant with depth of anaesthesia (Hubel & Wiesel, 1962).
Receptive fields tend to be larger as the recording site is moved away from representation of the area centralis to that of the peripheral retina (Hubel & Wiesel, 1962) and the proportion of units lacking orientation sensitivity increases (Joshua & Bishop, 1970). In a sample of 85 units obtained 30° from the area centralis some 30% were not orientation selective. Such contrasts between the peripheral and central populations cannot, however, be totally responsible for discrepancies between the observations of Hubel & Wiesel and others because the former have recorded units well away from the area centralis and most of the latter have recorded units within 15° of the area centralis alone.

The marked contrast between the totally concentric retinal and totally linearly selective cortical unit population recorded by Hubel & Wiesel is thus reduced by the discovery of concentric and circular fields at the cortical level by other workers. The contrast is lessened further now that nonconcentric units have been recorded within the cat retina. Hubel & Wiesel have neither recorded nor taken into account the nonconcentric units described by other investigators. In view of the demonstration of, non-orientation selective, directional units in the cat retina by Rodieck & Stone (1965), Stone & Fabian (1966), Spinelli & Weingarten (1966) and in the cat L.G.N. by Kozak, Rodieck & Bishop (1964), as well as of bar and edge shaped fields in the cat retina (Spinelli, 1967), it appears that most of the properties of retinal units so far described have been observed at the cortical level but that the converse is certainly not true.

Comparison of Cat and Rabbit Primary Visual Cortex; The Units

Many of the cortical units of the rabbit possess properties similar to those of units recorded in the retina just as has recently been found to be the case in the cat. Certain differences have, however, been observed between the cortical and retinal representatives of overtly similar unit classes. In my own series of experiments it is apparent that on-off fields are much more common than concentric types in the cortex and that not many of this group possess the directional or local edge sensitivity which is invariably ascribed to them in the retina. The cortical directional and orientation sensitive units are more responsive to moving stimuli than to flashed light spots, to which only 50% will fire. The firing sector of directional units is reduced relative to the retina so that a stimulus with a component of movement in the null direction will not induce
A response. Many orientation selective units require an extended edge for the response to be dependable and the necessity of using a bar or slit as a stimulus is much more often apparent than appears to be the case in the retina.

Some of these features of unit behaviour appear in the L.G.N. units (Takahashi et al., 1969). It is possible, therefore, that units with properties in common with the retinal classes were afferent fibres from the L.G.N. The units of my own experimental series were recorded from the monocular field representation so that the binocularity criterion for cortical neurons was not available as is the case in the retina. The most convincing argument that these units represent cortical cells is that their action potential shapes were similar to those of visual units with properties which have not been reported in the retina or L.G.N. and which are thus assumed to be of cortical origin.

The small, medium and very large field homogeneous on-off units formed a large proportion of the units with receptive fields appearing only at the cortical level in the rabbit. The most interesting of the purely cortical units are members of the orientation selective class. The directionally selective orientated bar sensitive unit has not previously been reported at other levels and suggests class convergence. Of greater significance, however, is the observation that several of the orientated edge and bar selective units were selective for oblique orientation, so far only observed for 45° inclination to the horizontal, which has not been observed at the retinal level by Levick (1967) or Oyster (1968).

It thus appears that both rabbit and cat cortex possess concentric, circular and directional receptive fields which do not require orientated bar or slit stimuli. Edge, bar and slit sensitive fields are also common to the two species although in the rabbit they are much less numerous and do not possess either the selectivity for, or variety of, orientation observed in the units of the cat cortex.

The apparent readiness of many of the rabbit cortical units to respond to either stationary or moving light spots does not differentiate them from those recorded in the unanaesthetised cat (Spinelli, 1967). Even complex orientation sensitive units will map well to a moving spot in that animal but the map is different to that obtained with a bar.

None of the classes of cortical field other than the orientated edge and bar units have been found to show any degree of selectivity for straight edges or stimulus orientation. The units respond with velocity selectivity to stationary or moving spots, discs, squares, rectangles etc. Some respond exclusively to black stimuli on a white background and not to the reverse contrast. Although the small field and medium field on-off units reveal an inhibitory surround of
straight forward kind, it is to be noted that the very large fields show evidence of something akin to hypercomplex properties in that, as stimulus size increases, their response may decrease before an extension equal to the receptive field diameter is reached.

**The hierarchical organisation of the visual cortex**

The hierarchical theory of cortical organisation put forward by Hubel & Wiesel (1962; 1965; 1968) for the cat and monkey was based upon the need to account for the formation of orientation selective units from an L.G.N. population consisting only of concentric neurons.

In the rabbit, however, the full hierarchical system of orientation selective unit generation is an unessential hypothesis on the basis of the available evidence because:

a. 11% of the retinal ganglion cells in the visual streak are selective for vertical or horizontal orientation.

b. 80% of the cortical orientation selective units have properties explicable by the convergence of the output of the above cells after its passage through the L.G.N.

c. No higher order cells of the hypercomplex class, with properties explicable only in terms of the convergence of orientation selective units of parallel or orthogonal selectivity, have been identified.

The 20% of the cortical orientation selective units which respond to oblique line stimuli, also observed by Ogawa et al. (1968), may be representative of an undetected retinal population or they may be generated 'de novo'. Their properties are akin to those of the cat simple units and require similar explanation in terms of the convergence of L.G.N. afferents in the event of their being synthesised in the cortex.

In view of the limited population of orientated edge, bar and slit selective units in the rabbit area 17 it is convenient that certain features of the hierarchical concept of Hubel & Wiesel may stand in isolation from the property of orientation selectivity and be sought as common factors in the organisation of the visual cortex of other species. The operations carried out in the cat visual cortex are divided by Hubel & Wiesel into two general processes; the stages of simple and lower order hypercomplex unit formation are regarded as processes for the increase of selectivity while the interdigitating stages of complex and
higher order hypercomplex unit formation are seen as processes for the generalisation of selectivity in visual space.

Increase in selectivity of the rabbit visual cortex units relative to those of the retina has been indicated to be of limited extent in the previous section (p. 127) and some of the change must be attributed to intra-class convergence in the L.G.N. (Takahashi et al., 1969). At present cross-class convergence is only required to explain the synthesis of the directional orientated bar selective unit from orientation and directionally selective L.G.N. afferents in the manner suggested for the equivalent class in the cat (Pettigrew et al., 1968). Amongst the more bizarre units of Arden et al. (1967, Fig. 1D) or Ogawa et al. (1968, Fig. 7, 8) are to be found no features which the simple convergence of L.G.N. afferents to the cortex cannot account for. The great variety of units apparent at the retinal level in the rabbit is thus not accompanied by the development of a proportionately larger variety of sophisticated units at the cortical level.

In contrast, although no spatial generalisation appears to occur in the rabbit L.G.N. (Levick et al., 1969) there is plenty of evidence for it at the cortical level. The medium and large field ON-OFF units and the occasional large field directional and orientation selective units (e.g. Arden et al., 1967, Fig. 1B) require explanation in terms of convergence of L.G.N. afferents representing adjacent but different regions of visual space. Again intra-class convergence suffices. Even the so called hypercomplex features of the very large field units suggest the concatenation of a number of smaller receptive fields with surround inhibition.

No rabbit cortical receptive fields have been discovered to possess any kind of selectivity which necessitates the postulation of convergence of first or second order cortical cell outputs such as is required for the explanation of complex and hypercomplex unit properties in the cat. This is a marked distinction between the species but it refers only to the minimum theoretical requirements for the ‘wiring up’ of the units. In the cat there is some evidence that the complex units really do have inputs remote from the cortical afferents (Derney, Baumgartner & Aderjani, 1968) but no similar study has been made on the rabbit. The size of the receptive fields of the compound units recorded by Arden et al. (1967) and Ogawa et al. (1968) suggest that they may be formed by the convergence of first order cortical cells but this is not a necessary condition. In general, therefore, the results from the rabbit thus fail to indicate the nature of the units, which must exist, of the same order of connection as the complex and hypercomplex units of the cat. It is unlikely that such units have not been
recorded so that it appears that either they possess properties similar to the first order units or that their optimum trigger features have not yet been established.

A 'tidy dichotomy', cannot be drawn between the cat and rabbit on the basis of their simple orientation selective units. At present these must be regarded as being both synthesised in the cortex and relayed from the retina. Spinelli's report of elongated receptive fields with inhibitory flanks in the cat retina indicates, in view of Blakemore's observation that such units appear to arrive at the cortex (personal communication), that they may play some part in accounting for the orientation selective properties of the cortical cells; they are not regarded as imperilling the hierarchical concept because of their rarity. Conversely the cortical synthesis, as well as relaying, of orientation selectivity appears to be a possibility in the case of the oblique orientation units in the rabbit. The small percentage of orientation selective units in the rabbit, 15-17%, compared with cat, 70%, is again an unsatisfactory basis for a distinction. The difference between the two species is thus, at present, best summed up in the general statement that the observed processes of selectivity increase and generalisation at the cortical level in the rabbit may be entirely accounted for, without an hierarchical organisation, in terms of cross class and intra class convergence of no more than a few incoming L.G.N. fibres at one stage.

This conclusion is similar to that of Ogawa et al. (1968) who wrote that, in the rabbit, 'Cortical neurons do not effect further analytical processing on the visual information conveyed along the specific afferent fibres, but rather exert some integrative action on it.' It is thus of interest to note that the very large field on-off units of the rabbit may be representative of part of the output of its cortex. Their large amplitude, long duration, action potentials and location in layer V and VI suggests that they represent the pyramidal cells of this region which are known to give rise to the cortical efferents (Gioeli & Guthrie, 1967; Globus & Scheibel, 1967). The relatively uncomplicated receptive field properties of the units is in keeping with the above statements on the nature of processing in the rabbit area visual I but the sampled efferents are not necessarily the most sophisticated products of the cortical processing. Hubel & Wiesel (1967) have pointed out that simple, complex and hypercomplex units are all to be found in the callosum of the cat.

In conclusion it is to be noted that the classification of receptive fields into simple, complex and hypercomplex categories cannot be transferred to the rabbit accompanied by the implications of hierarchical cortical synthesis which
has been acquired in the case of the cat and monkey. This restriction is additionally necessary because, at the retinal level alone, the directional, local edge and uniformity detectors must be regarded as at least complex units. The contrast sensitivity of the surrounds of local edge units (Levick, 1967), the selective suppression of directionality from the surround of the directional unit (Oyster, 1968) and the total inhibition of the response to extended stimuli which is effected by the inhibitory receptive field of many of the units even merit the description of 'hypercomplex' for their receptive fields as has been suggested in the case of certain frog retinal units (Hubel & Wiesel, 1965).

**Columnar organisation**

The statement by Arden, Ikeda & Hill (1967), to the effect that the columnar arrangement of cortical cells in the rabbit is the same as that already described for the cat, is misleading. It is based upon the observation that the cells encountered within a single electrode insertion have their receptive fields located in roughly the same region of visual space. The column as understood by Mountcastle (1957) or Hubel & Wiesel (1963) requires common topographic and submodality specificity in its constituent units, 'topographic representation does not by itself constitute a columnar system ... in the retinotopic projection the representation is continuous; there are no sudden jumps as the surface is traversed.' Hubel & Wiesel (1968). The columnar system is superimposed on the retinotopic projection as an arrangement of functionally defined cell aggregations occupying cortical volumes whose boundaries divide the cortical surface into mosaic form. Columns have been described in somatic cortex (Powell & Mountcastle, 1959), auditory cortex (Abeles & Goldstein, 1970), motor cortex (Welt, Aschoff, Kameda & Brooks, 1967) and, of course, in the cat and monkey visual cortex.

The presence of columns is characteristically revealed when an electrode encounters a long series of cells arranged in depth in the cortex with some feature common to their stimulus requirements. In the cat visual area 1 the orientation selectivity of the cells is the same. No long series of orientation selective units have been encountered during penetrations of the rabbit visual cortex. In fact, other than a common location in the visual field, no feature common to all or even the majority of units encountered during an electrode insertion has been noted in the rabbit.

The fact that units with different properties are encountered during one
electrode penetration is not of itself adequate evidence for the absence of the columnar organisation. Units with a variety of characteristics are routinely encountered during an electrode penetration in the cat because there appears to be no columnar organisation with respect to bar, slit, edge or directional properties, although in the monkey there appear to be hints of 'nests' of these classes superimposed on the orientation columns. Furthermore, the recent reports of nonorientation selective circular and directional receptive fields in the cat cortex indicate that these units must appear within orientation columns (confirmed by Blakemore, personal communication) so that at least a few of the cells encountered will be without the common orientational selectivity of the columns in which they are recorded. In the cat, however, such cells are rare; in the rabbit it is the cells with common features that are rare.

The rabbit visual cortex may readily be seen to possess the radial fibre fasciculi and cell columns which have been suggested as the possible anatomical basis of the functional columns (Hubel & Wiesel, 1963: 1968). The work of Globus & Scheibel (1967) on the rabbit cortex has shown that the histological features of Golgi preparations are organised vertically into a modular structure such as has been observed in the cat cortex by Sholl (1956) and Colonnier (1966). Cortical histology thus indicates the potentiality for interaction between cells possessing a physiologically defined community of receptive field location in visual space. It is hard to imagine that this is not accompanied by some physiological indication of organisation related to the interaction, even if it is not so overt as the common orientation selectivity found in the cat.

Ogawa et al. (1968), while denying the presence of columns in the rabbit cortex, have claimed that if more than one orientation selective unit happens to be recorded within an electrode insertion then the receptive field axes are parallel or perpendicular. This arrangement is similar to that reported in the dual orientation columns of the cat area VIII (Hubel & Wiesel, 1965) but in the rabbit it may usually be accounted for by the limited variety of orientation selectivity available. No common preferred directions were noted for the directional units.

The number of units obtained during an electrode 'stab' in the rabbit is much more limited than in the case of Hubel & Wiesel's recordings from the cat. In view of the larger variety of properties found in the rabbit cortex perhaps only two in ten units encountered during a good electrode insertion will be of similar class. The features common within a class of the units of an anatomical column will thus be harder to detect. Blakemore (1970) has, however, estab-
lished the presence of two classes of column in the cat on the basis of smaller samples of units than used by HUBEL & WIESEL.

OGAWA et al. (1968) have described the cortical units from the region of visual streak under the optic nerve head as possessing oval receptive fields in which the major axis runs parallel to the streak. This ovality appears to be similar to that evidenced in the retina (page 60) and is independent of the functionally defined receptive field axis. It must not be construed as a feature indicating columnar organisation when encountered in successive neurons.

Some Speculations

Is Visual Cortex Organisation Uniform in the Rabbit? All of the currently available reports on rabbit cortical units are concerned with the properties of the region receiving the representation of the lateral visual field. It is natural to wonder whether the apparent absence of the hierarchical and columnar organisation characteristic of the cat and monkey visual cortex is peculiar to the representation of the lateral field in the rabbit or whether it extends throughout the whole visual cortex and is indicative of a marked species difference.

The lateral field of the rabbit is overtly equivalent to the 'temporal crescent' of the cat but the corresponding retinal regions are not organised in similar fashion. The extension of the rabbit area centralis to form the visual streak means that, whereas in the cat a twelve fold decrease in ganglion cell density accompanies a movement of 30° peripheral from the decussation line, in the rabbit an excursion of up to 90° lateral along the retinal equator reveals an increase in density.

Similarly, the single units recorded from the central visual streak have much more in common with those of the area centralis of the cat than with those of the corresponding retinal region; the receptive field centres are small, the surrounds are more powerful than those of the peripheral units and the density of nonconcentric units is highest in both species.

There is good behavioural evidence which confirms the above indications that the central region of the visual streak is adapted as a fixation area similar to the temporal region adjacent to the decussation line - or the area centralis of the cat. A rabbit will rotate its head about the long axis and in the horizontal plane to bring images of interest to the region of enhanced resolution in the central visual streak under conditions not involving either forward locomotion or depth perception; if the latter are involved then the nasal field is used.
In the retina there is no indication that differences should be expected between the single unit properties of the central and temporal visual streak. The ganglion cell density does not differ greatly in the two regions and the cell diameter population is very similar.

On the other hand, an element of the lateral field representation in the rabbit cortex receives only one third of the area that is devoted to an equivalent region of the nasal field. This difference might be regarded as a basis for not accepting the unit population of the cortical representation of the lateral field as potentially similar to that of the nasal region. In the cat, however, there is a one hundred and twenty fold reduction in the cortical area representing a square degree of visual field when passing from the projection of the area centralis to that of a region 30° peripheral to it. In spite of this, orientation selective units (Joshua & Bishop, 1970: Whitteridge, personal communication) predominate, arranged in columns (Blakemore, personal communication), within at least 50° of the area centralis projection. The main features of cat cortical organisation thus appear to be uninfluenced by a change in areal magnification which is 40 times greater than the disparity noted between the nasal and lateral field representations in the rabbit cortex. It is thus improbable that this factor would be the basis of a difference in the organisation of the two regions which would make the units of the lateral field representation untypical of the cortex dealing with the nasal field.

The apparent interchangeability of the lateral and nasal fixation areas during visual behaviour not involving binocular vision has not been investigated by psychological techniques in the rabbit. In view of the above findings it appears that the lateral and nasal field are both capable of detailed form vision in spite of the absence of the hierarchical and orientation column system in the cortex corresponding to the lateral field and its probable absence from that of the nasal field. The organisation and potentiality of the cortex dealing with the two fixation areas must of course differ as a result of the ipsilateral projections to the binocular area.

Is the Absence of Both the Orientation Columns and Hierarchy in the Rabbit Related to their Functional Association? Every variety of column which has been found in the visual cortex of either cat (Blakemore, 1970: Hubel & Wiesel, 1962, 1965) or monkey (Hubel & Wiesel, 1968) is organised with respect to the receptive field orientation. The reported column varieties depend upon the connections of the ipsilateral eye which superimpose second order features
A. HUGHES

such as eye dominance, disparity and direction upon the orientation columns. Orientation selectivity is also common to the units in the tentative columns or 'nests' organised around such features as bar, slit or directional selectivity (Hubel & Wiesel, 1962, 1968). In view of Hubel & Wiesel's hypothesis that the orientation columns are the means of assembling the hierarchical series of cortical receptive fields it appears significant that, although orientation selective units are present, both the orientation selective hierarchy and the orientation selective columns are absent or poorly developed in the case of the rabbit. The possibility of other more subtle organisational features within an anatomical column of the rabbit cortex has already been discussed and there is no indication that binocular columns will be absent.

The superimposed binocular characteristics of columns in the cat cortex have been argued by Blakemore (1970) to play a part in the control of eye movement; specifically he relates the direction columns to conjugate, and the depth columns to both depth perception and disjunctive eye movements. It has been shown in a previous section that at least the conjugate voluntary eye movements are present in the rabbit, contrary to current opinion (e.g. Collewijn, 1970), but vergence movements have not been observed. Depth perception has, however, been demonstrated in this animal. The binocular area of the rabbit cortex may thus be revealed to possess both eye dominance and more sophisticated varieties of binocular column. The probable presence of eye dominance columns in the primitive unit population of the American opossum has been noted by Christensen & Hill (1970) in the absence of any sign of orientation selectivity.

Quantitative Indications of Species Difference In a discussion about neurological characteristics relating to behavioural capacity, Lasley (1949) has said, 'We must seek the clue to behavioural evolution in the number and interconnections of the nerve cells . . . . not in their gross structural arrangement.' The increase from 7.5 to 1,330 mm² in striate cortex area which accompanies the striking improvement of visual discrimination and generalisation between the rat and monkey (Table III) is a crude indication of species difference which takes no account of the disparity in the cortical representation of the central and peripheral field, or of species differences in cortical thickness, neuron density, and size of visual field. Information now available in the literature enables calculation of the neuronal density per square degree of the visual field in the cortex at the projection of the visual axis. An index of cortical provision for a region, of common function in different species, is thus obtained which is
suitable for interspecies comparison and relates to the area whose performance is that usually expressed in behavioural and physiological data.

The results of the calculation, which are given in Table III, indicate that in the representation of the square degree of visual field about the fixation axis the monkey possesses 23 times as many cortical neurones as the cat but exceeds the provision in the rabbit by some 880–2,700 times, depending upon whether the nasal or lateral fixation area of the latter is selected for comparison. The cortical provision for the central field in the cat is thus closer to that of the monkey than to that of the rabbit whose cortical neurone density per square degree it surpasses by a factor of 38–115.

The cortical neuronal density per square degree of visual field may be regarded as the product of two useful components; the retinal ganglion density per square degree and the number of cortical neurones available for each retinal ganglion cell. Although these factors are probably not totally independent, they do appear to correlate with separate behavioural features.

The visual acuity of a species appears to be in closer relationship to retinal than to cortical cell density. The receptor mosaic is of similar density in cat, rabbit and monkey and is relatively uniform across the retina of one species. Within the region of central vision, however, the degree of convergence from receptors to ganglion cells varies considerably between species and may be seen to parallel the differences in their behaviourally determined acuity. Thus between the rat and macaque convergence varies 30 fold and acuity 37 fold (Table III). The retinal ganglion cell density per square degree on the visual axis consequently also varies with the visual acuity. The presently available information on the rat does not indicate such a close relationship as is found in the case of the monkey, cat and rabbit. A similar association has been suggested to be present in a series of nocturnal and diurnal primates (Ordy & Samorajsky, 1968).

The ratio of cortical cells to retinal ganglion cells dealing with the same element of the central visual field appears to provide an anatomical index which correlates well with the physiological organisation of the visual cortex in different species. In view of the tendency for the tectal connections to decrease in importance upon ascending the phylogenetic series, an interspecies comparison of the index might give misleading results if considerably different proportions of retinal ganglion cells fail to supply the L.G.N. In the rabbit, the small medial division of the optic tract passes directly to the superior colliculus but the majority of the tectal input is derived from the fibres of the
<table>
<thead>
<tr>
<th>Animal</th>
<th>Code</th>
<th>Area of Area 17 mm²</th>
<th>Superior Colliculus Area mm²</th>
<th>Ratio of Areas of VI/S. Coll.</th>
<th>Cortex Magnification mm²/sq.deg.</th>
<th>Area 17 Cortical Thickness mm.</th>
<th>Area 17 Neurone Density No./mm²</th>
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<td>45.10⁴</td>
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Data for quantitative indications of species difference

The information included in this table for the rabbit is mainly derived from material presented elsewhere in the text. Information for other species has been taken from figures quoted in the text or measured from diagrams in the following works. In many cases the information is unreliable or based on too few instances but is included in order to enable some estimates of the order of magnitude of species differences to be carried out at this time.

Area of Striate Cortex

(M) DANIEL & WHITTERIDGE, 1961; (C) BILGE, BINGLE, SENEVIRATNE & WHITTERIDGE, 1967; (Rt) ADAMS & FORRESTER, 1968.

Area of Superior Colliculus


Cortical Magnification mm²/sq. deg.

(M) DANIEL & WHITTERIDGE, 1961: ROLLS & COWEY, 1970; (C) BILGE, BINGLE, SENEVIRATNE & WHITTERIDGE, 1967; measurements made along horizontal of this map were extrapolated into central field by fitting curve to the ganglion cell count of STONE, 1965, and L.G.N. Magnification factor of SENEVIRATNE, 1963, to give 2 mm² for 0-1° or 2.3 mm² on basis of g.cell count and cortical area for 0-5°; lower value accepted. Direct measurement 2 mm², MANDL (BURNS, 1962). (Rt) ADAMS & FORRESTER, 1968.
### Table III

<table>
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<td>37:1</td>
<td>30:1</td>
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**Area 17 Thickness**

(M) HUBEL & WIESEL, 1968; (C) HUBEL & WIESEL, 1965. Measured from their drawings which are corrected for shrinkage. (R) ADAMS & FORRESTER, 1968.

**Neurone Density in Area 17**

(M), (C), (R) & (Rt) Cragg, 1967. Absolute values corrected for shrinkage.

**Ganglion Cell Count/sq. deg.**

(M) ROLLS & COWEY, 1967. In this paper it is demonstrated to be a reasonable hypothesis that there is one ganglion cell for each cone, at least. The included cone count data was therefore used to derive an estimate of the effective ganglion cell density for the foveal region. (C), STONE, 1965; (R) HUGHES & WHITTERIDGE, (in preparation) & BROWN, 1965 for mm/° on retina.

**Acuity**

(M) ROLLS & COWEY, 1970; (C) SMITH, 1938; (R) VAN HOF, 1967 AV. of 4; (Rt) HERMAN, 1956 AV. of 3 for 80% criterion.

**Receptors/Ganglion Cell**

(M) ROLLS & COWEY, 1970; (C) CHEVITZ, 1891; (Rt) LASHLEY, 1939.
superior quadrigeminal brachium which branch to innervate the L.G.N.,
(gioli & Guthrie, 1969); a similar arrangement obtains in the cat (barris,
ingram & ransom, 1935). There is also electrophysiological evidence that the
majority of the input to the superior colliculus is in the form of collaterals of
both fast and slow fibres in the rabbit (lederman & noell, 1968) and cat
(hayashi, sumitomo & iwama, 1967). It is thus probable that similar proportions
of retinal ganglion cells project to the L.G.N, in the monkey, rabbit & cat and
that the functional implications of the index may be of significance.

Table III shows that between the cat and monkey the cortical provision for
each retinal ganglion cell on the visual axis increases only twofold whereas the
retinal ganglion cell concentration is increased tenfold. Conversely, between
the rabbit and the cat the retinal ganglion cell density increases twofold whereas
the cortical provision for each retinal ganglion cell is increased 15-67 fold. The similarity of cortical unit organisation in the cat and monkey and the pre¬
viously demonstrated difference between their organisation in the cat and rabbit
is therefore accompanied by a corresponding similarity and difference in the
respective cortical provision of neurones for each retinal ganglion cell on the
visual axis. It is understandable that the synthesis of units for some 15 classes of
orientation column in each element of visual field should lead to a much greater
provision of cortical neurones per retinal ganglion cell in the cat and monkey
cortex than is the case in the rabbit where extensive multiplication of cortical
representation does not occur.

It should be noted that the cortical provision for each ganglion cell on the
visual axis of the rat is only 1/11 of that for a cell in the corresponding part of
the rabbit temporal retina in spite of the lower retinal ganglion cell density per
square degree in the rat. The presence of a purely concentric unit population
in the retina of the rat (brown & rojas, 1965; partridge & brown, 1970;
hughes, unpublished observations) might lead to the conclusion that its cortical
unit organisation would be like that of the monkey or cat. If the predominantly
orientation selective cortex which is characteristic of the cat and monkey
requires a large cortical provision of neurones for the synthesis of orientation
selective units in at least fifteen varieties of orientation column then it appears
unlikely that the rat cortex, with only 1/167 of the cortical provision in the cat,
will be similarly organised. It is possible that the rat cortex will be organised
in the unsophisticated manner of the American opossum (christensen & hill,
1970).
**Series/Parallel Information Processing** Information transfer in the visual system is carried out in a fashion which combines the features of serial and parallel processing. A parallel system is able to handle information in numerous separate channels simultaneously and because of this it is many times faster than a serial system. The high speed of the parallel system is, however, gained at the 'cost' of a greatly increased number of components. On the other hand the economy of components which is effected by serial sampling of an input matrix is accompanied by both the risk of missing events occurring during the dead time between sampling and a lower rate of information transfer.

The primate fovea attains an ideal parallel organisation in that the individual channels of the receptor mosaic appear to be retained from the receptors to the optic nerve. In the area centralis of other species, and in the periphery of all, the extensive parallel processing of visual information at the receptor level is not continued at later stages; instead, a considerable economy in the number of central components required for the analysis of the incoming data is effected by the convergence of the input to successive stages. In all species so far examined there is a gradient of convergence from the central retina, where parallel processing and detail vision are optimal for the species, to the peripheral areas in which convergence is most prominent and sensitivity to movement and light is greatest. The reduced resolution of parallel processing in the peripheral retina introduces the need for serial sampling in detail vision so that objects of interest in the peripheral visual field may be brought onto the visual axis for examination. If a blind area is present to the rear of the head then serial sampling is also required for peripheral vision. The time required for the necessary eye and head movements reduces the speed of information acquisition in the fashion characteristic for the replacement of a parallel by a serial process.

The balance between serial and parallel processing varies considerably from one species to another. The extensive blind area and the restricted area centralis in cat and monkey mean that peripheral and central vision are organised in a predominantly serial fashion. It has been established at some length in an earlier section that the use of the binocular and lateral fixation areas in the rabbit also necessitate serial analysis of the visual environment by means of eye and head movements. It was pointed out, however, that such movements occur less frequently than in cat and monkey and that in the freeze condition they are totally abolished. It is in this state that the potential for parallel processing in the rabbit visual system is fully exploited in a manner understandable in terms of the animal's way of life. The large monocular field elimi-
brates the risk of missing events occurring during the sampling dead time which is present if serial scanning is required to give all-round peripheral vision. Delay in detection of predators resulting from such a sampling error could be disastrous in the case of an animal depending upon running away for its survival. The remarkable emphasis on parallel processing in the rabbit visual system is best revealed, however, by the horizontal extension of the area centralis to enable a detailed simultaneous analysis of 360° of visual horizon at the retinal level without the giveaway eye and head movements which feature so markedly in animals depending upon serial sampling of their visual environment for detail vision.

A square degree of visual field in the representation of the visual streak on the rabbit cortical map occupies an area varying not much more than fivefold from the decussation line to the T 60° meridian. For comparison, the representation of a square degree in the cat area 17 is reduced about 120 times in area for a passage of only 30° peripheral of the area centralis. The relative uniformity of the rabbit visual streak representation demonstrates that, except for the extreme temporal field, the emphasis on parallel processing obtaining in the retina is not surrendered by differential convergence or expansion in the retinocortical pathway. It is the retention of an extensive field of parallel processing at the cortical level, in conjunction with the small area of the visual cortex, which brings about the limited cortical provision for each retinal ganglion cell that was demonstrated for the rabbit in the previous section.

Only massive convergence of almost the whole output of the visual streak would enable adequate provision to be made within the limited visual cortex of the rabbit for the representation of even a portion of the visual streak on the same scale as that of the cat area centralis. As an alternative to the abandonment of parallel processing at the cortical level, let us imagine that the rabbit cortex be expanded to fulfil our requirements. If the area centralis is defined for both rabbit and cat as the area in which the retinal ganglion cell density exceeds 2,000/mm² then it contains 5% of the total count of retinal ganglion cells in the cat and 50% of the total in the rabbit. In absolute terms this is 5,000 cells in the cat but 150,000 in the rabbit – 30 times as many. If similar proportions of these fibres project to the L.G.N, in the two species then the area of the rabbit visual area I would have to be increased some 25 times for an analysis of the output of the visual streak to be performed by cortical tissue organised in similar fashion to that which deals with the output of the cat area centralis. The 80 mm² of the rabbit area 17 already represents 20% of the neocortex or
10% of the total available cortex (BLINKOV & GLEZER, 1968).

Area 17 is not the only region of cortex predominantly devoted to visual functions. Table IV contains information derived from SOLNITZKY & HARMON (1946) concerning the relative extent of the striate and extrastriate regions in certain selected mammals.

Conflict with data in Table III results from the use of different sources. It appears from this table that the tendency for increase in the absolute area of area 17 upon ascending the phylogenetic scale is associated with an increase in relative area of the summed extrastriate visual cortex (areas 18 & 19). This tendency increases the disparity in cortical provision for an element of visual field between the rabbit, with one extrastriate area, and the higher mammals if the corresponding extrastriate regions are included in the estimate. The disparity would be even greater if the presence of the CLARE-BISHOP area (HUBEL & WIESEL, 1969) and the inferotemporal area (GROSS, BENDER & ROCHA-MIRANDA, 1969) were to be taken into account in the cat and monkey respectively.

The possession of limited extrastriate cortex might be thought to be necessarily linked with phylogenetic status from an examination of the above table. Recent evidence suggests, however, that ecological factors may play an important part in the determination of the relative development of the striate and extrastriate regions. According to DIAMOND & HALL (1969), the grey squirrel possesses a cortical organisation akin to that of the phylogenetically distant but similarly arboreal tree shrew. The clearly laminated L.G.N. projects to the striate area alone while the adjacent lateroposterior nucleus projects to a relatively enormous area of temporal cortex which has been electrophysiologically defined as visual. This region includes VII which is quite small. An examination of the evoked potential maps of the rabbit cortex (WOOLSEY, 1958), or of the hedgehog (DIAMOND & HALL, 1969), reveals that the border of VII abuts the auditory cortex in a fashion quite unlike that of the grey squirrel.

### Table IV

<table>
<thead>
<tr>
<th>Area</th>
<th>Visual Cortex mm²</th>
<th>17</th>
<th>18+19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepus</td>
<td>107</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>Felis</td>
<td>388</td>
<td>67</td>
<td>33</td>
</tr>
<tr>
<td>Cercopithecus</td>
<td>2,268</td>
<td>41</td>
<td>59</td>
</tr>
<tr>
<td>Man</td>
<td>10,490</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>

10% of the total available cortex (BLINKOV & GLEZER, 1968).
where a considerable distance intervenes between the two areas. There is no space in the rabbit and hedgehog for a projection of the lateroposterior nucleus to a visual area outside of VII.

These findings may go some way in accounting for the apparent discrepancies in single unit organisation between the ground squirrel and rabbit which are indicated by the work of Michael (1967). The ground squirrel appears to resemble the cat and monkey in that the retinal projection to the L.G.N. consists almost entirely of concentric units while the deeper collicular units, of imputed extrastriate cortical origin, show orientation selectivity in their central and flanking areas. The grey squirrel does not possess a visual streak but the uniformity of its ganglion cells distribution in horizontal and vertical is sufficient for the animal to be regarded as carrying out a predominantly parallel analysis like the ground squirrel. This feature and the retention of some arboreal habits by the ground squirrel suggest that the visual cortex of this animal may be organised in similar fashion to that of the grey squirrel. If this is the case, then the large temporal area of extrastriate visual cortex may facilitate a cortical unit organisation more akin to that of the cat than to that of the rabbit in spite of the emphasis on parallel processing in the retina and the small size of the primary visual cortex.

The extrastriate visual areas may no longer be thought of as a set of ascending stations linked in serial order to one another as appeared to be the case for cat VI, VII and VIII (Hubel & Wiesel, 1965). In the cat, VII and VIII both receive projections from VI (Hubel & Wiesel, 1965) but each subdivision obtains a separate thalamic input so that they must all be regarded as operating to some extent in parallel. An increase in the extrastriate representation, as seen in the grey squirrel, does not necessarily mean that the tasks carried out by area 17 will be lightened because behavioural capacity in addition to that present in an animal with a restricted representation may be introduced. In the case of the tree shrew and squirrel, however, it does appear from behavioural investigation of visual discrimination following discrete lesions that the extensive temporal visual area confers discriminative powers on the dextrate animal that are lacking in a similarly treated hedgehog (Diamond & Hall, 1969) so that we must countenance the possibility that the properties of the rabbit cortical unit organisation are related to the poor development of the extrastriate regions as well as to the limited extent of the primary visual cortex.

The apparent absence of the orientation selective hierarchy and orientation columns, as well as the limited variety of orientation selective units in the
cortical representation of the lateral field have been suggested on previous pages to be interrelated with and ultimately attributable to the small ratio of cortical to retinal ganglion cells. The large population of units with varied selectivity at the retinal level in the rabbit may be regarded as possibly 'easing the burden' on the limited cortical system by leaving generalisation as its main task. The above indication that the paucity of cortical provision for retinal ganglion cells in the fixation areas arises in the rabbit as a result of the stress on parallel analysis links these special features of the single unit organisation to the same factor. It is found, therefore, that many characteristics of the rabbit single unit population, cortical organisation, and eye and head movement pattern may be interpreted as forming part and parcel of the adaptation to parallel processing which has been revealed by the topographical analysis of anatomical organisation at the various levels of the animal's visual system. As Barlow (1961) has said:

'It is foolish to investigate sensory mechanisms blindly – one must also look at the ways in which animals make use of their senses. It would be surprising if the use to which they are put was not reflected in the design of the sense organs and their nervous pathways – as surprising as it would be for a bird's wing to be like a horse's hoof.'

Conclusion On the basis of his studies of visual discrimination in the octopus, rat and other vertebrates, Sutherland (1968) has concluded that,

'Although the similarities in the way in which visual discrimination operates are much more striking than the differences, detailed investigation has revealed some differences ....... which suggest that we are dealing with small variations in some common basic mechanism.'

Arden (1963) has based a similar suggestion on physiological data,

'It may be that similar functional organisation occurs in the visual system of all vertebrates, but the less encephalised the animal, the lower the level in the chain of sensory neurones at which sophisticated analyses of the incoming information are made.'

Certain aspects of visual processing are clearly carried out in a fundamentally similar fashion in all species so far examined. The selectivity of the retinal and cortical units reveals that information in the visual image is subjected to a process of filtering in its passage through the visual pathways and even in the monkey eye the function of the retina is not to 'transmit information about the point to point distribution of light and dark in the image'. In mathematical
terms, the process of filtering is equivalent to the convolution of the visual signal with a weighting function which is physically represented by a class of retinal or cortical unit. The variation in the unit population between species is evolutionarily determined so that the selectivity of the filtering process is presumably matched to the requirements of the animal and the nature of its environment according to similar principles to those which govern the choice of one specific set of the large class of orthogonal functions rather than another when the maximum separation of certain components of a specific class of signal is required by means of convolution.

The lack of insight into the functioning of the cat or monkey visual cortex means that when comparing its unit properties with those of the rabbit we have no basis for recognising which differences are trivial and which are not. The situation is analogous to one in which we have been presented with two sets of coefficients and asked their significance; unless we know that one set indicates the magnitude of a series of sine and cosine waves and the other the magnitude of a set of Tchebychev polynomials, we have no chance of recognising that the coefficients represent the same signal analysed by similar processes into different sets of orthogonal components. Whether the variation in retinal and cortical unit properties between species is sufficient, of itself, to establish corresponding variations in their behavioural capacity thus remains to be seen. It seems probable, however, that the growing body of comparative work on visual discrimination will demonstrate this to be the case, as has already been established with respect to visual acuity and spectral sensitivity.

The extent to which the optimal stimulus for a given unit class corresponds with some specific feature of the visual environment has been an important problem in retina and cortex. A common assumption concerning the organisation of sensory filters has been described by Barlow as the 'password' or 'key-feature' hypothesis (1961).

'Specific classes of stimuli act as 'releasers' and evoke specific responses; these classes of stimuli are thought of as 'passwords' which have to be distinguished from all other stimuli, and it is suggested that their detection may be the important function of sensory relays.'

The decision as to whether a given class of units abstracts a 'key-feature', or 'password', at a given stage of processing must be based upon a detailed knowledge of the optimal stimulus for the unit and for the behavioural 'releaser'. Ambiguity in the response of the unit requires careful consideration of whether it would be apparent in the natural visual environment. Without close
RELATION BETWEEN ANATOMY AND PHYSIOLOGY

matching of the behavioural and unit properties, however, the identification of key-features is as premature as Maturana’s attempt to classify visual systems into determinate and indeterminate forms (1964) on the basis of the properties of the retinal units alone.

The concept of ‘key-features’ or ‘functional natural invariants’ perhaps originated with Barlow’s suggestion that certain units of the frog retina act as fly-detectors (1953) but is better known in its application to all the unit classes of the frog retina by Maturana, Letvin, McCulloch & Pitts (1960). The fate of the concept of invariants in relation to the visual system of the frog illustrates well the problems arising in its application to the mammalian visual cortex. In their stimulating paper, Maturana et al. (1960) begin by asking,

‘Does the retina perform an analysis and abstract the meaningful parameters that will permit the recognition of the universals or is this analysis performed only later in the visual centres? Any mechanism that permits the recognition of prey and enemy in as direct a manner as possible is of great advantage in survival.’

Their conclusions are somewhat ambiguous. On the one hand, Letvin et al. (1959) remark that the single unit, ‘operations have much more the flavour of perception than of sensation . . . . . We have been tempted, for example, to call the convexity detectors ‘bug-perceivers’.

While, on the other hand, they clearly indicate appreciation of the need for further processing in the tectum (Maturana et al., 1960) by the interaction of the four classes of retinal unit before invariants can be formed,

‘In a system like this, a unique combination of the four qualitative contexts in a certain spatial relation may define a class of objects . . . . . Obviously, this is a way in which the universals ‘prey’ and ‘enemy’ can be recognised . . . .’

The recent work of Grüsser & Grüsser-Cornehls (1966) (see also Butenandt & Grüsser, 1968) has shown that the prey catching of the frog reveals discriminatory features which require further processing of the retinal unit output for the explanation of its properties. Ingle (1968) has confirmed this finding and also reports that the orientation response is elicited by stimuli which generate only weak firing from class II, bugdetector, retinal units. Thus he concludes,

‘frogs possess such abilities as ‘constancies’ and ‘gestalt processes’, which cannot be predicted on the basis of known retinal electrophysiology.’
The ambiguity of response of the frog retinal units has led Grüsser, Finkelstein & Grüsser-Cornehls (1968) to go further than this; thus they point out that all four classes of frog retinal unit will respond to a 3° black spot moving at 5°/sec. and conclude, ‘It is evident that in the different classes of retinal neurones no ‘functional natural invariants’ are formed out of the set of possible natural stimuli as was assumed by Maturana, Letvin et al.’

In the case of cortical cells the use of the concept of invariants has not been overt. Hubel & Wiesel have been very cautious about attributing specific perceptual roles to cortical units but their working hypothesis has been summarised (1968) for the cat and monkey, ‘It is clear that any small region of the striate cortex analyses some small part of the visual field in terms of the direction light-dark contours . . . . a detection of the movement of the contours, a registration of the type of the contour (light against dark, edge, and the like), and, at the hypercomplex level, a detection of any change in direction (curvature) of the contours.’

Certain properties of the cortical units in the cat have led Spinelli & Barrett (1969) to question this conception of the role of simple and complex units, ‘The crucial question, then, centres on whether units with line shaped receptive fields are to be considered ‘detectors of lines’ and nothing else, i.e. nonlinear elements; or as operators with a more or less linear transfer characteristic capable of responding to a greater variety of stimuli in different ways.’

They conclude that the cortical units cannot be thought of as purely detectors of lines because out of 165 all could be stimulated by a small moving spot of light. Creutzfeldt & Itô (1968) have pointed out that in their experiments the majority of cortical neurones cannot distinguish a dark spot or bar in one part of their field from a spot or bar of light in another. They question the possibility of applying the concept of well defined functional selectivity to classes of such units; the optimal stimulus requirements are regarded as arising fortuitously.

On this basis the applicability of the concept of invariants has been questioned in at least the primary stages of cortical visual processing (Creutzfeldt & Sakmann, 1969), ‘No convincing correlations have yet been found between certain optimal stimuli of higher order neurones and correspondent ‘units of perception’. Therefore the question arises as to whether the hypothesis is correct that different stimulus aspects (‘invariants’) are processed in separate channels.
This hypothesis is often not stated clearly and is only implicit in the methods of investigation and presentation of data. It is the case, however, that the majority of visual stimuli are extended in visual space and are not small spots of light so that their contours may be effective as stimuli without the ambiguity revealed under experimental conditions by the above authors. Creuzfeldt & Ito (1968) also fail to take into account the specificity indicated in the columnar organisation of orientation selectivity which must arise by organised ‘wiring up’ during development because it is present before the eyes open in the kitten (Hubel & Wiesel, 1963).

The orientation selectivity and enhancement of contour evinced in the cat and monkey cortical units does not necessarily mean that the cortex must be regarded as extracting a cartoon-like representation of the visual environment in the firing hyperspace of the neuronal population. The discovery of the spatial frequency selectivity of cat cortical cells (Campbell, Cooper & Enroth-Cugell, 1969) and of similar characteristics in the human visual system (Blake-More & Campbell, 1968) provides an alternative concept. The latter authors have tentatively suggested that the visual input is recoded in terms of its component spatial frequencies in each orientation dimension in order to provide a compact and potentially size independent code for the analysis and store of visual information. In such a system the separate channels for processing different stimulus aspects remain but are related in a more abstract fashion to the components of the retinal image than has previously been envisaged in theories of ‘natural functional invariants’. A number of objections to this theory readily spring to mind but it will have played an important role if it stimulates the application of concepts from the rapidly expanding field of visual information processing to the analysis of visual cortex function.

Momentarily neglecting the above considerations, we might hazard the guess that the small percentage of orientation and bar selective units and their relative simplicity of organisation in the rabbit suggest that, relative to the cat and monkey, movement rather than contour extraction may play the major role in separating figure from ground. The ‘hypercomplex’ properties of some of the very large field ON-OFF units in the rabbit cortex ensure that they signal the presence of moving stimuli only within a specific angular size range and a given region of visual space. It is, however, clear from the material presented in this symposium that a sharp distinction cannot be drawn between the monkey, cat and rabbit on the basis of either their cortical units or their performance in elementary form discrimination. In this context it is important to note that the
The greatest specialisation of the retinal units is confined to the visual streak. The majority of concentric, directional and local edge units in this area possess very powerful surrounds and respond only to stimuli subtending angles of no more than 1° and moving at less than 4°/sec. These units analyse the image of the visual horizon and by their size and velocity selectivity are appropriately suited to the sole detection of the images of distant objects e.g. another rabbit at 20 m and moving at 4 m.p.h. produces an image just small enough and slow enough to fire a streak concentric unit. It will be clear that any improved behavioural capability of the rabbit relative to the cat and monkey which results from the specialisation of the visual cortex to deal with this kind of information will not be observed in behavioural experiments emphasising classic form analysis. In this respect rabbit behaviour remains unexplored.

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RELATION BETWEEN ANATOMY AND PHYSIOLOGY


--- Receptive fields of single optic nerve fibers in a mammal with an all-cone retina.


RELATION BETWEEN ANATOMY AND PHYSIOLOGY


RELATION BETWEEN ANATOMY AND PHYSIOLOGY

