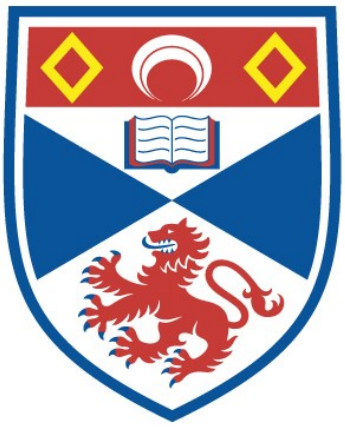
SPECTRAL PROPERTIES OF DARK-ADAPTED PLAICE RETINAL GANGLION CELLS

Peter Hammond

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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by

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and

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SUPERVISOR'S CERTIFICATE

I certify that Peter Hammond has fulfilled the conditions laid down under Ordinance No. 16 of the University Court, St. Andrews, and is accordingly qualified to submit this thesis for the Degree of Doctor of Philosophy.

DECLARATION

I declare that the work reported in this thesis is my own and has not been previously submitted for any other degree.

CURRICULUM VITAE

I graduated in Physiology at University College, London in 1964. The work reported here was performed between 1964 and 1967, while in tenure of a St. Andrews studentship awarded by the University. The first eighteen months of this period was taken up by the development of a suitable preparation, and preliminary experiments with colourless visual stimuli. The latter period was concerned with colour vision of plaice, the results of which are detailed in this thesis.

ACKNOWLEDGEMENTS

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CONTENTS

SUMMARY

1. The spectral, spatial and temporal properties of receptive fields of dark-adapted, on-off retinal ganglion cells in the intact eye of the plaice, have been analysed by recording responses from their axon terminals in the superficial layers of the contra-lateral optic tectum with indium micro-electrodes.

2. Two cell-types have been identified on criteria of discharge patterns. The first type gives spectrally opponent "on-off" responses to coloured stimuli, with no subdivision of receptive fields into centre and periphery. "On" and "off" response-components are mutually inhibitory.

The second type gives slow-adapting, "on-off" or "off" responses for different stimulus positions within the receptive field, with centre-surround or adjacent field patterns. Only "on-off" centre, "off"-surround cells, or "off"-centre, "on-off" surround cells have been found. "On-off" centre cells exhibit mutual antagonism between field centre and surround. "Off"-centre cells possess inhibitory centres. This cell-type gives only weak opponent, or possibly non-opponent responses.

3. Most cells of each type receive rod input in addition to input from cones. At stimulus intensities suprathreshold

for cones, response-components give spectral maxima in one or more of four wavelength ranges; blue, 440-460 mμ; blue-green, 470-490 mμ; green, 510-540 mμ; and orange, 560-590 mμ. No cells give red sensitivity maxima. At low stimulus intensities all cells with rod input give a single spectral peak between 520 and 530 mμ.

INTRODUCTION

At the retinal level in the vertebrate eye a large number of receptors converge upon a smaller number of bipolar cells, which converge in turn on single ganglion cells. Thus each ganglion cell "sees" a small part of the total visual field of one eye, the receptive field, and is responsive to visual stimuli placed within that area. In addition there is a parallel divergence by which a single receptor inputs to a large number of ganglion cells. This divergence results in considerable overlap of receptive fields. By virtue of both convergence and divergence, and the excitatory or inhibitory inputs from discrete receptor populations via bipolar cells, a point to point representation of the entire visual field is encoded as impulses in the ganglion cell axons running in the optic nerve.

The ganglion cells are the final common pathway to the optic nerve through which retinal visual information must pass. They therefore assume great significance since their responses must convey, in spatial and temporal parameters, the necessary information for central resolution of the visual image. Analysis of response patterns at this level in the visual pathway provides indirectly much

information on the nature of earlier retinal processes, for only recently has it proved possible to study such processes directly.

Features of retinal ganglion cells

1. Response types. Since Hartline, the properties of retinal ganglion cells have been widely studied in amphibia and terrestrial vertebrates, and more recently in fish. Hartline (1938; 1940a,b), recording from single units in the optic nerve of the frog, was able to identify three types of retinal ganglion cell: those responding only to retinal illumination, those responding only to extinction of illumination, and those responding both at presentation and extinction, termed on, off and on-off respectively. In all more recent studies ganglion cells have been found to fit essentially into one of these three types.

2. Receptive field sensitivity and inhibition. In the isolated frog retina Barlow (1953) observed that retinal ganglion cells were maximally sensitive at the receptive field centre, showing at the centre a plateau of high sensitivity, with a fall-off in sensitivity towards the periphery. In on-off units the rate of this fall-off was

different for the "on" and "off" components of the response. In off units subliminal stimuli summated over the whole receptive field, the strength of the response depending, within limits, only upon the total quantity of illumination. This finding was also true for the central portion of the receptive fields of on-off ganglion cells, but an additional phenomenon - inhibition - was apparent on illumination of the peripheral field or beyond the periphery. Further, Barlow found that the on-off ganglion cells were highly sensitive to movement.

3. Receptive field configurations. Evidence for the centre-surround receptive field arrangement of on-off retinal ganglion cells was first obtained in the cat by Kuffler (1953). He observed that receptive fields were roughly circular in shape and could be subdivided into two concentrically-arranged regions. These gave opposite responses when separately stimulated. Units were either "on"-centre or "off"-centre, with an antagonistic surround giving the opposite response. Essentially similar results have been obtained from a wide range of vertebrates in response to colourless stimuli (Hubel, 1960; Hubel & Wiesel, 1960; Jacobson & Gaze, 1964; Kuffler, Fitzhugh & Barlow, 1957; Rodeick & Stone, 1965b; Wiesel, 1960). In fish in particular, the "on" and "off" zones of ganglion

cells exhibiting spatial segregation of receptive fields are commonly adjacent rather than concentric (Jacobson & Gaze, 1964).

4. Movement detection. Barlow (1953) initially observed that the on-off retinal ganglion cells of the frog are sensitive to movement. This property has since been studied in the optic nerve and tectum, and at the retinal level. Movement sensitive units have also been identified in the lateral geniculate and cortex of higher vertebrates. In many instances it has also been shown that units are sensitive to the direction of movement. Directionally selective units are present at the retinal level in some animals. In others, this facility is found only in higher order cells. Directionally selective retinal ganglion cells have been identified in the rabbit (Barlow & Hill, 1963; Barlow, Hill & Levick, 1964; Barlow & Levick, 1965), in pigeon (Maturana & Frenk, 1963), in the ground squirrel (Michael, 1966a), and by inference from tectal recordings in frog (Lettvin, Maturana, Pitts & McCulloch, 1959) and tectal and optic nerve recordings in goldfish (Jacobson & Gaze, 1964). On the other hand such units have not been found at the retinal level in cat (Rodeick & Stone, 1965a,b; Rodeick, 1965). In this animal they have been identified both in the lateral geniculate and cortex (Hubel, 1959;

Hubel & Wiesel, 1959, 1962), and in the lateral geniculate of the rabbit (Arden, 1963b).

In goldfish Cronley-Dillon (1964) gives an account of one directionally-selective retinal ganglion cell type, based on recordings from the terminal arborisations of optic nerve axons in the tectum. In addition, he describes a second type with centre-surround receptive fields, that respond selectively to centrifugal or centripetal movement but which cannot be interpreted as directionally-selective in the true sense.

Colour vision

1. The opponent colour theory. The opponent colour theory of Hering (transl., 1964) envisages two opponent colour mechanisms in the retina, and a colourless black-white machanism concerned with luminosity functions. The two opponent colour mechanisms, red-green and yellow-blue, are considered each to ellicit retinal responses both for long and short wavelengths. In each case responses from the two ends of the spectrum are subtracted at the retinal level.

This theory supercedes the original concepts of Young embodied in the trichromacy theory. It is on the opponent theory that previous results and the results presented in this thesis are considered.

2. The retinal "S" potential. The first evidence that colour-dependent responses could be recorded electrophysiol: ogically from the retina was put forward by MacNichol & Svaetichin (1958). Using KCl micro-pipettes they recorded slow potential changes from the isolated retinae of several species of marine fish, to which they designated the term "S" potential (see also Svaetichin & MacNichol, 1958; MacNichol, 1966). Two types of "S" potential were identified; the luminosity or "L" response, showing hyperpolarisation at all wavelengths and associated with the giant horizontal cells of the outer plexiform layer; and the chromatic or "C" response, showing hyperpolarisation to long wavelengths and depolarisation to short wavelengths. The origin of the latter potential is uncertain, but probably stems from the bipolar or amacrine cells of the inner plexiform layer.

In deep sea fish, with achromatic vision, the "L" response alone is found. Amongst shallow water fish, all give the "L" response, together with the red-green or yellow-blue "C" responses, or both (MacNichol & Svaetichin, 1958).

3. Opponent responses from retinal ganglion cells. Studies of "S" potentials in fish, which originate at an earlier level in the visual pathway than retinal ganglion cells, indicated that the ganglion cells were likely to

be opponent colour-coded. MacNichol and co-workers recorded responses of single retinal ganglion cells in the isolated goldfish retina and showed that one type of on-off ganglion cell was indeed colour-coded (Wagner, MacNichol & Wolbarsht, 1960, 1963; MacNichol, Wolbarsht & Wagner, 1961; Wolbarsht, Wagner & MacNichol, 1961a,b; MacNichol, 1966). They identified two classes of opponent cell; those giving "on" responses to wavelengths shorter than 550 mμ and "off" responses to longer wavelengths; and those showing the converse, i.e. "off" responses to wavelengths shorter than 550 mμ and "on" responses to longer wavelengths. In many of these units no true centre-surround receptive field arrangement was found. Receptive fields could be subdivided only into regions more likely to give "on" responses and regions more likely to give "off" responses. A second on-off type, showing the classic centre-surround configuration of Kuffler, was identified, but lacked opponent colour properties, i.e. the spectral sensitivities of the field centre and surround appeared to be identical.

In line with the characteristics of "S" potentials these authors postulated that the "on", "off" and inhibitory aspects of retinal ganglion cell responses result from two discrete inputs from different populations of receptors, one excitatory, the other inhibitory, (Wolbarsht et al, 1961a).

The "on" response is ascribed to the excitatory input to the ganglion cell. The inhibitory input is considered to hyperpolarise the cell during retinal illumination. It accounts both for the inhibitory aspect apparent in responses, and for the "off" discharge which is a post-inhibitory rebound when the hyperpolarising influence is removed. It is in the light of these postulates that the properties of plaice retinal ganglion cells will be considered.

Jacobson (1964), recording from single units in goldfish tectum, identified the two opponent types postulated by Hering (viz. red-green and yellow-blue) and a further opponent type with red-blue sensitivity. Red-green units gave photopic sensitivity maxima at 630-651 mμ and 497-517 mμ; sensitivity maxima for yellow-blue units occurred between 552-605 and 462±14 mμ; and between 605-651 and 462±14 mμ for red-blue units.

Witkovsky (1965) measured the spectral sensitivity of retinal ganglion cells in the carp (Cyprinus) both in the dark-adapted and light-adapted state. When dark-adapted only on or off units were found. An additional wavelength-dependent on-off type appeared when light-adapted. All units gave scotopic sensitivity curves with a single spectral peak at about 520 mμ, which agreed well with the absorption

spectrum of rod pigment 523 isolated by Dartnall from carp retina. Photopic sensitivity maxima for on and off units occurred in one of four wavelength ranges - blue, green, orange and red - a number of units showing one or more secondary maxima. Most on-off units were orange-"on", blue-"off" or vice versa and showed characteristic opponent colour responses.

Witkovsky's most interesting findings are that a number of off units with predominant orange maxima, which gave secondary maxima in the blue or green, became on-off units when selectively bleached with red light. Presumably this is due to reduction in sensitivity of the inhibitory orange component, though selective adaptation with red or green light produced no wavelength shift of the orange peak. This indicates that the orange sensitivity is not due to mixed red-green input, but rather to a single input, presumably from red-green twin cones as described by Marks (1965) in goldfish.

4. Opponent colour units in the lateral geniculate. In higher vertebrates Hubel & Wiesel (1960) have made some preliminary investigations on opponent retinal ganglion cells of the spider monkey, recording from the optic nerve. Michael (1966) has recorded opponent units in the optic nerve of the ground squirrel. Apart from these authors, research

into colour systems of higher vertebrates has centred on recordings from the lateral geniculate nucleus. Monkeys have been used for the majority of these experiments since the visual system of this primate shows the closest correlation with that of man. Such work has advanced in parallel with that previously described for goldfish, and in conjunction with behavioural studies.

In the macaque monkey, De Valois and co-workers define four classes of opponent cell in the lateral geniculate, which they classify according to whether colour stimulation evokes an increase (+) or decrease (-) in the spontaneous firing frequency of the cell (De Valois, 1960, 1965, 1966; De Valois & Jones, 1961; De Valois & Abramov, 1966; De Valois, Abramov & Jacobs, 1966). These classes are +R-G, +G-R, +Y-B and +B-Y, where R, Y, G and B respectively denote whether effects are maximal to red, yellow, green or blue stimuli. Occasionally the above authors obtained records from cells which showed excitation or inhibition, each with sensitivity maxima in two spectral regions. This result provides an interesting comparison with the properties of retinal ganglion cells subsequently detailed for the plaice. Strong post-inhibitory "off" discharges are characteristic of fish retinal ganglion cells (see later). They were not observed in lateral geniculate cell responses in the macaque monkey.

On the other hand, Jones (1966) found no evidence for opponent colour units in the lateral geniculate of the owl monkey which possesses what is possibly a pure rod retina. Instead, on and off cells fall into one of three classes distributed around means of 500, 530 and 560 mμ. respectively. However, this particular animal gives both scotopic and photopic luminosity functions and a Purkinje shift. This suggests that two or more rod populations, in the absence of cones, are sufficient for colour discrimination.

Recently the remarkable work of Wiesel & Hubel (1966) on the rhesus monkey revealed three cell types in the dorsal layers of the lateral geniculate, that code spatial, intensity and colour variables of the visual input. One type demonstrates both centre-surround receptive field patterns and opponent colour responses. The sensitivities of the field centre and surround are maximal in different spectral regions. Cells of this type were either red "on"-centre, green "off"-surround; red "off"-centre, green "on"-surround; green "on"-centre, red "off"-surround; green "off"-centre, red "on"-surround, or occasionally blue "on"-centre, green "off"-surround. Like lateral geniculate cells previously described, and unlike fish retinal ganglion cells, the expression of the inhibitory component as an "off" discharge, in addition to inhibition, was rare.

The second cell type lacked spatial segregation of the receptive field, but invariably showed opponent colour responses of two varieties, either green-"on", blue-"off" or blue-"on", green-"off". In these respects they resemble the opponent on-off cells found by MacNichol and others in goldfish retina.

The third type possessed receptive fields with centre-surround arrangement, either "on"-centre or "off"-centre with an inhibitory surround. However the spectral sensitivities of the centre and surround were identical. These cells resemble the non-opponent, centre-surround cells also found in goldfish at the retinal level.

Some cells of both the first and third type showed evidence of rod input, in addition to that from cones. No evidence for rod input was found in the second cell type, namely that lacking centre-surround receptive field configurations.

Hubel & Wiesel's interpretations were that the third cell type was concerned with spatial information, the second with colour, while the first type combined spectral with spatial information. Plaice retinal ganglion cells exhibit many similarities with these three types. Comparisons are made in the Discussion.

5. Spectral properties of single cones. The nature and spectral sensitivity of rod pigments is well-documented since they have been extracted and isolated in solution. Several authors have measured the absorption spectra of single rods (e.g. Brown & Wald, 1964). It has long been thought that there are three cone types in the retina, each containing a single colour-sensitive photopigment - the basis of the Young trichromacy theory. However it has not proved possible to isolate cone pigments. Until recently evidence for three cone types and three cone pigments has been entirely of an indirect nature such as from behavioural colour discrimination experiments, Rushton's reflection densitometry measurements on protanopes and deuteranopes, and from the properties of colour-coded retinal ganglion cell and lateral geniculate cell responses.

Marks (1965), using microspectrophotometric techniques, was first able to measure the difference spectra of single goldfish cones, by passing light of different wavelengths through the cone outer segments. He identified three cone types, each containing a single visual pigment, with wavelength maxima at 455±15 mμ, 530±5 mμ or 625±5 mμ. Occasional maxima found at 480 and 570 mμ were interpreted as secondary photo: products and as the composite difference maxima of red-green twin cones respectively.

Similar methods applied subsequently to primate cones gave absorption maxima at 445, 530 or 570 mμ, i.e. blue, green and orange, but failed to show red maxima (Marks, Dobelle & MacNichol, 1964). These results were confirmed by Brown & Wald in human and monkey retinae (1963), and in human retinae (1964).

Finally, Tomita (quoted by MacNichol, 1966) has succeeded in recording potentials from single carp cones and identified three cone types with sensitivity maxima which agree well with Marks' values for goldfish.

Retino-tectal projection

Anatomical methods have shown that in fish the retinal ganglion cell axons run in the optic nerve to the contra-lateral optic tectum, with complete decussation in the optic chiasma. Detailed maps of the projection from the retina to the contra-lateral tectum have been obtained electrophysiologically. Jacobson & Gaze (1964) demonstrated an ordered retino-tectal projection in goldfish, following stimulation of the eye through air. The anterior retina (posterior visual field) projects to the posterior tectum, the dorsal retina to the ventral tectum and so on. Similar maps were obtained for black bass, bluegill, carp and goldfish (Schwassmann & Kruger, 1965) when the eye was

stimulated through water; and for the optic tecta of several other vertebrate classes, e.g. amphibia - frog (Gaze, 1958), reptiles - alligator (Heric & Kruger, 1965), and birds - pigeon (Hamdi & Whitteridge, 1954). These results are comparable with the projection from the retina to the superior colliculus in mammals (Hamdi & Whitteridge, 1953).

In fish, unlike other classes, receptive fields of ganglion cells in different retinal regions appear to be remarkably uniform in size and density (Jacobson & Gaze, 1964; Schwassmann & Kruger, 1965). There is no evidence of small receptive fields in the foveal region, or enhancement of the area of tectal representation for any part of the retina.

In frog, Maturana, Lettvin, McCulloch & Pitts (1960) demonstrated not only an ordered retino-tectal projection of all the cell types they identified, but in addition an orderly depth-sequence at which the axons of the various types terminate in the superficial fibre layers of the tectum. In fish neither Jacobson & Gaze (1964) nor Schwassmann & Kruger (1965) could detect any such depth relationship.

The problem in hand

The preceding description indicates that the retinal ganglion cells are a conveniently simple level for an electrophysiological analysis of the way in which visual information is encoded in the visual pathway. Their properties have been studied in terms of intensity, temporal and spatial variables, movement and directional movement discrimination, and finally colour. The properties of these cells per se have indirectly revealed much about the nature of earlier visual processes, for until recently it has not proved feasible to record directly from the receptors themselves.

1. Spectral sensitivity determination. Most studies of retinal ganglion cells to date have been designed to analyse the properties of large representative samples in terms of only one or two of the above stimulus parameters. For example, spectral sensitivity measurements on each cell have been obtained simply by determining stimulus intensities required either for threshold or constant response at a number of wavelengths through the spectrum, without systematic attempts to measure the intensity-response relationships at each wavelength (e.g. Witkovsky, 1965; Wiesel & Hubel, 1966; and papers by MacNichol, Wagner & Wolbarsht). Throughout

this thesis "intensity-response" functions should strictly be termed log intensity-response functions, but the former terminology has been adopted for simplicity.

Whilst the threshold method is reliable, alone it yields little information on the nature of interaction, if any, between excitatory and inhibitory inputs to the cell. The constant response method is unreliable in it's present form, where no intensity-response data is provided, for there remain the following unanswered questions:

1. Is the slope of the intensity-response curve through the point of constant response the same at all wavelengths for a given cell? - If not:

2. To what extent do inhibitory and/or excitatory interactions between the ganglion cell inputs responsible for the "on" and "off" discharges affect the slope of this curve, and thus the intensity relative to threshold which this constant response represents?

In view of these questions it is a dangerous assumption to make, as a number of workers have done, that a constant response to different stimulus colours is necessarily at constant intensity above threshold. Without having examined intensity-response relationships in detail at each wavelength it would seem unlikely that this is so. In fact, from the shape of curves of absorption spectra of visual pigments,

and even assuming that the Weber function:

$$\frac{\Delta I}{I} = \text{constant}, \qquad \text{where } I = \text{intensity},$$

holds over the whole visual intensity range from threshold to saturation, it is likely that the slope of this relation: ship varies with wavelength.

Both Hartline (1938), recording from single optic nerve fibres (ganglion cell axons) in the frog, and De Valois (1960) from single monkey lateral geniculate cells, show sigmoid-shaped intensity-response functions to white light stimuli. Even reasonable agreement with the Weber relationship holds only for a narrow intensity range mid-way between threshold and saturation. Hartline observed in addition a suppression of response magnitude at stimulus intensities above saturation, 'though it is questionable whether this is a super-saturation effect or the expression of an inhibitory process at these very high intensities.

Mkrticheva & Samsonova (1966) in a more detailed analysis of "silent tectal neurones" in the frog, give results similar to those of Hartline which show suppression of the response at high stimulus intensities. The former authors however failed to indicate whether they were recording from the axon terminals of retinal ganglion cells or from intrinsic

tectal neurones. Changes in responses from threshold to maximal are shown to occur over narrow and wide intensity ranges for their fast and slow adapting types respectively, with strong suppression of the response to supramaximal stimulus intensities. However, Mkrticheva & Samsonova make no mention of whether cells analysed were on, off or on-off. If on-off cells were included in their classification, the suppression effects may well be the expression of interaction between excitatory and inhibitory inputs.

2. Energy-quantum relationship of monochromatic light. Changes occurring in visual pigments of photoreceptors when illuminated are related to the number of light quanta, not to their energy (Hecht, Schlaer & Pirenne, 1942). Since the energy of quanta of light is related to wavelength by the Planck equation:

$$E = \frac{h.c}{\lambda}$$

where E = energy
h = Planck's constant
c = velocity of light
and λ = wavelength of light

it is immediately apparent that, for the extreme ranges of the visible spectrum, blue light quanta possess almost twice as much energy as quanta of red light.

Despite this, all previous investigations of the chromatic properties of retinal ganglion cells, with the

single exception of Witkovsky's (1965) work on carp, have been based on equal energy spectra. As a result, blue stimuli contained only about half as many quanta as red light stimuli. This has led to distortion of the true form of spectral sensitivity curves.

3. The plaice. Whilst the properties of retinal ganglion cells of amphibia, mammals and fresh water fish have received considerable attention, there is no data available for marine fish. In this latter case, apart from Svaetichin & MacNichol's (1958) early work on the retinal "S" potential, results have been confined to spectral sensitivity measurements from purely behavioural experiments (e.g. Blaxter, 1963), and to the identification and isolation of visual pigments.

The experiments reported here were designed to test whether plaice retinal ganglion cells are colour-sensitive, and if so to characterise the spectral, spatial and temporal properties of a small number of such cells in great detail. This work was intended to fill a gap in knowledge of ganglion cells. It has been treated comparatively between marine fish on the one hand and fresh water fish on the other. Analogies have been drawn with LGN cells of higher vertebrates.

Blaxter (1967, personal communication) informs me that

larval plaice possess a pure single cone retina. Both rods and cones occur in the retina of the adult. The rods develop at metamorphosis. Engstrom & Ahlbert (1963) reported the presence of single and twin cones distributed throughout plaice retinae, and also triple cones which are restricted to a specialised region of the dorsal retina. Nicol (1965) found that in addition to migrations of the retinal pigment layer in plaice, depending on the state of light adaptation, cones and rods also show migratory movements. Under illumination the pigment layer is fully extended and the rods extend fully into the pigment; cones are fully retracted against the external limiting membrane. Nicol interprets this as a mechanism by which rods are preserved in the dark-adapted state. In darkness the pigment layer is retracted, the cones are extended towards the pigment; the rods become fully retracted against the external limiting membrane for optimal function.

Since MacNichol & co-workers (1961, 1961a, 1963, 1966) showed that certain on-off retinal ganglion cells in goldfish give opponent colour responses, experiments in plaice have been confined to ganglion cells of this type. Experiments were performed in the dark-adapted state for simplicity of manipulation. Following preliminary analysis for units giving opponent responses to equal quantum spectra, detailed

intensity-response functions were obtained at intervals through the spectrum for some twenty units. Receptive fields of a few units were investigated in detail.

The shortcomings of the methods are considered later, but the prime considerations throughout were to maintain fish as physiologically normal as experimental conditions would permit. To accomplish this the methods of Maturana et al (1960) and Jacobson & Gaze (1964) were adopted, rather than record from the isolated retina or penetrate the eye as most authors have done. Units were recorded from the superficial fibre-layers of the contra-lateral optic tectum with extracellular micro-electrodes. The sole operative procedure consisted of exposing the brain and removing the cerebral hemispheres. For a number of reasons it was necessary to stimulate the eye through air, rather than through water (see later). The results are compared with those of Jacobson & Gaze (1964) who used a similar technique. This method appeared to be adequate, since the receptive field configurations and properties were the main features of interest, rather than the measurement of their absolute sizes.

MATERIALS & METHODS

Animals

Ninety-four adult plaice (Pleuronectes platessa, L.), 10-13 inches in length, were used. Several factors determined the choice of plaice as the experimental animal:

1. Plaice are available locally throughout the year.
2. They live well in captivity and under experimental conditions.
3. Their flatness facilitates stereotaxic clamping, and they remain immobile for long periods of time.
4. The eyes are bulbous and consequently easily held immobile.
5. Operative procedures are simple.

In a few pilot experiments, skate (Raia batis) and Cottus were used. While operative procedures were simple, the accompanying haemorrhage was greater than in plaice. These species were more difficult to clamp stereotaxically and maintain under experimental conditions.

The first 46 fish were used in a number of ways, to develop a suitable preparation and in preliminary experiments with white-light stimuli:

1. Preparation of serial transverse sections of plaice

brain, stained for nerves, as histological background.

2. Determination of a suitable anaesthetic.
3. Development of stereotaxic clamping apparatus.
4. Maintenance under experimental conditions, by sea water perfusion of the gills.
5. Development of suitable operative procedures, including decerebration.
6. Preliminary investigations of types of tectal unit responsive to black and white, stationary and moving visual stimuli; mapping of the retinal projection from the left eye to the right optic tectum.

These earlier experiments are considered here only insofar as they have a direct bearing on experiments with a further 48 fish, concerned with the spectral properties of retinal ganglion cells.

Anatomy

The anatomy of the plaice is peculiar in that the fish lies on its left (eyeless) side. The left eye has migrated to the right (ocular) surface during development. The brain is twisted on its axis so that the right optic tectum overlies the left tectum when exposed from the ocular surface (Fig. 1). It is necessary to penetrate blindly to record from the underlying left tectum, making location of the micro-electrode

uncertain. Therefore all units were recorded from the right tectum in response to stimulation of the left eye.

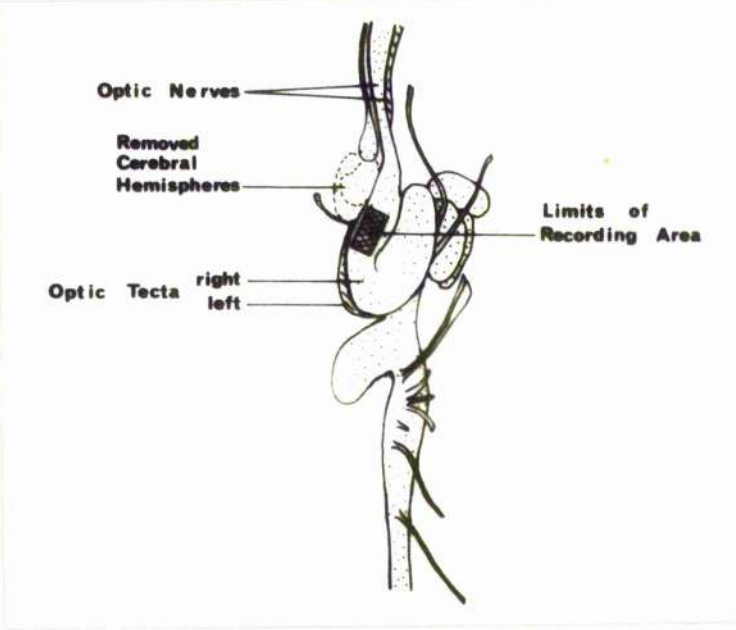

Fig. 1. Diagram of plaice brain as seen from the ocular surface. The visual field of the left eye available for stimulation projected to the area marked on the right tectum, with conventional antero-posterior and dorsi-ventral retino-tectal projection patterns (see text). All units described were recorded from within these approximate limits. A few units recorded from the visible portion of the underlying left tectum showed similar projections and properties. (Scale X5).

It was anticipated from the asymmetry of the fish that there might be differences in responses of units recorded from the two optic tecta. No evidence was found in support of this view from the few units recorded from the left tectum, responsive to stimulation of the right eye.

Anaesthesia

Intra-peritoneal injections of solutions of various concentrations of Urethane in 0.9% saline were most unreliable for maintaining fish at constant levels of anaesthesia. The heaviest doses of Urethane which could be administered without killing fish were effective for 20 mins. or less. Doses sufficient to suppress reflexes also suppressed all tectal neural activity.

Gill-perfusion with sea water containing concentrations of Urethane adequate to suppress reflexes, whilst maintaining fish at reliable levels of anaesthesia, invariably abolished all tectal neural activity.

The method of anaesthesia adopted was to immerse fish in a 1/5,000-1/10,000 solution of MS222 (Sandoz) in aerated sea water. Reflexes were suppressed after 3-7 mins. immersion, and respiratory movements ceased approximately 2 mins. later. Fish were then immediately transferred to a bath of sea water, clamped stereotaxically, and the gills continually perfused with aerated sea water. The depth of anaesthesia remained adequate sufficiently long for operative procedures to be completed.

Stereotaxic clamping

Fish were mounted, ocular side uppermost, on a matt black sheet of perspex (to minimise reflection of stray light onto the eyes) and clamped between this and four, horizontal, transverse bars shaped to fit the ocular surface of the animal. These bars were placed immediately behind the eyes, behind the gills, across the body and across the apex of the tail. Preparations were orientated with the antero-posterior axis of the animal parallel to the longitudinal axis of the perspex sheet. The left eye was centred over a locating mark scribed on the sheet. The whole arrangement was fixed into a shallow, rectangular perspex bath by four locating screws. The gills were perfused, at a controlled rate of flow, with aerated sea water, by gravity-flow from a reser: voir. Excess sea water was removed from the bath by gravity-flow through an outlet tube. The level of this outlet could be adjusted so as to maintain most of the fish in sea water, with only the eyes and the ocular surface of the skull in air.

Brain exposure and decerebration

The optic tecta and cerebral hemispheres were exposed from the ocular surface by removal of skin, muscle and skull-bone between the third and fourth tuberosities with

a small dental burr. The gelatinous substance surrounding the brain, and the cerebral hemispheres were removed by suction. Haemorrhage was usually slight. In the few cases where it was excessive, tectal neural activity was subsequently absent and fish were destroyed. The dura and tectal blood supply remained intact. The brain was kept moist by the surrounding fluid within the cranial cavity.

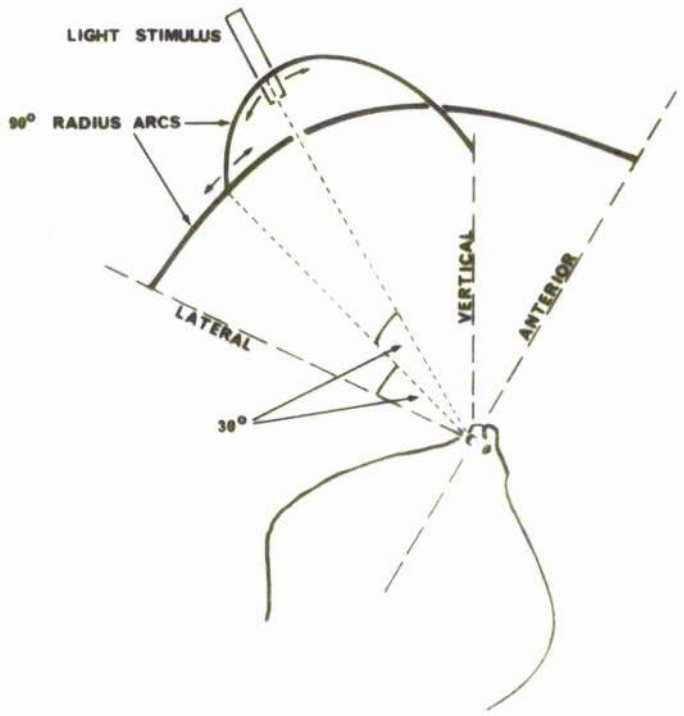

Fig. 2. Diagrammatic representation of the stimulus arrangement. The visual stimulus was contained in a light-proof tube and mounted radially by a slide on the vertical arc. The vertical arc was mounted by a slide at the base onto the horizontal arc, so as to pivot around the vertical axis. The fish was orientated so that the left eye was positioned at the effective centre of the sphere described by the arcs, and clamped directing 30° forwards (anterior) and 30° upwards (dorsal).

The left eye was clamped projecting approximately 30° forwards and 30° upwards by a system of needles (Fig. 2), so that the portion of the visual field available for stimulation projected to that part of the right tectum visible and accessible from the ocular surface (see Fig. 1). This procedure was adopted, rather than immobilisation by curarisation, to avoid the tedious necessity of giving 48 hours notice to the Home Office prior to each experiment.

Visual stimuli

In pilot experiments designed to test whether the retino-tectal projection and types of tectally-recorded units were similar to those found in other fish and vertebrates, a variety of white-light stimuli were used.

Colour stimulus. Details of the visual stimulus technique used to study the spectral properties of retinal ganglion cells are illustrated in Figs. 2 & 3. The components of the visual stimulus were housed in a light-proof tube. This was mounted radially by a slide on the vertical of a pair of 90°, 17 cm.-radius arcs set in the horizontal and vertical planes and describing a sphere with the left eye at the effective centre (Fig. 2). The vertical arc was attached, by a slide at the base, to the horizontal arc. Each arc was calibrated in degrees.

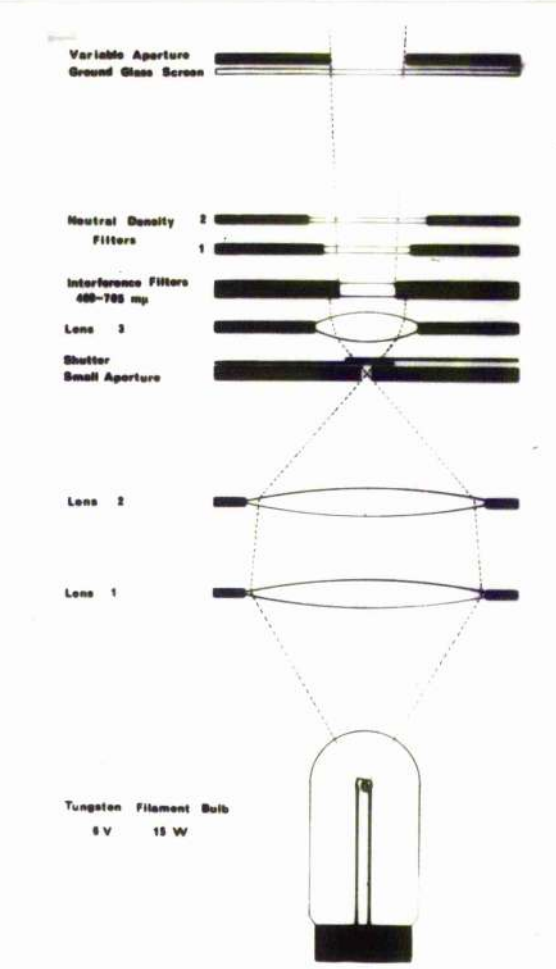

Fig. 3. Details of the components of the visual stimulus, mounted in a light-proof tube. The final variable aperture was positioned 15.0 cm. from the eye and produced uniformly illuminated, circular spots of light subtending angles of 0.5, 1, 3 or 6 degrees in air at the corneal surface.

The light source consisted of a 6V. 15W. plane-filament tungsten bulb (Phillips 13347 C), powered from a mains constant voltage transformer stepped down by a 240:6V. transformer. Light was focused by a pair of bi-convex lenses (Lenses 1 & 2, Fig. 3) onto a small fixed aperture, more-or-less collimated by a third bi-convex lens (Lens 3, Fig. 3). Light then passed in turn through interference filters and neutral density filters onto a fine ground-glass screen, to provide uniform illumination and to defocus the filament

image. Uniformly-illuminated circular spots of light subtending 0.5, 1, 3 or 6 degrees at a distance of 15 cm. from the eye, were obtained by adjusting the variable aperture placed immediately in front of the screen.

The light-concentrating lens system was necessary to provide a wide range of suprathreshold stimuli, despite transmission losses due to the optical components of the stimulus. Achromatic lenses were unnecessary since narrow bandwidth interference filters were used.

It was necessary to work with the eye of the fish in air, since it is difficult to cover the eye with water without flooding the exposed brain. In water the near point is about 14 cm. Thus in air this value is reduced and the stimulus final aperture is presumably within the range of accommodation. No correction was made in the final analysis for the resultant refractive error when a normally aquatic eye is stimulated through air (but see Discussion). Initial attempts were made to overcome this. A water-filled quarter-sphere, constructed from a 10 litre round-bottom flask with an external radius of 14 cm., was positioned above the eye (Fig. 4). The flask section was sealed with two thin perspex half-discs. The horizontal half-disc was inset 0.5 cm., so that the eye could be positioned at the effective centre of the quarter-sphere. Sea water covering the eye was held by surface tension against the underside of the horizontal

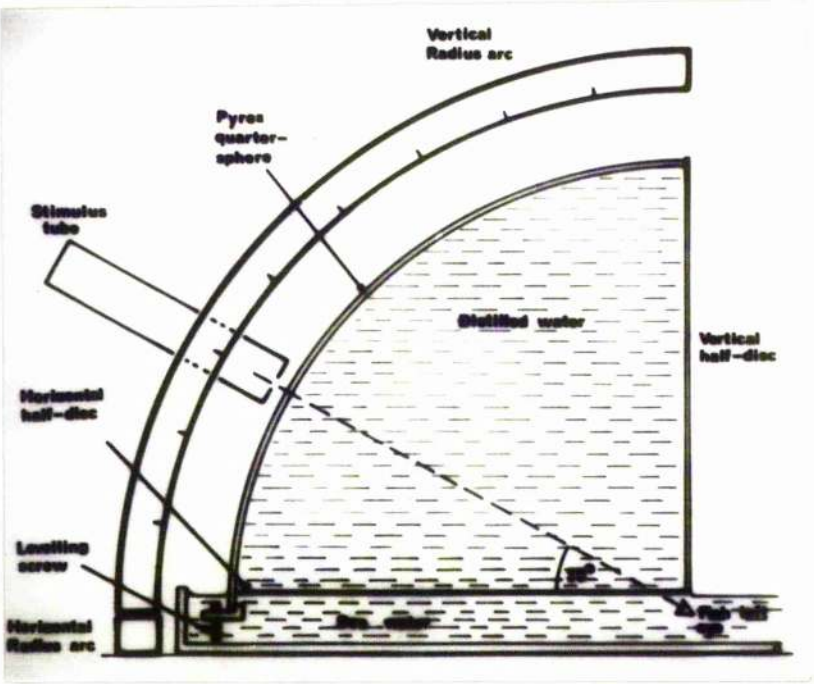

Fig. 4. Diagram of the water-filled quarter-sphere used in an attempt to simulate stimulation of the eye through water. The stimulus beam passed normally through the spherical surface. The eye was positioned in sea water at the effective centre of the sphere, which was adjusted by three screw feet mounted along the perimeter of the horizontal half-disc. Refractive errors due to this half-disc were small, since the disc was thin, but the back-reflection from the surface of the horizontal half-disc varied appreciably with the angle of incidence of the stimulus light path.

half-disc. Stimuli were presented normally at the spherical surface of the quarter-sphere. Since the horizontal half-disc was thin, refractive errors and differences in the real and apparent positions of the stimulus due to this half-disc were small. On the other hand the back-reflection from it varied substantially, depending on the angle of incidence of the stimulus light path, giving large errors in the actual intensity of illumination incident on the eye. For this reason alone the technique was abandoned.

Fifteen narrow bandwidth interference filters (Barr & Stroud), between 409 and 705 mμ, were standardised with a calibrated phototube (RCA 935, Fig. 5). An approximately equal quantum spectrum was obtained by regulating the light intensity of the source with a 5Ω heavy duty potentiometer in series. Interference filter characteristics, and correction

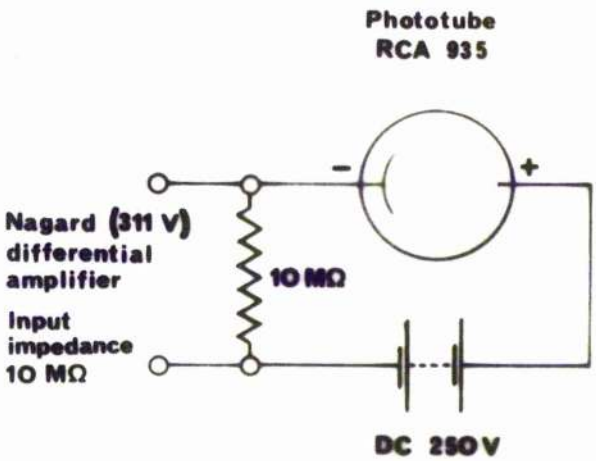

Fig. 5. Circuit diagram for the phototube used to calibrate interference and neutral density filters. Signals were led to the high-impedance, differential DC input of a Nagard oscilloscope (pre-amplifier type 311V) with calibrated gain.

factors required to give equal quantum emission within ±2.5% are given in Table 1. The required reduction of light intensity of the source was maximal for red filters and minimal for blue filters. This has an advantage over standardisation with neutral density filters, since for a tungsten source relatively more red light is emitted at low intensity and relatively more blue light at high intensity. As a result the shift of spectral peaks and distortion of bandwidths of interference filters are minimised.

TABLE 1

Interference filter characteristics

λ (mμ)	Bandwidth (mμ)	Transmission (%)	Log Rel. Quantum Emission
409	13.7	25	- 0.15
422	14.3	15	- 0.12
439	12.0	16	- 0.19
472	18.2	27	- 0.25
484	19.0	19	0
508	12.5	37	- 0.08
536	9.8	26	- 0.01
558	14.0	45	- 0.20
574	15.3	22	- 0.05
586	17.0	46	- 0.14
600	18.0	48	- 0.06
622	11.5	25	- 0.07
653	13.0	49	- 0.12
675	18.5	48	- 0.21
705	19.0	45	- 0.25

Filters were calibrated (as described in the text) to give approximately equal quantum emission (column 4). Corrections given in this column were applied throughout in spectral sensitivity analyses, to correct relative quantum emission of stimuli to within $\pm$2.5%.

Transmission peaks and bandwidths of the interference filters were checked with a spectrophotometer (Unicam SP 600). Deviations of less than 6 mμ from the manufacturers' specifications were found.

Neutral density filters (Kodak Wratten) provided intensity reduction in steps of 0.1 log unit over a range of 3 log units. They were calibrated through the visible spectrum with a spectrophotometer (Unicam SP 800), and cross-checked at each interference filter wavelength with the phototube. No significant differences were found between values obtained by the two methods. The values obtained with the spectrophotometer are given in Table 2 (Appendix) for each interference filter wavelength. These values have been used throughout.

Light flashes of variable duration and frequency could be controlled by a mechanical relay-driven shutter at the point of focus of the light path (Fig. 3). The durations of onset and offset of the stimulus were not more than 10-15 msec. The relay was activated by a 1 sec., square DC pulse from a modified Tektronix 161 Pulse Generator, triggered from a Tektronix 162 Waveform Generator. The same trigger pulse was used to trigger the sweep of a Tektronix 502A oscilloscope (Fig. 6). Flash durations of one second were chosen to give complete separation of responses.

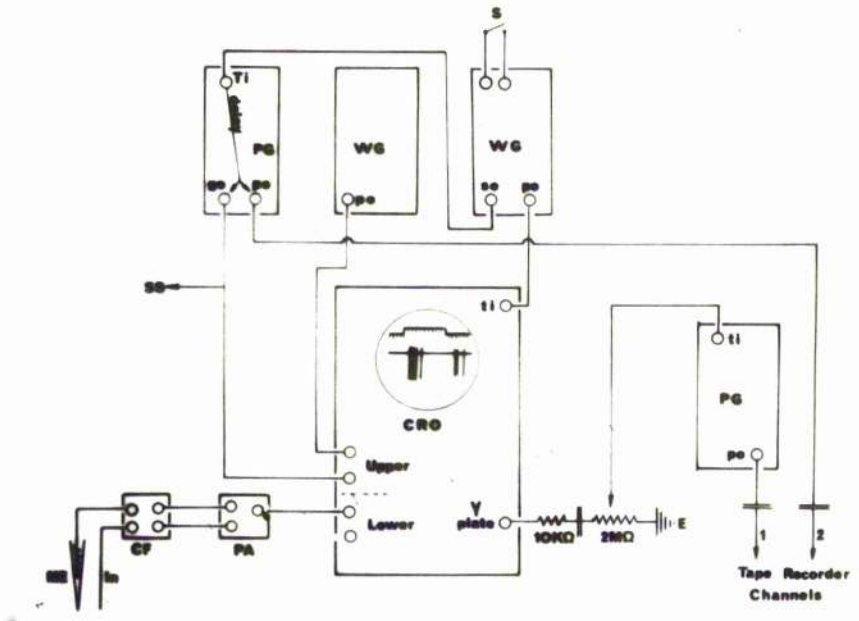

Fig. 6. Recording arrangements, reading from top left:
Pulse Generator 1 (PG): Ti - sawtooth trigger in; s.o. - gate out (1 sec. square DC pulse/2 sec. to 'scope monitor channel and to stimulus-shutter relay - SG); p.o. - pulse out, 1 sec. DC pulse per 2 sec. capacitively coupled to tape recorder channel 2.
Waveform Generator 1 (WG): p.o. - DC pulse out, frequency 10/sec. to 'scope monitor channel (time mark).
Waveform Generator 2 (WG): continually triggered on opening camera shutter - S; s.o. - neg. sawtooth out, frequency 0.5/sec.; p.o. - trigger pulse out, 0.5/sec. to scope time base trigger - ti.
ME - micro-electrode; In - indifferent silver electrode; CF - cathode follower (Fig. 8); PA - differential pre-amplifier.
Oscilloscope (CRO): Y plates of lower channel were coupled as shown to the trigger input - ti - of the second pulse generator - PG - and adjusted to accept only the largest spikes.
Pulse Generator 2 (PG): each large spike triggered a 2 msec. DC pulse - p.o. - fed capacitively to the tape recorder channel 1.

Recording

The responses of single units to stimulation of the left eye were recorded from their axon terminals in the superficial layers of the right (upper) tectum with platinum black tipped, indium micro-electrodes. Reasons for inferring that tectal recordings were from retinal ganglion cells are considered in the Results section.

1. Micro-electrodes. The electrodes used were a form of the indium micro-electrode described by Dowben & Rose (1953) and modified by Gesteland, Howland, Lettvin & Pitts (1959).

A. Micro-pipettes with long flexible tapers were drawn on a Palmer electrode puller from fine Pyrex glass capillary tubing (Corning).

B. Tips were broken off to give tip diameters of 2–4 μ, by butting them up against a glass slide under a microscope.

C. An alloy of Wood's metal and indium, with approximately the same coefficient of expansion as Pyrex glass, was prepared. A mixture of 70% Wood's metal and 30% indium was found by trial and error to be most suitable. The alloy was melted and drawn by suction into polythene tubing with an internal diameter slightly smaller than that of the Pyrex glass used, and allowed to solidify. The polythene was

stripped off when the alloy was required. Cylinders of the alloy, 0.5 cm. in length, were inserted into the shafts of micro-pipettes and pushed as far as the shank with plungers consisting of lengths of 22 gauge tinned-copper wire. Micro-pipettes were warmed over a small heating coil until the alloy cylinder melted. Using the wire as a plunger, the metal was forced through the taper as far as the tip. The wire was sealed into the alloy as a contact. Brief re-heating of the tip resulted in extrusion of alloy as a small ball which was knocked off by tapping the wire contact. Electrodes were stored in this form until required for use, and were examined microscopically to detect any air pockets in the metal column within the taper.

D. Micro-electrode tips were plated with platinum black by the following method:

i. Wash by dipping into distilled water.

ii. Clean by dipping into a freshly-prepared solution of snot remover (50% conc. H_2SO_4; 50% 100 vol. H_2O_2). It was found in practice that this solution successfully etched away alloy within the tip, rendering it unnecessary to use the HCl/H_2O_2 etch recommended by Gesteland et al (1959).

iii. Wash in distilled water.

iv. Plate. The most successful plating solution used had the following composition:

50% of 0.1% chloroplatinic acid solution,

30% dil. agar solution to bind the platinum tip, 20% distilled water, and a few drops of dil. lead acetate solution to poison the platinum black and prevent catalytic action on tissues.

A gold wire anode was placed in the plating solution and connected through a 1.5V. dry cell to the micro-electrode. The electrode tip was dipped repeatedly into the plating solution, thus closing the circuit. Tip resistances were monitored with a valve-voltmeter modified to read resistance directly. A small platinum-black cap was formed on the micro-electrode tip, and plating was continued until the tip resistance was reduced to less than 10 Kα.

The use of a gold anode, rather than platinum, successfully reduced the rate of deposition of platinum black. Deposition was thus easily controlled and finely divided. This had the advantage of giving low-resistance electrodes with a minimal size of platinum cap, and consequently facilitated penetrations.

v. Wash in distilled water.

Optimal recording characteristics were given by electrodes with tip diameters of 2–4 μ, and a cylindrical platinum black cap 4–8 μ in diameter. Such electrodes combined tip resistances low enough (less than 10 Kα) to give spike:noise discrimination ratios of at least 3 to 1,

with tip dimensions small enough to isolate single units. The indifferent electrode was a silver wire placed on the head; the surrounding sea water was earthed.

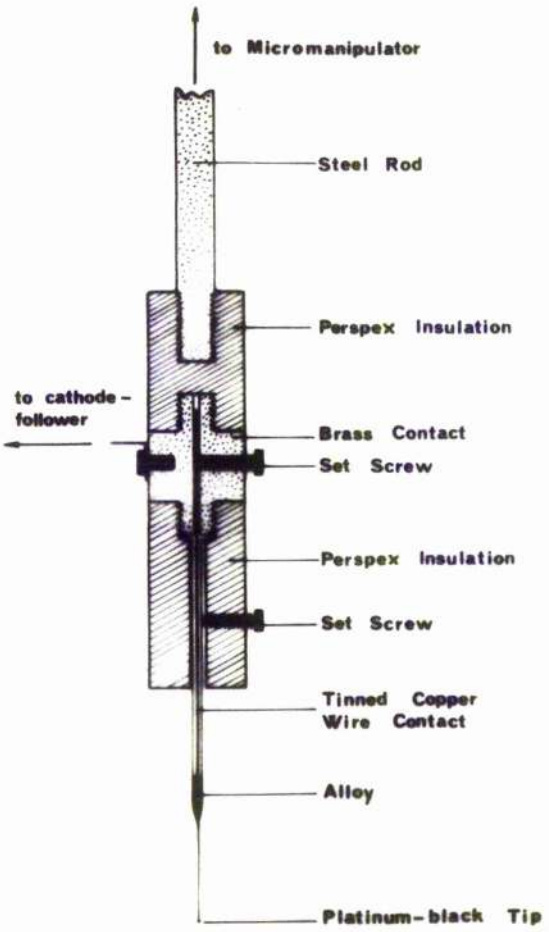

Fig. 7. Holder for indium micro-electrodes. The glass micro-pipette was clamped by a screw in the insulated section, and the wire contact by a screw in the brass mid-section. Signals were led from the contact to the diff: erential input of an AC-coupled cathode follower (Fig. 8). (The electrode tip is disproportionately enlarged for clarity.)

The micro-electrode was mounted in an insulated holder (Fig. 7) and positioned vertically over the right tectum by a lathe bed mounted horizontally on a micro-manipulator. It was lowered by a vertical micrometer drive until the electrode tip made contact with the tectal dura, assessed by a 10-15-fold reduction in the recording noise level.

Further advance dimpled the tectal surface under the microelectrode tip, with a slight increase in noise which fell to the previous level as soon as the dura was penetrated. Penetration resulted in breakage of numerous electrode tips but attempts to overcome this, either by removal of the dura or by making small incisions, were invariably accompanied by haemorrhage and loss of neural activity. The electrode was advanced in search of single units responsive to stimulation of the left eye. When such a unit was located, the electrode was adjusted to record impulses optimally, often accomplished by lightly springing the electrode taper by slight lateral movement.

2. Amplification and display. Impulses were channelled through a differential, AC-coupled cathode follower (Fig. 8), amplified (pass-band 100 c/s-1 kc/s) and displayed oscillographically (Tektronix 502A). Oscillographs were recorded on stationary or moving photographic paper (Ilford NS6) and simultaneously recorded on tape (Fig. 6).

In the earlier experiments the Y-plates of the CRT were capacitively coupled to the tape recorder input. This resulted in attenuation of spikes to give a poor spike:noise discrimination ratio on playback. Subsequently, the Y-plates were capacitively coupled and connected through a 2 MΩ potentiometer, to earth and to the input of a Tektronix

161 Pulse Generator (Fig. 6). The potentiometer could thus be set so that only those impulses above a certain amplitude triggered the pulse generator, i.e. impulses from a single unit could be selectively isolated. Each trigger pulse was converted to a 2 msec. DC pulse, fed capacitively to the tape recorder input.

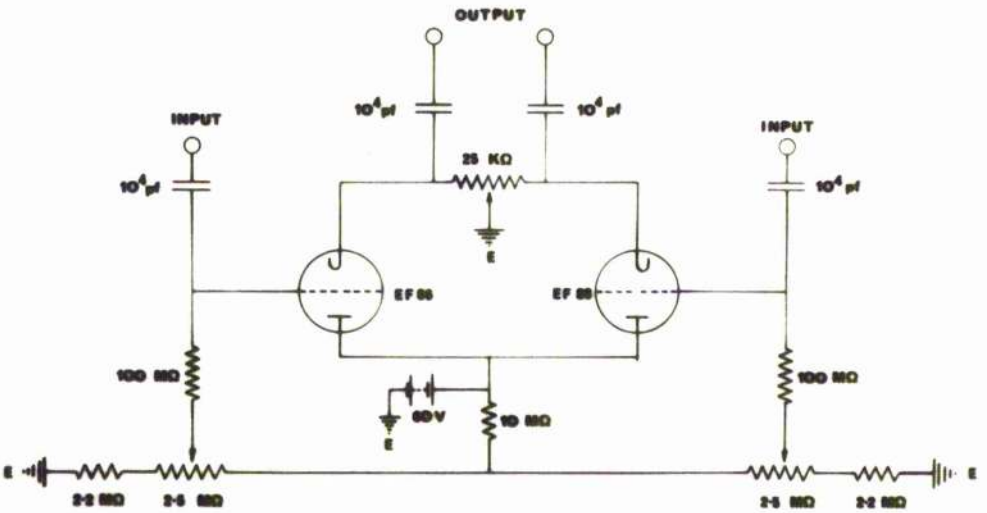

Fig. 8. Circuit diagram for the AC-coupled, differential cathode follower, gain 0.92. The output was led to a diff: erential AC pre-amplifier. (Circuit diagram kindly designed by C.J. Roemmele Esq.,)

On completion of recording from a unit, spikes in each response were counted automatically on a scaler (Echo N530F), from tape recordings converted to 2 msec. DC pulses of suitable amplitude. The responses obtained from each unit were counted either <u>in toto</u> or for a standard time interval.

Stimulus programmes

Fish were dark-adapted for at least 30 minutes. A single unit, giving "on-off" responses to coloured light stimuli, was isolated in the superficial tectum. The receptive field centre was determined by preliminary tests. Units were subjected to one or more of the following programmes.

Programme 1. This programme was used to study the early recorded units of the two types identified (Types 1 and 2, see Results). Starting with a blue filter, peak 409 mμ, responses were recorded to visual stimulation with 1 sec. flashes of light, delivered every 4 or 5 secs., at a fixed quantum intensity level, usually the maximum available. Responses were recorded for each colour-filter wavelength, in order through the spectrum, at the same quantum intensity level. Response series obtained are subsequently termed "equal quantum spectral response series". The full available range of spot sizes was used for different units. However the smallest stimulus spot (0.5°) was used preferentially, since it gave maximal discrimination of responses spatially, and minimal reduction in the level of dark-adaptation.

Since Type 1 units showed no centre-surround receptive field arrangement, the stimulus spot was positioned at the

geometric centre of the receptive field. In Type 2 units, with the classic centre-surround pattern, stimuli were positioned in the transitional portion of the field to give mixed "on-off" responses.

Programme 2. Units recorded more recently were analysed in greater detail. Again starting with the blue filter, peak 409 mμ, responses to 1 sec. flashes of light were recorded, at intensities reduced in steps of 0.2 or 0.4 log units over a 3 log unit range. This was repeated for each interference filter, in order through the spectrum. The flash frequency was increased to 1 per 2 sec. in order to obtain complete runs within the time for which units could be held, apparently without reduction in the level of dark-adaptation (see later).

For Type 1 units stimuli were presented at the geometric centre of the receptive field. For Type 2 units stimuli were positioned so as to give the purest "on" or "off" responses possible from the field centre; where units could be held long enough, a similar series was also obtained for a stimulus positioned in the surround. Intensity-response curves were plotted at each stimulus-wavelength, and subsequently are collectively termed "spectral intensity-response series".

In most cases the smallest stimulus spot was used. This gave optimal isolation of field-centre and field-surround

responses. Detailed analyses involved some 900 responses per unit.

Spectral sensitivity curves for the "on" and "off" components of the response, were constructed from series of intensity-response curves by reading-off from these curves the log relative intensity of the stimulus at each wavelength required to give the same number of spikes in the response. The correction factors given in Table 1 were applied before sensitivity curves were plotted.

In both programmes an average of 5 responses was taken at each stimulus wavelength and intensity, as a necessary smoothing of response variability. For some units a return series was recorded and in all cases random checks were made at the end of a run, for a few wavelengths.

Programme 3. On completion of the previous programme, units were tested for movement and directional sensitivity, to coloured stimuli moved across the receptive field in the horizontal and vertical planes.

The effect of increasing the size of centrally-positioned stimulus spots was investigated for some units with centre-surround receptive fields, at a number of wavelengths through the spectrum. Receptive fields of several units were invest: igated in detail with 0.5° stimulus spots of different colours.

RESULTS

A. GENERAL EXPERIMENTS

Origin of tectally-recorded spikes

It is inferred from the criteria of Maturana, Lettvin, McCulloch & Pitts (1960) for frog tectum, from Kuffler's (1953) criteria for discriminating cell and axon spikes, and from the work of Jacobson & Gaze (1964) on goldfish tectum, that the extracellular unit recordings described here were from the terminal arborisations of retinal ganglion cell axons. These units were encountered only in the most superficial layers of the tectum, almost immediately beneath the dura, and could usually be recorded, much reduced in amplitude, with the electrode tip pressing against the dura before penetration. Spikes were characteristically tri-phasic, with the predominant phase negative (Fig. 9) and satisfied the following criteria for single unit recordings. Only units giving a clear spike:noise discrimination ratio, and audibly distinguishable, were analysed. Spikes were commonly attenuated at high firing frequencies; similar phenomena were observed by Kuffler (1953) in single unit recordings from cat retina. Limits of receptive fields, and boundaries between receptive field sub-divisions, were well-defined. Patterns of spike trains were reproducible. Once a unit

had been isolated, slight movement of the electrode tip invariably attneuated or magnified all spikes, without differentiation of discharges into spikes of different amplitude. Such single units could often be held for several hours.

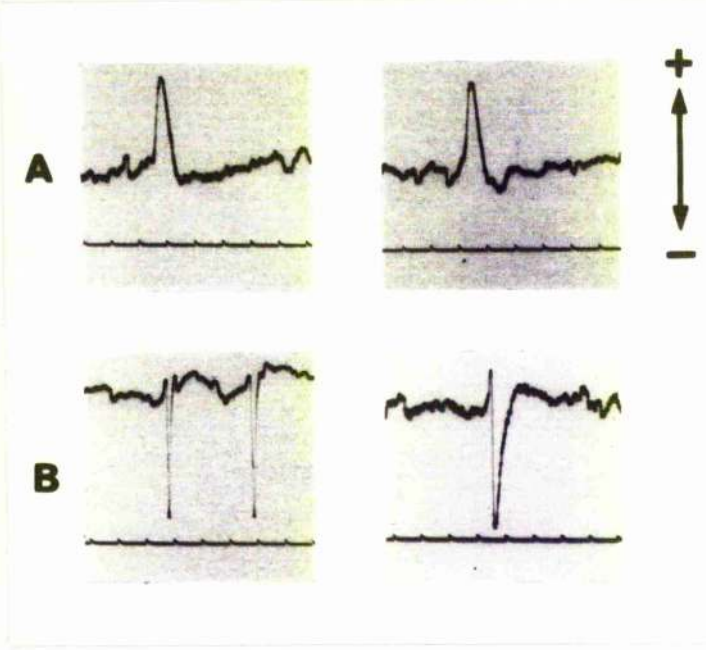

Fig. 9. Cell spikes (A) and axon spikes (B) recorded extracellularly from the superficial tectal layers. Time mark - 1 msec. Gain in B is approximately 10 times gain in A. Cell spikes are predominantly positive and of long time course. Two forms of axon spike are shown, depending on the location of the electrode tip relative to the source of potential. Spikes in the lower left hand record are typical - triphasic and predominantly negative - with a fast rise time. (Amplifier pass-band: 80 c/s - 10 kc/s.)

Infrequent recordings from cells resulted in spikes of much larger amplitude, longer time-course and opposite predominant polarity (Fig. 9). This is substantiated by histological evidence indicating that the superficial tectum is only sparsely populated with cell bodies. These spikes could be held only transitorily and showed a rapid decline in amplitude, presumably due to cell damage.

The response patterns of single units recorded from the superficial tectum of the plaice are similar to those

recorded from the optic nerve. They are remarkably similar to responses of goldfish retinal ganglion cells (Wagner, MacNichol & Wolbarsht, 1960; MacNichol, Wolbarsht & Wagner, 1961; Wolbarsht, MacNichol & Wagner, 1961a,b). Further they are comparable to units identified in other vertebrate classes (see Discussion).

Variation in spike height

In all units recorded there was a more-or-less rhythmic variation in spike height. However, from the spike:noise discrimination ratio and patterns and frequencies of firing, it was clear that these recordings were from single units. Rhythmic variations could be abolished by vascular occlusion. Spike amplitudes were commonly attenuated when firing frequencies increased (compare with Kuffler, 1953).

Often during the course of recordings from a unit there was a gradual decrease in spike amplitude. This may be due to movement, since signal-to-noise ratios could be improved by fractional adjustment of the electrode. On the other hand, when electrodes were withdrawn on completion of recording from a unit an adherent shell of tissue was often 'burnt' onto the tip, despite the addition of lead acetate to the plating solution to poison the catalyst. Gesteland et al (1959) suggest that, by increasing the separation between the electrode tip and the source of spike potential,

this shell might account for the observed reduction in spike amplitude.

Tectal layering and recording depth

No reliable estimates of recording depth could be obtained simply from readings on the vertical micrometer advance, since electrode penetrations caused dimpling and drag of the tectal surface. However, the orderly sequence of unit types was clearly demonstrated by successively advancing the micro-electrode during a single penetration. On and off units were encountered most superficially. Two on-off types, lying in close, but discrete, deeper layers were preliminarily identified by the nature of their "on" and "off" discharge patterns. The first on-off type, subsequently termed Type 1, gave fast-adapting, phasic "on-off" discharges. Type 2 on-off units gave slow-adapting "on-off" discharges, and were isolated slightly deeper in the tectum than Type 1 units.

Deeper penetrations revealed a broad, silent band beneath the superficial layers. At still greater depths additional spike activity, often rhythmic in nature and presumably of intrinsic tectal origin, was found. A few unsuccessful attempts were made to categorise the properties of these units. They are certainly more complex than the

superficial units and have retinal projections too large to assess with the present stimulus arrangements.

Retino-tectal projection

Attempts were made to map the projection from the left retina on an enlarged photograph of the right tectum. Blood vessels on the tectal surface showed considerable variation in course in different fish, but for any particular animal served as reliable landmarks for the location of recording sites. Lateral movement of the electrode over the tectal surface was accomplished by a lathe bed mounted on the micro-manipulator. This gave calibrated movement in steps of 10 μ in two directions in the horizontal plane, parallel to the longitudinal and transverse axes of the fish. Coordinates were taken for two widely-separated points on the tectal surface, with the electrode tip positioned above prominent junctions of blood vessels. Single units of the various types were isolated in the superficial tectum, and the coordinates of each recording site noted. Recording sites were subsequently plotted on the appropriate tectal photograph. Coordinates of these sites were related to the two original landmarks by simple geometry. The magnif: ication of the photograph was taken into account.

Coordinates of the stimulus positions were read in degrees from the horizontal and vertical arcs, for each

recording site, for stimuli positioned at the centres of receptive fields.

This technique should have provided accurate maps of the retino-tectal projection patterns of those parts of the visual field and right tectum simultaneously accessible with the present stimulus and recording arrangements. The accuracy of such maps was however limited, since the tectum underwent considerable lateral movement during electrode penetrations. Despite this, it was clear that the retino-tectal projection of all types of unit was ordered. The posterior retina (anterior visual field) projected to the anterior tectum, with corresponding anterior-to-posterior, ventral-to-dorsal and dorsal-to-ventral projections.

B. SPECTRAL EXPERIMENTS

Preliminary considerations

Experiments were designed to establish the existence and properties of colour-sensitive elements in the retina of plaice, a marine teleost. Such elements have been identified in numerous fresh-water teleosts. MacNichol & others have shown that in goldfish certain on-off retinal ganglion cells give opponent colour responses. Thus in plaice attention was centred on on-off ganglion cells,

studied in the dark-adapted state. Under such conditions it is easier to manufacture and manipulate stimuli free from spurious secondary stimuli, e.g. stray reflected light from the stimulus and extraneous sources, and movements of the operator.

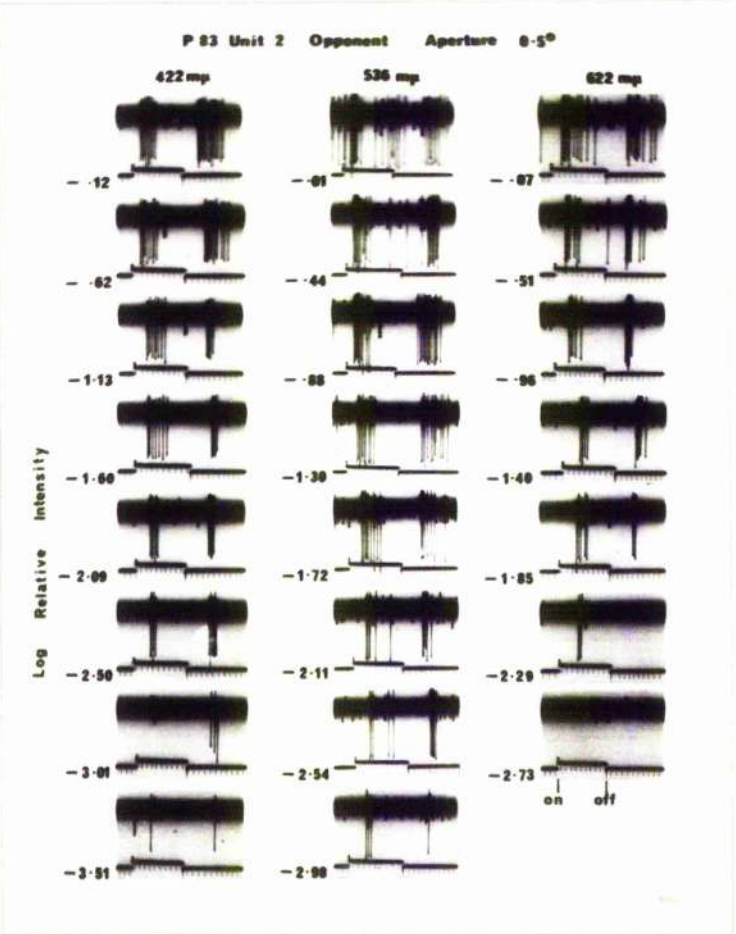

Fig. 10. Responses of a Type 1 on-off unit to stimulation at three wavelengths and different intensities with a 0.5° stimulus spot positioned at the receptive field centre. For illustration, records showing responses nearest to the average of 5 at each intensity have been shown. Top records are for maximum available stimulus intensity at each wavelength. The log relative quantum intensity of the stimulus is given at the left of each record. Upward deflection of lower trace indicates stimulus-on, and downward deflection stimulus-off. A 100 msec. time mark is superimposed on the lower trace. Stimuli consisted of 1 sec. flashes of light delivered at 2 sec. intervals. No spatial zonation of receptive fields; responses are mixed "on-off" at all wavelengths (but note isolation of responses by intensity modulation in second from bottom records of columns 1 & 3).

Preliminary experiments with black and white stimuli revealed two on-off ganglion cell types with quite different discharge patterns and receptive field arrangements:

Type 1. Brief, phasic "on-off" discharges in response to stimuli placed anywhere within the receptive field (see Fig. 10). No centre-surround field arrangement, i.e. "on" and "off" response-components could never be evoked in isolation by using spatial variables of the stimulus alone. (This was sometimes possible by intensity modulation, see Fig. 10.)

Type 2. Slow-adapting "on-off" discharges, invariably showing centre-surround or adjacent receptive field arrangements, i.e. "on" and "off" discharges could be separately evoked by spatial positioning of the stimulus within the receptive field, (see Fig. 11). Type 2 units have receptive fields larger than those of Type 1 units, with axon terminals ending slightly deeper in the tectum (see p. 51). For both the "on" and "off" responses, an initial high-frequency burst of impulses is followed, after a short latency, by a second lower-frequency discharge lasting for many seconds or even minutes. In experiments this was terminated by the offset of the stimulus or the onset of the succeeding stimulus.

Ganglion cells were classified throughout subsequent experimental work into one of the two types described above.

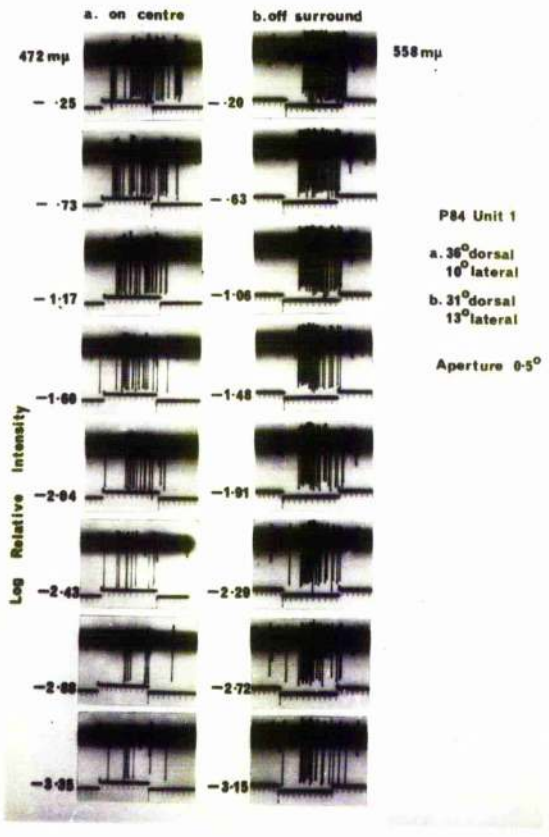

Fig. 11. Records of responses of a Type 2 "on"-centre unit to a 0.5° stimulus spot positioned a) at the field centre, b) in the surround. The initial high-frequency, short-latency burst of the "on" response is absent at low intensities, whereas the longer-latency, sustained discharge is present at all intensities, indicative of both cone and rod input to the cell. Top records are for maximum available stimulus intensity at each wavelength. The log relative quantum intensity of the stimulus is given at the left of each record. Upward deflection of lower trace indicates stimulus-on, and downward deflection stim: ulus-off. Time mark is 100 msec. Stimuli consisted of 1 sec. flashes of light delivered at 2 sec. intervals.

Equal quantum spectral response series (Programme 1)

1. Type 1 units. Series of responses to equal quantum spectra were obtained from 12 Type 1 units. All units gave opponent responses through all or part of the spectrum. Opponent responses of two units to equal quantum spectra are illustrated in Fig. 12. The first of these is shown graphically in Fig. 13. In some, the peak of one response component occurred in the green, whilst the other component peaked at either end of the spectrum (e.g. Unit P64, Figs. 12 & 13). In others, the "on" response was greatest towards

one end of the spectrum, whilst the "off" response peaked towards the opposite end (e.g. Unit P69, Fig. 12).

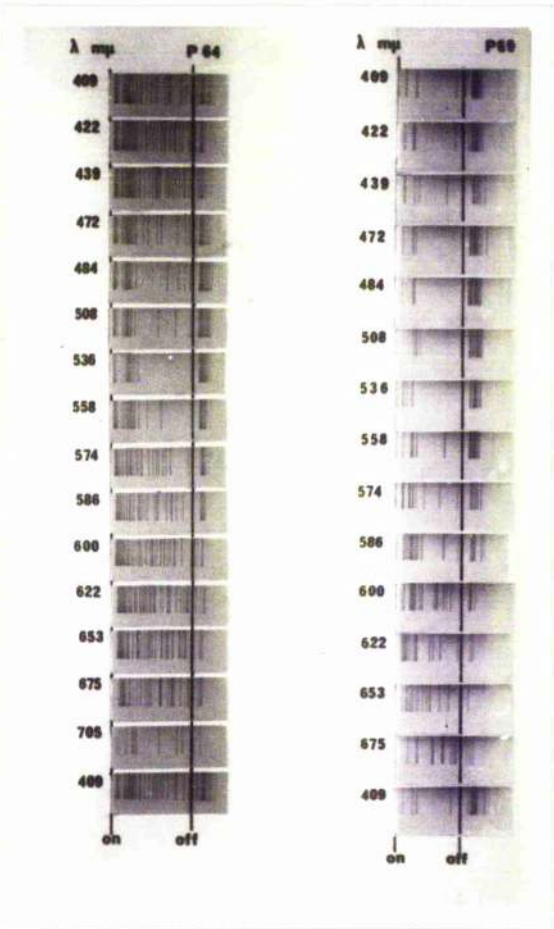

Fig. 12. Opponent responses of two Type 1 on-off units to an equal quantum stimulus through the spectrum. Intensity, maximum available, thus showing some phasic activity in common with other Type 1 units. 3^{o} stimulus spot positioned at the receptive field centre. Stimuli were 1 sec. flashes of light delivered at 5 sec. intervals. Spikes are re: presented as 2 msec. pulses from playback of tape recordings.

At the receptive field centre either the "on" or "off" component of the response was stronger at all or most wave: lengths, but with a marked variation in the "on":"off" ratio in different spectral regions. Stimulus testing in the more peripheral field indicated that the sensitivity of the stronger component at the field centre fell-off more rapidly than that of the weaker component towards the periphery (see p. 99 , Detailed analysis of receptive fields).

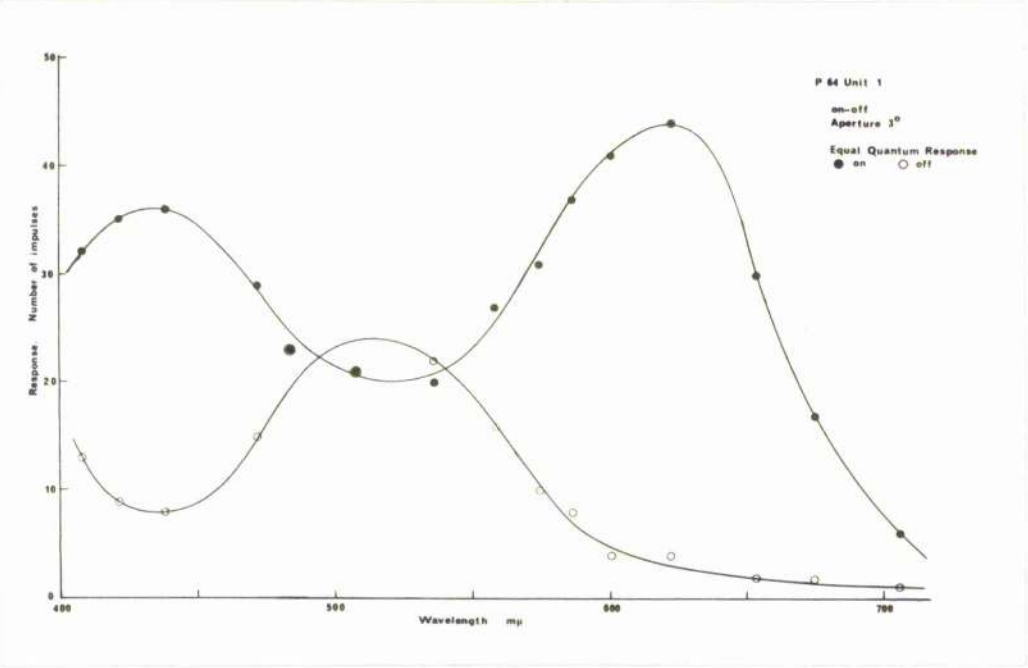

Fig. 13. Graphical representation of responses recorded from Unit 1, P64, as illustrated in Fig. 12, plotted against wavelength to bring out the opponent nature of the unit. Each point is an average of 5 responses.

2. Type 2 units. Response series to equal quantum spectra were obtained for four Type 2 units, with stimuli positioned to give mixed "on-off" responses. By contrast with Type 1 units, these units showed at best only weak opponent responses, with much less-pronounced differences in the "on":"off" ratio for equal quantum stimuli of different wavelengths.

3. Comment. The opponent nature of units was more marked in light-adapted eyes and at high stimulus intensities, as expected. Low-intensity, white light background illumination

applied during the testing of two units, enhanced the opponent response.

This programme indicates that Type 1 units, at least, are opponent colour sensitive. But without additional intensity-response data it gives little indication of the spectral sensitivities and types of input to the ganglion cells. The following experiments in which spectral, spatial and temporal properties of a few units have been studied in great detail, form the bulk of this thesis.

C. SPECTRAL SENSITIVITY DATA (Programme 2)

Preliminary considerations

1. Determination. Spectral sensitivities can be determined by measuring the stimulus intensities required either for threshold or for constant response (in this situation a standard number of spikes in the "on" or "off" response component) at each wavelength through the spectrum. The latter method was used for the following reasons:

1. Retinal ganglion cells receive inputs from several cone-types and from rods. In the dark-adapted state threshold measurements indicate only rod peaks.

2. Most units studied exhibit some spontaneous activity in darkness, in the absence of visual stimulation. Thus

minimal changes in firing patterns are masked. Thus without making elaborate measurements of the statistical regularity of spikes it is impracticable to measure threshold accurately.

3. Threshold-intensity measurements are unreliable since, at the appropriately low stimulus intensities, the preparation is susceptible to the slightest variations in general background luminance.

Intensity-response curves are classically sigmoidal see Introduction p. 20). Thus experimentally, if one works on the more or less linear portion where Weber functions apply, it is sufficient to determine at each test-wavelength the stimulus intensities required to give responses just greater, and just less, than the constant response. The intensity necessary to give a constant response can be obtained to a close approximation by linear interpolation from these measurements.

In subsequent experiments on the spectral sensitivity of retinal ganglion cells, intensity-response functions were determined at each wavelength over a wide intensity range. It was found that intensity-response curves were rarely sigmoid, and seldom linear over an appreciable range. Moreover they revealed much more information on the nature and interaction of inputs to ganglion cells than would have been obtained working solely from the previous assumption.

2. Limitations of the stimulus technique. In order to obtain complete spectral intensity-response series within the time units could be held, it was necessary to increase the frequency of stimulation to 1 sec. flashes of light delivered at 2 sec. intervals. The technique was standardised as far as possible. For any particular wavelength flashes were delivered in groups of five at each intensity, followed by a standard interval of darkness lasting 5 secs. (the time required to change neutral density filters to obtain the subsequent intensity level.)

Each group of five responses at any particular wavelength and intensity invariably showed some adaptation. The first "on" response was usually the strongest. Where this was so the corresponding "off" response was usually weaker than subsequent responses. For constant response to different wavelengths, one can make the assumption that this adaptation follows a similar course. Thus the averaging of any group of five responses leads to no appreciable error in the determination of spectral sensitivity.

Experiments were performed with the smallest light spots available (0.5°), to give minimal effects on dark-adaptation. However, responses were not always maximal to the highest stimulus intensities used at some wavelengths (see Figs. 14 & 21). It might be argued that at these intensities (some 3 log units above threshold), coupled

with high stimulation frequencies, sensitivity is decreased by reduction in the level of dark-adaptation. That this is not the case was shown by results from a few experiments in which stimulus intensities at a given wavelength were reduced without the 5 sec. standard interval in the flash-frequency, i.e. filters were changed in the 1 sec. period of stimulus-off. Responses were weaker and occurred with greater latencies at the higher intensity. Therefore the effect is <u>not</u> due to reduction of dark-adaptation, but to inhibition. This was borne out by subsequent experiments.

Spectral intensity-response series were thus complicated by the inhibitory interaction of the response- components. This was particularly so in Type 1 units, where it was not possible to isolate the components. As a result the spectral sensitivity of one component was partially suppressed in the range of peak sensitivity of the other component. Intensity-response series were further complicated in units receiving both cone and rod input. Thus, for example, where cone peaks occurred in the blue and/or orange, sensitivity curves were commonly broadened in the green due to rod input (see Fig. 22a). For these reasons spectral sensitivity functions were normally obtained at a constant response level below that where inhibition was marked, i.e. on the linear portion of the falling phase of intensity-response curves (e.g. Fig. 15). This minimised distortion and shift of peak sensitivity.

In a few units constant response levels were taken on the rising phase of intensity-response curves, i.e. at high stimulus intensities where inhibition was marked (e.g. Fig. 15), to show the effect of inhibition on spectral sensitivity.

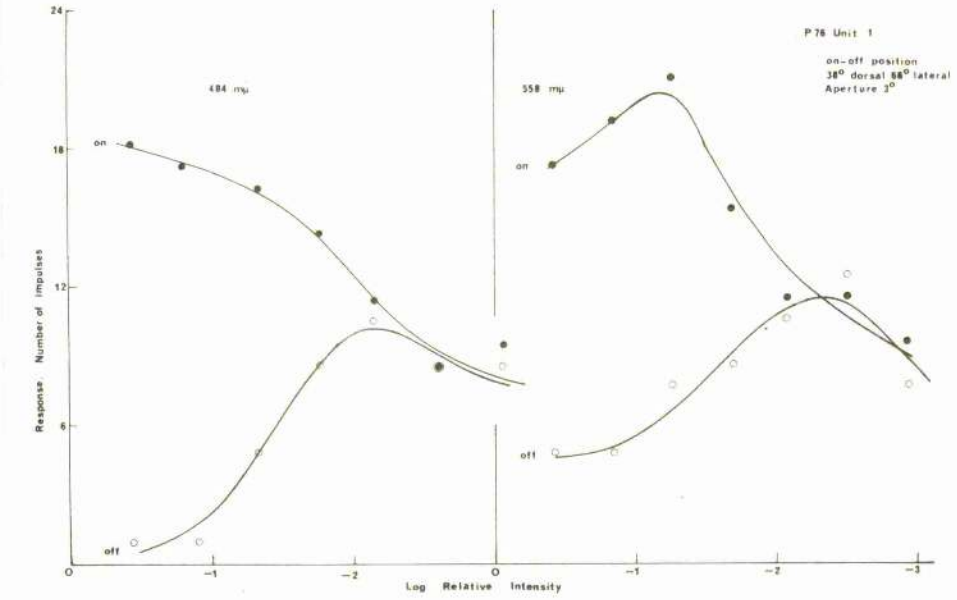

Fig. 14. Intensity-response curves for the "on" and "off" response-components of a Type 1 unit, at two wavelengths from the spectral series. This unit gave predominant "on" responses at all wavelengths to a 3° stimulus spot positioned at the receptive field centre. Each response-component showed marked inhibition (rising phase of curves) at high stimulus intensities, maximal for colour filters in the peak spectral sensitivity range of the opposite component. Stimuli were 1 sec. flashes of light delivered at 5 sec. intervals. Each point is an average of 5 responses.

Spectral sensitivity (Programme 2)

Type 1 units

1. Spectral intensity-response series. Typical intensity-response records for a Type 1 unit, at three wavelengths from the spectral series, are shown in Fig. 10 (p. 54). Responses were rapidly-adapting, brief bursts of spikes except at the highest stimulus intensities used at each wavelength. Complete series were obtained for seven Type 1 units, and partial series for a number of further units, in response to stimulus spots positioned at the centres of receptive fields.

The opponent nature of responses in such records was seldom marked, but as mentioned previously can be brought out more clearly by light-adapting the eye. It becomes apparent when spectral sensitivity functions are determined for each unit (see below).

Intensity-response curves were plotted at each wavelength for both the "on" and "off" components of the response. They show a smooth transition from wavelength to wavelength through the series. Typical curves for a Type 1 on-off unit are shown in Fig. 14, at two wavelengths from the spectral series. In this unit the "on" component of the

response was strongest at the receptive field centre. Three out of seven fully-characterised units gave stronger "on" responses, whilst four gave stronger "off" responses for this stimulus locus.

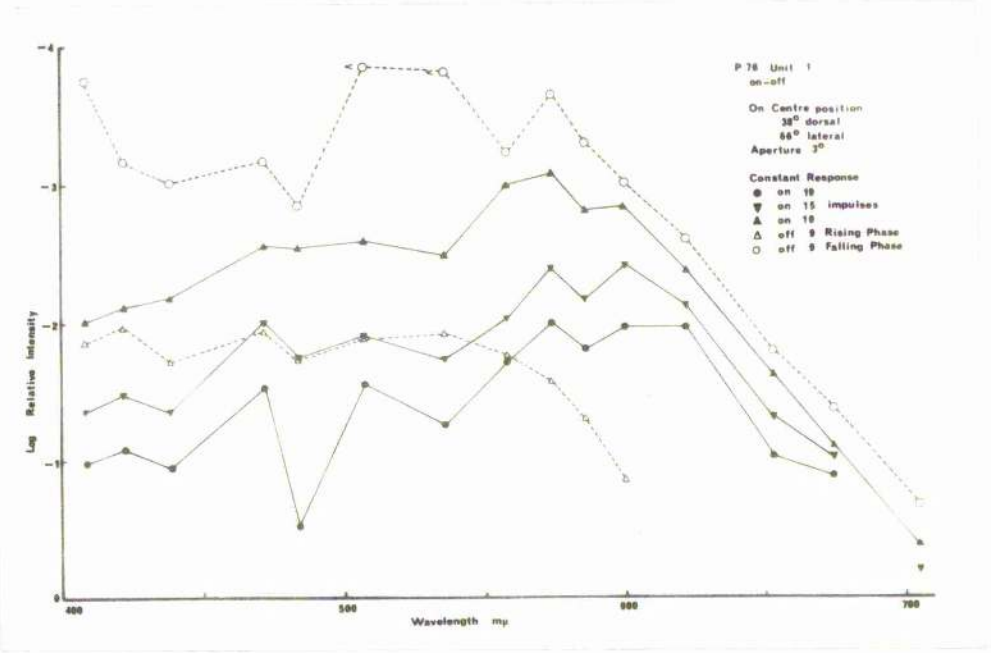

Fig. 15. Spectral sensitivity of the same Type 1 unit as in Fig. 14, abstracted from intensity-response series at several constant response levels for the "on" and "off" components. This unit was of the orange-"on", green-"off" type. Open triangles, taken for constant response levels on the rising (inhibition) phase of the "off" component intensity-response curves, indicate suppression of the green-"off" peak by inhibition from the "on" component.

2. Spectral sensitivity curves. In most fully-characterised units it was possible to plot constant response spectral sensitivity curves for both components of the response. In two, discrepancies in the intensity-response curves of the weaker component through the series were so

great that no attempt was made to plot the spectral sensitivity of this weaker component.

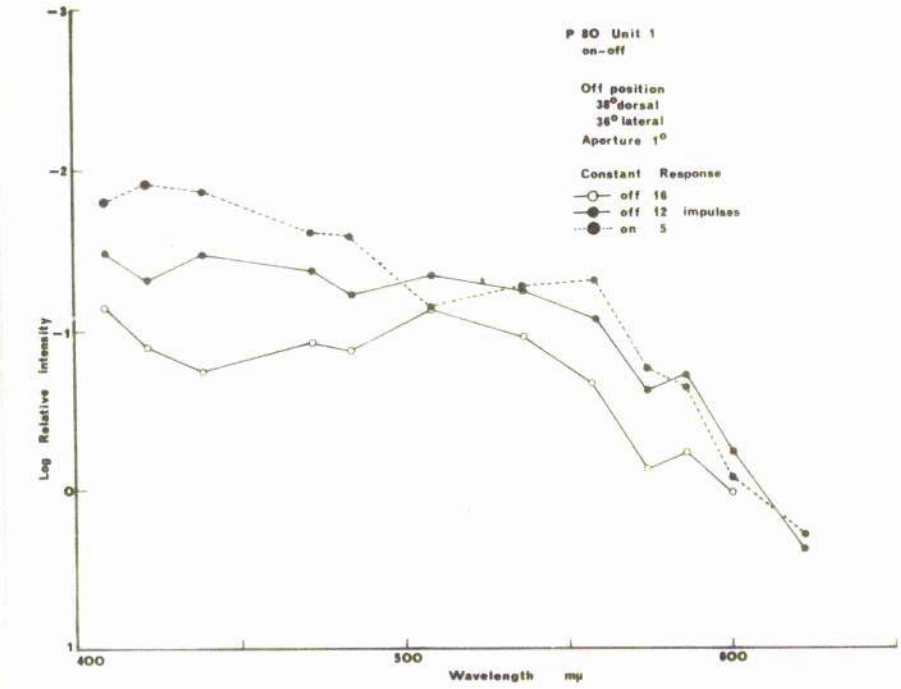

Fig. 16. Spectral sensitivity of a green-"off", blue-"on" Type 1 unit, which gave predominant "off" responses to a 1^{0} stimulus spot positioned at the geometric centre of the receptive field.

All Type 1 units gave spectrally-opponent "on-off" responses. It was apparent from intensity-response series and from latency measurements that the two response-components were mutually antagonistic. There was partial suppression of both responses at high stimulus intensities (Fig. 14). Response latencies were greater where suppression was marked, than at lower stimulus intensities where suppression was

less-marked or absent. Inhibition by the dominant central response was more marked than that due to the weaker component. Suppression in sensitivity of the "on" component was maximal at wavelengths corresponding to the spectral sensitivity peak of the "off" component, and vice versa.

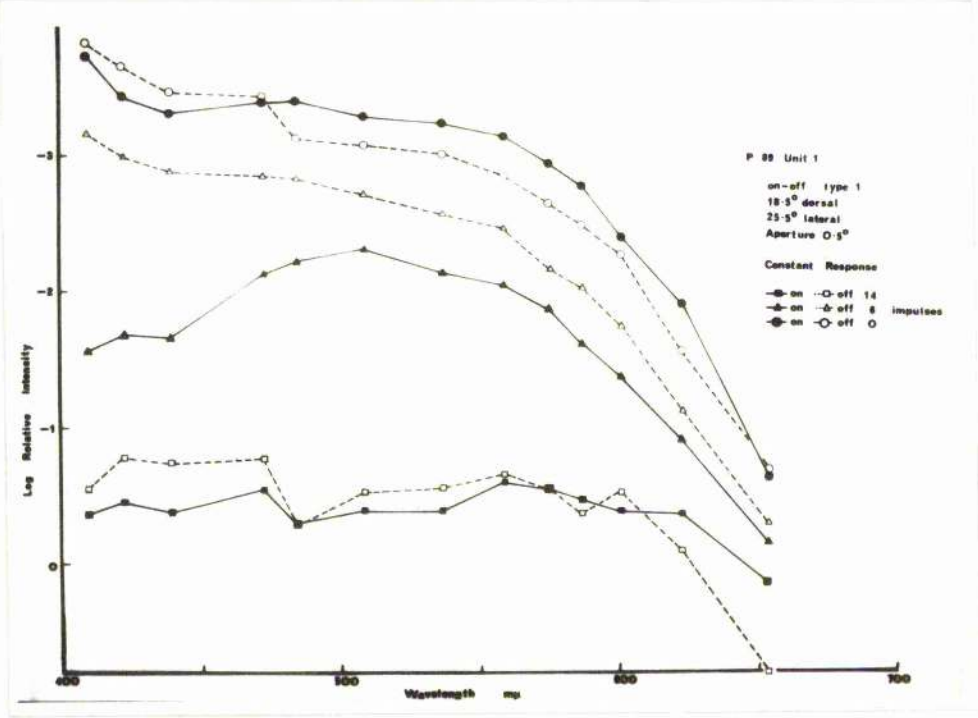

Fig. 17. Spectral sensitivity of a Type 1 unit, showing enhanced sensitivity in the green at low stimulus intensities due to rod input (see later). Sensitivity curves of several units were indeterminate at high stimulus intensities, apparent in this Figure and suggestive of inputs from several cone-types.

Spectral sensitivity curves for three Type 1 units are illustrated in Figs. 15-17. In Fig. 15 the spectral sensitivities of the "on" and "off" components of the same unit as in Fig. 14, are plotted at several constant response levels. Sensitivity peaks are indicated in the orange for

the predominant "on" component, and in the green for the "off" component. At low stimulus intensities (subthreshold for cones) the green "off" peak was more pronounced, indicative of rod input. A second unit was green-sensitive for the predominant "off" response, and blue-sensitive for the weaker "on" component (Fig. 16). A third unit, with indeterminate spectral sensitivity at high stimulus intens: ities, is shown in Fig. 17.

3. Atypical units. One unit showed the field arrange: ment and discharge pattern typical of Type 1 units (Fig. 18). However, spectral intensity-response series (Fig. 19) and spectral sensitivity curves (Fig. 20) for the "on" and "off" response-components were quite different.

Intensity-response curves were complicated by several peaks falling within the stimulus intensity range (Fig. 19). For the predominant "off" response, blue filters gave a single peak, maximal at about 440-450 mμ. For filters more into the green a second peak was expressed at higher stimulus intensities, maximal at 530-550 mμ, with attenuation of the blue peak. A third peak, maximal at about 600 mμ, was expressed for orange filters. Colour filters inter: mediate between those wavelengths illustrated in Fig. 19 showed a smooth transition of peaks from wavelength to wavelength. Similar peaks occurred in intensity-response curves for the weaker "on" component.

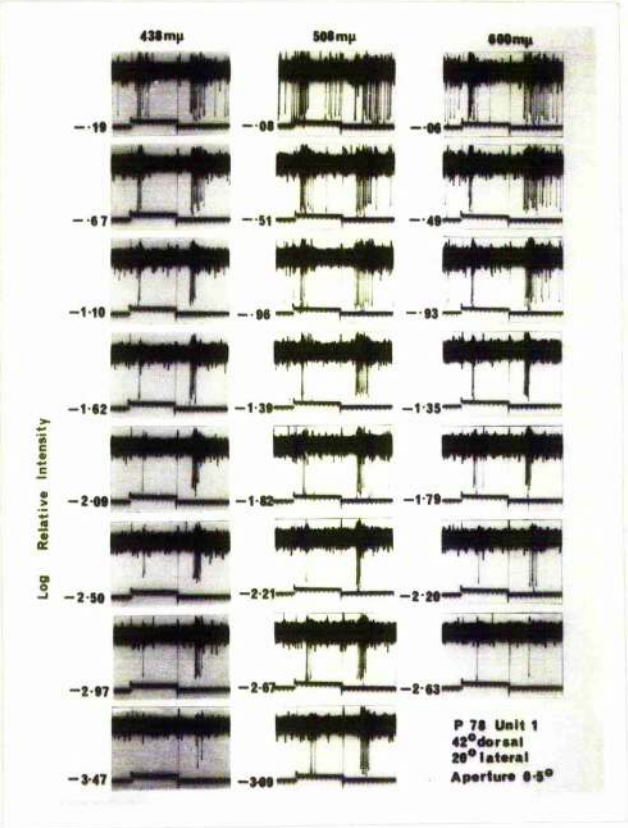

Fig. 18. Responses of the atypical Type 1 unit described, which exhibited a series of response peaks, within the stimulus intensity range, at different wavelengths. Responses at different intensities are illustrated for three wavelengths, for a 0.5° stimulus spot positioned at the receptive field centre. Top records are for maximum available stimulus intensity at each wavelength. The log relative quantum intensity of the stimulus is given at the left of each record. Upward deflection of lower trace indicates stimulus-on, and downward deflection stimulus-off. Time mark is 100 msec. Stimuli were 1 sec. flashes of light delivered at 2 sec. intervals.

At high stimulus intensities spectral sensitivity curves indicated sensitivity peaks in the blue, green and orange for both "on" and "off" response components, with the same wavelength maxima as for peaks expressed in the spectral intensity-response series. These peaks were not apparent at low stimulus intensities, where the spectral sensitivity curve for each component showed a single peak in the green (Fig. 20).

Several Type 1 units (and also some Type 2 units) gave a single peak within the stimulus intensity range, similar

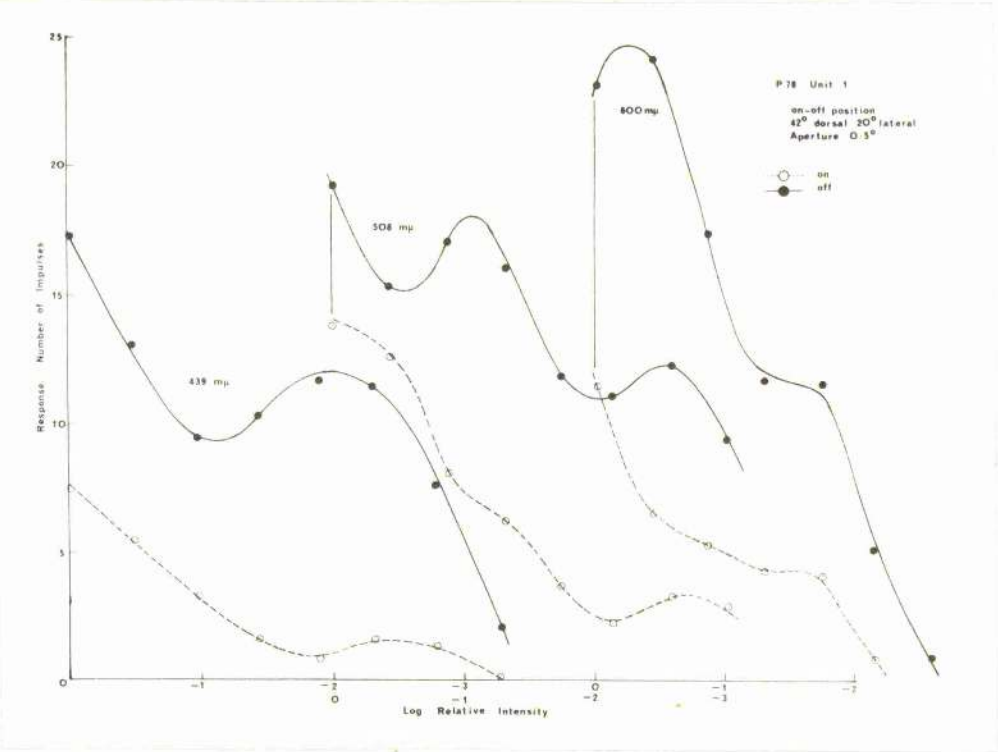

Fig. 19. Intensity-response curves at three wavelengths for the "on" and "off" response-components of the atypical Type 1 unit shown in Fig. 18. The series of peaks expressed within the stimulus intensity range were maximal at the same wavelengths as the spectral sensitivity peaks for the unit (Fig. 20a,b). Intensity-response curves at wavelengths intermediate between those illustrated showed a smooth transition of peaks from wavelength to wavelength. Each point is an average of 5 responses.

to the intensity-response curve at wavelength 439 mμ for the above unit (Fig. 19). With the exception of one Type 2 unit, in no other case was a multiple-peak system encountered in spectral intensity-response series. Of course, intensity-response peaks and spectral sensitivity peaks show the same wavelength maxima, typical of either cone or rod input. Therefore, where such peaks occur in intensity-

response curves, they have been interpreted as the expression of specific inputs to ganglion cells.

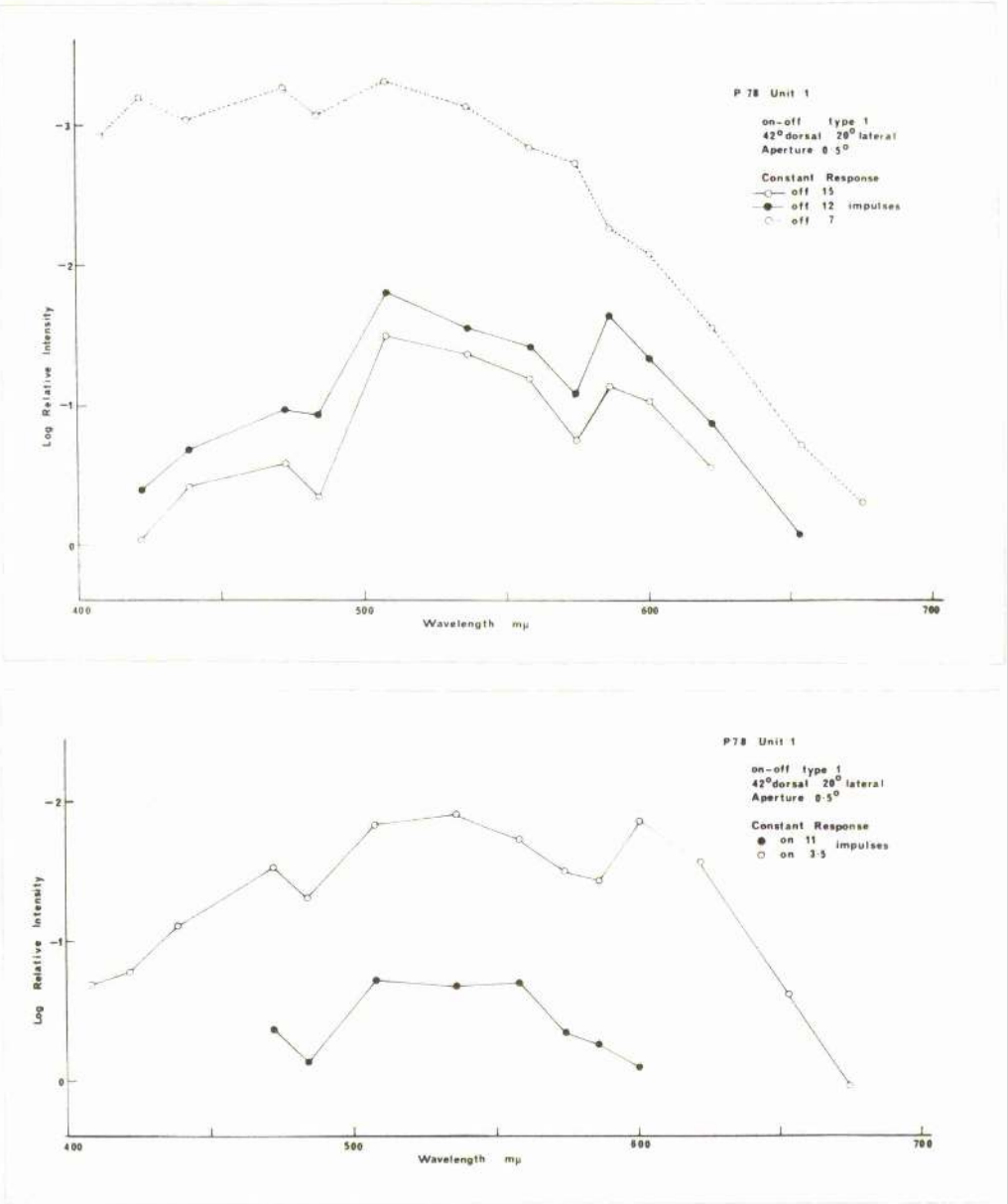

Fig. 20a,b. Spectral sensitivity of the atypical Type 1 unit of Figs. 18 & 19; A) "off" component; B) "on" component. Each component gave spectral peaks in three regions at high stimulus intensities. At low intensities these peaks were strikingly absent, with spectral sensitivity and peak characteristic of rod input.

The rising phase of curves at slightly higher stimulus intensities is presumably the expression of inhibitory action on the specific input.

4. Conclusion. The following Type 1 units have been recorded in detail and clearly identified:

			No. of units
"On" response predominant:	orange-"on"	green-"off".	1
	green-"on"	orange-"off".	2
"Off" response predominant:	green-"off"	blue-"on".	1
	blue-"off"	green-"on".	1
	green-"off"	orange-"on".	1
	blue/green/orange "on-off"		1

Type 2 units

1. Spectral intensity-response series. Type 2 units show centre-surround or adjacent receptive field arrangements, similar to those described by Kuffler (1953) in cat retina. They give one response to illumination of the receptive field centre and the opposite response to illumination of the surround. Consequently it has been more difficult to obtain complete data for this type, since it was necessary to obtain intensity-response series for two stimulus positions in the receptive field; at the field centre and in the

surround. Complete data has been obtained for both stimulus positions in four units; and series for the central response only, in a further four units.

In all units, with the exception of one "off"-centre unit (see section 3. Atypical units), the stimulus could be positioned in one part of the receptive field to evoke pure "off" responses. Activity during stimulus-on was completely suppressed, except at low stimulus intensities. At these intensities a few spontaneous impulses appeared during illumination.

Although it seemed likely that solely excitatory regions of receptive fields were present, for they have been found in many other vertebrate types, when stimuli were positioned appropriately, mixed "on-off" responses were always ellicited. Only at the lowest stimulus intensities used was the associated "off" component absent. The implications of this latter component are considered in the Discussion.

Unit types essentially similar to Kuffler's were identified; either "off"-centre, "on-off" surround; or "on-off" centre, "off"-surround, with a preponderance of "off"-centre units.

Records typical for Type 2 units are shown in Fig. 11a,b for the centre and surround responses of a Type 2 "on-off" centre unit, at wavelengths giving the clearest intensity-response patterns. In a number of units, as is clearly shown for the "on" component of the centre response for this unit, the initial high-frequency, short latency burst of spikes was absent at lower stimulus intensities, indicative of both cone and rod input to the unit (see later section).

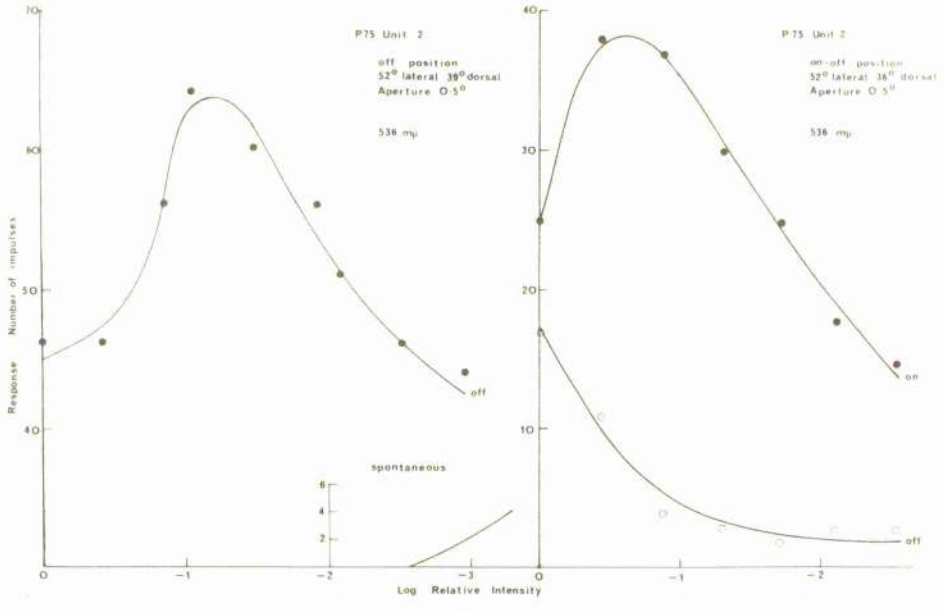

Fig. 21. Typical Type 2 unit intensity-response curves for the "off"-surround response, and for the "on" and "off" components of the centre response at one wavelength, for the unit illustrated in Fig. 11. Each point is an average of 5 responses.

Intensity-response curves were plotted at each wavelength, for the "on" and "off" components of the "on-off" response,

and for the "off" response. Curves were also plotted for the spontaneous activity occurring during the "on" of the stimulus, when stimulating the "off"-field. Typical curves for the "on" and "off" components of the "on-off" centre response, and for the "off" response and spontaneous activity of the field surround, are shown in Fig. 21 for a Type 2 "on-off" centre unit.

2. Spectral sensitivity curves. Constant response spectral sensitivity curves were plotted for a number of units, for each component of centre and surround responses, (Figs. 22-24 & 27-28). Sensitivity curves were also plotted for "spontaneous zero" of each unit, when stimulating the "off"-field.

"Spontaneous zero" was measured from the spontaneous activity "intensity-response" series. It is defined as the minimum stimulus intensity required at each wavelength, when stimulating the "off"-field, to give complete suppression of spontaneous discharges from ganglion cells during stimulus-on. In units from which complete intensity-response series were obtained both for the "off"-field and for the "on-off" field, the "spontaneous zero" spectral sensitivity curve was found to parallel closely the spectral sensitivity of the "on-off" response (e.g. Fig. 22a,b,c). This is a puzzling feature, for one would expect the

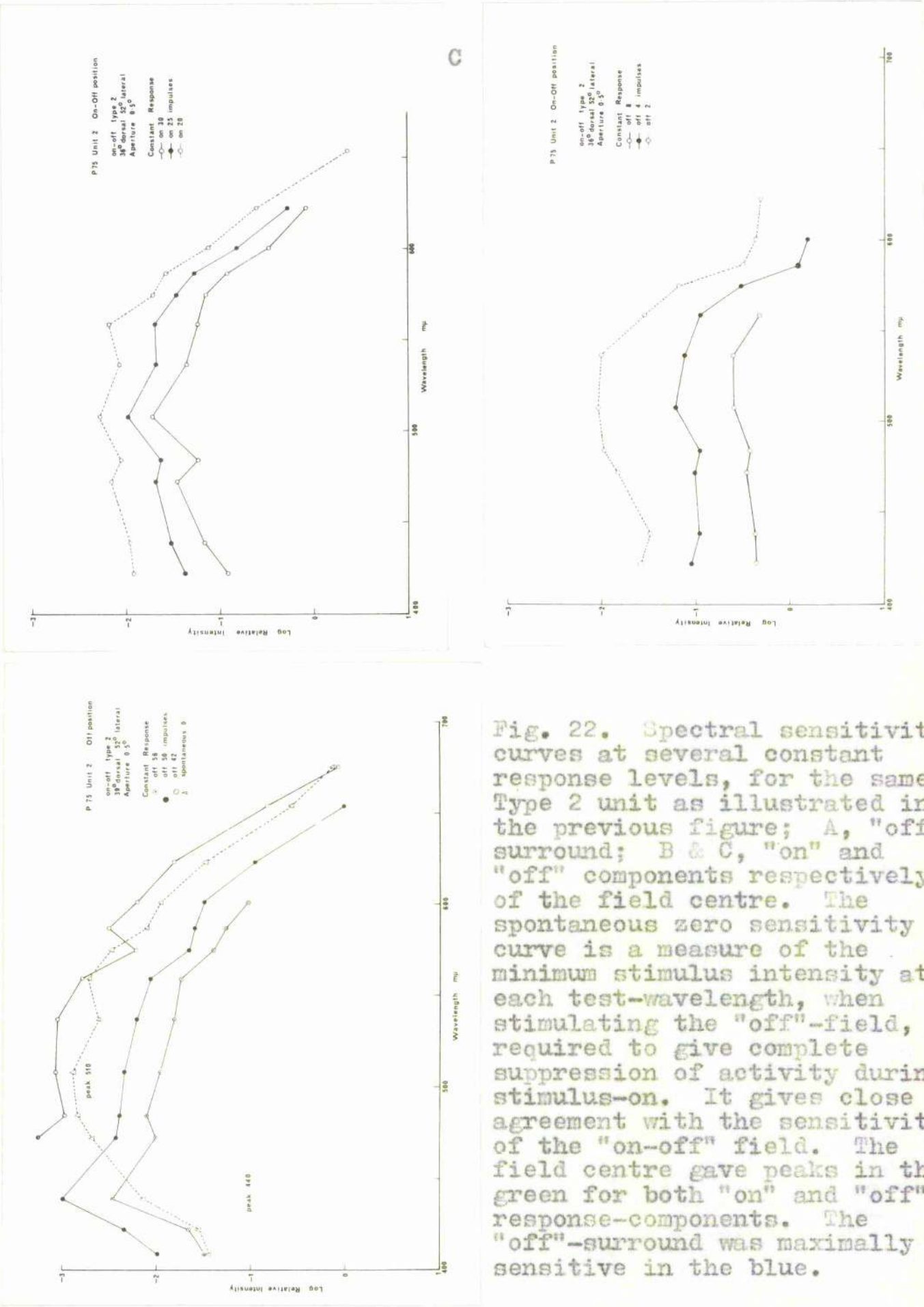

Fig. 22. Spectral sensitivity curves at several constant response levels, for the same Type 2 unit as illustrated in the previous figure; A, "off"-surround; B & C, "on" and "off" components respectively of the field centre. The spontaneous zero sensitivity curve is a measure of the minimum stimulus intensity at each test-wavelength, when stimulating the "off"-field, required to give complete suppression of activity during stimulus-on. It gives close agreement with the sensitivity of the "on-off" field. The field centre gave peaks in the green for both "on" and "off" response-components. The "off"-surround was maximally sensitive in the blue.

"spontaneous zero" sensitivity to reflect the sensitivity of the inhibitory "off"-field. None-the-less, the "spontaneous zero" sensitivity curve has been used as an estimate of the sensitivity of the "on-off" surround in those units which could be held long enough only for characterisation of the "off"-centre response (e.g. Figs. 23 & 24).

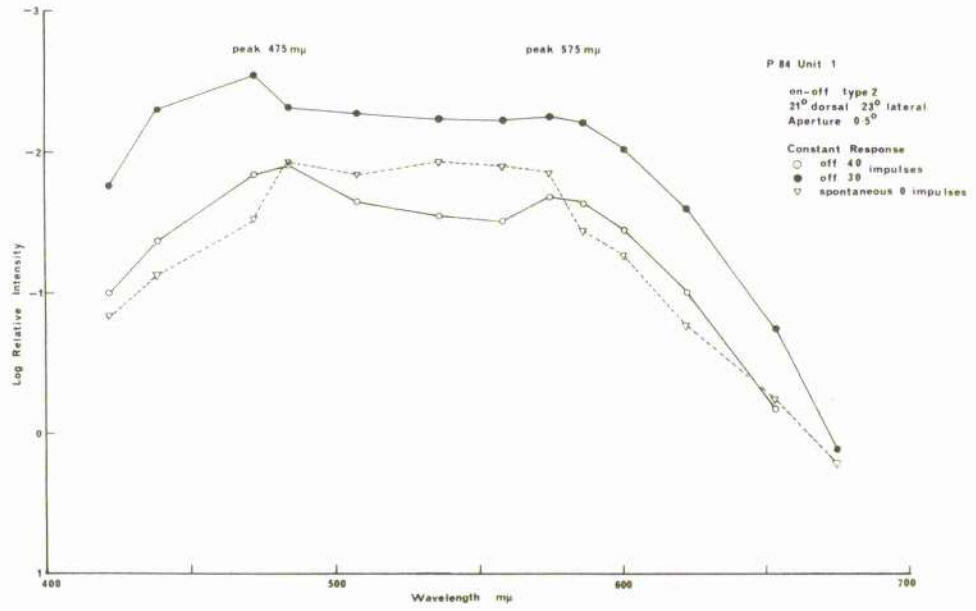

Fig. 23. Spectral sensitivity of the field centre of a Type 2 "off"-centre unit. This unit was orange-and-blue sensitive for the "off"-centre of the receptive field, and green-sensitive in the "on-off" surround.

Spectral sensitivity curves were generally broader than for Type 1 units, presumably because of the weak opponent nature of responses and the prominence of rod input. Response thresholds were lower. Spectral sensitivity

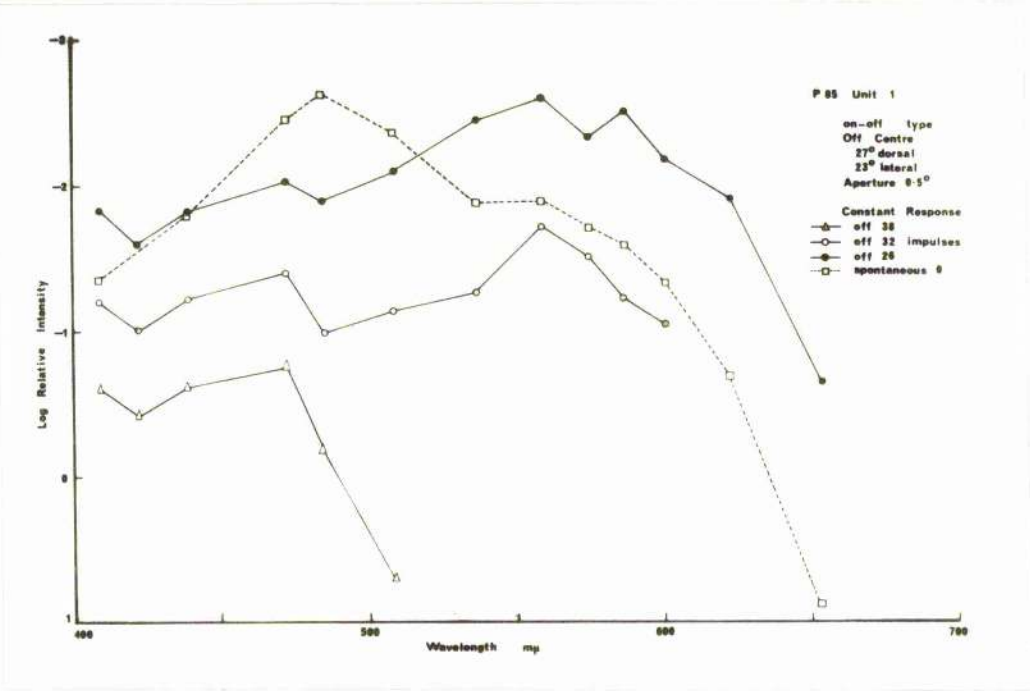

Fig. 24. Spectral sensitivity of a Type 2 "off"-centre unit. This unit was centre-sensitive in the blue and orange. At low intensity, the sensitivity was enhanced in the green, due to rod input. The "spontaneous zero" curve reflected the sensitivity of the surround.

curves at high stimulus intensities showed one or more peaks with maxima typical for cones. In several units either the centre or surround gave two such peaks at different wavelengths, e.g. blue and orange (see Figs. 23 & 24).

Most Type 2 units gave evidence of both cone and rod input. A reduction in stimulus intensity was followed by a change in the form of sensitivity curves. All units with rod input gave a single spectral peak in the green at low intensities. As with Type 1 units, where this was the case a single peak was also expressed in the intensity-response

series (e.g. Fig. 26, "off"-centre response).

In every unit the "on" and "off" components of the "on-off" response, whether this was the centre or surround response, showed the same spectral sensitivity. This finding has a number of implications which are considered in the Discussion.

In most units it was clear that the spectral sensitivities of the field centre and surround were different; i.e. peak "on-off" and "off" responses occurred in different spectral regions. In one or two units however (see Fig. 27, for example), peaks were indeterminate and it was not clear whether the sensitivities of the field centre and surround were different or identical. It is possible that the study of a greater number of Type 2 units might reveal two sub-types, opponent and non-opponent.

3. Interaction between inputs. In "off"-centre units there was strong mutual inhibition between the "on" component of the centre response and the "off"-surround response at high stimulus intensities (Fig. 21). In "off"-centre units the "on" component of the surround response showed marked inhibition from the centre, but inhibition of the "off"-centre response was comparatively weak (Fig. 26 and see also Section 5). Inhibition was most marked at the peak spectral sensitivity of the inhibitory component,

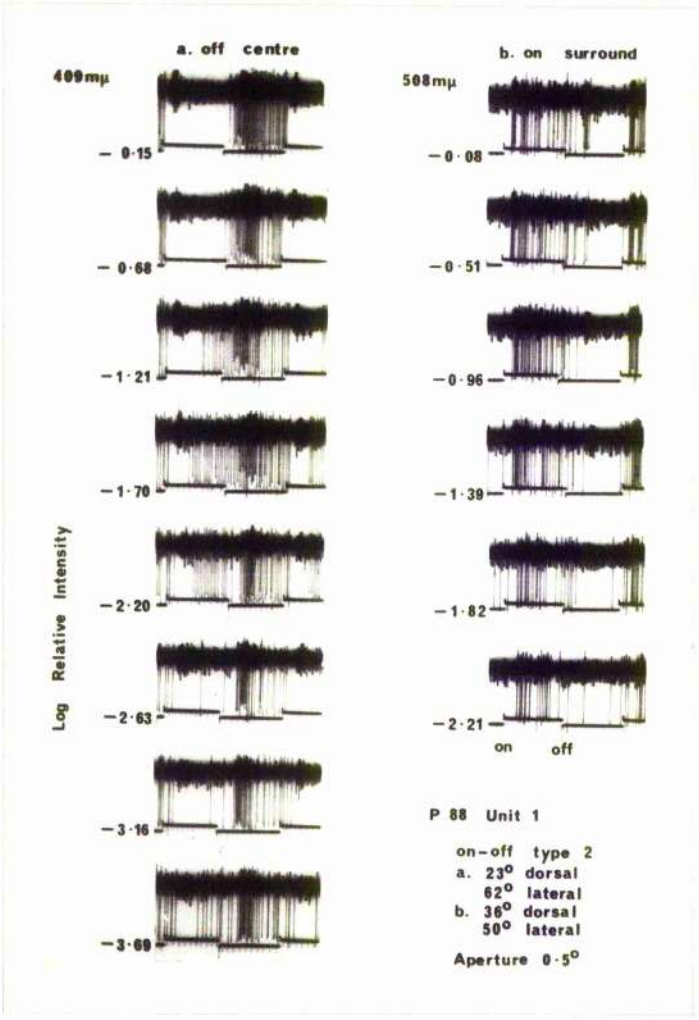

Fig. 25. Records of responses from the centre and surround of the receptive field of one atypical Type 2 "off"-centre unit; a) centre responses, b) surround responses, each at one wavelength and different intensities. Top records are for the maximum intensity available at each wavelength. Only in this one unit was the response from the "off"-field accompanied by an "on" component. The "on" component was completely suppressed at high stimulus intensities. Spikes during stimulus-on in the lowest left hand trace are of resumed spontaneous activity. Note also the suppression of the "on"-surround response at the highest intensities, and the weak accompanying "off" discharge. Upward deflection of lower trace indicates stimulus-on, and downward deflection stimulus-off. Time mark is 100 msec. Stimuli were 1 sec. flashes of light delivered at 2 sec. intervals.

and at high stimulus intensities. It was weak or absent at low intensities. Inhibition of the "off" component of the "on-off" response was not observed in any units studied. This is to be expected, and is considered in the Discussion. No examples of centre-and-surround summation were found.

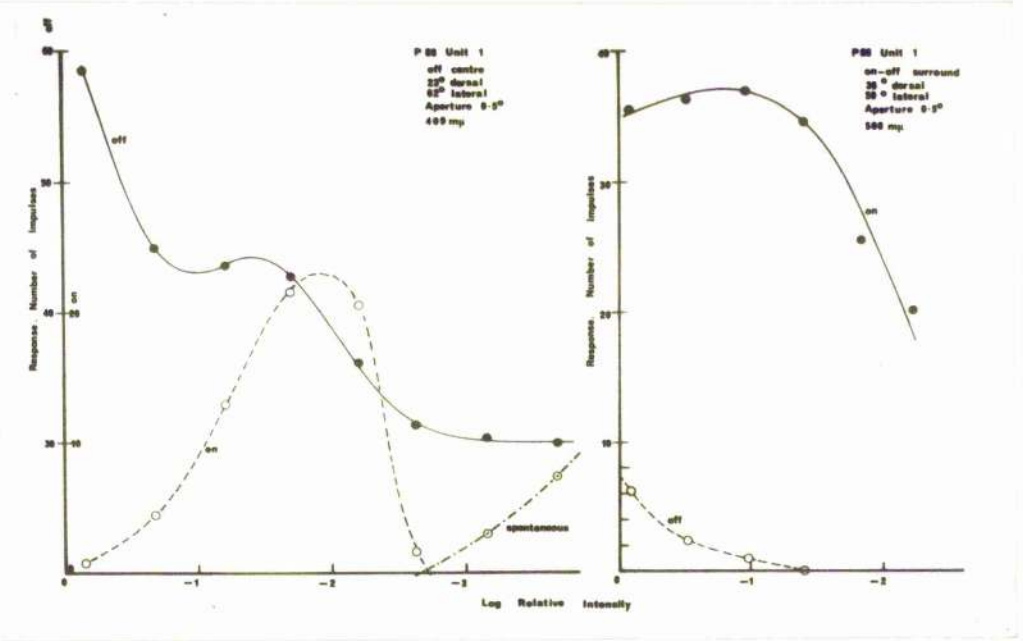

Fig. 26. Intensity-response curves for the centre and surround response-components of the atypical Type 2 unit of the previous figure. With the exception of the centre "on" component, these curves are typical for "off"-centre units; i.e. absence or weakness of inhibition of the "off"-centre response, strong inhibition of the "on"-surround component at high intensities and lack of inhibition of the weak accompanying "off" component. Each point is an average of 5 responses.

4. Atypical units. In one "off"-centre unit the "off" response was accompanied by an "on" component. This "on" component was completely suppressed by intense stimuli at all wavelengths, (Figs. 25 & 26); suppression was very

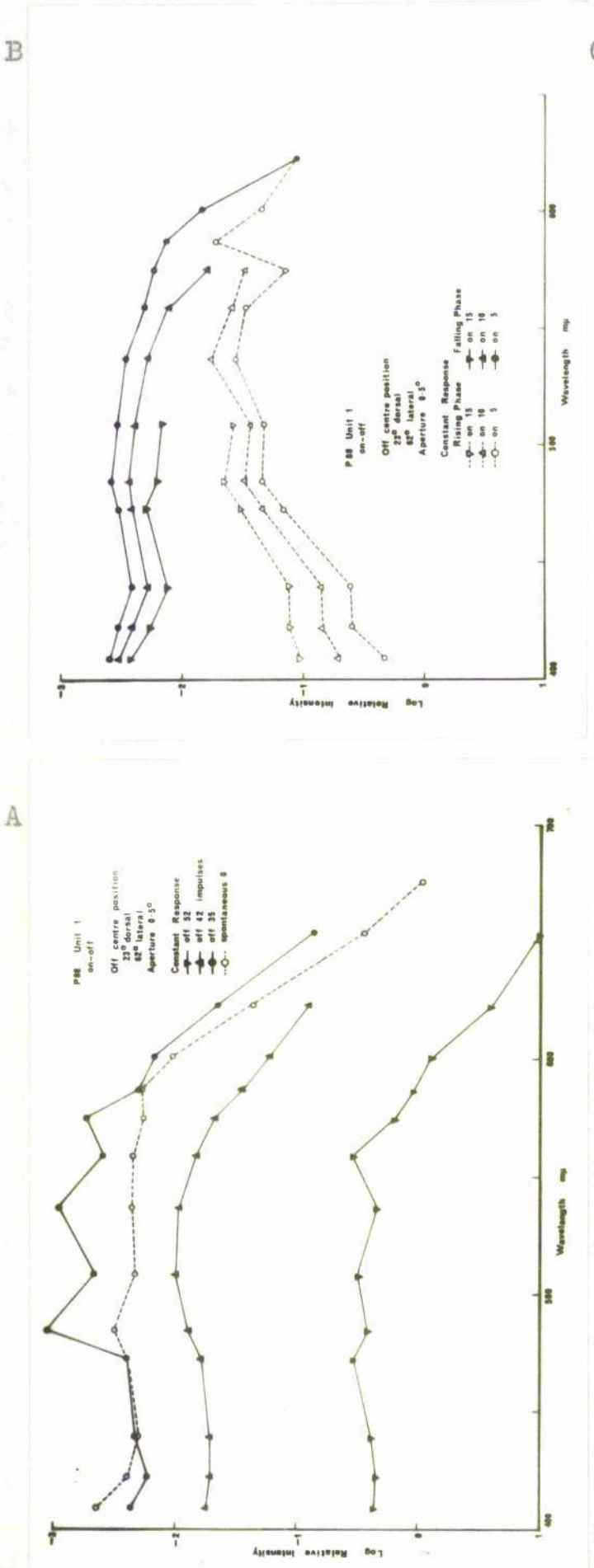

Fig. 27. Spectral sensitivity of the atypical Type 2 "off"-centre unit of Figs. 25 & 26 (see also text). A, "off"-centre response; B, "on" component of centre response; C, "on" and "off" components of surround response. Spectral sensitivities of the field centre and surround are indeterminate. It is not clear whether the unit is opponent or non-opponent. Inhibition of the "on" component of the centre response(B, broken curve) is maximal at the peak sensitivity of both centre and surround.

marked over a broad range of intensity, and maximal for green filters. The spectral sensitivity was similar to that of the accompanying "off"-centre response (Fig. 27a,b). Centre and surround responses both showed sensitivity to blue and green, but with an indication that green sensitivity was greater at high intensities in the surround and at low intensities for the field centre.

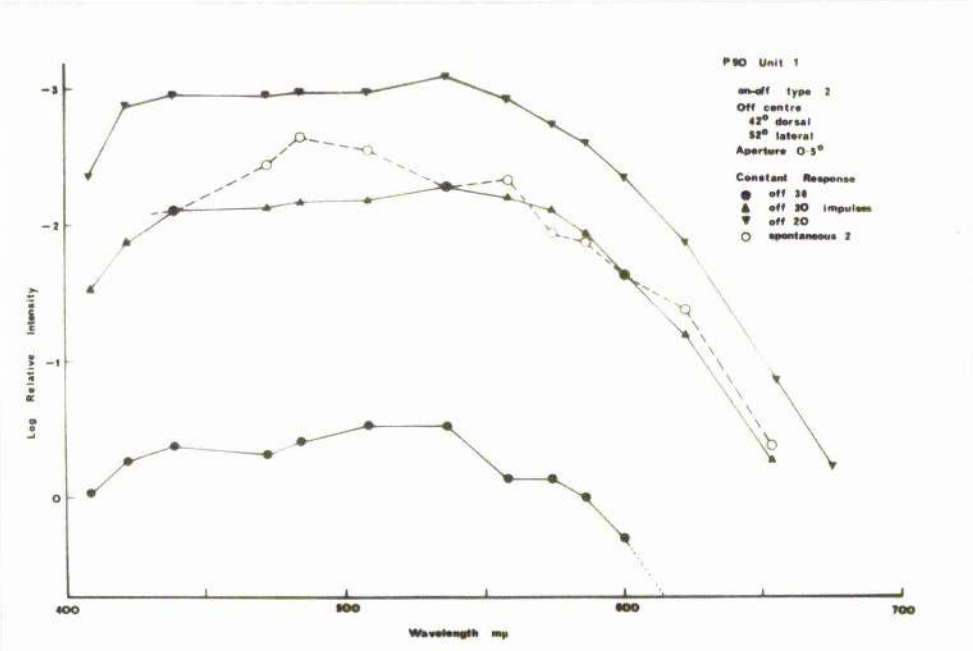

Fig. 28. Spectral sensitivity of the field centre of a Type 2 "off"-centre unit. At stimulus intensities suprathreshold for cones (lowest curve) there is a suggestion of three peaks in the ranges of typical cone absorption spectra (compare with the Type 1 unit of Fig. 20, p. 71). With reduction in intensity there is a change in sensitivity to give curves more typical of rod input. The "spontaneous zero" curve indicates blue-green sensitivity for the field surround.

The "off"-centre response of one Type 2 unit gave intensity-response series and spectral sensitivity somewhat

similar to the atypical Type 1 unit described earlier. At high stimulus intensities there is a suggestion of three spectral peaks - blue, green and orange. At intensities below the peak expressed within the intensity range used, spectral sensitivity curves indicated that responses were dependent on rod input alone.

5. Effect of increasing size of equal-intensity, centrally-positioned stimulus spots (Programme 3). In two "off"-centre units where the spectral sensitivity of both field centre and surround had been fully characterised, the effect of increasing the size of a centrally-positioned stimulus spot from 0.5° to 3° was investigated at a number of wavelengths through the spectrum. Stimuli having an approximately equal quantum output per unit area at each wavelength were used.

The first unit was tested at the highest stimulus intensity available for each wavelength; i.e. without interposition of any neutral density filters in the stimulating light path. Responses to a 3° spot were always weaker than responses to a 0.5° spot of the same wavelength and intensity per unit area, and their latencies were greater. Illustrative records are shown in Fig. 29, selected

from groups of five responses for each wavelength and spot size. Averaged values for the number of spikes in each group of "off" responses are given in Table 3, together with the "suppression factor" which has been defined as the ratio of the response strength for the small spot to that of the large spot.

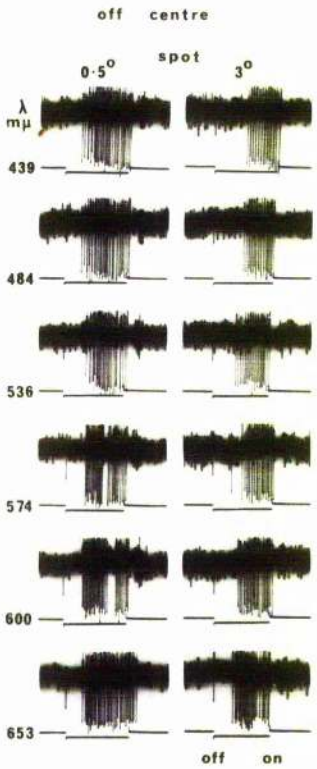

Fig. 29. Effect of increasing size of a centrally-positioned stimulus spot on the centre response of a Type 2 "off"-centre unit. Records for two spot sizes of the same intensity per unit area are shown at a number of wavelengths. Response strengths to the larger spot are decreased and latencies increased, indicating inhibition. Inhibition is maximal at the spectral peak (484mμ) of the field surround (see also Table 3). Note the pause between the initial and second phases of the response in two of the left hand records, indicating cone-rod input (see later). Downward deflection of lower trace marks stimulus-off. Stimuli were 1 sec. flashes of light delivered at 2 sec. intervals. Stimulus intensity is maximum available at each wavelength.

TABLE 3

Wavelength (mμ)	439	484	536	574	600	653
Response (no. of spikes)						
A. 0.5° spot	35	37	31	33	37	38
B. 3° spot	27	24	24	29	31	33
Suppression factor (A/B)	1.29	1.56	1.28	1.17	1.22	1.18

That this is inhibition we know from the increased latency of the response, a recognised phenomenon in other work. Moreover the intensity level at which responses were measured falls on the rising (inhibition) phase of intensity-response curves for the "off"-centre response of this unit. The inhibition and latency of the response are maximal in the range of peak sensitivity of the "on" component of the surround response, which is approximated by the "spontaneous zero" curve in Fig. 24.

The anomaly of this data lies in the fact that when the limits of the "off"-centre of the unit were determined with a small exploratory spot, even a 3^{o} spot positioned at the locus from which the above data was obtained fell well within the limits of the field centre. It is possible that the inhibition observed is due to light scatter from the stimulus, or to optical errors invoked by stimulation of an aquatic eye in air. But these possibilities are discounted by certain points raised in the Discussion.

A second unit, tested in an identical manner to the first, but with a 1 log unit neutral density filter interposed in the stimulus light path, showed exactly opposite effects. The larger spot evoked <u>stronger</u> responses at all wavelengths, (Fig. 30 & Table 4). These results are not incompatible with the concept of inhibitory surrounds, for

responses at this intensity level lie on the falling phase of intensity-response curves, where there is little or no inhibition apparent. In this case, provided stimuli fall within the field centre, stronger responses with shorter latencies are expected either for an increase in stimulus intensity, or for an increase in area of the stimulus at the same intensity.

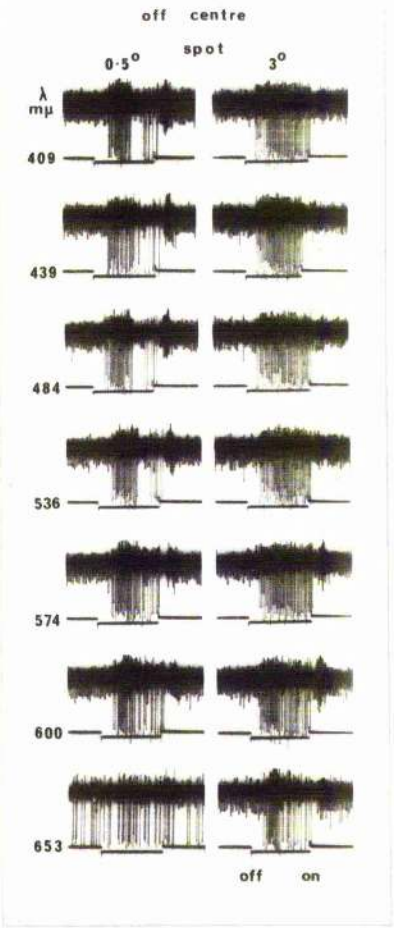

Fig. 30. Type 2 unit: "off"-centre responses to equal-intensity stimulus spots of two sizes at a number of wave: lengths. Downward deflection of lower trace indicates stimulus-off. Stimulus intensity: approximately 1 log unit below maximum. Stimuli were 1 sec. flashes of light delivered at 2 sec. intervals. Note: in the bottom left hand record illumination is insufficient to suppress spontaneous activity.

In Table 4 are given the averaged number of spikes from groups of five responses to 0.5° and 3° stimulus spots of equal intensity per unit area, at a number of wavelengths.

TABLE 4

Wavelength (mμ)	409	422	439	472	484	508	536	558	574	586	600
Observed response (no. of spikes)											
A. 0.5° spot	39	36	35	38	41	42	41	41	45	41	39
B. 3° spot	57	58	57	55	52	55	53	57	57	53	50
Expected response (no. of spikes)											
C. 3° spot	54	50	48	53	55	54	52	58	62	55	52
Observed/Expected B/D (%)	106	116	119	104	95	102	102	98	92	96	96

The area of the 3° spot was accurately measured to be 45 times that of the smaller spot. Thus on Hartline and Barlow's assumption that response strength depends only on the total quantity of illumination (see Introduction) equal responses to the two stimuli would be expected if the intensity of the smaller spot was 1.65 log units above that of the larger spot. (Both spot sizes fell well within the limits of the receptive field centre, which were deter: mined previously.) To test this theory the expected response strength (Table 4, line C) was estimated from intensity-response series obtained previously for a 0.5° stimulus spot, at an intensity level 1.65 log units above the point giving the observed response to a 0.5° spot (shown in Table 4, line A). The observed response to a 3° spot is given in

line B. The observed and expected responses to a 3^{o} spot at each wavelength are then compared and expressed as a percentage (line D). Deviations from the expected relation: ship are significant only for filters in the far blue, indicating pure excitation and responses depending only on the total quantity of illumination.

6. Conclusion. The following Type 2 units have been characterised and identified:

	No. of units
"On-off" centre, "off"-surround:	
green centre, blue surround	1
green centre, orange-and-blue surround	1
"Off"-centre, "on-off" surround:	
orange-and-blue centre, green surround	2
blue centre, green or blue-green surround	2
blue-green centre, green (+ blue) surround	1
green (+ blue + orange) centre, green surround	1

Rod-cone interaction (Programme 2)

At least for some animals with cone-rod retinae it is well-established that the responses of single retinal ganglion cells are influenced by inputs both from cones and rods. In plaice also this feature is apparent from

records of most ganglion cells of both on-off types.

Many techniques have been employed to test this premise. The most obvious line of evidence is the difference in spectral sensitivity of single units when light-adapted and when in the dark-adapted state. Alternatively Gouras & Link (1966) showed that for dark-adapted perifoveal monkey ganglion cells receiving mixed cone-rod input, cone-induced responses have shorter latencies. Thus at stimulus intensities suprathreshold for cones, it is the cones which determine response latency. At lower intensities latencies are greater and are determined by the more sensitive rod input.

Evidence for cone-rod input to dark-adapted plaice retinal ganglion cells, and the interaction of the two processes, is considered in terms of spectral sensitivity and response latency, determined from the same set of records for each cell.

1. Spectral sensitivity. In most units spectral sensitivity curves plotted for low constant response levels (i.e. at intensities subthreshold for cones) showed a marked difference to sensitivity curves for high stimulus intensities. With reduction of intensity there was a transition from curves showing one or more spectral peaks at wavelengths typical for cones, to those which exhibited a single peak

at 520-530 mμ, characteristic of rod input. This is seen particularly for the "off" component of the Type 1 unit of Fig. 20a, p. 71.

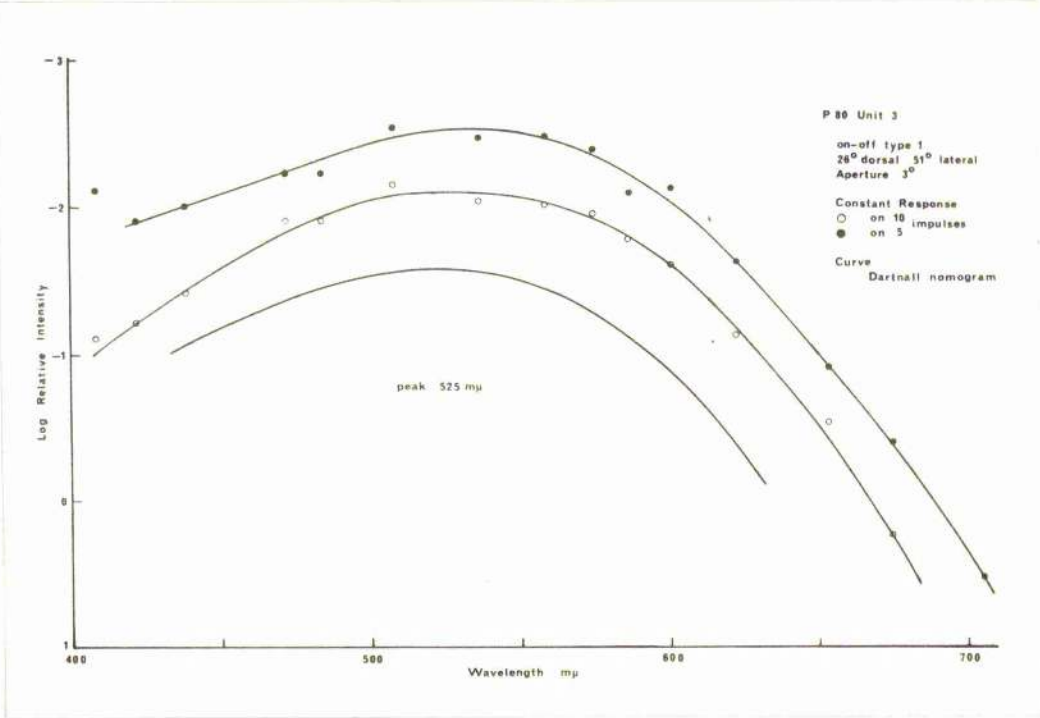

Fig. 31. Spectral sensitivity of the predominant "on" component of a Type 1 unit with cone-rod input. At low stimulus intensities curves showed a single peak in the green at about 520-530 mμ (upper two curves). These curves are compared with the empirically-derived absorption spectrum for a rod pigment with spectral peak at 525 mμ, constructed from the Dartnall nomogram (Dartnall, 1953).

The spectral sensitivity of a Type 1 unit is shown in Fig. 31 at two low-intensity, constant response levels, for the predominant "on" component at the field centre. Exper: imental curves are compared with the sensitivity curve for rod pigment 525, constructed from the Dartnall nomogram (Dartnall, 1953). In this and in other units, experimental

curves agree well with the Dartnall curve. The shift on the ordinate is immaterial since the sensitivity scale is relative only. At the extremes of the spectrum, the experimental curve is narrower than the fitted curve in some cases. This can be ascribed to absorption by the lens and ocular media, since Dartnall's measurements involved pigment extracts.

2. Response latency. The expression of cone and rod inputs in ganglion cell responses is most obvious in records from Type 2 units - those with sustained discharges where the response consists of two phases: 1, an initial, short-latency, high-frequency burst and 2, a longer-latency, maintained discharge. At high stimulus intensities, suprathreshold for cones as adjudged from spectral sensitivity peaks, the response is of short latency since both phases are present (see Fig. 11a, p. 56). At low intensity the initial burst is absent, which results in a step-increase in response latency.

As with Kuffler's (1953) records the initial burst at some intensities is followed by a transient pause before onset of the maintained phase (see Figs. 25b, 29 & 30). The intensity level at which the pause occurs corresponds to the trough immediately preceding peaks commonly expressed in intensity-response curves within the stimulus intensity

range used (see Fig. 26, "off"-centre response). Gouras & Link (1966) observe a transitory refractoriness following cone responses. This is almost certainly the explanation of the pause.

In several Type 2 units attempts were made to plot intensity-response curves at each wavelength for the initial and later phases of the response, in the hope of obtaining sensitivity curves with peaks characteristic for cones and rods respectively. This was not possible, however, because of the interaction between the inputs and the temporal overlap of the two discharge phases in the mid-region of the stimulus intensity range.

If latencies of all responses recorded through the stimulus intensity range at any one wavelength are measured and plotted as a latency histogram, good confirmation of mixed cone-rod input is obtained if the distribution is bimodal. Further, cone or rod input alone will be indicated by a unimodal distribution of shorter or longer latency respectively. No assumptions of characteristic or average cone or rod latencies can be made, for stimulus intensities used were limited to a 3 log unit range embracing cone thresholds and did not cover the total cone and rod intensity-response range. Hence histogram peaks are somewhat skewed.

Latency histograms of a large number of Type 1 and Type 2 units were plotted in this way. There is good correlation with spectral sensitivity curves for each unit in every case. Examples for both unit types are illustrated in Figs. 32-34.

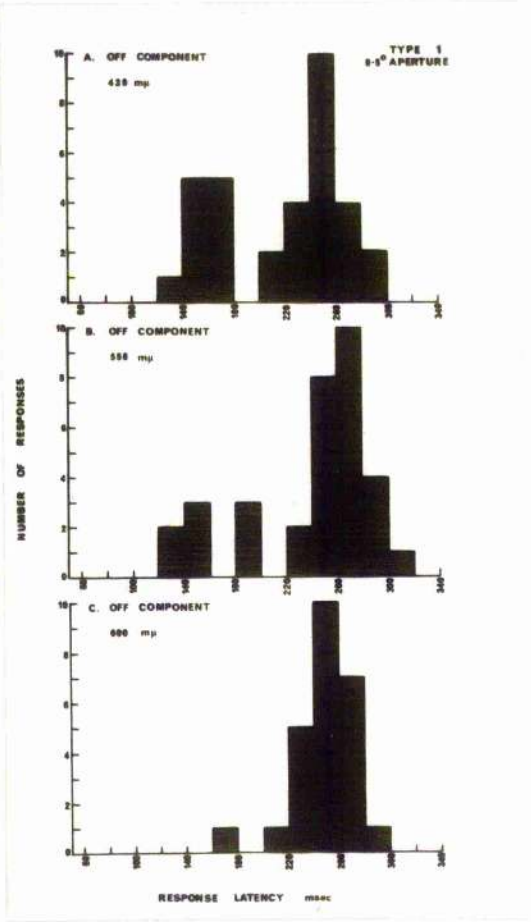

Fig. 32. Type 1 unit: response latency histograms. A, B & C - predominant "off" component at three wavelengths. The bimodal distribution in A and B indicates both cone (short latency peak) and rod (longer latency peak) influences on responses to blue and green stimuli. In the orange (C) only the rod peak is apparent.

Response-latency histograms for the "off" component of a Type 1 unit are shown for three wavelengths in Fig. 32. Spectral sensitivity measurements on this unit indicated blue and possibly green cone inputs for the predominant "off" component, with rod input apparent at low stimulus intensities. Latency histograms are clearly bimodal at

wavelengths 439 mμ (blue) and 558 mμ (green), but unimodal at 600 mμ (orange) where the single peak corresponds in latency to the second (longer latency) peak at the two former wavelengths. Inputs from blue cones, green cones, and rods are thus indicated, confirming previous findings.

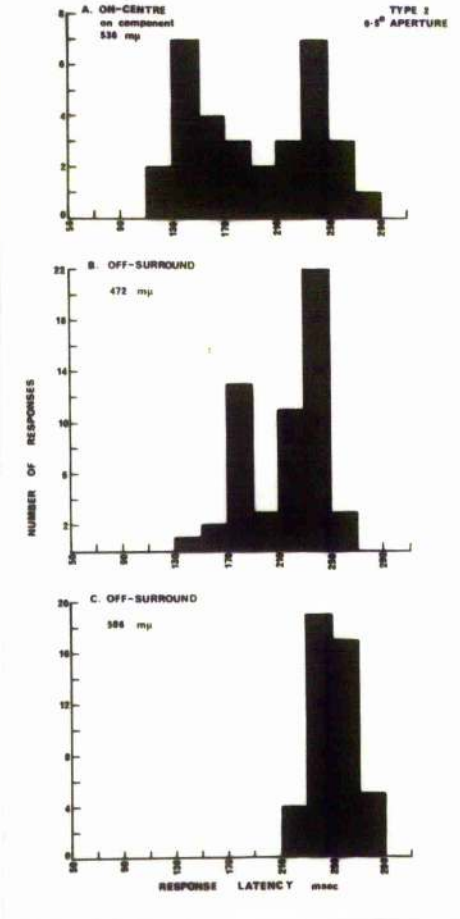

Fig. 33. Type 2 "on-off" centre unit: response latency histograms. A, "on" component of centre response; bimodal distribution for green filters indicates both green cones, and rods. B & C, "off"-surround response at two wavelengths; histograms are bimodal only for blue filters, indicating inputs from blue cones and from rods.

Latency histograms for Type 2 units show similar correlations with spectral sensitivity. The example chosen for illustration (Fig. 33) is the Type 2 "on-off" centre unit also shown in Fig. 22. The field centre of this unit was green-sensitive, but from spectral sensitivity alone

it was not possible to determine whether this was due only to cones, or to rods, or both. The surround was blue-sensitive, with rod input in addition. The latency histogram for the centre response (Fig. 33a) at wavelength 536 mμ (green) is clearly bimodal, indicative of green cones, and rod input. Similarly for the "off"-surround the latency histogram at 472 mμ (blue-green) indicates inputs from blue cones, and from rods (Fig. 33b), but for orange wavelengths (Fig. 33c) only rods are active.

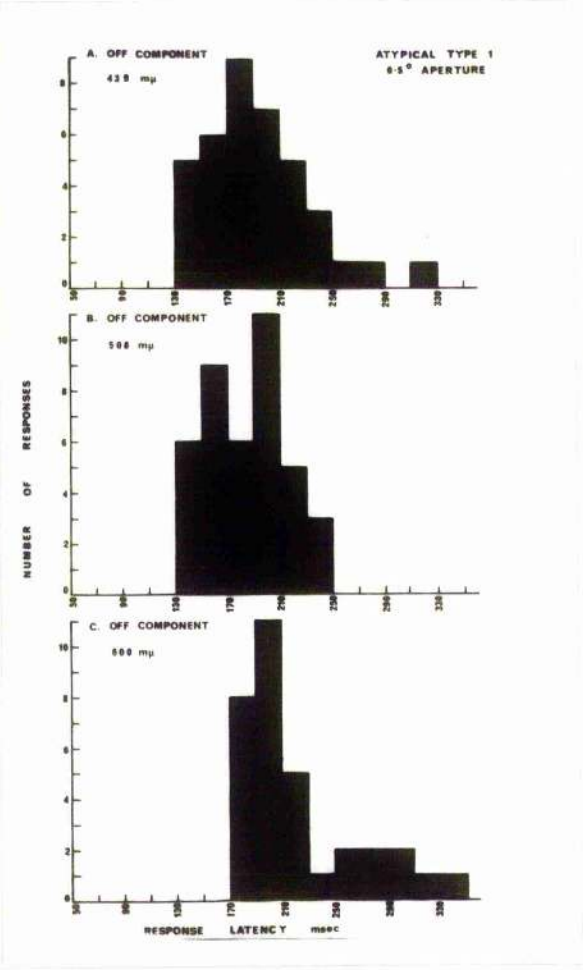

Fig. 34. Atypical Type 1 unit: response-latency histograms for the predominant "off" component at three wavelengths. Short-latency peaks at all three wave: lengths substantiate evidence from spectral sensitivity measurements (Fig. 20a) for inputs from three discrete cone types.

An interesting case is the atypical Type 1 unit of Fig. 20. Each response-component gave spectral peaks in

three regions. It was concluded that these were the expression of three cone inputs - blue, green and orange. For the "off" component at least (Fig. 20a), green sensitivity was enhanced at low stimulus intensities. This was attributed to rod input. The short-latency peaks of latency histograms at three wavelengths for this "off" component (Fig. 34) substantiate the conclusion that there are inputs from three cone types. Only in histograms A and C (Fig. 34) is there evidence for a longer-latency rod peak. However, where cones are active they, and not rods, determine response latency. Thus if the sensitivity of blue and green cones is higher than that of orange cones, rod response latencies would be masked at corresponding wavelengths.

3. Conclusion. On this evidence it is concluded that ganglion cells receive inputs from one or more cone types, and usually from rods also. In Type 2 units the short-latency component is presumably the expression of cone input to the cell, while the longer-latency more sensitive component is due to rod input.

Movement and directional sensitivity (Programme 3)

On completion of spectral sensitivity measurements, some units were tested for responses to movement in the horizontal and vertical planes. Stimuli consisted of steadily-illuminated, coloured apertures. With the present stimulus arrangement it was not possible to refine the velocity parameters of these tests, but all Type 1 and Type 2 units gave strong responses to movement.

Type 1 units. Type 1 units gave brisk discharges to movement in either direction and in either plane. These crude tests revealed no directional selectivity, unlike similar units identified in goldfish (Cronley-Dillon, 1964). The strength of discharges was governed in a rational way, depending on the intensity, size and colour of the stimulus. Strongest responses to movement of stimuli of different colours and equal quantum emission, were given at the peak spectral sensitivity of the predominant response-component.

Type 2 units. As with Type 1 units, every unit tested was highly-sensitive to movement, but in a manner governed by the centre-surround or adjacent subdivision of the receptive fields. "Off"-centre units respond to centripetal, and "on-off" centre units to centrifugal movement (compare

with Cronley-Dillon, 1964), without showing true directional selectivity. This feature is found only in Type 2 units with adjacent, rather than concentric, "on-off" and "off" receptive field zones.

Detailed analyses of receptive fields of Type 1 units

Of particular interest were the receptive fields of Type 1 units, those receiving both excitatory and inhibitory inputs, yet showing no segregation of receptive fields into centre and surround. Stimulation of an aquatic eye through air may lead to vast optical distortion and preclude the identification of such receptive field subdivisions. A few experiments, such as that described below, were aimed at elliminating this possibility.

The technique employed was to determine the receptive field centre by preliminary tests (i.e. the central position giving maximal responses) and then to record responses to stimulation at intervals across the receptive field, horizontally and vertically through the field centre. The smallest light spot available (0.5°) was used, to give maximal discrimination of responses. At each test locus, equal quantum stimuli of three wavelengths (439, 536 and 600 mμ) - chosen to correspond roughly with cone and rod peaks - were delivered as one second flashes of light.

The sector of the visual field available for stimulation is limited to the quadrant described by the paired arcs of the stimulus mounting, with the eye projecting 30° anterior (60° lateral) and 30° upwards (dorsal) as shown in Fig. 2. Diagrams of receptive fields are thus related to this quadrant by coordinates as follows:

0° dorsal, 0° lateral = anterior to eye in horizontal plane,

0° dorsal, 90° lateral = lateral to eye in horizontal plane,

and 90° dorsal = vertically above the eye.

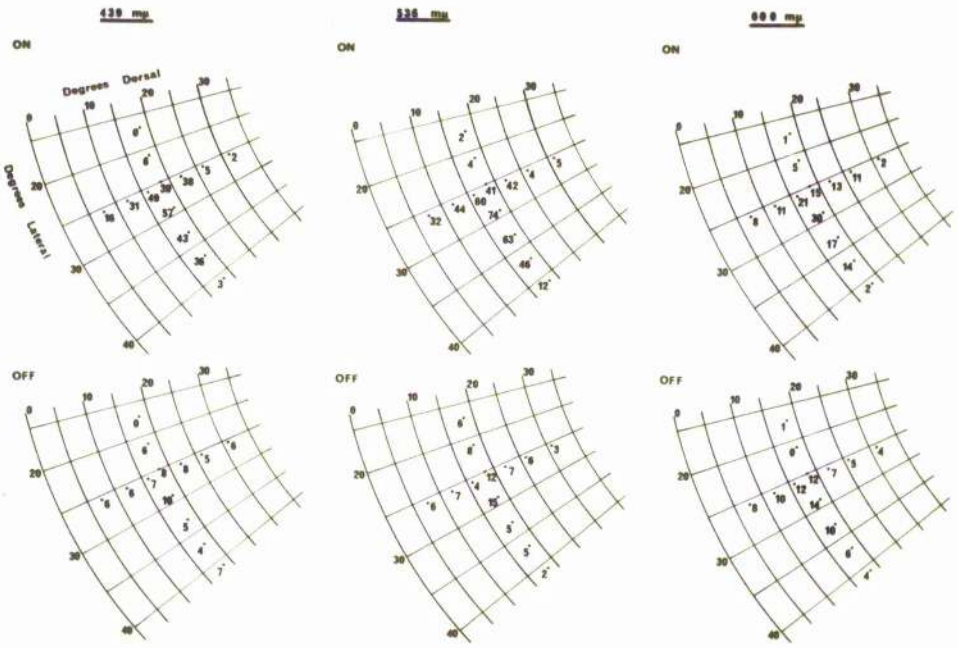

Fig. 35. Receptive field plot for a Type 1 green-"on", orange-"off" unit. Numbers of spikes in each component of the mixed "on-off" response are shown for three wavelengths at a number of stimulus loci, horizontally and vertically through the field centre. Diagrams represent a section of the sphere described by the radius arcs of the stimulus arrangement, with the eye centred at 60° lateral, 30° dorsal. Stimuli were 1 sec. flashes of light, aperture 0.5°, giving an equal quantum emission at each test wavelength.

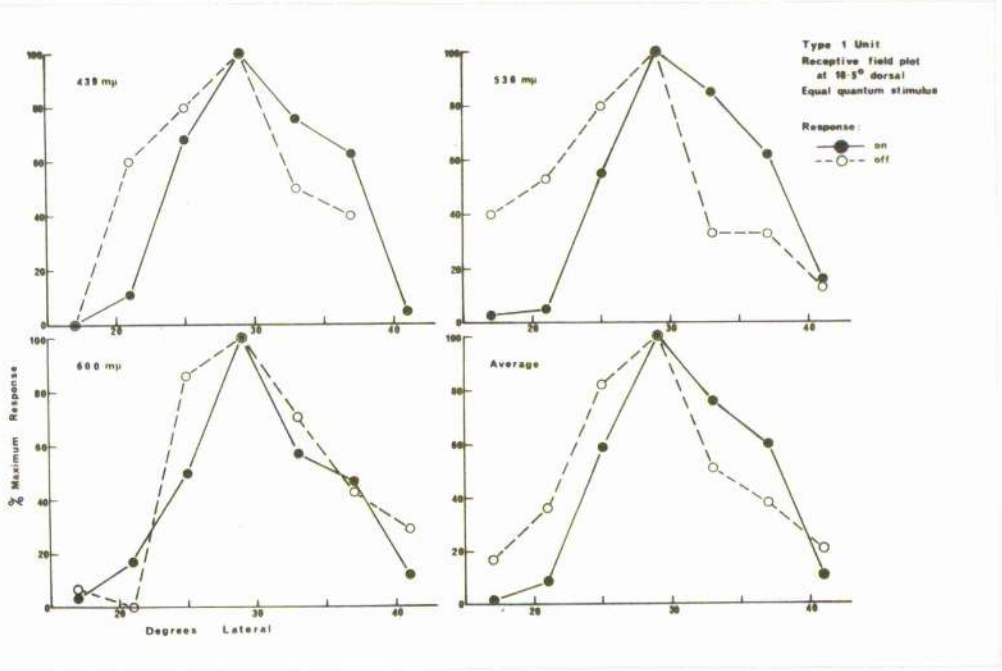

Fig. 36. Response profile for the Type 1 green-"on", orange-"off" unit of Fig. 35, horizontally through the receptive field centre. Both components are shown at three wavelengths, expressed as percentages of the maximal response at each wavelength. The average profile of these three wavelengths is shown in the bottom right hand graph.

The receptive field of a Type 1 unit is illustrated in Fig. 35. The number of spikes in the "on" and "off" components of the mixed "on-off" response are shown separately for each stimulus position and wavelength. This unit was of the green-"on", orange-"off" variety, giving a predominant "on" response at the field centre. Strengths of the "off" component at 536 and 600 mμ are not markedly different, since the orange-"off" peak occurred between these two wavelengths. For stimulation, a quantum intensity level about 1 log unit below the maximum available was used, since

at this level intensity-response functions had revealed little interaction between the response components. The receptive field was roughly circular in shape, some 30° in diameter.

The response profile of the receptive field, horizontally through the field centre, is shown graphically in Fig. 36 for each wavelength. "On" and "off" components are expressed as percentages of the maximal response from the field centre at each wavelength.

The fall-off in sensitivity of each component, towards the periphery, appears to be similar. There is some suggestion that the positions of maximal sensitivity of the "off" and "on" components lie just anterior and lateral to the field centre respectively. There is no indication of spatial segregation of the receptive field into adjacent or concentric "on" and "off" zones.

DISCUSSION

An attempt has been made to analyse the spectral, spatial and temporal properties of a relatively small number of on-off retinal ganglion cells in plaice in great detail, and to investigate the nature and interaction of the several discrete inputs to these cells. It is likely that there are further variations within each of the two cell types identified, but on the basis of discharge patterns every cell, without exception, fits one of the two recognised types.

Background Experiments

The retino-tectal projection in the plaice is similar to that found in other fish; goldfish (Jacobson & Gaze, 1964); black bass, bluegill, carp and goldfish (Schwassmann & Kruger, 1965); and in the optic tecta of several vertebrate classes; in amphibia - frog (Gaze, 1958; Maturana et al, 1960); in reptiles - alligator (Heric & Kruger, 1965); and in birds - pigeon (Hamdi & Whitteridge, 1954).

In plaice, tectal-layering of axon terminals of the several ganglion cell types appears to be well-defined. On and off cells terminate most superficially. The two on-off types terminate in discrete deeper layers. This is

more akin to the situation found in frog (Maturana et al, 1960). Jacobson & Gaze (1964) found no evidence of tectal-layering in goldfish.

Spectral sensitivity

1. Limitations of the stimulus technique. An obvious criticism of the present work is the stimulation of an aquatic eye through air. This shortcoming is difficult to obviate since stimulation through water, while ruling out refractive errors, brings in unknown errors in the intensity of light incident on the eye. Fish are naturally myopic even in their normal environment. As pointed out earlier, the near point in air must lie even nearer to the eye, so that the final aperture of the stimulus probably lies within the range of accommodation. No examination was made to test this, but it seems likely that reasonable image formation is possible in air, for boundaries of receptive fields and divisions between field centre and surround in Type 2 units were sharp. Moreover there appears to be little distortion in the shapes and configurations of receptive fields. These tend to be of the conventional circular or elliptical pattern. The presence of centre-surround or adjacent subdivisions of receptive fields of Type 2 units is in agreement with the work of others.

The prominent feature of stimulation through air is the enhanced visual angle which receptive fields of single ganglion cells subtend. Thus Type 1 units have receptive fields up to 30° in diameter. Type 2 units have larger receptive fields, often as much as 50° in diameter. These figures are of the same order as those of Jacobson & Gaze (1964), who recorded responses from goldfish tectum to stimulation of the eye through air.

At stimulus frequencies necessary for complete characterisation of units within the time for which they could be held, coupled with the intensities used, it is likely that one is not working with truly dark-adapted eyes; rather at a more-or-less constant level between full dark- and full light-adaptation. There was no way of overcoming this, other than using the smallest stimulus apertures available. Response variability made it necessary to average five responses at each wavelength and intensity setting. Errors were accounted for as far as possible by standardising both the sequence and time intervals of stimulus presentations.

2. Comments on spectral sensitivity. Peaks of constant response spectral sensitivity curves are not always reliable estimates of cone or rod input to units.

At stimulus intensities necessary to excite cones there is marked inhibitory interaction between inputs, resulting in distortion and apparent wavelength shifts of spectral peaks. This is particularly true of Type 1 units, those without centre-surround receptive fields, in which it has not proved possible to stimulate either the excitatory or the inhibitory input in isolation. In fact, wherever both "on" (excitation) and "off" (inhibition) discharges occur together there must be some interaction and sensitivity distortion, for both processes input to single units. To an extent this distortion can be overcome by working on the more-or-less linear portion of intensity-response curves, at intensity levels below those where inhibition is marked. However, this has involved working at intensity levels near to cone thresholds. Consequently cone peaks are weak. This explains why opponent responses are not as marked as in light-adapted eyes, quite apart from the effects due to migratory movements of cones (Nicol, 1965). But cone peaks are more faithfully defined, for to intense stimuli opponent responses are the resultant of inputs from discrete cone-populations and strong interaction between these inputs.

The inferred connections of cones to retinal ganglion cells of Types 1 and 2 are illustrated diagrammatically in

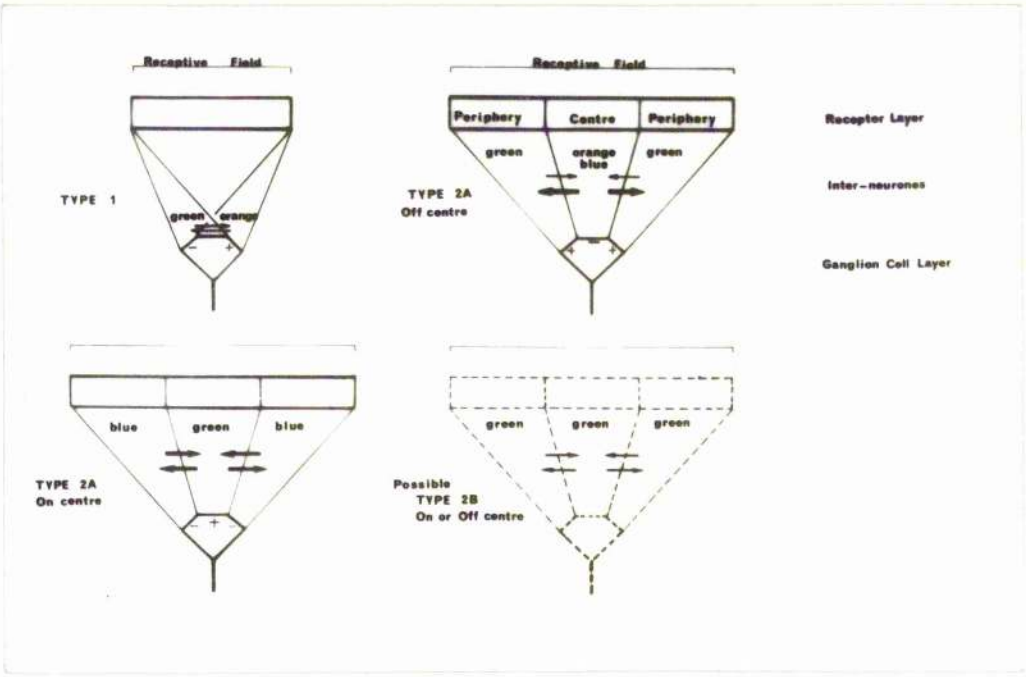

Fig. 37. Principal inferred connections of cones to retinal ganglion cells of Types 1 and 2. Type 1 units gave mutually-inhibitory opponent "on-off" responses to stimulation anywhere within the receptive field, i.e. no centre-surround field arrangement. The cell illustrated was orange-"on", green-"off". "Off"-centre Type 2 units showed strong surround inhibition; "On-off" centre Type 2 units showed strong centre and surround inhibition. Field centre and surround showed different spectral sensitivities. A further possible variation of Type 2 unit (either "on-off" or "off" centre) is shown, in which sensitivities of the field centre and surround are similar, since it was uncertain whether all Type 2 units were spectrally-opponent. (+ indicates excitatory input to the ganglion cell, i.e. from the "on"-field; - indicates inhibitory input from the "off"-field. Arrows indicate inhibition between the inputs, presumably earlier than the ganglion cell level; thick arrows indicate strong inhibition and thin arrows weak inhibition.)

Fig. 37. It would appear from the results reported here, and on the assumptions that retinal connections in fish are comparable to the Dowling-Boycott picture for primate retina (Dowling & Boycott, 1966), that the inputs to ganglion

cells are more complex than Wolbarsht & others (1961a) suggest from their work on goldfish. These latter authors suggest that the "on", "off" and inhibitory aspects of ganglion cell discharges can be explained in terms of two inputs, excitatory and inhibitory, and that the inhibitory aspect is associated only with the "off" discharge.

This explanation may hold for plaice also, but inhibition of the "off" discharge is also observed both in Type 1 units and in "on-off" centre Type 2 units. This effect may be due to a reduction in the inhibitory hyperpolarisation of the cell by the excitatory input. In terms of the Dowling-Boycott model on the other hand, it seems likely that inhibitory interactions between receptor populations from the field centre and surround occur, at least in part, at an earlier level than the ganglion cells, presumably in the horizontal and/or amacrine cell layers. This is indicated by arrows in Fig. 37; thick arrows indicate strong inhibition and thin arrows weak inhibition.

3. Spatial localisation of stimuli: comments on the "off" component associated with the "on"-field of Type 2 units.

The inability to demonstrate centre-surround or adjacent receptive field configurations in Type 1 units may be criticised on the grounds of poor spatial localisation of stimuli. Receptive field analyses, however, show very

clearly that both the "on" and "off" components of the response occur throughout the receptive field, and that the sensitivity of both is maximal at the field centre. If spatial localisation were poor one would not expect to detect clearly-defined centre-surround or adjacent fields in Type 2 units.

Again, in Type 2 units the inability to obtain pure "on" responses from the "on"-field may stem from the refractive error inherent in an aquatic eye situated in air and be due to light scatter from the stimulus. Inhibition from the "on"-field is invariably weaker than that associated with the "off"-field, possibly even absent in "off"-centre cells. Presumably it is inadequate to suppress "off" responses from the "off"-field, if light <u>is</u> scattered from the stimulus. This cannot be so for opponent cells, since the "on" and "off" components of the mixed "on-off" response invariably show the same spectral sensitivity, indicating that they both originate from receptors in the "on"-field. Two possibilities remain:

1. that there are two similar populations of receptors in the "on"-field, one having excitatory, and the other inhibitory influences on the cell. This is not likely, for such an arrangement has been found in no other vertebrate class.

2. The most likely explanation, as Dowling & Boycott (1966) suggest for similar phenomena observed by Wagner & others (1960) in goldfish, is that when stimulating the "on"-field, the associated "off" component is due to amacrine cells which extend across the receptive field and invade the "off" zone. In this case one would expect the "off" component of the mixed "on-off" response to mirror the spectral sensitivity of the "on" response, since both derive from the same set of receptors. This in fact is found in plaice.

As one might expect, the "off" component of mixed "on-off" responses of Type 2 units is most prominent at high stimulus intensities, and often absent at low intensities when pure "on" responses are obtained. Similar intensity-dependent "on-off" responses have been observed in goldfish (Wolbarsht et al, 1961a). Further, if the associated "off" component is due to amacrine spread from the "on"-field, absence of any inhibitory aspect on this component is to be expected.

4. Type 1 cells: function. Type 1 units appear to be similar to some opponent ganglion cells found in goldfish (MacNichol et al, 1961; Wolbarsht et al, 1961b), and comparable to one type of opponent cell recently described by Wiesel & Hubel (1966) in the lateral geniculate of the monkey.

By virtue of their opponent nature, Type 1 units are interpreted as being primarily concerned with colour-coding of visual information. Mutual antagonism between "on" and "off" components of the response would serve to enhance the colour contrast sensitivity. Since these cells exhibit no centre-surround receptive field arrangement, it is unlikely that they are concerned with the analysis of size or form, or directional movement discrimination. This latter has been confirmed by preliminary tests with appropriate stimuli.

5. Type 2 cells: function. Type 2 units tend to give much broader spectral sensitivity curves and only weak opponent responses. In some, spectral peaks are clearly-defined for field centre and surround, with peaks in different spectral regions. In others, spectral peaks are indeterminate and centre and surround colour sensitivity may be identical. In the latter case, weak opponent properties, and differences in centre and surround spectral sensitivity may be apparent only and result from the inhibitory interaction between field centre and surround.

Two classes of cell may emerge from the initial Type 2 classification, the non-opponent cells being similar to cells found by Wolbarsht & others (1961b) in goldfish. Both opponent and non-opponent lateral geniculate cells

with centre-surround field arrangements have been found in the monkey (Wiesel & Hubel, 1966). They are comparable in some respects to the Type 2 units described here.

Type 2 units are presumably concerned with size and form analysis, since they possess antagonistic centre-surround receptive fields. Opponent cells of this type are also capable of colour discrimination. Since rod input, in addition to cones, is more common in this type, they function over a wide spectral and intensity range. This and their high-frequency and maintained discharges suggest that these cells may also perform the important function of intensity discrimination. Type 2 units with adjacent "on" and "off" field zones are directionally selective to moving stimuli, whilst cells with concentrically-arranged fields respond preferentially to centripetal or centrifugal movement. These findings can be predicted for each unit from its receptive field configuration.

6. Cone-rod interaction. From spectral sensitivity determinations and their comparisons with empirical Dartnall nomogram curves, and from measurements of response latency, evidence for both cone and rod input is found in many units of both types. High stimulus intensities reveal sensitivity peaks at wavelengths typical for cones, for each response-component. For Type 2 units it is not uncommon for one

response-component to exhibit peak sensitivity to two well-separated wavelengths; i.e. inputs from two cone populations, for example blue and orange (compare with the work of De Valois & others on monkey LGN cells). In others, it is debatable whether the input is blue and green, for example, or blue-green. It is not expected that these peaks will approximate to cone absorption spectra, since there are intervening processes and, as mentioned earlier, the inhibitory interactions are considerable at intensities sufficient to stimulate cones. Moreover when stimulus brightness, rather than threshold, is measured in terms of ganglion cell responses, cones and rods are not independent of one another. Rod input to a cell will thus distort responses at stimulus intensities suprathreshold for cones.

Wherever rod input is apparent from the previous criteria, a peak is expressed in intensity-response curves within the stimulus intensity range used (e.g. Fig. 26). In some units a similar peak occurs at higher intensities. This is presumably due to cones since it is maximal at wavelengths corresponding to cone absorption peaks. At intensities subthreshold for cones, the spectral sensitivity of cells with rod input approximates closely to rod absorption spectra. At these intensities responses are independent of cones, interaction between inputs is slight, and distortion is therefore minimal.

7. Peak spectral sensitivity ranges. Spectral peaks for response-components at high stimulus intensities occur predominantly in one or more of three spectral ranges: in the blue (440-460 mμ); in the green (510-540 mμ); and in the orange (560-590 mμ). Peaks were found in the blue-green (470-490 mμ) in two units. The first and second agree tolerably well with peaks obtained by Marks (1965) for single goldfish cones; the first and third with Jacobson's (1964) data for tectally-recorded units in goldfish; and the second and third with the results of Witkovsky (1965) for carp retinal ganglion cells. Spectral peaks were never found in the red in any units analysed, which contrasts sharply with results for the fresh-water goldfish and carp. This finding is not surprising, for marine fish live in an environment where red light is largely absent. Both Marks (1965) and Witkovsky (1965) also obtained peaks in the same spectral region as the orange and blue-green peaks evident in plaice. These peaks were interpreted by Marks as red-green twin cones and secondary photoproducts respectively. It is possible that the absence of red sensitivity and the commonness of orange sensitivity in plaice is associated with the prominence of twin-cones, identified by Engstrom & Ahlbert (1963) in plaice retinae. In the two atypical cells recorded, which gave sensitivity peaks in the blue, green and orange, it is not possible to tell whether this

is due to three discrete cone inputs, or to triple cones which the latter authors identified in the specialised dorsal retina of plaice.

In all cells which gave evidence of rod input, spectral sensitivity curves and peaks at low stimulus intensities were in good agreement with the fitted absorption spectrum of rod pigment 525, constructed from the Dartnall nomogram (Dartnall, 1953). The indication is that in plaice the predominant rod pigment is of the "porphyropsin" rather than the "rhodopsin" type.

APPENDIX - TABLE 2

(Part A)

Characteristics of Neutral Density Filters

Spectrophotometer (Unicam SP 800) calibrations of Wratten neutral density filters at each interference filter wavelength. Values given are for optical density in log units.

Filter name	Wavelength (mμ) 409	422	439	472	484	508	536	558
0.1	0.11	0.11	0.10	0.09	0.09	0.09	0.09	0.09
0.2	0.27	0.25	0.24	0.23	0.23	0.22	0.22	0.21
0.3	0.41	0.39	0.37	0.34	0.34	0.33	0.32	0.32
0.4	0.53	0.50	0.48	0.45	0.44	0.43	0.43	0.43
0.5	0.65	0.61	0.59	0.55	0.55	0.54	0.53	0.53
0.6	0.77	0.74	0.72	0.67	0.67	0.65	0.64	0.65
0.7	0.93	0.89	0.83	0.81	0.80	0.78	0.77	0.76
0.8	1.06	1.01	0.97	0.92	0.91	0.88	0.87	0.86
0.9	1.23	1.18	1.13	1.06	1.03	1.02	0.99	0.97
1.0	1.28	1.23	1.18	1.12	1.11	1.09	1.07	1.06
1.1	1.39	1.34	1.28	1.21	1.20	1.18	1.16	1.15
1.2	1.55	1.48	1.43	1.35	1.34	1.31	1.29	1.28
1.3	1.69	1.62	1.55	1.46	1.44	1.42	1.39	1.39
1.4	1.81	1.74	1.66	1.57	1.55	1.52	1.50	1.49
1.5	1.93	1.85	1.78	1.67	1.66	1.62	1.60	1.59
1.6	2.05	1.97	1.90	1.79	1.77	1.74	1.71	1.71
1.7	2.21	2.12	2.02	1.93	1.91	1.87	1.84	1.83
1.8	2.34	2.24	2.16	2.04	2.01	1.97	1.94	1.93
1.9	2.51	2.41	2.31	2.18	2.14	2.11	2.06	2.04
2.0	2.48	2.38	2.31	2.18	2.15	2.13	2.10	2.09
2.1	2.59	2.49	2.41	2.28	2.25	2.22	2.19	2.18
2.2	2.75	2.63	2.55	2.41	2.39	2.34	2.32	2.30
2.3	2.88	2.77	2.67	2.52	2.49	2.46	2.42	2.41
2.4	3.01	2.89	2.78	2.63	2.60	2.59	2.53	2.52
2.5	3.13	2.99	2.90	2.74	2.70	2.66	2.63	2.62
2.6	3.25	3.12	3.02	2.86	2.82	2.78	2.74	2.74
2.7	3.41	3.27	3.14	2.99	2.95	2.91	2.87	2.85
2.8	3.54	3.39	3.28	3.10	3.06	3.01	2.97	2.95
2.9	3.71	3.56	3.43	3.25	3.19	3.14	3.09	3.06

continued over

APPENDIX - TABLE 2

(Part B)

Characteristics of Neutral Density Filters

Spectrophotometer (Unicam SP 800) calibrations of Wratten neutral density filters at each interference filter wavelength. Values given are for optical density in log units.

Filter name	Wavelength (mμ) 574	586	600	622	653	675	705
0.1	0.09	0.09	0.10	0.09	0.09	0.09	0.08
0.2	0.23	0.22	0.22	0.23	0.23	0.23	0.20
0.3	0.32	0.33	0.33	0.34	0.34	0.34	0.29
0.4	0.42	0.43	0.43	0.44	0.44	0.45	0.38
0.5	0.53	0.53	0.54	0.56	0.55	0.56	0.48
0.6	0.65	0.65	0.66	0.68	0.69	0.69	0.60
0.7	0.76	0.76	0.76	0.79	0.79	0.79	0.70
0.8	0.87	0.86	0.87	0.89	0.90	0.89	0.78
0.9	0.98	0.98	0.98	1.00	1.00	1.00	0.88
1.0	1.06	1.06	1.07	1.10	1.11	1.11	0.98
1.1	1.16	1.16	1.17	1.19	1.20	1.20	1.06
1.2	1.29	1.28	1.29	1.33	1.34	1.34	1.18
1.3	1.39	1.39	1.40	1.44	1.45	1.45	1.27
1.4	1.49	1.49	1.50	1.54	1.55	1.56	1.36
1.5	1.60	1.59	1.61	1.66	1.66	1.67	1.46
1.6	1.72	1.71	1.73	1.78	1.80	1.80	1.58
1.7	1.82	1.82	1.84	1.89	1.90	1.90	1.68
1.8	1.93	1.93	1.94	1.99	2.01	2.00	1.76
1.9	2.04	2.04	2.05	2.10	2.11	2.11	1.86
2.0	2.11	2.12	2.14	2.22	2.26	2.26	2.02
2.1	2.20	2.21	2.23	2.31	2.35	2.35	2.10
2.2	2.34	2.34	2.35	2.45	2.49	2.49	2.22
2.3	2.43	2.44	2.47	2.56	2.60	2.60	2.31
2.4	2.53	2.54	2.57	2.66	2.70	2.71	2.40
2.5	2.64	2.65	2.67	2.78	2.81	2.82	2.50
2.6	2.76	2.77	2.79	2.90	2.95	2.95	2.62
2.7	2.87	2.88	2.90	3.01	3.05	3.05	2.72
2.8	2.98	2.98	3.00	3.11	3.16	3.15	2.80
2.9	3.09	3.09	3.12	3.22	3.26	3.26	2.90

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