J. Physiol. (1968), **195**, *pp.* 535–556
With 13 *text-figures*
Printed in Great Britain

SPECTRAL PROPERTIES OF DARK-ADAPTED RETINAL GANGLION CELLS IN THE PLAICE (*PLEURONECTES PLATESSA*, L.)

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(*Received* 22 *March* 1967)

SUMMARY

1. Spectral, spatial and temporal properties of receptive fields of dark-adapted, on–off retinal ganglion cells in the intact eye of the plaice, were analysed by recording from their axon terminals in the superficial layers of the optic tectum with indium micro-electrodes.

2. Two cell-types were identified. The first gave fast-adapting, spectrally opponent on–off responses without centre-surround subdivisions of the receptive field. On and off response-components were mutually antagonistic. The second type gave slow-adapting on–off or off responses for different stimulus positions within the receptive field, with centre-surround or adjacent field configurations. Only on–off centre cells, showing mutual antagonism between field centre and surround, or off centre cells with inhibitory centres, were found. These cells had weak opponent or non-opponent properties.

3. Most cells of each type received inputs both from cones and rods. At stimulus intensities suprathreshold for cones, response-components gave spectral peaks which have been classified into one of four wave-length ranges; blue, 440–460 nm; blue-green, 470–490 nm; green, 510–540 nm; and orange, 560–590 nm. No cells analysed gave sensitivity maxima in the red. At low stimulus intensities all cells with rod input gave a single spectral peak between 510 and 530 nm.

INTRODUCTION

The responses of single retinal ganglion cells have been widely investigated in amphibia and mammals. In goldfish, properties of retinal ganglion cell types have been studied in response to black and white stimuli

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(Jacobson & Gaze, 1964), to movement (Cronley-Dillon, 1964), and to colour (Wagner, MacNichol & Wolbarsht, 1960; MacNichol, Wolbarsht & Wagner, 1961; Wolbarsht, Wagner & MacNichol, 1961*a*, *b*; Jacobson, 1964). Witkovsky (1965) recorded responses from opponent ganglion cells in the carp (*Cyprinus*).

In marine fish, however, retinal ganglion cells have received little attention. Results so far obtained have been confined to spectral sensitivity measurements from behavioural experiments, and identification and isolation of visual pigments. Histologically, in plaice retinae Engstrom & Ahlbert (1963) have identified single cones, twin cones, and rods; triple cones occur in a specialized region of the dorsal retina. J. H. S. Blaxter (1967, personal communication) finds only single cones in the retinae of larval plaice; the rods develop at metamorphosis.

Experiments reported in this paper were confined to on–off retinal ganglion cells and designed to test for spectral opponency. Discharge patterns and receptive fields of a small number of units were characterized in great detail, in fish maintained as physiologically normal as experimental conditions permitted. To accomplish this, rather than record from the isolated retina or penetrate the eye, ganglion cell responses were recorded from their axon terminals in the contralateral optic tectum. Comparisons are made with similar cells found in fresh-water fish, and analogies drawn with LGN cells of terrestrial vertebrates.

METHODS

Adult plaice (*Pleuronectes platessa*, L.), 10–13 in. (25–33 cm) in length, were used. Fish were anaesthetized by immersion in a 1/10,000–1/5000 solution of MS. 222 (Sandoz) in sea water and clamped between a Perspex plate, and four shaped bars, placed transversely across the upper (ocular) surface of the body. The gills were continuously perfused with aerated sea water. The brain was exposed from the ocular surface and the cerebral hemispheres were sucked out. Haemorrhage was usually slight. The tectal circulation and dura remained intact. Following decerebration fish were allowed to recover from the effects of the anaesthetic.

Visual stimulus. The stimulus was mounted radially 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 (Figs. 1, 2). The eye was immobilized by needles, projecting 30° anterior and 30° superior to the transverse and horizontal planes respectively through the eye, 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.

The light source was a 6 V, 15 W planar-filament, tungsten bulb, powered from a mains constant voltage transformer stepped down by a 240:6 V transformer. Light was concentrated and focused by a pair of biconvex lenses on to a small aperture, more or less collimated by a third lens and passed in turn through interference filters and neutral density filters on to a fine ground glass screen. Uniformly-illuminated circular spots of light, subtending 0·5, 1, 3 or 6° in air at a distance of 15 cm from the eye, were obtained by adjusting a variable aperture placed immediately in front of this screen.

Fifteen Barr & Stroud interference filters were used. Bandwidths varied from 10 to 19 nm. Transmission peaks ranged from 409 to 705 nm, and were checked with a spectrophotometer (Unicam S.P. 600). Filters were standardized with a calibrated phototube: approximately equal quantum emission was obtained by regulating the intensity of the light source. Correction factors were calculated to give equal quantum emission correct to within $\pm 2{\cdot}5\,\%$. Kodak Wratten filters provided intensity reduction in steps of 0·1 log units over a range of 3 log units. They were calibrated at each wave-length with a spectrophotometer (Unicam S.P. 800) and cross-checked with the phototube.

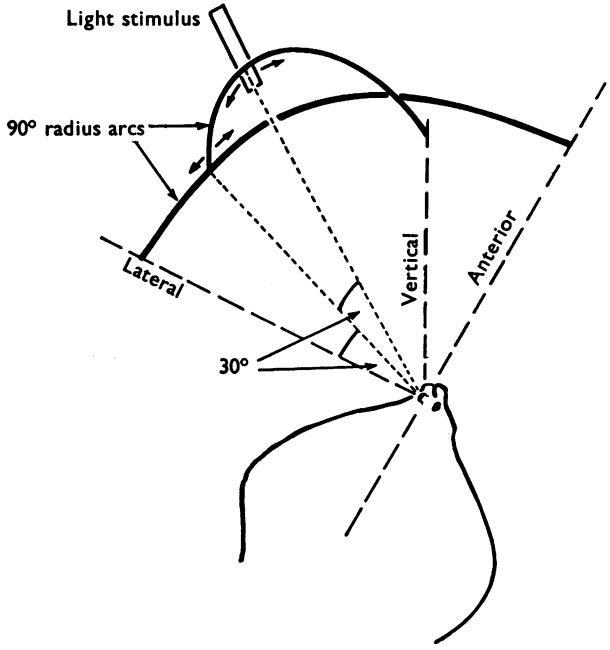

Fig. 1. Diagrammatic representation of the stimulus arrangement. The visual stimulus was mounted radially by a slide on the vertical arc. The vertical arc was mounted by a slide at the base on to the horizontal arc, so as to pivot around the vertical axis. The eye was clamped directing 30° forwards (anterior) and 30° upwards (dorsal).

Light flashes of variable duration and frequency could be controlled by a relay-driven mechanical shutter at the point of focus of the light path. Light intensity rose to peak value in 10–15 msec with a similar decay time at the end of a flash; 1 sec flashes were chosen to give complete separation of the on and off components of each response.

Recording. Fish were dark-adapted for at least 30 min, by which time recovery from anaesthesia was complete. The eyes were in air, without correction for refractive error. The cornea was prevented from drying by periodically bathing with sea water. Responses of single units to stimulation of the left eye were recorded from the superficial layers of the right (upper) tectum with platinum-tipped, indium micro-electrodes drawn from Pyrex glass (Gesteland, Howland, Lettvin & Pitts, 1959). Optimal recording characteristics were obtained from electrodes having tip diameters of 2–4 μ, with a cylindrical platinum-black cap 4–8 μ in diameter. Such electrodes combined tip resistances of 10 kΩ or less, with tip

dimensions small enough to isolate single units. Impulses were channelled through an a.c.-coupled cathode follower, amplified, displayed conventionally, and recorded simultaneously on moving film and as 2 msec pulses on magnetic tape. The indifferent electrode was a silver wire placed on the head. The surrounding sea water was earthed.

The micro-electrode was positioned vertically over the tectum and advanced in search of a single unit giving on-off responses to flashes of light. When such a unit was identified, the electrode was adjusted to record impulses optimally. The centre of the receptive field of the unit was determined by preliminary tests.

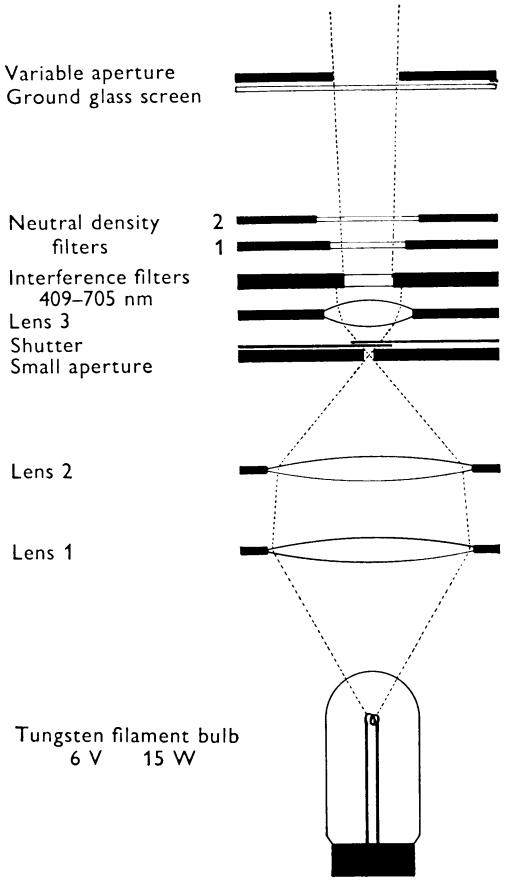

Fig. 2. Details of the components of the visual stimulus mounted in a light-proof tube.

In the earlier experiments 1 sec flashes of light were delivered at 4 or 5 sec intervals, at a fixed quantum intensity level. Responses were recorded at a number of wave-lengths through the spectrum. Latterly the flash-interval was reduced to 2 sec in order to obtain complete data within the time units could be held. Then, starting with a blue filter, peak 409 nm, responses were recorded to stimulation at the receptive field centre with five 1 sec light flashes at the maximum available intensity, and at intensity levels reduced in steps

of 0·4 log units over a 3 log unit range. The five responses at each intensity were averaged, as a necessary smoothing of response variability. This sequence was repeated for each interference filter, in order through the spectrum. In some cases a return series was recorded, and in all cases random checks were made at the end of a run for a few wave-lengths, without showing any substantial differences in response patterns. Usually the smallest light spot (0·5°) was used to give optimal discrimination of response-components and minimal effects on dark-adaptation. Where units with centre-surround fields could be held long enough a similar series of responses was obtained for a stimulus positioned in the surround. Receptive fields were mapped out in detail for a few units. In some the effect of increasing stimulus size was investigated. The whole analysis involved some 900 responses per unit.

Spikes in each response were counted automatically on a scaler (Echo N530F) from the tape recordings converted to 2 msec d.c. pulses of suitable amplitude. All responses from a given unit were counted either *in toto*, or for a standard time interval triggered by the first spike in the train.

RESULTS

Recording site. Units were recorded from the superficial fibre-layers of the right tectum in response to stimulation of the left eye. Their properties were similar to those of units recorded from the optic nerve. Spikes were characteristically triphasic, predominantly negative, and could usually be held for several hours. On Kuffler's (1953) criteria for discriminating cell and axon spikes, these recordings are of axonal, rather than cellular origin. Diphasic spikes of much larger amplitude and opposite predominant polarity were occasionally recorded, and could be held only transitorily. Presumably these are of cellular origin and this is substantiated by histological evidence indicating that the superficial tectum is only sparsely populated with cell bodies.

The following factors ensured that recordings were from single units. Only units giving a clear spike:noise discrimination ratio, and audibly distinguishable, were analysed. Small rhythmic fluctuations in spike height could be abolished by vascular occlusion. Spikes were commonly attenuated at high instantaneous firing frequencies; similar phenomena were observed by Kuffler (1953) in single units recorded from cat retina. Limits of receptive fields, and boundaries between receptive field subdivisions, were well defined. Patterns of spike trains were reproducible. Once a unit had been isolated, slight movement of the electrode tip invariably attenuated or magnified all spikes, without differentiation of discharges into spikes of different amplitude.

Response types were isolated in different tectal strata. On and off units were found most superficially. Two on–off types were identified, lying in discrete deeper layers. The retino-tectal projection of all these types of unit was ordered. The posterior retina (anterior visual field) projected to the anterior tectum, with corresponding anterior-to-posterior, dorsal-to-ventral and ventral-to-dorsal projections. Deeper penetrations revealed a broad silent layer, and deeper still more complex activity, pre-

sumably of intrinsic tectal origin. Since both the layering and retino-tectal projection of the superficial unit types were ordered, recordings must be from a unique region of the ganglion cell axons. The axon terminal arborizations, as suggested for frog tectum (Maturana, Lettvin, McCulloch & Pitts, 1960), are the most plausible possibility.

Résumé of on–off unit types. Two types of on–off unit (1 and 2) were identified. Type 1 units gave spectrally opponent, mixed on–off responses to coloured stimuli positioned anywhere within the receptive field, without demarcation into on and off zones. Type 2 units possessed centre-surround or adjacent receptive fields, either on–off centre, off-surround, or off-centre, on–off surround. In these, opponent properties were weak or absent. Most units of each type received both cone and rod input.

Type 1 *on-off units*

These units occurred in the more superficial on–off layer. Receptive fields ranged up to 30° in diameter. On and off discharges were characteristically brief, compact bursts of impulses. Figure 3 shows typical intensity–response records for one unit. Receptive fields analyses indicated no centre-surround receptive field arrangement. Even small stimuli evoked mixed on–off responses at every locus within the receptive field, with occasional exceptions at low stimulus intensities near to threshold (Fig. 3). The extent of the receptive field of each unit was determined. At the geometric centre of these limits either the on or off component was predominant, but with a marked variation in ratio for different wave-lengths. Both components were maximally sensitive at the receptive field centre. The sensitivity of the predominant centre component fell-off more rapidly than the weaker component towards the periphery.

Opponent responses of two Type 1 units to equal quantum spectra are compared in Fig. 4. In some, one component peaked in the mid-region of the visible spectrum (e.g. column 1, Fig. 4). In others, peak on and off responses occurred at opposite ends of the spectrum (e.g. column 2, Fig. 4). The opponent nature of units was more marked at stimulus intensities well above cone threshold, and could be enhanced by low-intensity, white light background illumination.

The responses of seven units were studied over an intensity range of 3 log units at each colour-filter wave-length (Fig. 3). Intensity–response curves were plotted at each wave-length for both the on and off components of the response, and are collectively termed 'spectral intensity–response series'. Typical intensity–response curves for one unit, at two wave-lengths from the spectral series, are shown in Fig. 5. In this unit the on component was predominant at the receptive field centre. Stimulation at the receptive field centre evoked stronger on responses in three

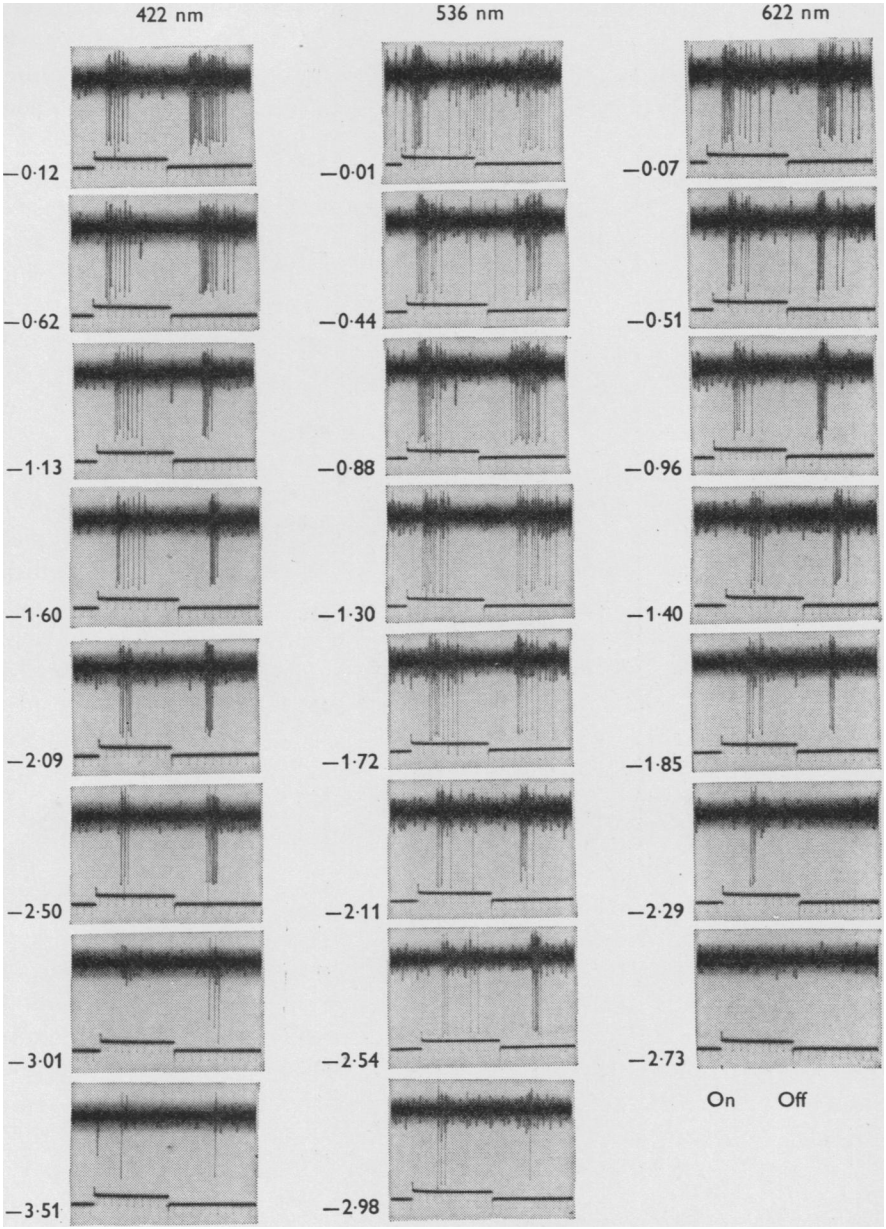

Fig. 3. Responses of a Type 1 on–off unit to stimulation at three wave-lengths with a 0·5° stimulus spot positioned at the receptive field centre. Top records are for maximum available stimulus intensity at each wave-length. The log relative quantum intensity of the stimulus is given at the left of each record. Upward deflexion of lower trace indicates stimulus-on; downward deflexion, stimulus-off. Time mark on lower trace is 100 msec. Stimuli were 1 sec flashes of light delivered at 2 sec intervals. Second from bottom records in left and right column show pure off and on responses obtained at intensity levels near to threshold.

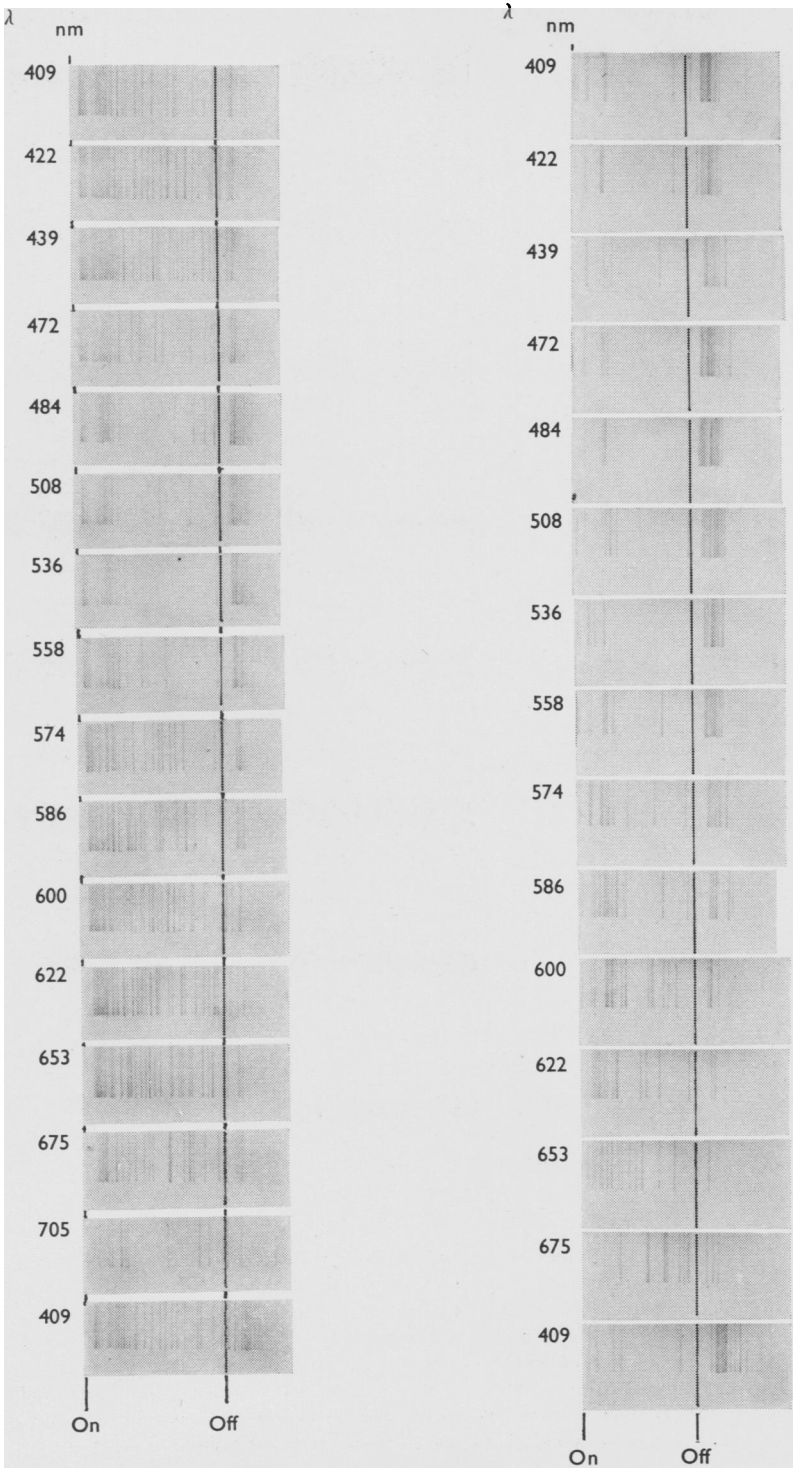

Fig. 4. For legend see opposite page.

units and stronger off responses in the remaining four units. The response components were mutually antagonistic. Suppression of the weaker component by the dominant input was always more marked than the converse (see Fig. 5).

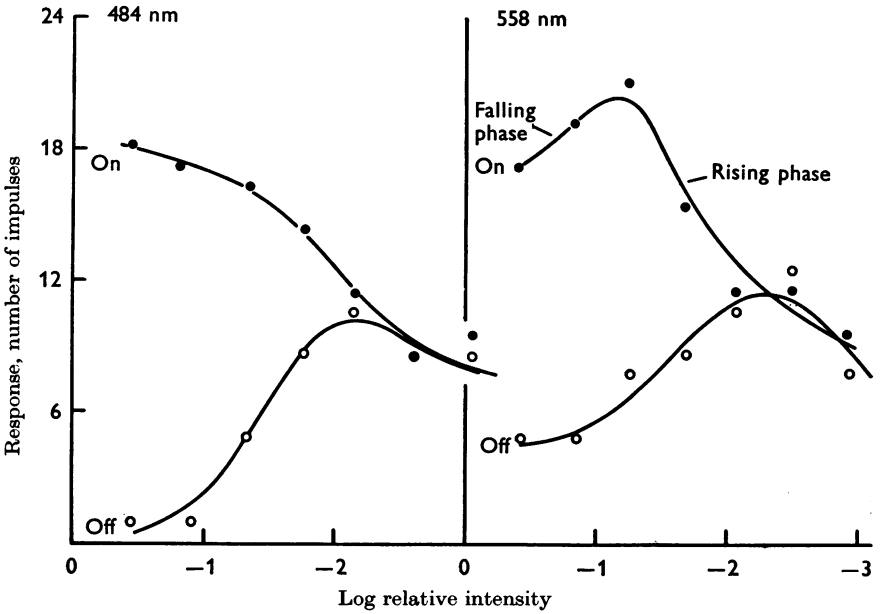

Fig. 5. Intensity–response curves for the on and off response-components of a Type 1 unit at two wave-lengths from the series. This unit gave predominant on responses at all wave-lengths to a 3° stimulus spot positioned at the receptive field centre. Each response-component showed marked suppression (falling phase of curves) at high stimulus intensities, maximal for wave-lengths at the peak sensitivity of the alternative component. Stimuli were 1 sec flashes of light delivered at 2 sec intervals. Each point plotted is an average of five responses.

Spectral sensitivity curves were constructed for the on and off response-components of each unit. The log relative intensity of the stimulus required to evoke the same number of spikes in the response at each wave-length was read off from the appropriate intensity–response curve. Correction factors mentioned previously (see Methods) were applied to these

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Legend to Fig. 4.

Fig. 4. Opponent responses of two Type 1 on–off units to equal quantum stimuli through the spectrum. Intensity: maximum available. 3° stimulus spot positioned at the receptive field centre. Stimuli: 1 sec flashes delivered at 5 sec intervals. Spikes are represented as 2 msec pulses from playback of tape recordings.

intensity values before sensitivity curves were plotted. Spectral sensitivity curves for a Type 1 unit are shown in Fig. 6.

Responses of Type 1 units to equal quantum spectra at a single intensity level illustrate their opponent nature clearly. From spectral sensitivity data (e.g. Fig. 6), however, opponency and spectral peaks are ill-defined. At best only a tentative assessment of colour sensitivity can be made for Type 1 units, as follows:

On response predominant: orange-on, green-off (1);
green-on, orange-off (2).
Off response predominant: green-off, blue-on (1);
green-off, orange-on (1);
blue-off, green-on (1).

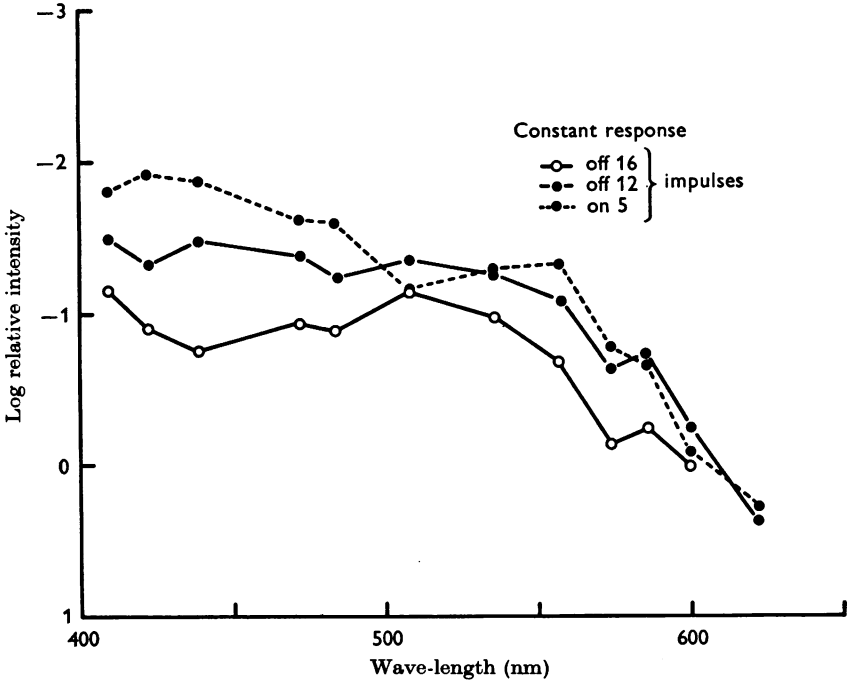

Fig. 6. Spectral sensitivity of a Type 1 unit, abstracted from intensity–response series for the on and off response-components, at several constant response levels. This unit gave predominant off responses to 1° stimulus spot positioned at the receptive field centre. Equal quantum stimuli through the spectrum indicated that the unit was green-off, blue-on, but spectral sensitivity curves, abstracted from intensity-response data, are poorly defined (see text).

In Type 2 units (see later), where receptive fields are subdivided into excitatory and inhibitory zones, sensitivity curves and spectral peaks are better defined, since it is possible to stimulate each input in near isolation.

With Type 1 units this is not the case and intensity–response curves reveal strong interaction between excitatory and inhibitory inputs. The on component of the response is maximally suppressed in the range of peak sensitivity of the off component, and vice versa. For this reason spectral sensitivity curves were normally measured at low intensity levels where the interaction between inputs was less marked (i.e. on the rising phase of intensity–response curves), to minimize the distortion in sensitivity. Weak spectral peaks (presumably due to cone influences) result at these low

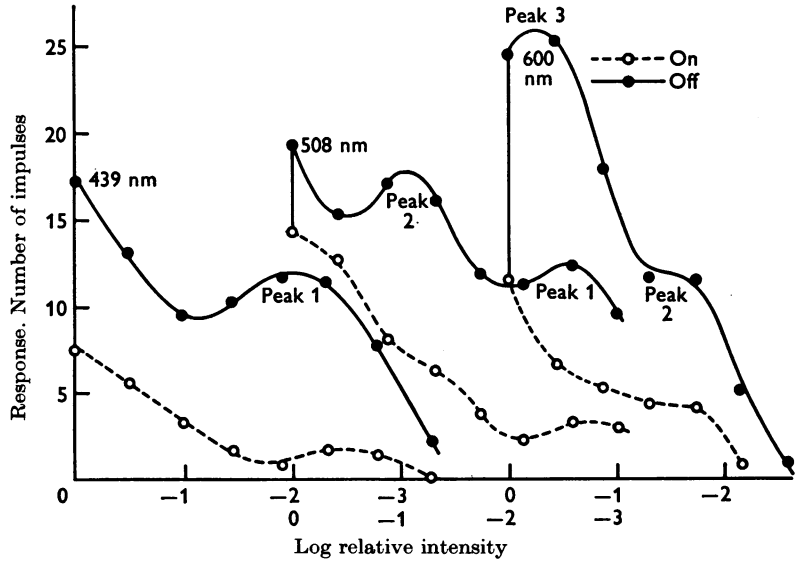

Fig. 7. Intensity–response curves at three wave-lengths for the on and off response-components of the atypical Type 1 unit described in the text. The series of peaks (1, 2 and 3) in these curves were maximal at the same wave-lengths as the spectral sensitivity peaks (Fig. 8). Each point is an average of five responses.

intensities. Sensitivity is further complicated in units receiving inputs both from cones and rods. In dark-adapted eyes stimuli suprathreshold for cones also stimulate rods. Thus where 'cone' peaks occur in the blue or orange, spectral sensitivity curves are influenced by rod input and broadened in the green.

One unit showed the field arrangement and discharge pattern typical of Type 1 units, but gave atypical intensity–response series (Fig. 7) and spectral sensitivity (Fig. 8*a*, *b*). Intensity–response curves were complicated by a series of peaks falling within the range of stimulus intensities used. For the predominant off component, blue filters gave a single peak which was maximal at 440–450 nm (peak 1, Fig. 7). A second peak

occurred at higher stimulus intensities using green filters, maximal at 530–550 nm (peak 2, Fig. 7), with attenuation of the first peak. Orange filters gave a third peak, maximal at about 590 nm (peak 3, Fig. 7). Colour filters intermediate between the wave-lengths illustrated showed a smooth

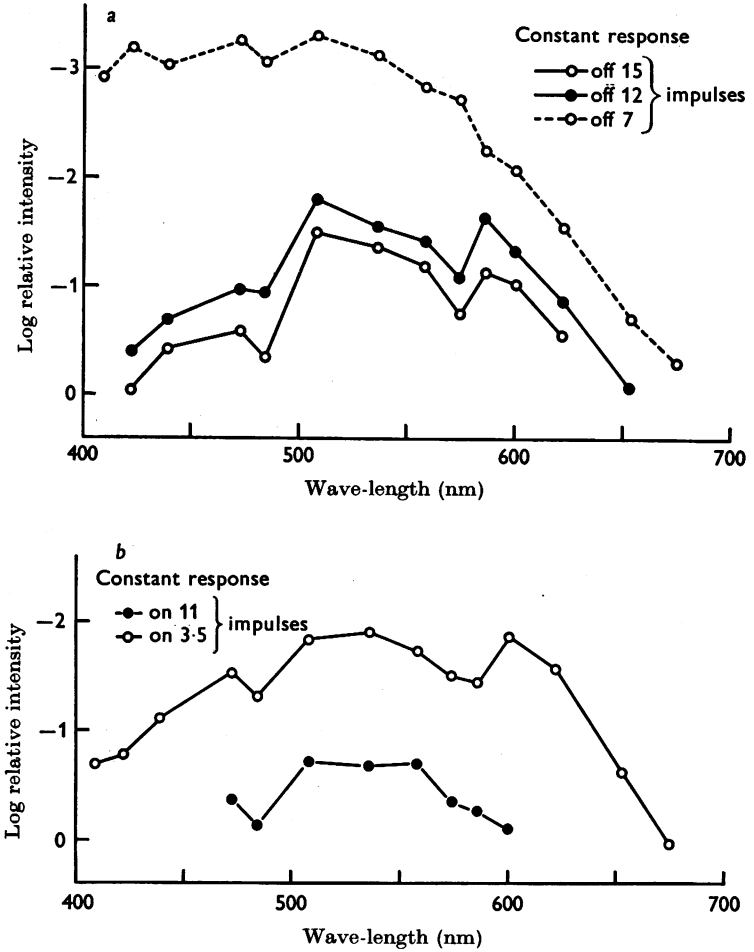

Fig. 8. Spectral sensitivity of the atypical Type 1 unit: (*a*) off component, (*b*) on component (see also Fig. 7). Each component gave spectral peaks in three regions at high stimulus intensities. These peaks were absent at low intensity, where spectral sensitivities and peaks were indicative of rod input (top curve in '*a*').

transition of these peaks from wave-length to wave-length. Spectral sensitivity curves, at constant response levels corresponding to high stimulus intensities, indicated spectral peaks in the blue, green and orange for each response-component (Fig. 8*a*, *b*). These peaks were not apparent at low

stimulus intensities where sensitivity curves showed a single peak in the green, indicative of rod input (upper curve, Fig. 8*a*).

Other Type 1 (and Type 2) units gave a single peak in intensity–response series, similar to the intensity–response curve at wave-length 439 nm for the above unit (Fig. 7). In no other case was a multiple-peak system encountered. Where such peaks occur they have been interpreted as the expression of specific components in inputs to ganglion cells, for spectral sensitivity maxima occur at similar wave-lengths.

Type 2 on–off units

Type 2 units were encountered deeper in the tectum than Type 1 units. Their receptive fields were larger, sometimes as much as 50° in diameter. On and off discharges were characteristically slow adapting and spontaneous firing occurred in darkness. Each discharge consisted of an initial, high-frequency, short-latency burst of impulses, followed by a lower-frequency maintained phase (Fig. 9). The maintained phase of each discharge was terminated by stimulus-off or by the onset of the succeeding stimulus. Responses of Type 2 units to equal quantum spectra, for stimulus positions evoking mixed on–off responses, indicated only weak opponent properties, much less-marked than for Type 1 units.

Type 2 units commonly showed the classic centre-surround receptive field pattern, though in two cases field regions were adjacent, not concentric. With a single exception stimuli could be positioned in one zone of the field to give pure off responses. All activity during stimulus-on was suppressed except at low intensities, when spontaneous firing was resumed. Responses from the other receptive field zone were invariably mixed on–off at high stimulus intensities; pure on responses were obtained only at low intensities. The significance of this is discussed later. Eight units were studied in detail; two were on–off centre, off-surround; the remainder were off-centre, on–off surround. Essentially, therefore, there are three parameters to be considered: the on and off components of the on–off field, and the off response from the off-field.

A spectral intensity–response series was obtained for each of these units, for a stimulus positioned at the field centre. Where units could be held long enough, a series was also obtained for a stimulus positioned in the field surround. Intensity–response curves were plotted at each wave-length, for the on and off components of the mixed on–off response, for the off response and for spontaneous activity occurring simultaneously during stimulus-on. Typical curves for the surround and centre responses of an on–off centre unit are shown in Fig. 10. In on–off centre units there was strong mutual antagonism between the on-component of the centre response and the off-surround response, at high stimulus intensities

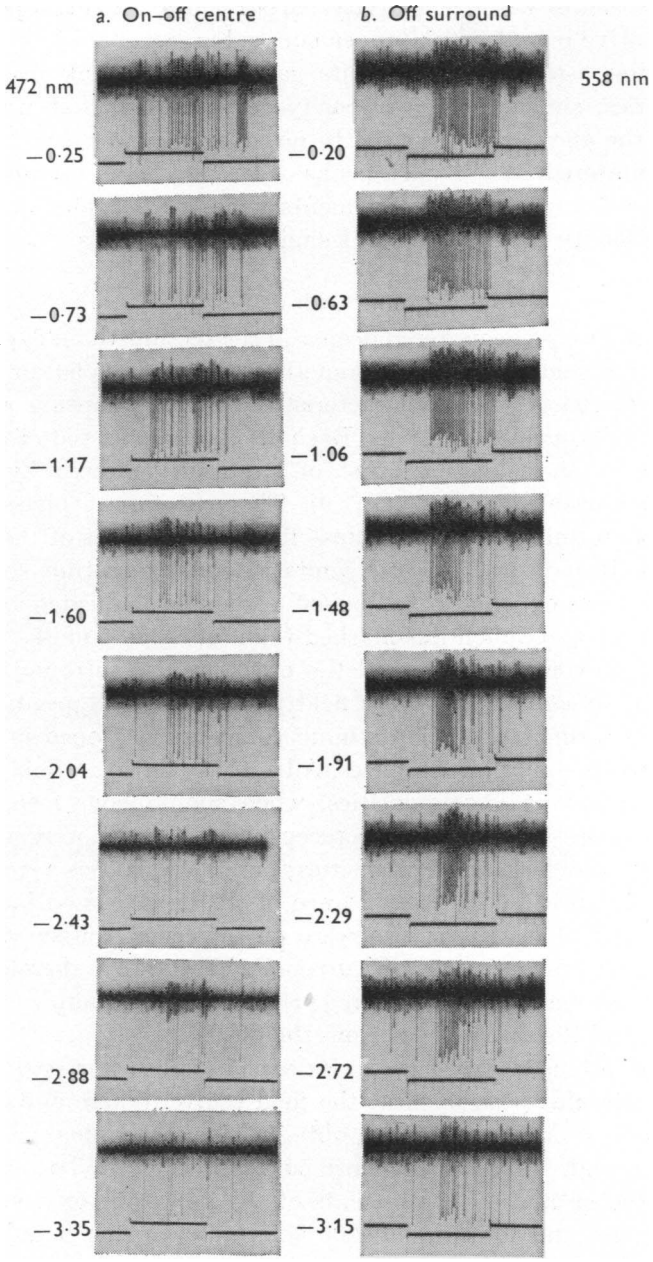

Fig. 9. Responses of a Type 2 on–off centre unit to a 0·5° stimulus spot positioned: (*a*) at the field centre, (*b*) in the surround. Note short-latency, high-frequency burst of impulses and longer-latency, maintained phase. Top records are for maximum available stimulus intensity. The log relative quantum intensity of the stimulus is given at the left of each record. Upward deflexion of lower trace indicates stimulus-on; downward deflexion, stimulus-off. Time mark is 100 msec. Stimuli were 1 sec flashes of light delivered at 2 sec intervals.

(falling phase of curves, Fig. 10). In off-centre units the on-component of the surround response showed marked inhibition from the field centre. Inhibition was most marked for wave-lengths at the peak sensitivity of the inhibitory component. In particular there was apparently little or no suppression of the off-component of the on–off response (see Fig. 10) in any unit studied. No examples of centre-surround summation were found.

The effect of increasing the size of centrally-positioned stimulus spots of constant intensity per unit area was investigated in two off-centre units. In one, at an intensity corresponding to the falling (suppression)

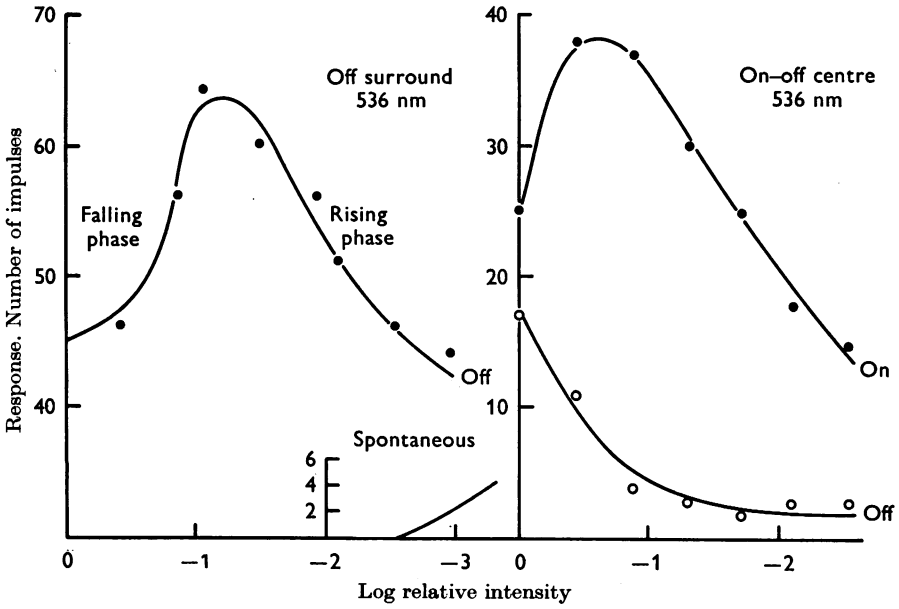

Fig. 10. Intensity–response curves for the off-surround response, for spontaneous activity, and for the on and off components of the centre response of a Type 2 on–off centre unit. Each point is an average of five responses.

phase of intensity–response curves obtained for the small spot, larger spots evoked weaker responses. In the second, at an intensity corresponding to the rising phase of intensity–response curves, within the limits of the receptive field centre larger spots enhanced the response. The response to a small spot having the same total quantum output as the large spot, was deduced at each wave-length from the appropriate intensity–response curve. The strengths of this response and that to the large spot were in good agreement at every wave-length. Thus at the field centre at intensities below those where there is marked suppression from the field surround, the response is dependent only upon the total quantity of illumination.

Constant response spectral sensitivity curves were plotted for the centre and surround response-components of each unit. When stimulating the off-field, the lowest stimulus intensity required to suppress spontaneous activity during stimulus-on was measured at each wave-length. This is expressed as the 'spontaneous zero' sensitivity curve (Figs. 11*a*, 12). In every unit where both centre and surround sensitivity were characterized, the 'spontaneous zero' curve approximated to the spectral sensitivity of the on–off field (see Fig. 11). Thus where off-centre units could be held long enough only to characterize centre sensitivity, the 'spontaneous zero' curve was used as an estimate of surround sensitivity (see Fig. 12). Spectral sensitivities of one on–off centre, and one off-centre, unit are illustrated in Figs. 11 and 12. The on–off centre unit (Fig. 11) was green-sensitive for both the on and off components of the centre response. The off-surround response was blue-sensitive, with sensitivity broadened in the green due to rod input. The off-centre unit (Fig. 12) was orange-and-blue sensitive at the field centre, and green-sensitive in the surround. Most Type 2 units gave evidence of both cone and rod input. All gave strong responses to movement. Units with adjacent field subdivisions were directionally sensitive. Clear-cut spectral sensitivity measurements were obtained for the following 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)

and tentative measurements for two further off-centre units:
- blue-green centre, green (+blue) surround (1)
- green (+blue+orange) centre, green surround (1)

Cone-rod interaction

Spectral sensitivity curves at constant response levels corresponding to high and low stimulus intensities, were quite different for most units. At high-intensities curves exhibited one or more peaks, classified by the

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Legend for Fig. 11.

Fig. 11. Spectral sensitivity of the Type 2 unit illustrated in Fig. 10, at several constant response levels; (*a*) off-surround; (*b*) and (*c*) on and off components respectively of the field centre. Both the on and off components of the field centre response gave spectral peaks in the green. The off-surround was maximally sensitive in the blue. Note the approximation of the 'spontaneous zero' sensitivity to that of the field centre.

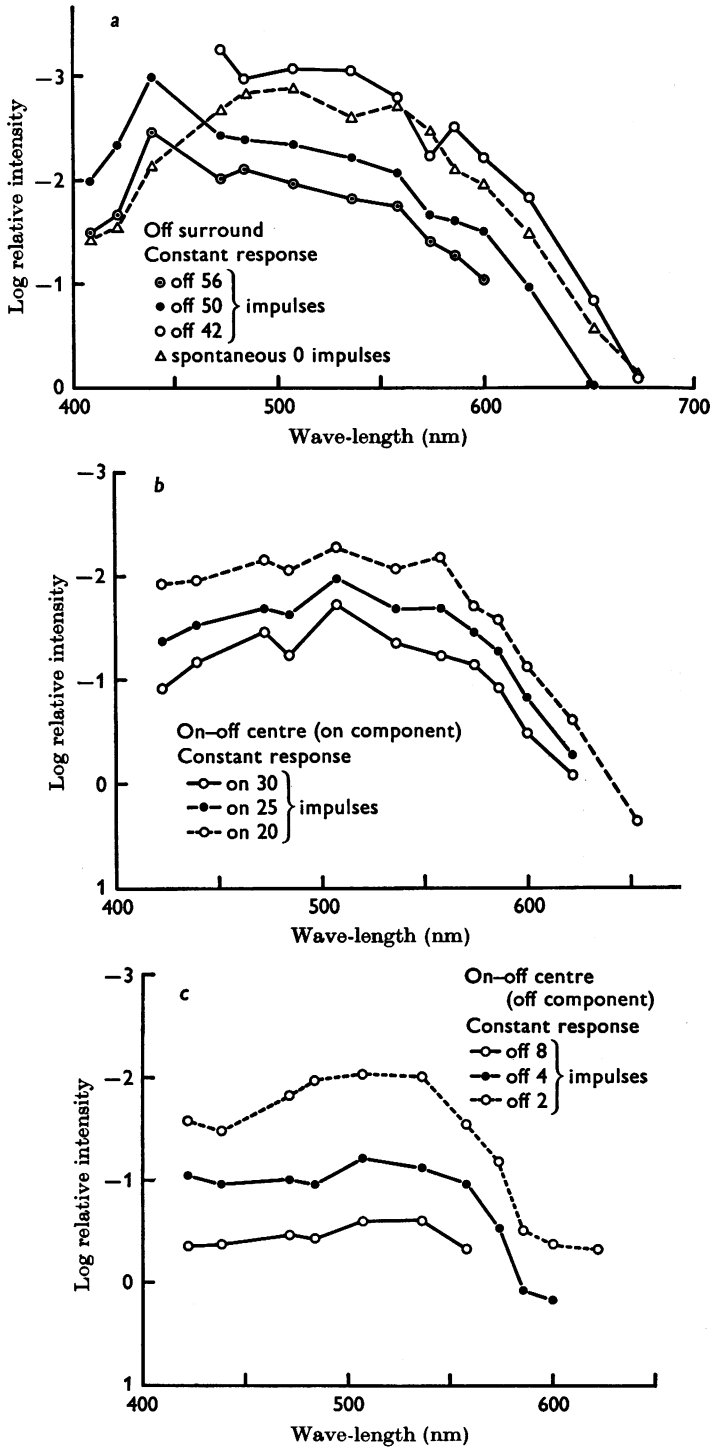

Fig. 11. For legend see opposite page.

following wave-length ranges: blue, 440–460 nm; blue-green, 470–490 nm; green, 510–540 nm; or orange, 560–590 nm. These ranges are more typical of cone input. At low intensities curves for most units gave a single sensitivity peak between 510 and 530 nm. These curves gave good correlation with the fitted curve constructed from the Dartnall nomogram (Dartnall, 1953) for a rod pigment with spectral peak at 525 nm (Fig. 13). Deviations from the Dartnall curve can possibly be ascribed to pre-retinal absorption.

Evidence for cone-rod input was borne out by latency measurements. Gouras & Link (1966) observe that, for dark-adapted monkey ganglion cells, rod-induced responses have longer latencies. Thus for stimuli suprathreshold for cones, the cones determine response-latency. This is

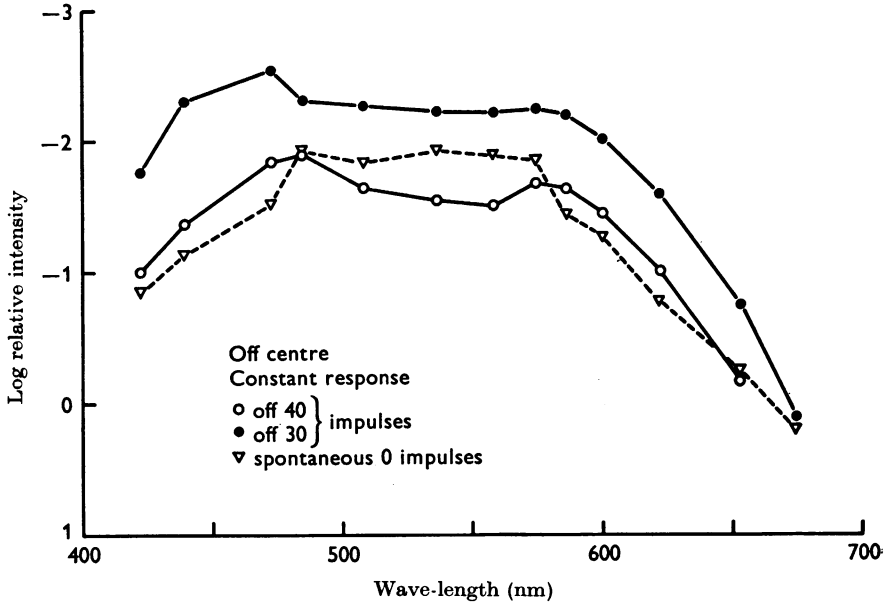

Fig. 12. Spectral sensitivity of the field centre of a Type 2 off-centre unit. The field centre was orange-and-blue sensitive. The on–off surround was green-sensitive.

seen for the on-component of the centre response of the unit shown in Fig. 9, where spectral sensitivity curves indicated cone-rod input. At high stimulus intensities the high-frequency, short-latency burst is followed by a longer-latency sustained phase. At lower intensities the short-latency burst is absent; only the sustained phase remains and this results in a step-increase in latency. There was interaction and temporal overlap of the two phases of the response at some intensities. Thus it was not possible to plot separate intensity–response, and spectral sensitivity curves for each input. However, in units where spectral sensitivity curves indicated

cone and rod input, latencies of all responses recorded over the 3 log unit range of intensity at a particular wave-length showed a bimodal distribution, with short and long latency peaks. The response–latency distribution in units which from spectral sensitivity curves appeared to receive only cone input, was unimodal about a short-latency peak. Units with cone-rod input gave unimodal distributions about a long-latency peak, at wave-lengths where cone sensitivity was low.

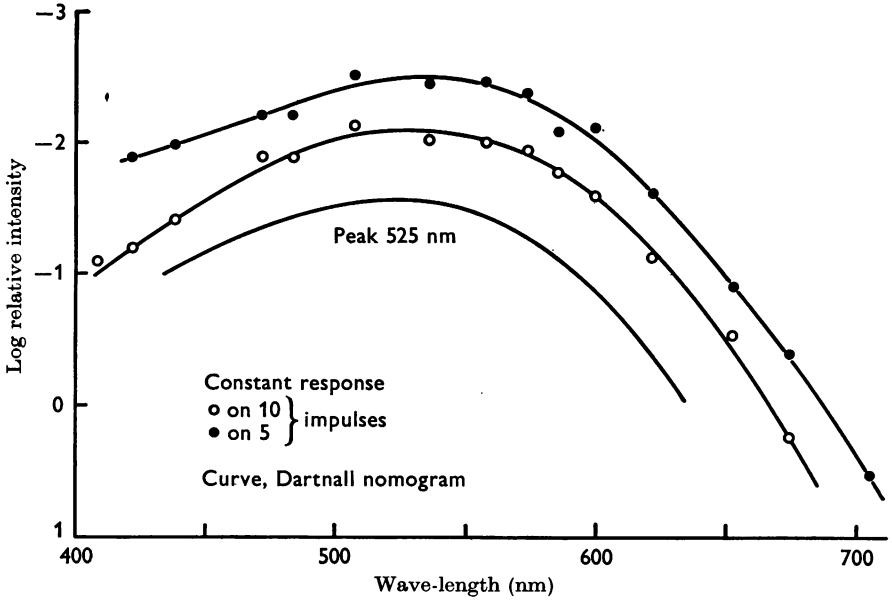

Fig. 13. Spectral sensitivity at low stimulus intensities for the predominant on component of a Type 1 unit with cone-rod input. Sensitivity curves give a single peak in the green, maximal at about 525 nm. The smooth curve is constructed from the Dartnall nomogram for a rod pigment with a spectral peak at 525 nm. The experimental and Dartnall curves are in good agreement.

DISCUSSION

In the plaice there is a tectal layering of retinal ganglion cell types, and an increase of receptive field size with depth at which axons terminate in the superficial tectum. This situation is comparable to that found in the frog (Maturana *et al.* 1960). The superficial on, off and Type 1 on–off units found in plaice appear to correspond to the on, off and brief on–off responses identified by Jacobson & Gaze (1964) in their layers *B* and *C* of goldfish tectum, but with more marked evidence of layering. Discharge patterns of Type 2 units correspond most closely to those of the sustained on and off units found by these authors in layer *D* of goldfish tectum. The

retino-tectal projection is similar to that found in goldfish (Jacobson & Gaze, 1964), and in black bass, bluegill, carp and goldfish (Schwassmann & Kruger, 1965).

Wolbarsht *et al.* (1961*a*) suggest that the on, off and inhibitory components of ganglion cell discharges can be explained in terms of discrete excitatory and inhibitory inputs from one or more receptor populations. The on discharge is ascribed to the influence of the excitatory input. The inhibitory input is considered to hyperpolarize the cell during retinal illumination, expressed as the inhibitory component of the response. The off discharge is a post-inhibitory rebound when the hyperpolarizing influence is removed. Their explanation may hold for plaice also, for the observed suppression of the off discharge could be due to reduction of the inhibitory hyperpolarization by the excitatory input.

The large size of receptive fields can be attributed partly to refraction at the corneal surface of a normally aquatic eye placed in air. Field sizes are of the same order as found for goldfish eyes stimulated through air (Jacobson & Gaze, 1964). It is unlikely that stimulation through air leads to spatial distortion, for receptive field boundaries, and boundaries between field subdivisions in Type 2 units, are sharp. Fields are commonly of the classic circular or elliptical pattern. Receptive field analyses of Type 1 units indicate that both response-components are maximally sensitive at the field centre, with a different fall-off in sensitivity towards the periphery. Thus the inability to demonstrate centre-surround receptive fields is not due to poor spatial localization. Again, in Type 2 units the on and off components of the mixed on–off response from the 'on'-field show the same spectral sensitivity. Hence for those units which are clearly opponent the associated off component must originate from the 'on'-field: it cannot be due to stimulus-spread into the off-field. If spatial localization were poor, one would similarly expect to record mixed on–off responses from the off-field, an occurrence observed only in one unit. To account for the mixed on–off response it is possible that receptors in the on-field have both direct excitatory and inhibitory influences on the ganglion cell. This seems unlikely, for although the on component of the mixed on–off response shows marked suppression, there is never corresponding suppression of the associated off component. The most plausible explanation, as Dowling & Boycott (1966) suggest for similar phenomena observed by Wagner *et al.* (1960) in goldfish, is that when stimulating the on-field, the off component is due to spread via amacrine cells extending into the off-field. This could explain why the off component occurs only at high stimulus intensities. On this theory, as is observed, the spectral sensitivities of both components of the on–off response would be identical.

Type 1 units appear to be similar to some opponent cells found in the

goldfish (MacNichol *et al.* 1961; Wolbarsht *et al.* 1961*b*), 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 properties, Type 1 units are interpreted as being primarily concerned with colour-coding. The mutual antagonism between the on and off components of responses would serve to enhance the colour-contrast sensitivity. Since receptive fields of these units show no subdivision, it is unlikely that they are concerned with size and form analysis, or directional movement discrimination.

Type 2 cells give only weak opponent responses. In some, spectral peaks are well defined and centre and surround sensitivities are clearly different. In others, peaks are indeterminate and centre and surround sensitivities may be identical. Opponent properties in the latter case may be apparent only, resulting from interaction between field centre and surround. Two classes may emerge from the initial Type 2 classification, the non-opponent class being similar to cells found by Wolbarsht *et al.* (1961*b*) in goldfish. Type 2 units are comparable to both opponent and non-opponent, centre-surround, geniculate cells found in the rhesus monkey (Wiesel & Hubel, 1966). They are presumably concerned with size and form discrimination over a wide spectral and intensity range, for most units receive cone-rod input.

Spectral sensitivity curves at high stimulus intensities were distorted by interaction between inputs, and broadened in the green in units receiving rod input. Peaks, however, were more typical of cone input and occurred predominantly within one or more of three spectral ranges; blue, 440–460 nm; green, 510–540 nm; and orange, 560–590 nm. Blue-green peaks (470–490 nm) were found in two units. Red peaks were never found. Blue and orange peaks agree tolerably well with Jacobson's (1964) data for units recorded from goldfish tectum; and green and orange peaks with the results of Witkovsky (1965) for carp retinal ganglion cells. Marks (1965) measured the difference spectra of single goldfish cones and occasionally obtained orange and blue-green peaks. These he interprets as red-green twin cones and secondary photopigments respectively. Twin cones are prominent in plaice retina (Engstrom & Ahlbert, 1963). Since in plaice red maxima have not been identified whilst orange peaks occur frequently, it is tempting to suggest that these are due to inputs from twin cones.

Spectral peaks at low stimulus intensities occurred invariably between 510 and 530 nm in units with rod input. Sensitivity curves agreed well with empirically-derived rod absorption spectra (Dartnall, 1953).

I wish to thank Dr G. A. Horridge for invaluable criticism and encouragement, and J. Stevenson Esq. for assistance in preparing the figures. This work was performed while in tenure of a post-graduate award from the University of St Andrews.

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