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THE D–WAVE OF THE ELECTRORETINOGRAM OF PERCH ORIGINATES IN THE CONE PATHWAY

Milena Milošević[1], A. Bajić[2], and **Z. Gačić[1]**. [1]*Center for Multidisciplinary Studies, University of Belgrade*, 11000 Belgrade, Serbia, [2]*Faculty of Biology, University of Belgrade*, 11000 Belgrade, Serbia.

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In response to the sufficiently long duration of photo stimuli during light offset, a positive potential called the d-wave of the electroretinogram (ERG) in cone-rich retinas (B r o w n, 1968). The d-wave is believed to be generated from cone-driven secondary retinal cells, such as the OFF bipolar cells (S t o c k t o n and S l a u g h t e r, 1989; N a a r e n d o r p and W i l l i a m s, 1999). It has been suggested that in zebrafish, during the transition from light to dark adaptation, the b-wave represents a function of both rod and cone systems (R e n and L i, 2004). The positive d-wave, on the other hand, represents mainly, if not exclusively, cone functions (A n d j u s, 2001; R e n and L i, 2004).

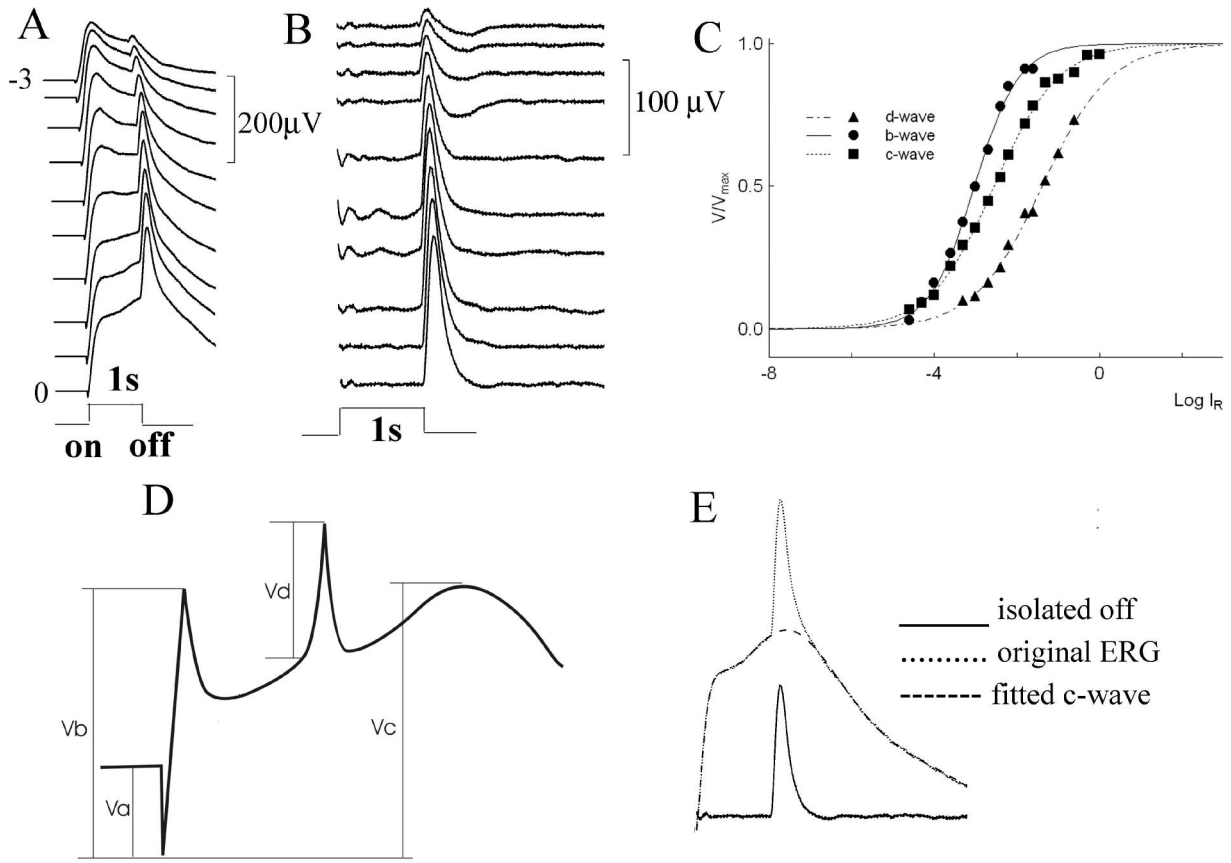

Fig. 1. Relationship of normalized amplitude of response V/Vmax and log intensity of stimulation. A: A series of ERGs obtained with incremental stimulation on the eye of perch. Intensity of stimulation was 282 µW/cm^2, duration 1 s. B: Isolated off – responses of previous series. C: Amplitude/intensity relations in the perch for b-wave (solid circles), c-wave (solid squares) and off-response(solid triangles). Fitting according to the basic model of N a k a and R u s h t o n (1966). D: Amplitudes of measured ERG components. Va: a-wave, measured from zero to a-wave minimum; Vb: b-wave, measured from minimum of a-wave to maximum of b-wave ("peak to peak"); Vc: c-wave measured "peak to peak"; Vd: d-wave, measured from breaking point of c-wave to maximum of d-wave.

In order to show that the d-wave could be an indicator of cone-dominated retinas, we performed experiments on perch (*Perca fluviatilis*). Animals were electrofished in the floodplain zone of the Danube River (kilometer 1136). The fish were kept in captivity for at least 15 days in order to acclimatize to the experimental conditions (dark with room controlled temperature of 15°C). Perch were anesthetized (phenobarbital sodium) and curarized (tubocurarine) following procedures recommended by H a m a s a k i *et al.* (1967), adjusting the dosage soas to induce respiratory arrest. Artificial respiration was provided continuously by forcing aerated and temperature-controlled water through the gills. The immobilized fish were positioned laterally on a plastic platform inside a light-proof Faraday cage. After removal of the cornea, lens and most of the vitreous, the *in situ* eyecup was filled with Ringer solution. Electroretinogram potentials were detected with non-polarizable silver chloride electrodes (Ag-$AgCl_2$, World Precision Instruments, Inc., model EP2), the active one being introduced into the interior of the saline filled eyecup. The reference electrode was in the retro-orbital space. The signal was conducted to a computer via a differential preamplifier and a PCI-20428W-1 AD-converter (8-bit; 125-Hz sampling rate). Photic stimuli were delivered by a single-beam optical system using an 8 V 50 W tungsten-halogen lamp as the light source, and providing independent control of intensity (neutral density filters) and duration (electromagnetic shutter, UniBlitz model T132) of the test flashes. Light intensities were calibrated and checked by placing the active surface of a custom-made radiometer probe in the position usually occupied by the eyecup preparation. When comparing intensity/amplitude relations in different preparations, relative intensity (IR) scales were used, plotting ERG amplitude voltage against attenuation extent in log units.

After 1 h of dark adaptation, ERGs were recorded. Figure 1A shows responses obtained with a 1-s (ts) "white" flash ranging in intensity from 0.282 μW/cm^2 (-3 log intensity units) to 282 μW/cm^2 (0 log intensity units). In this series, the c-wave is masked by the d-wave, and immeasurable directly from the ERG. In order to reconstruct the c-wave, we removed the samples at intervals of [ts, ts+1s] and fitted the resulting curve with Chebyshev rational functions of higher orders (ninth or tenth). The criteria for selection of the fitting function were slope of the b-wave and its amplitude. The amplitude of the c-wave was then measured from the fitted curve. A series of isolated off-responses (Fig. 1B) was obtained by subtracting the fitted curve from the original ERG response (method shown in Fig. 1E).

The stimulus intensity-amplitude relation was checked by fitting experimental data with the basic model: (N a k a and R u s h t o n, 1966), where V_0 is the normalized voltage (V/Vmax) of the ERG signal (Vb, Vc or Vd; method of measuring shown in Fig. 1D), I_0 is the stimulating light intensity corresponding to V_0= 1/2, and exponent a is constant (Fig. 1C). The slopes (parameter a values) of normalized log profiles were 0.8057 for the b-wave, 0.5288 for the off-response, and 0.5603 for the c-wave. The saturation level for the b-wave was reached at a relatively low stimulus intensity of 7 μW/cm^2 (-1.6 log intensity units, Fig. 1C). The saturation level for the c-wave was reached with 40 times higher stimuli than in the case of the b-wave, 282 μW/cm^2 (0 log intensity units, Fig. 1C). The saturation level of the d-wave was never reached, even when maximal intensity stimuli were applied as in cone-driven horizontal cells of eel retina (B y z o v *et al*., 1998). The obtained results are in accordance with the previous finding that the d-wave represents cone functions (A n d j u s, 2001; R e n and L i, 2004).

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