Spectral distribution of the negative potential in the cichlid electroretinogram

D.M. Bell

Department of Biological and Allied Health Sciences, Fairleigh Dickinson University, Madison (New Jersey 07940, USA), 16 March 1981

Summary. A negative potential occurring in the ERG of certain cichlid fishes shows a unique set of properties when evoked by monochromatic light. Spectral distributions determined for *Cichlasoma octofasciatum* and *Hemichromis bimaculatus* show a blue (460–478 nm) maximum, no Purkinje shift upon light adaptation, and secondary peaks correlated with the species' behavioral preferences in reproduction and parental care.

Bell[1] recently reported the occurrence of a negative potential in the local electroretinogram of the cichlid fish *Cichlasoma octofasciatum*. This 'cichlid negative response' (CNR) has also been seen in other cichlid ERG'S by the author, but not in other families of teleosts commonly used for visual analysis, i.e., the cyprinids and centrarchids. The CNR elicited by white (tungsten) light shows an occurrence dependent upon stimulus duration, dominating the ERG at durations longer than 70 msec. At shorter stimuli, the positive b-wave dominates to generate the classical ERG waveform. At about 70 msec duration, the CNR and b-wave appear to be bucking each other, resulting in a composite waveform with little positive or negative deflection from baseline.

The CNR shows another interesting and non-paradigmatic property - its spectral response curve differs greatly from curves described for other ERG waveforms, and also shows spectral variation between 2 species of the family Cichlidae. These variations appear correlated with the nuptial coloration and behavioral preferences of the species investigated.

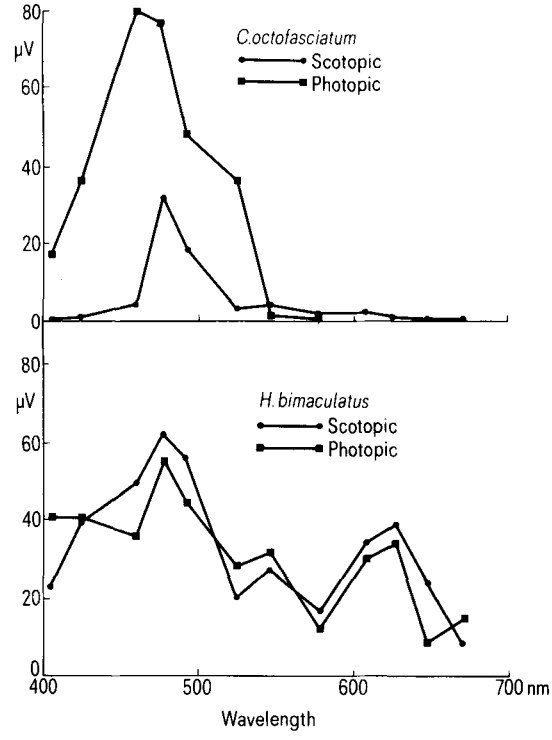
CNR'S were recorded from 2 cichlid species, *Cichlasoma octofasciatum* and *Hemichromis bimaculatus*. The former cichlid shows blue-black nuptial colors, while the latter assumes a brilliant red coloration during courtship and spawning. ERG'S were recorded as described in the first report of the CNR, using salt-agar wick electrodes apposed to the cornea and trunk. Unanesthetized fish were strapped to a styrofoam block with a moist gauze wrapping. CNR magnitudes were measured from the baseline of the persistent CRT trace. Light stimuli were generated by a B & L grating monochromator, collimated, adjusted to equal energy with a neutral density wedge, and focused at the pupillary area of the eye. Photopic preparations received a stimulus energy of 0.4 μV and scotopic preparations a stimulus energy of 40 μV (as measured with an Eppley thermopile and Keithley microvoltmeter).

The mean CNR amplitudes and standard deviations for 10 preparations of each species are charted in the table. The spectral curves generated from these responses are shown in the figure. Several features of the spectral distribution of the CNR are immediately evident. Both species show maximum average amplitudes near 478 nm, and this peak does not shift appreciable with adaptation state. The curves for *Cichlasoma* are monophasic, showing little response past 525 nm in this experimental protocol. *Hemichromis* has triphasic curves, with inflections at 478, 546, and 623 nm in both adaptation states. The response of *Cichlasoma* increases 2-fold upon light adaptation; the responses of *Hemichromis* are of similar amplitudes despite the change from dark to light-adapted states.

In interpreting these spectral responses, 2 conditions must be kept in mind. The curves represented by the joined data points are response curves, wherein variable responses are generated by exposure to equal energy stimuli. Due to the higher energy per quantum of the shorter wavelengths, the blue maxima are conservatively represented. Action spectra, the number of quanta necessary to elicit a criterion response, have not yet been completed, due to the greater number of determinations needed for regression analysis. 2nd, the absence of CNR occurrence past 525 nm in *Cichlasoma* in this experimental protocol does not mean that the capability for producing this potential is not present; CNR's in the red wavelengths have been seen by

Means and standard deviations for CNR's for *C. octofasciatum* and *H. bimaculatus* at selected wavelengths after dark (scotopic) and light (photopic) adaptation

	C. octofasciatum		*H. bimaculatus*	
	Scotopic	Photopic	Scotopic	Photopic
405	0.0(0.0)	16.7(36.1)	22.5(16.6)	41.3(9.5)
426	1.7(4.1)	35.0(76.1)	38.8(30.1)	38.9(19.3)
460	4.2(6.6)	80.0(62.5)	48.8(14.4)	35.0(12.9)
478	32.5(25.8)	75.8(86.7)	62.5(10.4)	55.0(31.1)
492	18.3(22.1)	48.3(50.1)	56.3(37.7)	45.0(14.7)
525	3.3(8.2)	35.8(59.9)	20.0(26.1)	27.5(18.5)
546	4.2(6.7)	3.3(6.1)	27.5(42.7)	31.3(12.5)
579	2.5(4.2)	0.0(0.0)	16.3(19.7)	13.8(17.0)
607	2.5(6.1)	0.0(0.0)	33.8(40.3)	30.0(31.9)
623	1.7(4.1)	0.0(0.0)	38.8(29.0)	33.8(22.9)
646	0.8(2.0)	0.0(0.0)	23.8(29.3)	8.75(8.5)
670	0.0(0.0)	0.0(0.0)	8.8(17.5)	15.0(12.9)



Spectral response curves for *C. octofasciatum* and *H. bimaculatus* after scotopic and photopic adaptation.

this author a higher stimulus intensities. This implies that the CNR is present across the visible spectrum in both species tested, but differs in the relative ease of elicitation (and presumably in relative visual significance).

Despite these precautions in interpretation, these spectral response curves are sufficiently different from paradigmatic expectations to be considered significant variations (and elaborations) of the basic vertebrate visual response patterns.

a) Neither set of curves shows a Purkinje shift upon light adaptation, a property common to most retinal potentials. This implies that the CNR does not originate from the proximal retina, but from more distal cell layers.

b) The monophasic *Cichlasoma* peak and the triphasic *Hemichromis* peaks correspond with known or suspected areas of vertebrate cone activity. They match well with cyanopsin, iodopsin, and Dartnall's[2] pigment 467.

c) The latency of the CNR (circa 80 msec) is longer than that of the b-wave and the proximal negative response. Its origin by this criterion would be expected to be in the more distal retina, possibly involving interactions at the amacrine cell layer. The occasional appearance of spike bursts (2–4 spikes) at the peak of the response supports this inference, since only amacrine and ganglion cells spike.

d) The spectral responses correspond well with the expression of nuptial colors in the species tested, although the *Hemichromis* red sensitivity is not monophasic. The persistence of the blue peak in *Hemichromis* may indicate that the blue sensitivity via this response is common to the cichlid family, and that the enhancement of other spectral sensitivities might be a more limited phenomenon.

The occurrence and spectral distributions of the CNR in these cichlid species, coupled with their well-documented dependence upon color cues in territorial and reproductive behaviors (Baerends and Baerends van Roon)[3], demonstrate an additional information processing and wavelength discrimination capability in the vertebrate retina. The behaviors of these fishes also indicate that these sources of retinal information may be primary determinants of visual orientation responses critical to the maintenance of the species.

1 D.M. Bell, Experientia *35*, 342 (1979).
2 H.J. Dartnall, J. Physiol. *116*, 257 (1952).
3 G.P. Baerends and J.M. Baerends Van-Roon, Behavior, suppl. 1 (1950).

Does tenotomy of skeletal muscle alter Z-line width in older animals?[1]

Dorothy Chase, W.C. Ullrick, K.A. Dasse, D.J. Burke and A.H. Goldberg

Boston University School of Medicine, 80 East Concord Street, Boston (MA 02118, USA), 27 April 1981

Summary. Results of unilateral achilles tenotomy on older (350–410 g) male rats, showed that the general width of Z-lines in the tenotomized muscles was not significantly altered by this procedure. Streaming of the Z-lines and rod formations was still present, as in younger rats.

A variety of pathological conditions[2,3] as well as tenotomy[4,5] are known to alter the morphology of vertebrate skeletal muscle Z-lines. The changes more frequently cited are Z-line streaming and rod formation. A few years ago this laboratory reported that tenotomy caused a generalized widening (+15%) in all the Z-lines of the affected muscles, a study done in young (150–300 g) growing rats[6].

It has been suggested that the Z-line changes which occur in tenotomy represent a proliferation of Z-material as a step in the development of new sarcomeres[7–9], presumably in response to a sudden loss of tension in the muscle. The question immediately arises then: would the Z-line changes still be present if the tenotomy procedure was carried out in older animals with very slow or zero growth rates? The following study was designed to answer this question.

Materials and methods. Achilles tenotomy, including removal of a 2–3 mm segment of tendon, was performed on 1 leg of 350–410-g male albino rats, with the unoperated leg serving as the control. The animals were sacrificed by decapitation 1, 2, and 3 weeks after tenotomy, and the soleus muscles excised and placed in ice cold buffer (0.1 M KCl, 1 mM $MgCl_2$, 5 mM EGTA, 5 mM sodium pyrophosphate, pH 6.8).

Under the dissecting microscope, fibers were dissected free, tied to 3-cm fragments of wooden applicator sticks at approximately rest length, and then placed at 4 °C in 4% glutaraldehyde in buffer (7.5×10^{-2} M KCl, 7.5×10^{-4} M $MgCl_2$, 7.5×10^{-3} M Na_2HPO_4, 7.5 ± 10^{-3} M KH_2PO_4, pH 7.0). The samples were then post-fixed in 1% OsO_4 for 1 h, dehydrated in a graded series of ethanol and embedded in a mixture of araldite 502 and dodecenyl succinic anhydride. Longitudinal thin sections were cut at 60–70 nm on a Porter-Blum MT 2 microtome, and mounted on 200-mesh copper grids. The sections were then stained with 1% PTA, 10% uranyl acetate, and Reynold's lead citrate and examined with a Siemens Elmiskop I electron microscope.

Effect of tenotomy on soleus muscle Z-line, A-band width, and sarcomere length in older rats

Paired muscles*	Duration of tenotomy (weeks)	Sarcomere length (μm)**	A-band width (μm)**	Z-band width (nm)**
C^{68}	1	2.33	1.13	100.58
E^{68}	1	2.03	1.22	102.92
C^{69}	1	1.77	1.12	109.94
E^{69}	1	1.80	1.13	107.60
C^{72}	2	1.98	1.08	91.23
E^{72}	2	1.69	1.30	102.14
C^{73}	2	1.93	1.05	115.40
E^{73}	2	1.61	1.15	110.72
C^{74}	3	1.99	1.17	128.65
E^{74}	3	1.82	1.07	102.14
C^{75}	3	1.96	1.10	106.82
E^{75}	3	1.75	1.10	107.60
Mean difference		0.21	0.05	3.25
SD of mean difference		0.13	0.10	12.58
p-value, paired comp.		< 0.05	> 0.05	> 0.05

* Specimens labelled 'C' represent control muscle; 'E' contralateral tenotomized muscle. ** Values represent means of measurement within each muscle.