CHROMATIC PROPERTIES OF THE RETINAL AFFERENTS IN THE THALAMUS AND THE TECTUM OF THE FROG (*RANA TEMPORARIA)*

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Abstract—In order to clarify physiological mechanisms underlying colour-specific visually guided behaviour, we measured spectral sensitivities of On-fibres projecting to the thalamus and class 2 and 3 fibres passing to tectum opticum. In addition we recorded responses of these fibres to moving coloured papers with known spectral reflectancies. The latter method, here called paper colourimetry, allowed us to change the relative stimulations of the blue-, green- and red-sensitive photoreceptors in any direction desired. Under the photopic conditions used the tectal fibres were driven exclusively by red-sensitive receptors, while the thalamic fibres received strong On-inputs from both red- and blue-sensitive receptors. Due to a partly antagonistic interaction between these inputs the On-fibres acted in a dichromatic way, responding with specific extended low-frequency discharges to all relative increases in blue receptor stimulation, e.g. to a great reduction in red stimulation combined with unchanged blue stimulation. Thus they have functional characteristics which could serve a visual system showing colour constancy.

Frog   Retina   Ganglion cell   Thalamus   Tectum opticum   Colour vision.

INTRODUCTION

The frog *(Rana temporaria)* has been shown to possess true colour discrimination both in its optomotor responses to rotating striped cylinders (Birukow, 1939), in its specific positive phototaxis towards blue (Muntz, 1962b), and in its pairing behaviour during the breeding season. In spring, when the throat of the *Rana temporaria* males becomes bluish, while the females are yellowish or reddish, the males prefer red and yellow female dummies to blue ones (Kondrashev *et al,* 1976).

Microspectrophotometric measurements and electrophysiological recordings have further shown that the frog retina possesses characteristic wavelength-discriminating mechanisms. Three visual pigments are described: a blue-sensitive pigment (λ_{max} 432 nm) in the "green rods", a green-sensitive pigment (λ_{max} 502 nm) in the regular red rods and in the accessory components of the double cones, and a red-sensitive pigment (λ_{max} 575 nm) in the single cones and the principal members of the double cones (Liebman and Entine, 1968; Donner and Reuter, 1976). In this study we need not concern ourselves with the cellular location of the visual pigments and thus we will just refer to three photoreceptor mechanisms, a blue (-sensitive), a green and a red one.

Our knowledge is less solid when we come to the ganglion cell classes and afferent fibre projections mediating the chromatic information from the recep-

tor mosaic to the brain of the frog. Muntz (1962a) stressed the importance of the blue-sensitive On-fibres projecting to the nucleus (rather neuropil) of Bellonci in the dorsal thalamus, while Reuter and Virtanen (1972), measuring ganglion cell thresholds against strong yellow backgrounds in eye-cup preparations, found that several cell classes which apparently project to tectum opticum receive blue-sensitive input and may have a capacity for transmitting chromatic information.

In this study we have measured spectral sensitivities and recorded responses to moving paper stimuli with "natural" non-saturated surface colours. The logic of the latter method (here called paper colourimetry) may be summarized as follows:

(1) In an eye with three receptor types with ' different visual pigments the primary effect of any light reflected from a coloured surface can be defined by a point in a three-dimensional colour space. The axes of this space are the quantum catches in each of the three receptor types (relative to the catches produced by a perfectly white surface). This mapping from the physical to the physiological establishes when very different physical stimuli will have equivalent physiological effects.

(2) An optic fibre driven exclusively by one receptor type will not respond to colour changes within planes perpendicular to the axis for that receptor.

(3) A fibre driven by two (non-summating) receptor types will respond to all changes except those represented by lines parallel to the axis for the third receptor.

Our results indicate that (in photopic conditions) the tectal fibres satisfy property (2) above, while the Bellonci fibres satisfy property (3).

MATERIAL AND METHODS

Preparation and recording

Common frogs *(Rana temporaria)* were obtained in late autumn from the regions surrounding Helsinki and Moscow and stored in darkness at 4°C. A few hours before an experiment they were paralyzed with 0.2 mg D-tubocurarine in 0.2 ml Ringer solution injected intravenously. During the experiments they were kept at 10-15°C fastened on a support and partly covered with moist gauze. If the frog moved a few drops of concentrated urethane were applied on the skin of its legs. The skull was opened (see Maturana *et al.,* 1960) exposing unilaterally the thalamus and the anterior part of the optic tectum. A few control experiments were carried out with recordings from ganglion cells in the excised and opened eyes as described by Bäckström and Reuter (1975).

Responses from the superficial layers of the anterior tectum and the neuropil of Bellonci in the dorsal thalamus were recorded with Woods alloy filled micropipettes as described by Maturana *et al.* (1960), Muntz (1962a) and Fite *et al.* (1977). As we clearly recorded from the same types of units as the authors above, their arguments for ascribing the responses to axonal arborizations of retinal afferents (and not tectal and thalamic cells) apply to this work as well (see also George and Marks, 1974). Recent histological evidence for the contralateral retinal projections to tectum and the neuropil of Bellonci has been presented by Fite and Scalia (1976). The axon terminals of retinal origin produce fast spikes, are driven by the contralateral eye, and have, compared with postsynaptic brain units, relatively small receptive fields (2-8 deg of visual field for the class 2 and 3 fibres in tectum and about 10 deg for the On-units in thalamus). Responses were occasionally photographed from the screen of a Tektronix 5103N storage oscilloscope.

In some cases we worked with multiunit recordings from a group of similar fibres, but by using a signal discriminator passing only high amplitude spikes to the loudspeaker it was usually possible to isolate the unit with the largest spikes. All units had their receptive fields in the binocular (rostral) part of the visual field of the investigated eye. Responses from possible ipsilateral units were excluded by covering the other eye.

Spectral sensitivity measurements

The light from a xenon arc first passed through two channels, one for On-Off stimuli, the other for steady backgrounds, each with arrangements for inserting filters and neutral density wedges controlling colour and intensity (Donner and Reuter, 1968). In the stimulus beam we used a series of narrow-band interference filters with half-bandwidths of 8-10 nm, in the background channel white light or an orange Wratten (22) filter or a yellow glass filter (Schott and Gen., GG 495) transmitting less than 1% below 550 and 470 nm respectively.

At a point where the stimulus and background channels united the light from both of them was collected into an optic waveguide, the other end of which formed a 5 mm opening in the centre of a screen (dia. 10 cm) which could be placed into any position in relation to the frog. Except for the waveguide orifice, this screen was covered with a white or grey paper which was separately illuminated by a tungsten source with or without filters so that it approximately matched the background light transmitted through the waveguide in colour and luminance.

After characterizing a single unit and localizing its receptive field, we placed the 10 cm screen at a distance of about 10 cm from the eye of the frog so that the light from the waveguide, which to the frog represented a 3-5° spot, stimulated the centre of the receptive field of the unit. The absolute intensities produced by the stimuli and backgrounds on the retina could not be reliably estimated, but the relative quantum intensities of the narrow-band stimuli were measured both with a vacuum thermocouple and an Airam UVM-8 photometer.

On and Off thresholds at various wavelengths were determined using 5 sec exposures at 20-30 sec intervals. The lowest intensity that repeatedly produced a discharge was determined by decreasing the intensity in steps of 0.2 or 0.1 log units until the cell ceased to respond. For control the last two steps were repeated.

Paper colourimetry

Principle. The stimulation procedure which we here refer to as paper colourimetry has much in common with both classical colourimetry where a subject matches the hues of two adjacent (physically different) fields and the silent substitution techniques introduced by Bongard (1955; Bongard and Smirnov, 1957) and Donner and Rushton (1959). These authors determined the characteristics of a stimulus which can replace a spectrally different one without eliciting an electrophysiological response (for recent applications, see Estevez and Spekreijse, 1982). We have also determined conditions for silent substitution but instead of the spectral filters used in the above experiments our paper colourimetry method uses reflecting surfaces resembling the unsaturated colour stimuli to which animals react in nature and in many behavioural experiments.

Stimulation procedure. Stimuli cut from thin matt papers (e.g. Indicolor®, Zürich) were presented on a flat surface illuminated by a tungsten source. The relative positions of the light projector, the paper and the mirror through which the frog saw the stimuli were adjusted so that the frog could see no direct reflexions from the paper even if this were glossy (for

a presentation of the stimulus situation, see Orlov and Kondrashev, 1978, Fig. 2).

The stimulation was carried out by replacing one coloured paper covering the receptive field with another. This could be done either by moving one large sheet over another or by moving a large sheet of one paper on which a small round piece (dia. 3-5°) of another paper was attached (the irrelevant paper edges were not seen by the frog). In a third stimulation variant one paper seen through a 3-5° hole in an overlying sheet was suddenly replaced by another paper. Frequent controls showed that replacement of one coloured paper by another sheet of the same paper elicited no response.

We investigated only units which passed our standard contrast sensitivity test, i.e. responded well to a 20% decrease or increase in neutral (grey) reflectance. For each unit we used the stimulus configuration and the rate and direction of movement producing the strongest responses to these neutral stimuli. In the experiments on which Figs 3 and 5 are based, we used very rapid movements (> 100° s^{-1})·

The experiments were carried out under moderate photopic conditions; the white illumination level reaching the stimulus plane was about 1000 lx, and during the intervals between the presentations of stimuli this surface was usually covered with a grey paper reflecting about 30%.

Reflectance spectra. Spectral reflectance functions $\rho(\lambda)$ of all papers were measured from 400 to 700 nm with a LOMO SF-10 spectrophotometer using magnesiumoxide as white reference (the reflectance of the MgO surface was taken as 100% at all wavelengths). Reflectancies at 380 and 390 nm were estimated by extrapolation. Figure 1(a) presents reflectance spectra of four papers: a blue-green (BlGr), a grey (N = neutral) with about 30% reflectance throughout the spectrum, and a pair of a green (Gr) and a purple (Pu) paper deviating in opposite ways from N. N was a standard background used in both spectral sensitivity (Fig. 2) and paper colourimetry (Figs 3, 4 and 5) experiments.

Calculation of relative receptor stimulation. In order to estimate how efficiently different papers stimulate a given receptor mechanism we have to know, except the reflectance spectra $\rho(\lambda)$ of the papers, the spectral energy distribution of the light source $S(\lambda)$ and the action spectrum of the receptor mechanism in question. Considering, as an example, the red receptor mechanism having the action spectrum $\check{r}(\lambda)$, the relative efficiency of a given paper, i.e. the R value of that paper, will be given by the equation

$$R = a_R \int_{380}^{700} S(\lambda) \rho(\lambda) \check{r}(\lambda) \, d\lambda. \qquad (1)$$
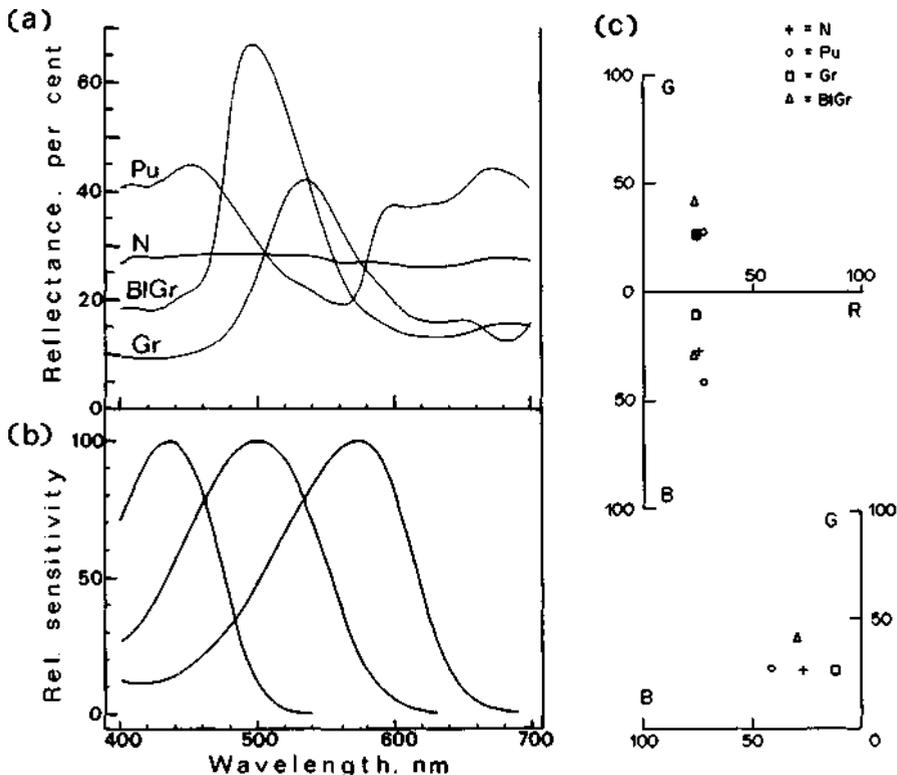
Fig. 1. Procedure for calculating the relative efficiencies with which four selected papers stimulate the blue, green and red receptor mechanisms. (a) The reflectance spectra of a purple (Pu), a grey (N), a blue-green (BlGr) and a green (Gr) paper. (b) The spectral sensitivities of the receptor mechanisms. (c) Plane projections of the frog colour space. The relative efficiencies of the papers were calculated [see equation (1)] from the spectra shown in (a) and (b), and from the spectrum of the incident light (the results are given in Table 1). The positions of the papers in the -colour space are shown in the projections in (c).

Table 1. The relative efficiencies with which the four papers considered in Fig. 1 stimulate the red (R), green (G) and blue (B) receptor mechanisms of the frog

Type of paper	Relative receptor stimulation		
	R	G	B
N	25.1	26.4	26.8
Pu	27.2	27.9	41.1
Gr	24.1	26.5	11.6
BlGr	23.2	40.2	28.1

These efficiencies were calculated from the reflectance spectra of the papers [Fig. 1(a)], the spectral sensitivities of the receptor mechanisms [Fig. 1(b)], and the spectrum of the incident light [see Equation (1)]. Note that three values deviate significantly from the rest: those giving the stimulation of B by Pu and Gr, and that giving the stimulation of G by BlGr; all the others keep within the same range (23-28).

Correspondingly, the relative efficiency for stimulation of the green and blue receptor mechanisms, the G and B values, are obtained by making the appropriate substitutions in Equation (1): $\breve{g}(\lambda)$ or $b(\lambda)$ for $\breve{r}(\lambda)$, and a_G and a_B for a_R. The latter symbols represent normalizing factors chosen such that the R, G and B values of a perfect (MgO) white having $\rho(\lambda) \equiv 1$ would be equal to 100.

For $S(\lambda)$, i.e. the spectrum of the incident light, we used that of a Planckian source with a colour temperature of 2856 K.

Action spectra of receptor mechanisms. Figure 1(b) shows the relative quantum sensitivity spectra (action spectra) used in this study to represent the red, green and blue receptor mechanisms of the frog. For defining the blue fundamental $b(\lambda)$ which is apparently determined by the output from the green rods we used a λ_{max} at 432 nm (Liebman and Entine, 1968) and a short-wavelength Ebrey-Honig nomogram (Ebrey and Honig, 1977) further assuming a maximum effective absorbance of 0.3 (Reuter, 1969). For the green fundamental $\breve{g}(\lambda)$ we used the absorption spectrum of frog rhodopsin with λ_{max} at 502 nm (Dartnall, 1953) assuming a maximum absorbance of 0.4 (Reuter, 1969; Govardovskii and Zueva, 1974). The λ_{max} of the red fundamental $\breve{r}(\lambda)$ lies at about 575 nm (Liebman and Entine, 1968). We used an Ebrey-Honig nomogram with that peak absorption to depict the short-wavelength part of its spectrum. For the long-wavelength part, where the absorption decrease seems to be considerably steeper than suggested by the nomogram, we fitted the curve to the electrophysiologically measured (early receptor potential) spectral sensitivity of frog cones (Govardovskii and Zueva, 1977; see also Goldstein and Wolf, 1973).

We carried out our experiments on frogs with intact eyes and the slight wavelength-selective absorption in the anterior ocular media of the frog (Govardovskii and Zueva, 1974) was taken into account when the above three fundamentals were determined.

Graphic representation of the colour space of the frog. Table 1 gives the R, G and B values, calculated as described above, of the four papers whose spectral reflectances are shown in Fig. 1(a). It can be seen that the papers BlGr, Gr and Pu have approximately the same R as our standard grey (N), and thus the red mechanism should respond very little or not at all when these papers replace the grey background.

The R, G and B values between 0 and 100 can be regarded as coordinates of the three-dimensional colour space of the frog, where each (paper)colour is then represented by a point. Figures 1(c), 4 and 5 are projections or planes of this colour space, representing two of the three parameters specifying each colour. Figure 1c shows the positions of the papers presented in Fig. 1(a) in the R–G, R–B and G–B planes. It can be seen that papers N, Gr and Pu occupy approximately the same position in the R–G plane but different positions in the R–B and G–B planes, while the papers N and BlGr occupy the same position in the R–B plane but different positions in the R–G and G–B planes.

Figure 1, equation (1) and Table 1 thus describe how the chromatic properties of paper surfaces are interpreted in terms of relative excitations of the three receptor mechanisms.

RESULTS

Responses to neutral stimuli

When the receptive field centre of a thalamic fibre is stimulated with white spots turned on and off, it responds with short On-discharges. As these cells have large receptive fields (dia. about 10°) they respond best to large spots and to increments in illumination produced by wide moving fields. A selective stimulation of retinal regions surrounding the receptive field centre often elicits Off-discharges (see below).

The tectal class 2 fibres respond optimally to moving small (dia. 2–4°) dark objects. They produce sustained responses to edges stopping in the centre of the excitatory receptive field (ERF), but as this ERF is surrounded by a strong inhibitory field they do not respond to large moving objects, nor to the turning on and off of diffuse stimuli. When only the ERF is illuminated they usually respond to both On and Off. The tectal class 3 cells respond with short high-frequency discharges to the turning on and off of both small and large stimuli (Maturana *et ai,* 1960; Bäckström and Reuter, 1975).

Spectral sensitivity

Figure 2 presents three spectral sensitivities measured against the same background, all units having their receptive fields in the same part of the anterior
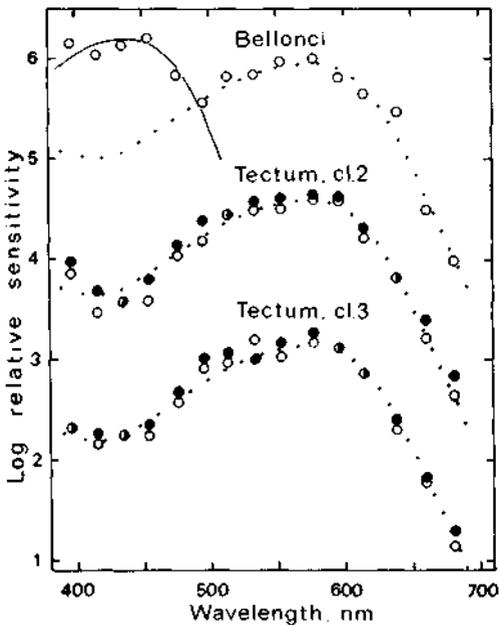
Fig. 2. Spectral sensitivities of an On-fibre projecting to the neuropil of Bellonci, and class 2 and 3 fibres projecting to tectum opticum. The On and Off thresholds are marked with open and solid circles respectively. The ordinate is log relative sensitivity (reciprocal of the relative quantum intensity at threshold). Nearly monochromatic spot stimuli were used. The adapting background surrounding the stimulus spot was our standard grey paper (N in Table 1) illuminated with 1000 lx, and the waveguide-mediated white background on which the stimulus was superimposed was adjusted to match this surround. To avoid overlap, the three spectral sensitivities are vertically displaced in relation to each other. The continuous and dotted lines represent the action spectra of the blue and red receptor mechanisms respectively (see Methods).

visual field of the left eye. The uppermost series of thresholds were measured from an On-fibre in the neuropil of Bellonci in the anterior thalamus and the middle and lower series from tectal class 2 and 3 fibres respectively.

Both the On and Off thresholds (open and solid circles) of the tectal units seem to reflect the spectral sensitivity of the red receptor (dotted lines) with a possible minute contribution from green receptors (cf. Bäckström and Reuter, 1975). The Bellonci unit, however, clearly shows inputs from both blue (continuous line) and red receptors.

Using orange, yellow and neutral backgrounds of various intensities we have measured photopic and mesopic spectral sensitivities of 15 thalamic and 25 tectal (class 2 and 3) units. Fourteen of the thalamic but only 2 of the tectal recordings revealed clear inputs from blue receptors. In four experiments with yellow backgrounds we measured from both thalamus and tectum in the same frog keeping the adaptational state and the stimulus position constant while changing the position of the electrode. In all four recordings the thalamic units showed distinct

inputs from blue receptors while the tectal units showed none.

In the two cases when we observed blue-sensitive inputs to the tectum we used strong orange and yellow backgrounds selectively suppressing the sensitivity of red and green receptors. Even then the blue-sensitive thresholds were, however, variable and elusive. As this contrasts to the clear blue-sensitive inputs found in recordings from class 2 and 3 units in yellow-adapted isolated eyecups (Reuter and Virtanen, 1972), we tested the hypothesis that the lack of blue inputs to tectum may be due to an effect of the drugs used in connection with recordings from the brain. We isolated eyes from two tubocurarine- and urethane-treated frogs and recorded from ganglion cells close to the retinal surface. Disproving the above hypothesis we found prominent blue-sensitive inputs to class 2 cells.

When we decreased the intensity of the background we found that the spectral sensitivities of both tectal and thalamic units became dominated by green receptors (apparently red rods).

Paper colourimetry

Some key tests. Spectral sensitivities of the type presented in Fig. 2 help us to identify the receptors contributing to ganglion cell thresholds. However, in order to clarify how the receptor mechanisms interact in driving the responses to changing colours and moving colour borders we turned to paper colourimetry. This technique makes it possible to investigate sub- and supra-threshold interactions between different chromatic mechanisms.

The basic strategy of paper colourimetry is illustrated in Fig. 3 which shows the responses of a well isolated Bellonci On-fibre (a–d) and some tectal class 2 (e–h) and 3 (i–l) fibres to the same series of stimuli. The background was in all cases grey and each recording represents a 3 s period during which another paper was first rapidly moved in over the receptive field and then retracted. The top recordings (a, e, i) show our standard neutral contrast test producing a 20% increase followed by a corresponding decrease. The Bellonci unit responded only to the intensity increase while the tectal units responded to both changes. For sensitive cells the threshold contrast was about 10%.

The lower recordings stem from tests employing the papers presented in Fig. l(a,c) and Table 1. The coloured papers Pu (b, f, j), Gr (c, g, k) and BlGr (d, h, l) were moved in over and away from the standard background N. As seen in Table 1 none of these movements produced significant changes in the stimulation of the red receptor. Pu and Gr changed the stimulation of the blue receptor without affecting the green receptor, while BlGr changed the stimulation of the green receptor without significantly affecting the red and blue ones (see Table 1). The Bellonci unit responded to the increase in blue receptor stimulation produced by Pu moving in and Gr
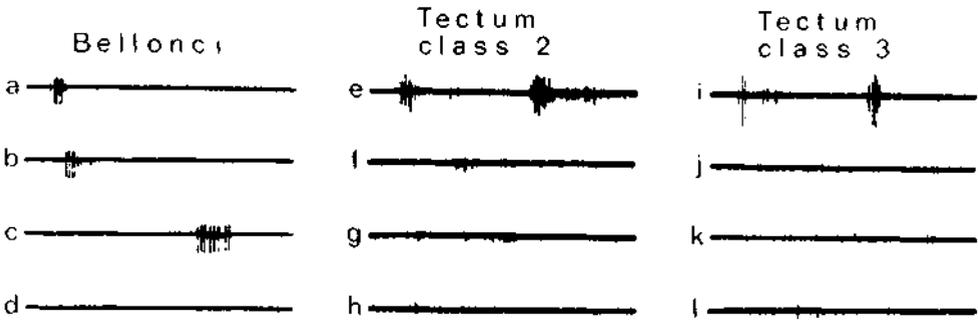
Fig. 3. Responses of a Bellonci On-fibre (a–d) and of tectal class 2 (e–h) and class 3 (i–l) fibres to the replacement of one coloured or grey paper with another. During the first half of each 3 s recording a test paper was moved in over the receptive field of the unit, and during the second it was retracted. The background paper was in all cases grey. The first row of recordings (a, e, i) shows responses to another grey paper producing a 20% increase in reflectance followed by a corresponding decrease. The lower recordings represent tests employing the papers presented in Table 1, the background being our standard grey (N) while the test papers were purple (Pu; b, f, j), green (Gr; c, g, k) and blue–green (BlGr; d, h, l). It is seen that only the Bellonci fibre responds to Pu and Gr, and none of the units detect BlGr. The size of the test paper was about 15° for the Bellonci unit and 4° for the tectal units.
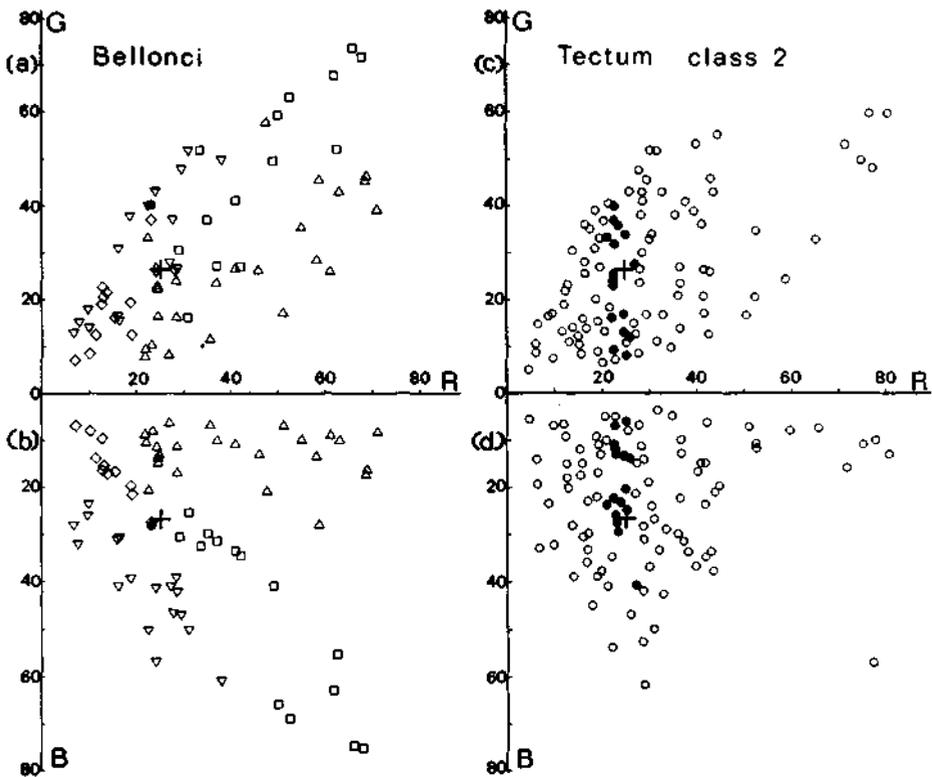
Fig. 4. The colour discrimination properties of a Bellonci unit (a, b) and a tectal class 2 fibre (c, d) tested with paper colourimetry. The solid circles mark positions (in the R–G and R–B planes of the frog colour space) of the test papers which could be interchanged with the standard grey background (N) without eliciting a response. The location of background N is marked with a cross. The positions of the response-producing papers are marked with open symbols, and for the Bellonci unit different response types are marked with different symbols: □ = long rhythmic discharges when the test paper replaced the background; Δ = long rhythmic discharges when the background paper was again uncovered; □ = short bursts to test paper replacing background, but no long discharges; ◊ = short bursts to background replacing test paper, but no long discharges. Observe that different response types are symmetrically arranged in the R–B plane but disordered in the R–G plane. Test paper sizes as in Fig. 3.

moving out, but not to the substitution of BlGr for N. The tectal units responded to none of these coloured papers. By classifying paper substitutions into response-producing and "silent" ones we can thus identify the receptors driving a given unit in a given adaptational state.

In the paper colourimetry experiments above we used the same light-adapting background as in the spectral sensitivity measurements presented in Fig. 2 (paper N, 1000 lx illumination), and the results are consistent: no marked green receptor effects are seen, the tectal fibres are driven mainly or exclusively by red receptors, while the Bellonci fibres show efficient inputs from both red and blue receptors (the tests in Fig. 3 were designed to demonstrate blue and green receptor inputs but they could not positively identify red receptor inputs: the red input to the Bellonci unit was evident from other tests). Due to their blue input the Bellonci fibres can detect moving purple-grey and green-grey borders not seen by tectal cells.

Investigation of colour space. Key stimuli of the type used in Fig. 3 give rapid and useful information but for a thorough understanding of the interactions between chromatic mechanisms we need more complete investigations of the colour space. It is for instance not clear from Fig. 3 whether or not a silent substitution could result from a decreased stimulation of the red mechanism compensated for by a simultaneous and matching increase in blue stimulation. In order to answer such questions and test the true colour border detecting capacities of the projections to tectum and thalamus we have carried out extensive experiments with hundreds of coloured papers.

Figure 4 shows the positions in the R-G and R-B planes [see Methods, Fig. 1(c)] of about 60 stimulus papers used for testing one typical Bellonci fibre (a, b) and one tectal class 2 unit (c, d). In these experiments all coloured papers moved over and thus transiently replaced our standard grey (N) the position of which is marked with a cross. The locations of the papers producing no response when replacing N are marked with solid circles, while the response-producing papers are marked with open circles (c, d) or with other open symbols to mark different types of responses (a, b; see Legend).

The vertically extended groups of solid circles in Fig. 4(c) and (d) indicate that, regardless of great changes in the stimulations of blue and green receptors, this class 2 unit remains silent as long as the stimulation of the red mechanism is not changed. For the Bellonci unit (Fig. 4a, b) the condition for silent substitution is clearly different: the only paper (shown in this Figure) which can substitute for N is BlGr, i.e. a paper which can replace our standard grey (N) without significantly changing the stimulation of the blue and red receptors [Fig. 4(b); Table 1].

Assuming that an increase in blue stimulation could compensate for a decrease in the stimulation of the red mechanism, or vice versa, we would expect

weaker (or absent) responses when moving from the neutral position (the cross) in Fig. 4(b) towards the lower left or upper right. The opposite was observed: to these replacements the Bellonci fibre produced particularly strong responses (see below). Thus the On-unit shows the properties of a dichromatic (red–blue) system, while the class 2 cell [Fig. 4(c, d)] operates in a monochromatic (red) fashion.

Extensive colour space mappings carried out with 15 tectal class 2 cells confirmed that they (under our standard adaptational conditions) were driven mainly or exclusively by red receptors. The same applied to 12 class 3 cells and 3 tectal class 4 cells (Off-cells). The latter observation confirms results presented by Prehn and Scheibner (1978).

Quite often our paper colourimetry experiments have revealed Bellonci fibres lacking efficient inputs from blue receptors. It is impossible, however, to estimate the proportion of these fibres in intact frogs as the blue mechanism is the first to suffer when the preparation deteriorates.

Chromatically specific response types

Probably our most interesting results stem from a systematic investigation of the response patterns produced by different relative changes in receptor stimulation, i.e. by different displacements within the colour space. Figure 5 shows representative responses of three cell classes to displacements in all main directions in the R–B plane. The position of the grey background is marked with a cross. The first part of each recording shows the response to a sudden movement of a coloured test paper into the receptive field, i.e. a centrifugal displacement from the cross, while the later part shows the response to the opposite movement.

It is clear that the Bellonci unit differed from the tectal class 2 and 3 fibres not only in its ability to detect colour changes not eliciting responses in tectal cells, but also in having a larger repertoire of response patterns. The tectal cells in Fig. 5 responded with short bursts to all increases and decreases in red receptor stimulation. They operated in a spectrally univariant fashion, i.e. the discharges were correlated only to the length and sign of the displacement along the R axis. The Bellonci unit, on the other hand, produced two kinds of discharges, relatively short high-frequency responses connected with an increase in stimulation of the red mechanism, and longer low-frequency discharges connected with a decrease in red stimulation and an absolute or relative increase in blue receptor stimulation (or with a pure increase in blue stimulation without significant change along the R axis). For most Bellonci units the discharges of the latter type were even longer than shown in Fig. 5 (due to technical limitations only two experiments with Bellonci cells were completely recorded in this way).

The Bellonci unit responded to all papers, but it can be seen that two main directions of displacement
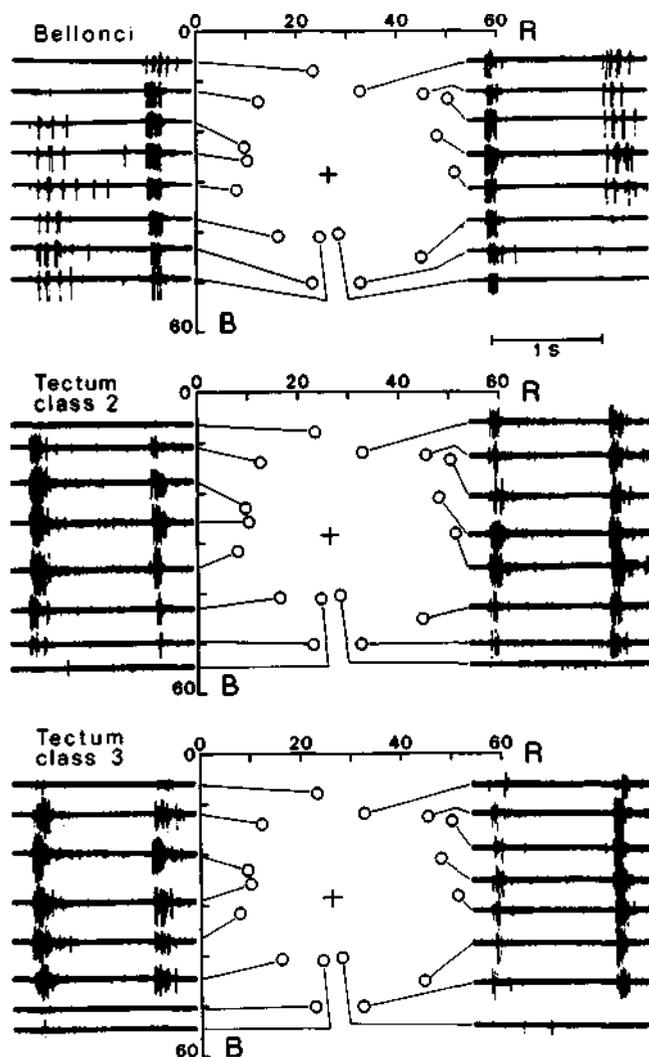
Fig. 5. Responses of a Bellonci On-fibre and tectal class 2 and 3 fibres to the same series of paper replacements. Each of the 1.63 sec recordings shows first the response of the unit to the test paper replacing the standard grey background and then the response to the opposite change. To simplify response patterns we used fast paper movements in both directions. Each response is connected with a circle marking the position of the test paper in the R–B plane. The position of the standard neutral background (N) is marked with a cross. Test paper sizes as in Fig. 3.

within the R–B plane, and the sectors between them, were "blind"; these directions were an upward movement along the vertical axis, i.e. decreased blue receptor stimulation without effect on red receptors, and a diagonal movement from the lower right to the upper left, i.e. a displacement along the neutral axis decreasing the stimulation of red and blue receptors to the same degree.

The seemingly complex response patterns of the Bellonci units shown by the recordings in Fig. 5, and schematically represented by different open symbols in Fig. 4(b), can be understood on the basis of two previously described observations and one new finding presented in this study.

It is well known, firstly that an increased blue receptor stimulation elicits long rhythmic On-discharges while an increased red receptor stimulation produces short high-frequency On-responses (Orlov, 1961; Muntz, 1962a; Fite *et al.,* 1977), and secondly that, when these stimuli are combined, the red-driven response type tends to win and suppress the blue-sensitive mechanism (Reuter, 1969; Kicliter *et al.,* 1981). Due to this latter-process all displacements substantially increasing the red receptor stimulation produces short responses independent of changes in blue stimulation.

The new observation made in connection with these experiments is that long low-frequency discharges are produced not only by increased blue stimulation but also by radically decreased red stimulation combined with unchanged or even slightly decreased,blue stimulation (see the third and fourth
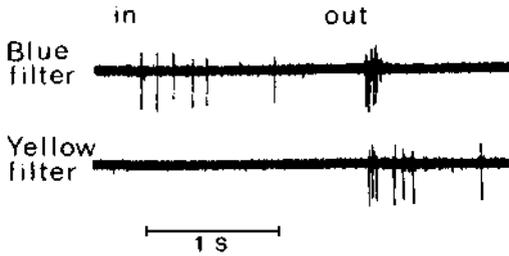
Fig. 6. Recordings from a Bellonci fibre the receptive field of which was covered by a white paper initially illuminated by an unfiltered tungsten source. The upper recording shows the responses to a blue filter rapidly introduced into, and then retracted from, the white beam illuminating the paper, while the lower recording is made during a corresponding introduction and removal of a yellow filter. The blue filter decreased the blue and red receptor stimulations by 55 and 89% respectively, while the yellow filter decreased them by 99.6 and 45% respectively.

recordings from above in the upper left part of Fig. 5). Thus these discharges signal an increase in the blue/red relation, an increased "blueishness", and not just an absolute increase in blue receptor stimulation.

Observe that the type of response elicited is not determined by the position to which we move in the R-B plane but rather by the direction in which we move. This may seem self-evident when we move diagonally along the neutral intensity axis; it is, however, equally true when we just change the R/B relation, i.e. when we move between the upper right and lower left. Thus the same grey (cross in Fig. 5) elicits a long "blue" response when it replaces a red paper but a short "red" response when it replaces a blue paper.

We designed an independent test for our finding that Bellonci fibres which traditionally are considered to be pure On elements can respond to a *decrease* in the stimulation of all receptors as long as the relative contribution by blue receptors increases. This was

done simply by suddenly introducing a blue frameless gelatine filter in a beam of white light diffusely illuminating the receptive field of a Bellonci unit. The introduction of this filter which decreased the red receptor stimulation much more than the blue one, did indeed produce an extended series of action potentials (Fig. 6, above), while its removal produced a short discharge typical for increased red stimulation.

This system for signalling changes in the blue/red relation seems to be asymmetrically organized, i.e. a great decrease in blue stimulation combined with a slight decrease in red never elicits a red response. This is borne out by the lower trace in Fig. 6, where the introduction of a yellow filter did not elicit any response, although the removal of that filter did produce a relatively long discharge signalling increased blueishness.

It is finally worth pointing out that just reducing the intensity of a given light, even red, in a spectrally non-selective way, does not change the relative stimulation of different receptor types and produces no discharge.

The organization of the receptive fields of Bellonci fibres

Independently of stimulus wavelength an On fibre remains silent when a diffuse stimulus, or a stimulus illuminating just the receptive field centre, is turned off. A stimulus illuminating only an annulus surrounding the ordinary excitatory receptive field can, however, produce an Off response. This is seen in Fig. 7, which first (upper row) shows On responses of a Bellonci fibre to large red and blue stimulus fields (dia. 30°). The cell also responds when we turn off annular stimuli (12° inner and 30° outer diameter) of these same red and blue lights (lowermost row in Fig. 7), and when we selectively turn off a corresponding annular part of a 30° field without changing the
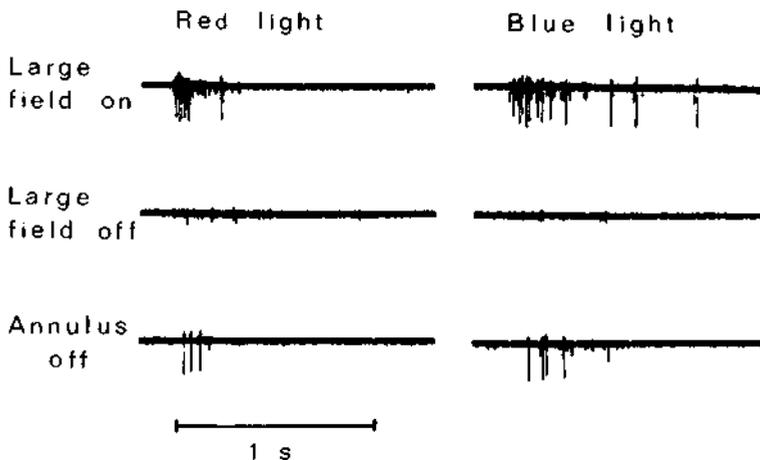
Fig. 7. Responses of a Bellonci fibre to large stimulus fields (dia. 30°) and annuli (12° inner and 30° outer diameter) concentric with the receptive field. The light beams projecting these stimuli on a white paper passed through either a red or a blue broadband filter (filters published in Nature to be used in conjunction with Zeki, 1980; without these filters the illumination was about 1000 lux at the plane of the paper).

centre illumination (not shown in the figure). These Off responses are, however, suppressed by simultaneous antagonistic Off signals in the centre if the whole 30° field is turned off (middle row).

Both red and blue receptors seem to contribute to the surround response; the red receptor contribution is obvious as the response can be elicited by red lights not affecting blue and green receptors, while the blue contribution is strongly suggested by the finding that the Off response to a blue annulus can be enhanced by illuminating the whole field with a relative strong continuous red background light-adapting the red receptors. This effect is difficult to reconcile with the idea that the response elicited by turning off a blue annulus is mediated by red receptors.

DISCUSSION

Chromatic properties of tectal and thalamic pathways

A central task in sensory physiology is to understand how different aspects of the information originating from a given (moving) object are processed in diverging and converging afferent pathways. To restrict ourselves to the visual system: how are such aspects as size, form, colour and direction of movement analyzed and integrated?

The directionally selective retinal ganglion cells of the ground squirrel seem to project to the superior colliculus which is homologous to the tectum opticum of lower vertebrates, while the opponent-colour cells project to the lateral geniculate in thalamus (Michael, 1973). The superior colliculus of the rhesus monkey may allow rudimentary colour-discrimination capacity but it is well known that most of the information needed for the colour-specific behaviour of this primate ascends the geniculostriate limb of the visual system (for a review, see Jacobs, 1981).

Muntz (1962a,b) and Kicliter *et at.* (1981) have presented strong evidence suggesting that the visual information needed for the phototaxis of the frog is mediated by the blue-sensitive On fibres running to the neuropil of Bellonci (both the fibres and the taxis show high blue-sensitivity and suppression by long-wavelength light). The importance of the dorsal thalamus in the frog's reactions to blue is also suggested by a lesion study (Kicliter, 1973) and by an electrophysiological investigation of the colour detector properties of different optic fibre terminals in the frog's brain (Orlov and Kondrashev, 1978). The latter study revealed clear blue-sensitive inputs only to the On fibres projecting to the neuropil of Bellonci.

The unique colour-coding role of these On fibres has, however, been questioned by Reuter (1969) and Reuter and Virtanen (1972; see also Grüsser-Cornehls and Saunders, 1981). They found that many ganglion cells obviously projecting to the optic tectum can be shown to receive inputs from blue receptors in addition to their inputs from red ones.

In order to demonstrate the blue-sensitive thresholds of these cells Reuter and Virtanen had, however, to use strong yellow backgrounds selectively depressing the sensitivity of the green and red receptors without radically affecting the blue ones. In later experiments using white background lights they were unable to record "blue" responses (Reuter and Virtanen, 1976).

The results presented in this study clearly suggest that the pathway through the neuropil of Bellonci is of special importance for the frog's discrimination between blue and long-wavelength light: spectral thresholds measured against identical backgrounds showed that the blue-sensitive input to the neuropil of Bellonci is much stronger than that to the tectal layers where the class 2 and 3 fibres arborize, and our paper colourimetry experiments demonstrated that only the Bellonci fibres detect coloured paper patches moving against a background which stimulates the red receptors equally but the blue receptors differently compared with the moving patch.

Possible differences due to type of preparation

These findings can be interpreted as a warning not to consider multireceptor contributions and opponent-colour responses observed against strong chromatically selective background illuminations as clear indications of biologically relevant colour-coding capacities.

A difference in background illumination can, however, hardly furnish a satisfactory explanation for the variance of the present results with those of Reuter and Virtanen (1972): in this study we found it surprisingly difficult to isolate the blue-sensitive mechanism in tectal recordings in spite of strong orange backgrounds. The isolated eye and whole frog preparations might differ in ways which could contribute to the lack of blue-sensitivity in the latter. Control experiments indicated that the drugs used to paralyze the "intact" frogs do not abolish the blue-sensitivity in isolated eyes. One possibility is that the blue input to class 2 and 3 cells in the retina of an intact frog is suppressed by a tonic efferent input to the eye. It is of course also possible that recordings from units at the retinal surface and recordings from axonal arborizations in tectum produce different sampling biases favouring different cell subtypes. Neither can we exclude that a significant portion of the class 2 and 3 units in the retina project to non-tectal brain nuclei.

Integration of spatial and chromatic information

The results presented in this study indicate that, in the frog, the system carrying chromatic information originates as a channel separate from the tectal pathway providing the main information needed for the frog's reactions to moving objects. The tectal pathway does not, however, carry all the spatial information: Ingle (1977) has found that frogs, after ablation of optic tectum, successfully orient in relation to stationary structures in the environment. He

attributes this behaviour to function of the retino-thalamic projection. This projection may thus carry both the chromatic and the spatial information needed for the frog's positive phototaxis towards blue.

Response properties of Bellonci units in relation to colour constancy

In this study we have confirmed earlier observations regarding the colour-specific responses of Bellonci On-fibres, and extended them by showing that the long blue-specific discharges (lasting 0.5–2 s after a border movement) are produced not only by absolute increases in blue stimulation but also by *relative* increases. Thus these cells show a specific response to increased blueishness irrespective of the absolute stimulus changes involved. They resemble the yellow-blueness comparators described in the monkey cortex and postulated as parts of a colour processing system showing colour constancy (Livingston and Hubel, 1984; see also the colour space with transformed axes described by Land, 1983).

Colour constancy could be loosely characterized as the capacity to recognize the colour of an object in a natural environment irrespective of changes in the spectral composition of the light source. At least some anuran amphibians show colour constancy in their behavioural responses (Gnyubkin et al., 1975) and they may be appropriate research objects for clarifying the mechanisms of this capacity. In this study we have deliberately used non-saturated surface colours like those to which the animals respond in nature (Kondrashev et al., 1976) and in the behavioural colour constancy experiments referred to above.

A qualitative model describing the response characteristics of Bellonci On-units

The model in Fig. 8 is an extension of a scheme presented by Kicliter et al. (1981). It reproduces some of the response characteristics shown by the receptive field centre of a Bellonci On-fibre. When the light is turned on both blue (B) and red (R) receptors cause depolarizations (+) and spike responses in the summating ganglion cell (G). These signals are rectified before entering the ganglion cell. Due to this rectification negative signals do not reach the ganglion cell, i.e. an increase in blue stimulation cannot compensate for a decrease in red stimulation, nor vice versa. Such a mutual annihilation is obviously also prevented by the different signal kinetics produced by the two filters (Fl and F2) inserted between the receptors and the ganglion cell.

In this model red receptor stimulation further affects signal transmission from the blue receptors through an antagonistic (—) contact close to the blue receptor, with the result that the extended blue-driven discharges are counteracted by a simultaneous increase in red stimulation. As this contact is not rectified a stimulus decrease selectively affecting the red receptor produces, indirectly and via the blue channel, a response indistinguishable from the response produced by a selective increase in blue stimulation. An important characteristic of the model is that this summation of non-rectified signals which could represent the activity of a typical colour-opponent cell, precedes the filters finally determining the response kinetics.

From colour opponency to colour specificity

In the receptive field centre of a Bellonci unit both red and blue receptors produce discharges at stimulus On, and the Off surround (not included in Fig. 8) is also driven by both receptor types. Thus these cells differ from classical simple and double colour-opponent cells (Daw, 1968), although it is obvious that their colour-discriminating capacity is based on colour-opponent processes in the retina. Because of this Kicliter et al. (1981) say that they possess indirect or gated spectral opponency. We may call the long and rhythmic responses of these cells colour-specific and suppose that they represent a colour-processing stage succeeding the colour-opponent level.

Retinal origins of blue-red opponency and strong blue inputs

The model in Fig. 8 does not represent an anatomical circuit. It correlates, however, with some published observations. Brown and Flaming (1977) observed that already the blue receptors (the green rods) in the toad retina are antagonized (depolarized) by a selective illumination of red-sensitive cones, and Witkovsky and Stone (1983) have described blue-red opponent-colour organized bipolar cells in the retina of the clawed frog *(Xenopus).*

What is the difference between the strong blue-sensitive input to the On ganglion cells projecting to
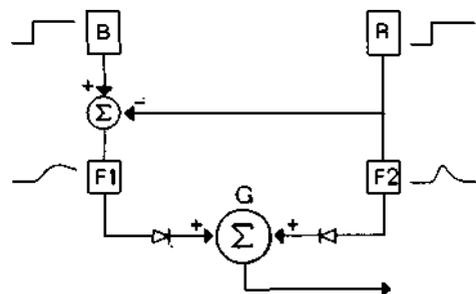
Fig. 8. Qualitative network model to account for the described response characteristics of the receptive field centre of a Bellonci On-unit. The On ganglion cell (G) sending its axon to the neuropil of Bellonci receives rectified inputs from blue- (B) and red-sensitive (R) receptors. The curves associated with the electronic filters Fl and F2 show how these filters distort the responses to step stimulations of B and R respectively. R is subtractively connected with the blue-sensitive channel at a point preceding Fl and F2, and thus R-signals depress B-signals when both receptor types are simultaneously stimulated. Further a relative *decrease* in the stimulation of R, combined with sustained B-stimulation, produces a response indistinguishable from the response to increased B-stimulation.

the neuropil of Bellonci and the weaker blue inputs to other ganglion cell classes? It is known that the excitatory receptive fields (ERFs) of all tectally projecting ganglion cells are surrounded by an antagonistic responding surround, the activity of which is apparent only under special stimulus conditions (Morrison, 1975; Donner and Gronholm, 1984). In this study we have described an Off periphery surrounding the ERFs of Bellonci On-fibres. This periphery is clearly homologous to the antagonistic responding surrounds of other ganglion cell classes.

Most interestingly Donner and Grönholm (1984) found that the blue input to all tectally projecting ganglion cells constitute a part of the antagonistic responding surround mechanism of these cells, not of their ERF proper. The blue input to the Bellonci fibres, however, contribute to On responses in the centre of the receptive field and to Off in the periphery; thus it seems to contribute both to the ERF proper and to the antagonistic surround of these cells.

We do not know the anatomical circuits driving the ERFs and antagonistic responding surrounds of frog ganglion cells but the above observations clearly suggest that the blue input is organized differently in thalamically and tectally projecting ganglion cells.

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