Influence of Photic Environment on the Form of the Fish Electroretinographic Off-Response

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ABSTRACT: Scotopic electroretinogram of dogfish shark (*Scylliorhinus canicula*) and eel (*Anguilla anguilla*) is characterized by a negative off-response, changing in sign under photopic condition. It increased under the effect of increased background illumination, but its amplitude never exceeded that of the b-wave. On the other hand, dark-adapted electroretinograms of two perch-like species, perch (*Perca fluviatilis*) and painted comber (*Serranus scriba*), exhibited a positive off-wave, exceeding the b-wave amplitude under bright photopic conditions.

KEYWORDS: b-wave; d-wave; off-response; eel; dogfish; perch; painted comber

The PII component of the electroretinogram[1] (ERG) is comprised of the b-wave and the dc-component and is thought to reflect bipolar cell activity.[2,3] Although the b-wave is generated to a large extent by Müller cells, the origin of the dc-component is unclear. Different forms of the off-responses in cone and rod ERGs are due to the rapid decay of the cone late receptor potential (RP), compared with the much slower decay of the rod late RP. Lowered stimulus intensity is the method for revealing the dc-component, even in nonmammalian species.[4]

Isolated eyecups were obtained from eyeballs (about 10 mm in diameter) of small-spotted dogfish sharks (*S. canicula*, caught by trawler nets in the South Adriatic). Under dim lights the eyecups were excised after rapid decapitation of the fish. The preparations were surgically deprived of cornea, lens and most of the vitreous. The eyecup was filled with elasmobranch Ringer's solution[5] and placed on a cotton-

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wool bed soaked with the same solution, in a plastic temperature-controlled chamber inside a lightproof Faraday cage. After mounting, the preparations were dark-adapted for an additional 30 min before actual ERG recording. In the case of photopic b-wave recording, the eyecup was continuously exposed to a 500 nm background illumination capable, at its onset, to evoke a b-wave response of saturating amplitude and reduce sensitivity more than 3,000-fold.

European eels (*A. anguilla,* electrofished during summer months in coastal waters running along Kotor Bay) were anesthetized (phenobarbital sodium) and curarized (tubocurarine) following procedures recommended by Hamasaki and colleagues,[6] adjusting the dosage so as to induce the arrest of respiratory movements. Artificial respiration was provided continuously by forcing aerated and temperature-controlled water through the gills. The immobilized eel was positioned laterally on a plastic platform inside a lightproof Faraday cage. The *in situ* eyecup was prepared in the same way as in the case of isolated preparations of the dogfish (removal of cornea, lens, and most of the vitreous humor), and it was filled with teleosts Ringer solution. The same procedures were applied in experiments performed on painted comber (*S. scriba*, caught by net in the South Adriatic) and perch (*P. fluviatilis*, electrofished in the flood-plain zone of the Danube).

ERG potentials were detected with nonpolarizable silver chloride (Ag-$AgCl_2$) electrodes, the active one being introduced in the interior of the saline-filled eyecup. The reference electrode was in contact with the cotton-wool bed underneath the isolated preparations of the dogfish, or in the retro-orbital space behind the *in situ* eyecup of the eel, painted comber, and perch. It was connected to the input stage of a directly coupled differential preamplifier, and responses were recorded by means of a Polaroid camera from a storage oscilloscope display (dogfish-shark, eel, and painted comber) or from GRASS P15 D.C. preamplifier to AD-converter PCI-20428W-1 and computer (perch). For acquisition and analysis, original software was used.

Photic stimuli were delivered by a single-beam optical system using an 8 V 50 W tungsten-halogen lamp as the light source in dogfish-shark, eel, and painted comber, and 12 V 50 W lamp in perch, providing independent control of intensity (neutral density filters), duration (electromagnetic shutter), and spectral composition (interference filters) of the test flashes. Light intensities were calibrated and checked by placing the active surface of the radiometer probe in the position usually occupied by the eyecup preparation. The attenuating effects of the interference filters were accounted for when comparing responses to flashes of different wavelengths. Unattenuated, the energy flux delivered by the test field was of the order of 2.4×10^{-2} mW/cm^2 and 28.2×10^{-2} mW/cm^2.

The three principal components of the rod-dominated vertebrate retina[1] were well represented (FIG. 1B): an initial negative deflection (a-wave), a relatively fast positive transient (b-wave), and a slow positive potential (c-wave). In spite of earlier beliefs that the dc component is absent in nonmammalian species,[4] the dc component can be revealed in the dogfish shark by manipulating the intensity and the duration of photic stimuli. It appears in its classical rectangular form in response to sufficiently long stimuli of an appropriate low intensity. At the end of a test flash, in dark-adapted preparations, a conspicuous negative indentation appears on the rising phase of the c-wave. As FIGURE 1A shows, this indentation, the negative d-wave, is best visible when the c-wave is of reduced amplitude due to an attenuation of the test flash. A conspicuous positive d-wave appeared, however, at the end of test flashes

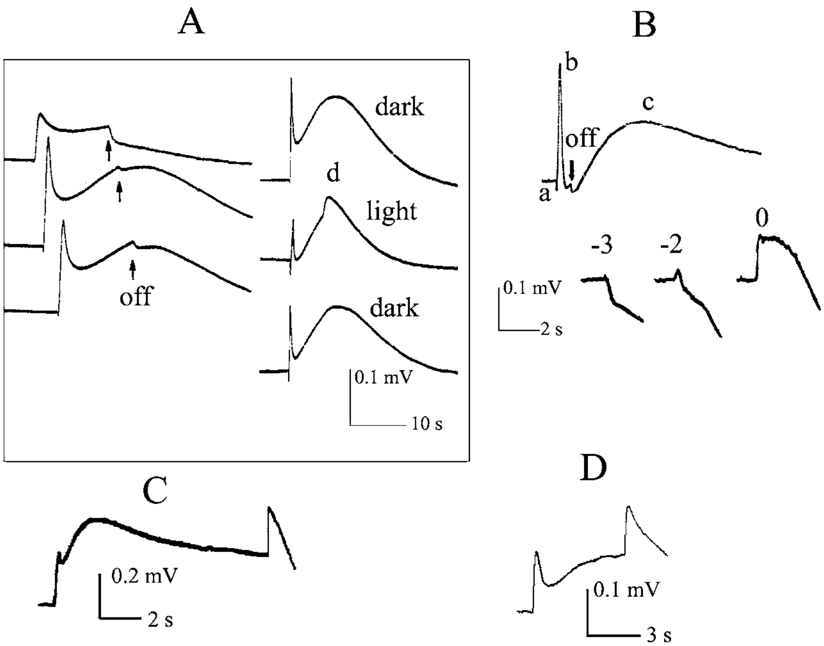

FIGURE 1. (**A**) (*Left*) At the end of a test flash, in dogfish (*S. canicula*) dark-adapted preparations, a negative off-response, indicated by *arrow*, appears on the rising phase of the c-wave. The negative d-wave is best visible when the c-wave is of reduced amplitude due to an attenuation of the test flash (increased from top to bottom trace). (*Right*) Upper trace: preparations of dogfish before bleaching (*dark*). Middle trace: after 30 min bleaching (*light*) the end of a test flash is signaled by a positive deflection, the d-wave (*d*). It appears on top of the c-wave, which outlasts the duration of the flash. Lower trace: d-wave disappears after dark re-adaptation (*dark*). Stimulus duration 5 sec. (**B**) (Top) Dark-adapted eel (*A. anguilla*) electroretinogram (1.35 sec test flash, off-response indicated by *arrow*). (Bottom) Illustration of the different intensity of illumination to the off-wave form and amplitude. Dark-adapted preparation of eel exposed to steps of incremental background illumination: $I_{B1} = 24 \times 10^{-6}$ mW/cm^2 (–3), $I_{B2} = 24 \times 10^{-5}$ mW/cm^2 (–2), and $I_{B3}=24 \times 10^{-3}$ mW/cm^2 (0). (**C**) Dark-adapted preparations of painted comber *S. scriba* ERGs in response to stimulus of 10 sec duration; stimulus light: white; stimulus intensity, I = 2.4 × 10^{-2}mW/cm^2. (**D**) Dark-adapted preparations of perch *P. fluviatilis* ERGs in response to light stimulus of 5 sec duration; stimulus light: white; stimulus intensity, I=28.2 × 10^{-2} mW/cm^2.

after light adaptation, and disappeared after dark re-adaptation (a phenomenon usually interpreted as based on cone function).

Electroretinograms obtained from *in situ* eyecup preparations of the dark-adapted eel, *A. anguilla* (body temperature 15°C, duration of light flashes 200 msec) were characterized, at saturating light intensities, by a prominent positive b-wave, reaching amplitudes of the order of 400–500 µV. It was preceded by a conspicuous, although much smaller (<100 µV), negative a-wave, and it was followed by a longer lasting “late negativity” phase, leading finally, beyond the termination of the light stimulus, to a salient, slow, and positive c-wave, which never reached amplitude of b-wave. A negative off-response was recorded at the termination of the light stimulus, but only when the duration of the latter was extended beyond the usual 200 msec (1.3 sec). In the case of light adaptation at the end of c-wave positive off-response appears. FIGURE 1B demonstrates the dependence of the off-wave form and amplitude on the intensity of illumination. Dark-adapted preparations of eel were exposed to the steps of incremental background illumination: $I_{B1} = 24 \times 10^{-6}$ mW/cm^2, $I_{B2} = 24 \times 10^{-5}$ mW/cm^2 and $I_{B3} = 24 \times 10^{-3}$ mW/cm^2. At the end of low intensity of stimuli (marked as "–3," FIG. 1B, bottom panel), negative indentation appears. In the case of higher intensity background illumination the end of a test flash is signaled by a positive off-response. However, at the end of I_{B2} positive off-response appeared which reached 110 μV after the end of I_{B3} (FIG. 1B, bottom panel marked by "0").

A bright light stimulus of 10 sec duration is used to record the ERG of the painted comber, *S. scriba* (FIG. 1 C). The a-wave and b-wave are followed by slow corneal-positive c-wave. After the termination of the stimulus, a d-wave develops. The ERG of the perch eye as elicited by a long (5 sec) light stimulus shows a-, b-, c-, and d-waves, the latter generated at stimulus offset (FIG. 1D). ERGs of the dark-adapted perch-like species *P. fluviatilis* and *S. scriba* contained a positive off-wave, exceeding the b-wave amplitude (FIG. 1 C and D).

The results obtained are related to the ecology of investigated fish species. Two perch-like species are predators with highly developed visual systems, well adapted to the high light illuminations in shallow water. On the contrary, information transmission in central nervous systems of eels and especially sharks are mostly based on the olfactory reception, while the visual system is adapted to scotopic conditions only.[7] Electroretinographic off-wave, as an indicator of the capacity of photopic vision, was thus more pronounced in perch and painted comber than in the other two species.

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