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SPECTRAL PROPERTIES OF AMACRINE CELLS IN TWO DICHROMATIC TELEOSTS

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Bao-Quey Huang and Mustafa B. A. Djamgoz (1990) Spectral properties of amacrine cells in two dichromatic teleosts. *Bull. Inst. Zool., Academia Sinica* **29**(1): 1–9. The purpose of the current study was to characterize the color function of the perch and cod retinas. The amacrine cells, an interneuron of both species, were evoked and tested their responses to different wavelengths of light. Intracellular recording on amacrine cells was performed. There are two types of responses of amacrine cells: sustained depolarizing and on-off transient, were observed. The results also showed the higher sensitivity at the red and green end of the spectrum in these two species respectively. The on-off transient response of cod amacrine cells suggested that they are likely to be the only colour coding interneurones in their retinal level. The most sensitive spectrum of perch amacrine cells is 400 nm. These results indicated that the amacrine cells of these two dichromatic teleosts may relate to the integration of color function.

Key words: Dichromates, Spectral sensitivity, Amacrine cells, Colour coding.

Spectral sensitivities are well known to be closely correlated to the natural habitats and some behavioural patterns in teleosts. Vertebrate retina can convey different spectral information from the photoreceptors *via* bipolar and ganglion cells, *via* the optic nerve to the central nervous system. Amacrine cells, as the final order interneurones in the retina, could function in visual processing and integration located in the inner nuclear layer (Djamgoz *et al.*, 1985a).

The microspectrophotometric (MSP) data demonstrated that both cod (*Gadus morhua*) and perch (*Perca fluviatilis*) have two spectrally distinct cones: green and blue sensitive (Huang, 1986) and red and green sensitive (Loew and Lythgoe, 1978). The dichromatic characteristics are thought to be unusual in teleosts and give the fish spectral discrimination over a narrower range. Both cod and perch have the same potential of dichromatic colour visions, but with wavelength discrimination over a different spectral band.

In terms of histological and physiological aspects, amacrine cells are generally considered as second order interneurones next to horizontal cells. If a microelectrode is penetrated from the photoreceptor side to the vitreous side, the responses of amacrine cells will appear after the S-potentials of horizontal cells. Therefore, amacrine cells may mediate signals between bipolar and ganglion cells. In this regard, Djamgoz *et al.* (1985a) had classified the respones of amacrine cell in roaches into three categories which are: (1) sustained depolarizing, (2) sustained hyperpolarizing and (3) on-off transient.

In order to investigate the characteristics of the spectral sensitivities of the dichromatic teleosts, the responses of the amacrine cell to different wavelength of stimuli were performed on both cod and perch. The result showed that the amacrine cell may have some functions on the color integration in the retinal level of these two dichromates.

MATERIALS AND METHODS

The present experiments were carried out on isolated retinae of Atlantic cod (*Gadus morhua*) and perch (*Perca fluviatilis*). Recordings were made from the amacrine cells.

A fully dark-adapted fish placed in a well aerated tank, was sacrificed and its retina peeled off. The isolated retina with the adhering vitreous body was placed with its receptor side upward in a transparent recording chamber. The isolated retina was surrounded by a ring of moist tissue paper and supplied with moist oxygen. Such a preparation could remain responsive to light for several hours. During the electrophysiological recordings, the retina was maintained in laboratory with an illumination level at about the cone threshold for the experimenters (Huang, 1986).

Micropipettes were drawn from 1 mm o. d. borosilicate glass with an internal

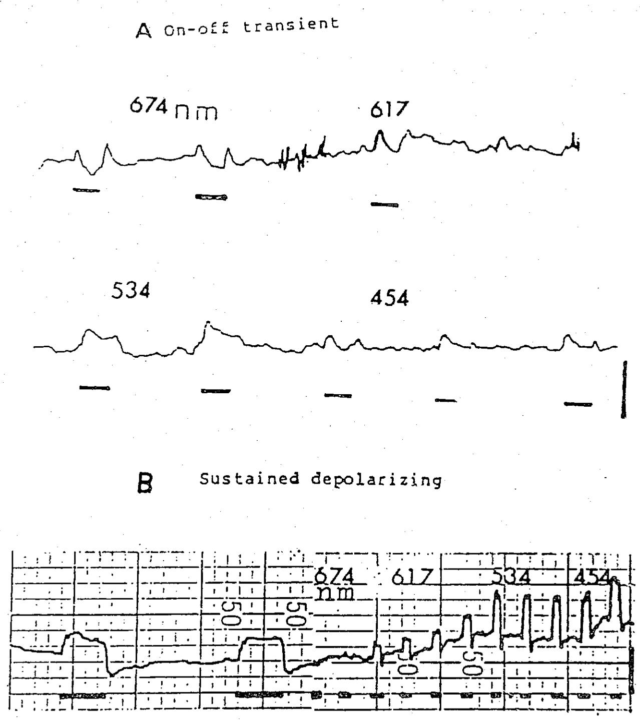

Fig. 1. A. Cod amacrine cell responses showing "on-off" transient responses to red (674 nm), orange (617 nm), green (534 nm) and blue (454 nm) light. Note hyperpolarization after "on" response to green and blue light.

B. Cod amacrine cell showing sustained depolarizations response. The first two faster sweeps were evoked by 617 nm flash to show the sustained depolarizing type.

Calibration: (A) 5 mV, (B) 15 mV. The horizontal bars under each response show the 200 ms duration of the test flash with intensity (I), log I=0, i. e. full intensity.

fibre on a modified Livingstone-type puller. They were filled with 2.5 M KCl and had tip resistance within the range of 50–80 MΩ. Due to the layered structure of the retinal neurones, it was possible to estimate the types of the recorded cells by the positions of the electrode tips (Downing, 1983).

The stimulation light used to identify and classify responses were derived from an optical system served by a 250 W tungsten-halogen lamp connected to a d. c. power supply. A light spot of about 0.5 mm diameter was focused on the retina from underneath and was flashed with an "on" duration of 200 ms during recording. The intensity and wavelength was changed by inserting different interference filters (Huang and Djamgoz, 1988).

RESULTS

Two types of the light-evoked amacrine responses were recorded from cod retina in the present experiments: (1) on-off transient responses, and (2) sustained depolarizing responses (Figs. 1A and 1B). 71% (5 / 7) of the recorded responses were on-off transient type. The "input-output" relationship between stimulus intensity and response amplitude was not a gradually rising relationship. Figs. 2A and 2B showed that these "input-output" relationships of the on and off responses of the on-off transient amacrine cells. The results indicated that the different intensities did not evoke the responses significantly different, particularly in the off responses (Fig. 2B) and also proved the higher

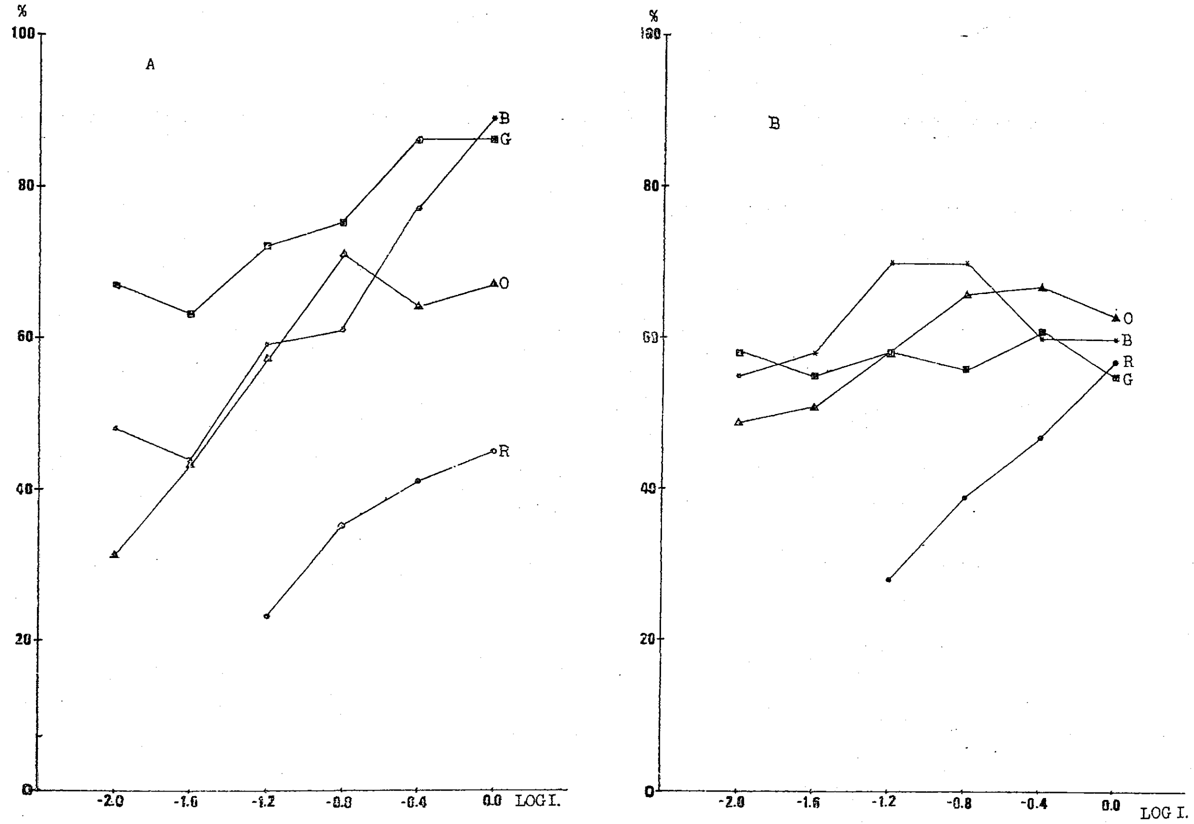

Fig. 2. V-log I relationships for the on (A) and off (B) responses obtained from three on-off transient cod amacrine cells responding to four different chromatic lights (R: 674 nm, O: 617 nm, G: 534 nm and B: 454 nm).

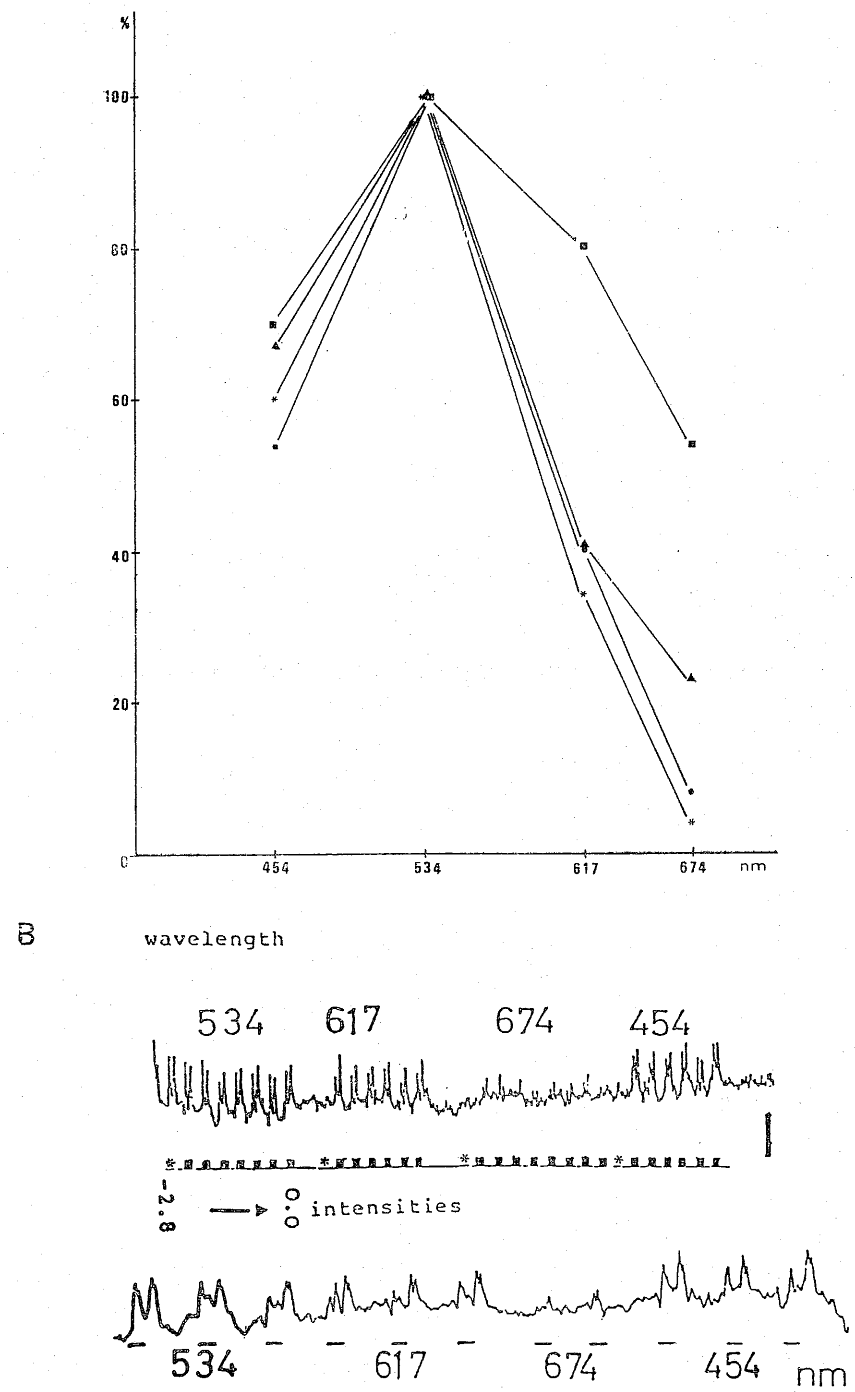

Fig. 3. A. Four sets of recordings obtained from cod transient amacrine cells (on-response) to show the higher sensitivity to green light (Normalized the maximal amplitude as 100%).
B. One set of recordings evoked by eight graded intensities (log I=−2.8 to 0.0, 0.4 steps) of four wavelengths from cod amacrine cell (upper). From the response to different wavelengths (log I=0.0, lower) recorded by faster sweep showed the neurone is an on-off transient unit. The vertical bar is 5 mV and the stimulus light duration (marked under each response) is 200 ms. *: log I=−2.8.

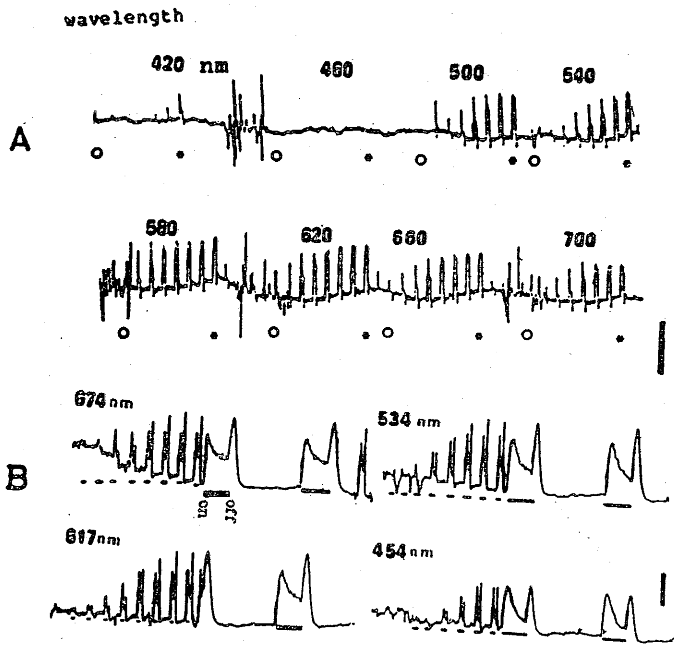

Fig. 4. Perch amacrine cell.
A. One representative recording of depolarizing-type cell. To each stimulus wavelength (420–700 nm, 40 nm steps), eight intensities (o: log I=−2.8 to *: log I=0.0, log I=0.4 steps) were continued applied with the same 200 ms duration.
B. The on-off transient responses do not show different waveforms to different wavelengths. (on-off duration: 200 ms under each response). One or two faster sweeps after each recording were to show the on and off transient response.
Calibration: 10 mV.

sensitivity to the green light in both on and off responses (Figs. 2A and 2B respectively).

Considering spectral sensitivity, cod transient amacrine cell response was shown greater sensitivity to green flash stimuli than to deep red stimuli (Fig. 3A), as was the case for the S-potential, although the waveforms (Fig. 3B) did not change to light of different colours. One of the recorded "on-off" transient amacrine cells (n=5) respond the cod retina demonstrated colour coding, and these components were hyperpolarizing to long wavelength and depolarizing to short wavelength (Fig. 1A). It is likely that this reflects different spectral adaptations of the retina. In other words, the blue-green sensitive retina of the cod has a depolarization sustained component at the blue-green end of the spectrum.

There were also two types of amacrine cell light-evoked responses found in perch retina: (1) sustained depolarizing and (2) on-off transient (Figs. 4A and 4B, respectively). Most of the recording cells (15/17) belonged to the latter type. Fig. 4A represented an example of the depolarizing sustained response of one cell. From the tracing of Fig. 4A, the most sensitive spectrum was in the range of 580–620 nm. In addition, the response to 420 nm had greater amplitude than those to 460 nm. The results were not sufficient to judge perch retina had another sensitive peak at the shorter spectrum of 420 nm.

Fig. 5 showed the relationship (V–LOG. I) between response amplitude (V) and stimulus intensity (LOG. I). Both

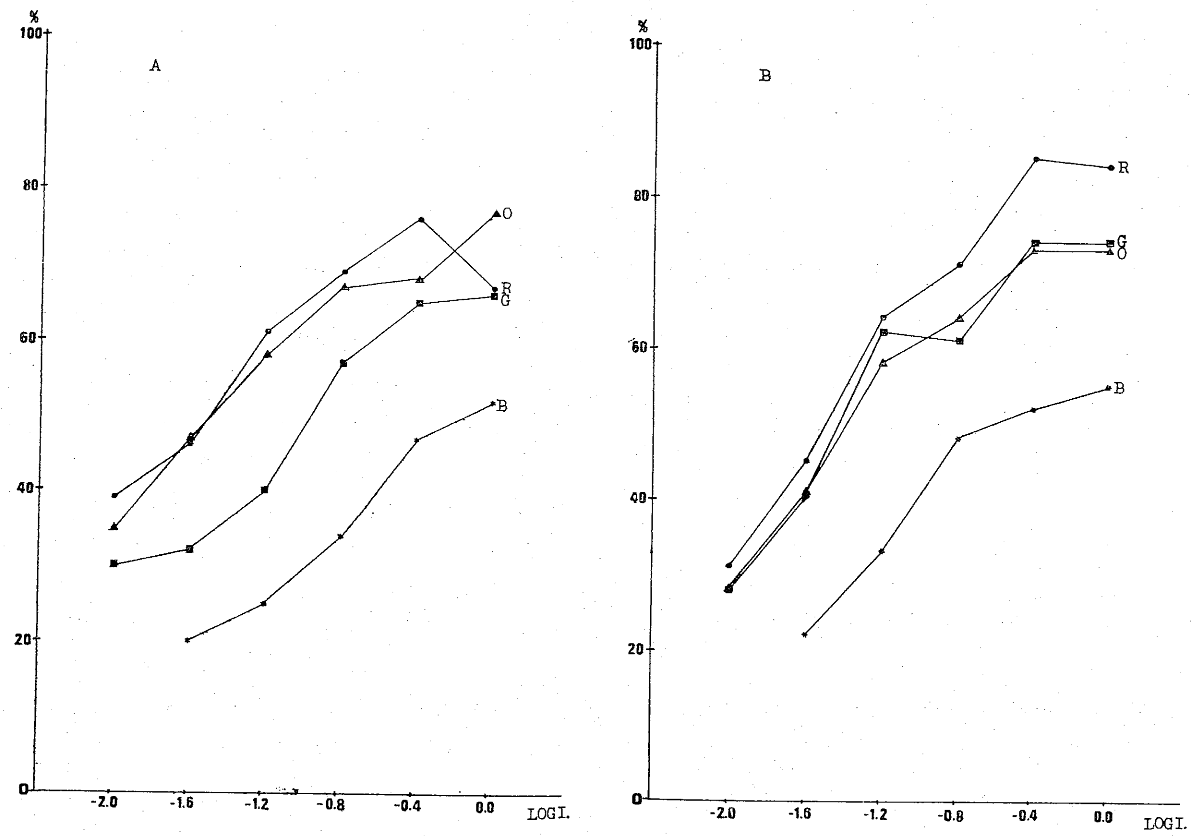

Fig. 5. V-log I relationships for the on (A) and off (B) responses obtained from six on-off transient perch amacrine cells responding to four different chromatic lights (R: 674 nm, O: 617 nm, G: 534 nm and B: 454 nm.)

the on (Fig. 5A) and off (Fig. 5B) responses of the transient type amacrine cells presented a similar tendency with the larger response amplitude to the stimulus of the longer wavelength. It was demonstrated a higher sensitivity to the red light in perch amacrine cells.

DISCUSSION

The spectral sensitivity at various surveys, recorded in different levels of visual system, and the visual behaviours, showed some disparities in some studied teleosts (Guthrie, 1983; Cameron 1982). From the results of the MSP studies, cod and perch were proved to be dichromatic: twin cones with λ_{max}=615 nm and 517 nm, single cones λ_{max}=535 nm and 446 nm, respectively (Loew and Lythgoe, 1978; Huang, 1986). From electrophysiological studies, the photoreceptor responses of both cod and perch retinas were reasonably compatible well with the results from visual pigments measured from MSP survey; i. e. systems are biased to their own dominant photoreceptors (twin cones) spectral sensitivity (Huang and Djamgoz, 1989), which indicated a twin cones dominate retina in these two dichromates.

The axonless amacrine cells form the second layer of the laterally organized interneurones. They function in visual processing and integration located in the inner nuclear layer with synaptic connections distributing in the inner plexiform layer (Kaneko, 1970; Werblin, 1974;

Djamgoz, 1978; Downing, 1983; Djamgoz *et al.*, 1985a). In the present study, it is clearly shown that the amacrine cells of both cod and perch have reasonably similar spectral sensitivities to the ERGs and the horizontal cell potentials (Huang, 1986; Huang and Djamgoz, 1988, 1989).

Both species of cod and perch had sustained depolarizing and "on-off" transient responses. Djamgoz *et al.* (1985a) found that three electrophysiological types of amacrine cells may be a homogeneous group of neurones, and that the different types of responeses may represent differences in synaptic connection, receptive field organization and functional specialization rather than any fundamental difference in their membrane properties. In addition, Djamgoz and Wagner (1987) advanced the view that such a subdivision of amacrine cell responses would be somewhat analogous to the functional classifications of horizontal cells. The present results were not sufficient to understand all of the functional significance of these electrophysiological characteristics, but showed some properties analogous to those of goldfish and roach (Kaneko, 1970; Djamgoz *et al.*, 1985a). These properties are:

(1) Spectral-dependent response

The transient response which is the main characteristic of amacrine cells, shows more sensitivity to longer wavelength stimuli in perch and to shorter wavelength stimuli in cod. The sustained components have also been regarded to encode chromatic information similarly in all units (Djamgoz *et al.*, 1985b). The present experiments did not obtain enough data from cod retina to prove it. The recording from perch amacrine cells did demonstrate the higher sensitivity to the longer wavelengths.

(2) Narrow operating ranges

The responses recorded from roach amacrine cells had been found to have sharply rising V-log I curve (I: intensity), and the operating ranges of transient components are less than 2 log units (Djamgoz, 1978; Djamgoz *et al.*, 1985a). The present results showed the similar property. In addition, their operating ranges (stimulus-response) were not as wide as S-potentials (Huang and Djamgoz, 1988) of their own horizontal cells. These on-off transient depolarizations appear to be driven by a high gain mechanism enabling them to have a certain level of illumination threshold (Djamgoz *et al.*, 1985a). In the cyprinid retina, considering colour coding of the sustained component of the "on-off" transient amacrine cells was found that 15% of these "on-off" transient amacrine cells depolarized and hyperpolarized to long and to short wavelengths respectively during their sustained components (Djamgoz *et al.*, 1985a). The responses of the transient amacrine cells of both species were also shown these characteristics, but the number of cells obtained were not sufficient to analyze the exact percentage.

(3) Rapid adaptation

The light stimuli are brighter than those necessary to evoke a saturation response would obtain a less maximal response amplitude. In addition, the background illumination would cause a slight membrane shift and suppress the response but the membrane potentials are able to recover very quickly (Djamgoz, 1978). These findings indicate that the transient responses of amacrine cells of both species might be capable of adapting rapidly as in roach.

(4) Colour coding

In the perch amacrine cells, a depolarizing response was more sensitive to

460 nm. In fact, the relative radiance of 420 nm and 460 nm are 0.117 and 0.271 (Huang, 1986), so the sensitivity difference between them must be even greater than those of the recordings. It is worth noting that the spectral sensitivity of photoreceptors measured by microspectrophotometry and by extracellular mass potentials or the S-potential measured by electrophysiological intracellular recordings did not show that perch retina had any spectral sensitivity peak at 420 nm. On the contrary, the behavioural studies showed a high sensitivity around 400 nm (Cameron, 1982; Guthrie, 1983) as the present results from amacrine cells. In cod transient amacrine cells, there was an interesting recording indicating that the sustained component was depolarizing to short and hyperpolarizing to long wavelength light stimuli. These results, in terms of colour coding, were analogous to the recordings from the sustained component of the roach transient amacrine cells. Similar colour-coded sustained components were also observed in the goldfish retina (Djamgoz *et al.*, 1985a). Since it has not been found any colour-coding S-potentials from cod horizontal cells, it seems that the amacrine cells in the cod retina may be the only colour coding interneurones.

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REFERENCES

Cameron, N. E. (1982) The photopic spectral sensitivity of a dichromatic teleost fish (*Perca fluviatilis*). *Vision Res.*, **22**: 1341-1348.

Djamgoz, M. B. A. (1978) Electrophysiological, biochemical and anatomical studies of the vertebrate (fish) retina. Ph. D. Thesis, University of London.

Djamgoz, M. B. A. and H. J. Wagner (1987) Intracellular staining of retinal neurones: Applications to studies of functional organization. In: Progress in Retinal Research. Vol. 6 p. 85-150. Pergamon Press, Oxford.

Djamgoz, M. B. A., J. E. G. Downing and H. J. Wagner (1985a) The cellular origin of an unusual type of S-potential: an intracellular horseradish peroxidase study in a cyprinid fish retina. *J. Neurocytol.*, **14**: 469-486.

Djamgoz, M. B. A., J. E. G. Downing, D. J. Prince and H. J. Wagner (1985b) Functional organization of amacrine cells in the teleost fish retina. In: Neurocircuitry of the Retina, Cajal Memorial (A. Gallego and P. Gouras, eds) Elsevier, New York.

Downing J. E. G. (1983) Functionally identified interneurones in the vertebrate (fish) retina: Electrophysiological, ultrastructural and pharmacological studies. Ph. D. Thesis, University of London.

Guthrie, D. M. (1983) Visual centre processes in fish behaviour. In J. P. Ewert, R. Capranica and D. J. Ingle (ed.) Advances in Vertebrate Neuroethology. Plenum Press., pp. 381-412.

Huang, B. Q. (1986) Visually evoked startle responses in the cod (*Gadus morhua*) Ph. D. thesis, University of Aberdeen U. K.

Huang, B. Q. and M. B. A. Djamgoz (1988) Spectral characteristic of S-potentials: An intracellular horseradish peroxidase study in perch (*Perca fluviatilis*). *Bull. Inst. Zool., Academia Sinica*, **27**: 183-193.

Huang, B. Q. and M. B. A. Djamgoz (1989) Spectral properties of electroretinograms in two dichromatic teleosts. *Bull. Inst. Zool., Academia Sinica* **28**: 41-48.

Kaneko, A. (1970) Physiological and morphological identification of horizontal, bipolar and amacrine cells in goldfish retina. *J. physiol.*, **207**: 623-633.

Loew, E. R. and J. N. Lythgoe (1978) The ecology of cone pigments in teleost fishes *Vision Res.*, **18**: 715-722.

Werblin, F. S. (1974) Control of retinal sensitivity. II. Lateral interactions at the outer plexiform layer. *J. General physiol.* **63**: 62-87.

變色視硬骨魚網膜之 Amacrine 細胞之色覺特徵

黃寶貴　　M. B. A. DJAMGOZ

為探討雙色視 (dichromatic) 鱸、鰐魚網膜之色覺功能，本實驗對網膜之最後層次的中間神經元 (即 Amacrine 細胞) 進行分離網膜之胞內記錄，結果顯示該兩魚種之 Amacrine 細胞對不同波長刺激均呈現兩類反應：(1) 持續性去極化 (Sustained depolarizing) 反應或 (2) on-off 瞬間 (transient) 反應，兩類反應均分別對紅光 (鱸) 及綠光 (鰐) 較為敏感。鰐魚網膜上之 Amacrine 細胞其 on-off 瞬間反應已證實具有明顯之色覺功能。鱸魚之 Amacrine 細胞內記錄亦證實具有除紅綠敏感波長外之敏感波長是 $\lambda_{max}=400$ nm；綜合上述結果顯示這兩種雙色視之硬骨魚之 Amacrine 細胞可能均具有較高階層之色覺整合功能。