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Absolute Sensitivity of the Electroretinogram and of the Optic Nerve Action Potential in the Perfused Feline Eye

Manami Kuze* and Günter Niemeyer†

*Department of Ophthalmology, Mie University School of Medicine, Mie Prefecture, Japan;
†Neurophysiology Laboratory, Department of Ophthalmology, University Hospital, Zürich, Switzerland

Purpose: To assess and characterize the electroretinogram (ERG) and the optic nerve response (ONR) at threshold stimulus intensity in the isolated perfused cat eye.

Methods: Eyes were enucleated from deeply anesthetized adult cats and arterially perfused with oxygenated serum-enriched tissue culture medium. Light stimuli of 20- to 400- msec duration from a xenon arc source, attenuated down to threshold intensities by neutral density filters, were delivered via a modified fundus camera in full dark adaptation. Vitreal ERGs and ONRs were amplified, digitized, averaged and analyzed using LabVIEW for Windows® software.

Results: The threshold intensities in log scot q/deg^2 per second for the negative scotopic threshold response (STR), for the ERG b-wave, and for the ONR were at 2.87 ± 0.35, 3.53 ± 0.35 and 1.78 ± 0.48, respectively.

Conclusion: The in vitro perfused mammalian eye preparation exhibits remarkably low thresholds for the ERG and particularly for the ONR near the intensity required for the human psychophysical threshold. **Jpn J Ophthalmol 2003;47:362–367**

Key Words: Electrophysiology, optic nerve response, perfused cat eye, scotopic threshold response, threshold stimulus intensity.

Introduction

The isolated arterially perfused eye preparation offers several advantages over in situ or in vivo preparation, including control over chemical inputs to the eye and exclusion of systemic variables and extraocular metabolism. The retinal pigment epithelium, the neural retina and the vascular system are maintained in their natural integrity, as well. Access to different levels of visual information processing is given by recording of the various components of the electroretinogram (ERG) and of the optic nerve response (ONR).[1–3] Recording of the ONR, which is not accessible in vivo, has yielded useful and subtle information in a number of metabolic and pharmacological studies.[3–5]

In the present study we set out to determine and characterize the ONR at threshold stimulus intensities in full dark adaptation in relation to the weakest stimulus intensities that elicit the scotopic threshold response (STR) and the PII component of the ERG. The in vitro sensitivity will be compared to previously reported in vivo data.

Material and Methods

Animals

The seven eyes used in this study were enucleated from five adult cats. The experiments were carried out in accordance with the regulations of the cantonal veterinary authority of Zürich and the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Details on the method have been published previously.[3–7]

Anesthesia

After premedication with atropine sulfate (0.02–0.04 mg/kg, SC), the animals were anesthetized first with an IM injection of ketamine hydrochloride (Ketasol®,

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Correspondence and reprint requests to: Manami KUZE, MD, Department of Ophthalmology, Mie University School of Medicine, 1-174 Edobashi Tsu, Mie Prefecture 514-8507, Japan. Tel.: +81-59-231-5027; fax: +81-59-231-3036; E-mail: m-kaze@clin.medic.mie-u.ac.jp

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10–20 mg/kg; Graeub, Bern, Switzerland) and then with pentobarbital hydrochloride IV (Nembutal®, 30–40 mg/kg; Abbott Laboratories, Chicago, IL, USA). Intubation was done after injection of gallamine triethiodide (Flaxedil®; Rhone-Poulenc Rorer, France) IV as a bolus for artificial ventilation (respiration pump 66IA, Harvard Apparatus, South Natick, MA, USA) with oxygen-enriched (30%) room air. Deep anesthesia was maintained by continuous pump-driven IV application of pentobarbital hydrochloride (9–16 $mg \cdot kg^{-1} \cdot hr^{-1}$) and gallamine thriethiodide (5–10 $mg \cdot kg^{-1} \cdot hr^{-1}$). Fentanyl citrate (Fentanyl®; Janssen, Baar, Switzerland, 0.05 mg bolus IV) was applied prior to each surgical procedure to ensure analgesia. The electrocardiogram, the transcutaneous oxygen saturation and respiratory CO_2 concentration were monitored continuously, and blood oxygen saturation above 90% and end-tidal expiration of 3%–4% CO_2 were maintained throughout anesthesia. Eyes were enucleated after atropine- and phenylephrine-induced mydriasis, and the animals were sacrificed after enucleation of the second eye by an IV bolus of pentobarbital hydrochloride.

Arterial perfusion, Stimulation and Data Acquisition

After canulation of the ophthalmociliary artery, the eye was perfused with oxygenated tissue culture medium with Earleís salts (TC 199, Bio Concept, Allschwil, Switzerland), containing also L-glutamine (200 nM), amikacin sulfate (63.9 µM, Amikin®, Bristol-Myers Squibb AG, Baar, Switzerland) and newborn calf serum (30% v/v, Newborn Calf Serum Bio Concept). The perfusate was buffered with HEPES (15 mM) and $NaHCO_3$ (26 mM) to a pH of 7.4 at 37º and oxygenated with oxycarbon (95% O_2, 5% CO_2) to reach a pO_2 of 400–450 mm Hg. The flow rate of the perfusate, driven by a constant hydrostatic pressure head, was continuously monitored.

Light stimuli from a 150-W xenon arc lamp providing a maximum of 11.54 log scot quanta [507 nm]/deg^2 per second at the cornea were applied by means of a modified fundus camera in Maxwellian view. Light flashes were attenuated by neutral density filters and by a double neutral density wedge for fine tuning. Stimuli of 50 to 400 ms in duration were presented at intervals set from 5 to 60 seconds, adequate to avoid desensitization. The criterion amplitude for the STR threshold was set as 5 µV; for the P II, as 20 µV. The ERG was recorded by the step intensity.

The ERG was recorded between dc-suitable Ag-AgCl-wick electrodes[7] inserted via the pars plana in the vitreous and on the sclera near the posterior pole, respectively (Figure 1). The ONR was recorded between an Ag-AgCl suction electrode at the cut end and an Ag-AgCl reference electrode on the surface of the optic nerve (Figure 1). The light-evoked signals were amplified (PARC 113, EG&G, Princeton, NJ, USA), band-pass filtered from 0.03 to 100 Hz (model 3750, Krohn-Hite, Avon, MA, USA), fed to a digital oscilloscope (Gould digital storage oscilloscope 4050, Gould, Cleveland, OH, USA), and also digitized, averaged when necessary, and stored by a standard PC using LabVIEW for Windows® software (Lab PC+, National Instruments, Ennetbaden, Switzerland; upgraded by P.A. Knapp, Msc., Alea solutions, GmbH, Zürich, Switzerland).[8]

Results

A typical ONR recorded in light adaptation is shown in Figure 2, presenting the ON-, a plateau- and the OFF-components with vivid oscillations. Figure 3 illustrates a typical low-intensity response series from a perfused eye dark-adapted for over 60 minutes: ONRs were recorded at intensities below the thresholds for the STR and the PII. In the ONR to a stimulus of 400 msec in duration, the ON- and OFF-components appeared at near threshold intensities as slow, rounded, non-oscillatory deflections of negative polarity (Figure 3), in contrast to pointed and oscillatory light-adapted responses (Figure 2). Under full dark adaptation and only at threshold we often observed OFF-responses larger than ON- responses.

This phenomenon was found to be independent of the position of the recording electrodes at the optic nerve. Very dim background illumination, such as dim red light below 0.1 lux, depressed the large OFF-component of the ONR but not the ON-component.

The ERG channel could record a typical STR, the amplitude of which increased with increasing stimulus intensity until the PII component evolved (Figure 3). At higher intensities, the b-wave (P II) became the dominating component of the ERG, much like in vivo.

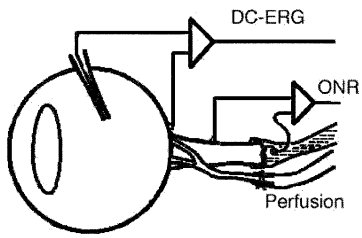

Figure 1. Schematic illustration of the electrode arrangement in the perfused cat eye. DC-ERG is recorded with best stability in the vitreous cavity with an electrode on the posterior sclera. ONR is recorded between a surface and a suction electrode.

Jpn J Ophthalmol
Vol 47: 362–367, 2003

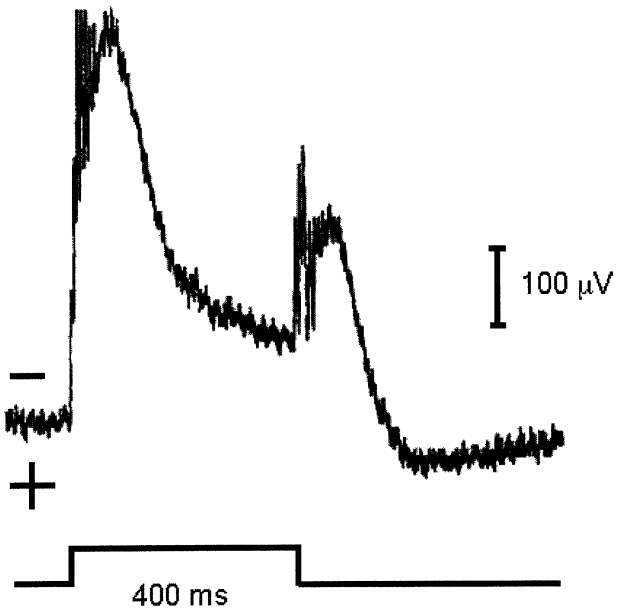

Figure 2. A typical ONR recorded at high intensity in a dark-adapted perfused cat eye. Calibrations: 400 ms and 100 μV. Positivity is displayed downward.

The threshold intensitiy for measurable ONR ON- and OFF-components was 1.78 ± 0.48 log scot q/deg^2 per second (mean ± SD), which is clearly below the threshold intensities for the STR and for the b-wave (Table 1). The ONR was elicited with the dimmest stimuli at about 1.8 log scot q/deg^2 per second, which is about 1.1 log scot q/deg^2 per second below the STR threshold, although some variations were present among preparations.

Within time windows of approximately 10 minutes, single or averaged ONRs showed fluctuations of the amplitude in the range of 2 to 5 μV (Figure 4). Fourier analysis failed to reveal any systematic rhythm such as a sine wave within this time window. We thought that these fluctuations in amplitudes are caused by noise inherent to very small signals like ONR.

Discussion

The perfused mammalian eye preparation, introduced by Gouras and Hoff, offers access not only to signals originating in the RPE and the neural retina but also to the mainly neural compound action potentials of the optic nerve.[2]

The action potential of the optic nerve, which is recorded directly from the in vitro preparation between a suction and a surface electrode is called the ''optic nerve response'' or ONR. It is a temporally dispensed field potential of negative polarity. One can easily imagine that to access the optic nerve is difficult in vivo, thus the ONR cannot be recorded in vivo. The recording from the isolated eye preparation is optimally suited to monitor the light-evoked mass response of the ''final stage'' of retinal information processing under light- as well as dark-adaptation. As a signal from the ganglion cells, the ONR can be compared with the rod-driven STR as another signal generated in the inner retina. The absolute sensitivity of the ONR in vitro is of interest in view of applications of the preparation to subtle pharmacological and metabolic investigations. We recorded the ONR and found that its threshold intensity was lower than the threshold intensitiy for the STR. The threshold intensity for the ONR was in fact close to the threshold of human psychophysical perception.[9] The nearest in vivo correlate to the ONR is the visual evoked cortical potential because the ONR is the final stage of the retinal information processing.

Origin and sensitivity of the ONR

The typical ONR is composed of a distinct rapid ON-component, followed by a slightly declining plateau-phase during illumination, and of a more complex and often polyphasic OFF-component (Figure 2). We interpret the complexity of the waveform of the ONR as resulting from extracellular summation of the potentials of all ongoing excitation and inhibition in the axons underlying the recording electrodes.[10] Two major conduction velocity groups in the ONR have also been demonstrated in vitro.[11] The various firing patterns of ON-center ganglion cells fit configurations of ONR observed under various stimulation conditions.[4] Looking at a sequence of ONRs at increasing intensity (Figure 3), growth in amplitude and quickening of the ON-component were accompanied by changes in configuration. The shape of the ON-component of the ONR changed from a rounded waveform at near threshold to a wedge- or spike-shaped oscillatory signal at higher intensities. The oscillation continued well into the plateau phase, which, we hypothesize, is probably related to grouped firing of ganglion cells.[12] The OFF-component was simply rounded at threshold but became polyphasic as stimulus intensity increased. This complexity is most likely related to the many coinciding potentials of at least the four major classes of retinal ganglion cells, Y-ON, X-ON, Y-OFF, X-OFF.[10,11] The plateau component of the ONR was probably reflecting maintained firing (tonic response) of ON-center ganglion cells, and the rapid components at ON and OFF can be attributed to phasic responses of ganglion cells of the Y type. Glial contributions deduced from recordings with K^+-sensitive microelectrodes revealed a much slower time course (Niemeyer, unpublished).

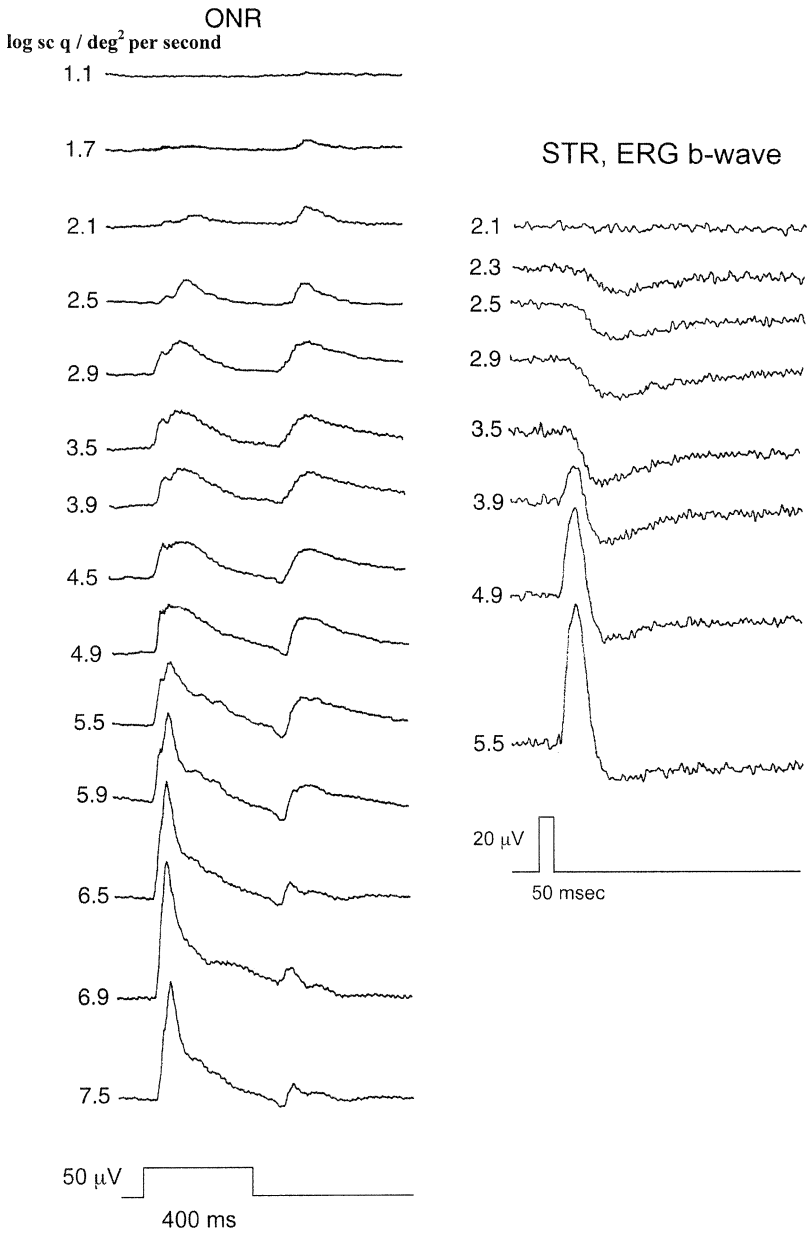

Figure 3. ONR, STR and ERG b-wave recorded from an arterially perfused cat eye. The duration of the stimulus was 400 msec for ONR and 50 msec for STR and ERG b-wave. The numbers on the left side are log scot quanta [507 nm]/deg^2 per second. Calibration and stimulus onset are indicated at the bottom of each row.

We also have shown that the threshold of the optic nerve's compound action potential to light is well below that of the STR in vitro.[13] The more proximal origin of the ONR compared to STR and the ERG b-wave probably accounts for its greater sensitivity, because signal amplification which occurs at several steps from the distal to the proximal retinal layers may explain the high sensitivity of the ONR. The synaptic connections between the rods and the bipolar cells exhibit a several fold gain, estimated as high as 50 for the goldfish retina.[14] Another amplification occurs at the bipolar-amacrine cell synapses. A second type of amplification of threshold signals is given by the divergence of the rod pathway through successive retinal layers: one rod synapses onto two or more bipolars and, in turn, onto more A II-amacrine cells, resulting in pooling of the rod signal in the proximal retina.[15]

Relation to previous threshold recordings

The STR threshold intensity and ERG b-wave threshold intensity observed in the present study are within the

Jpn J Ophthalmol
Vol 47: 362–367, 2003

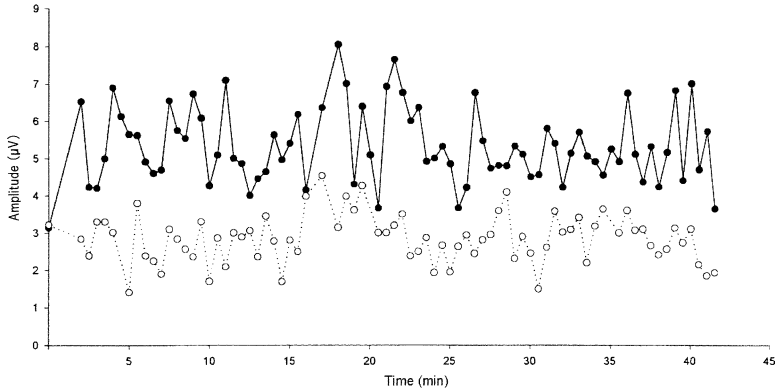

Figure 4. The amplitude of the ONR ON-component (closed circle) and the ONR OFF-component (open circle) at 1.4 log scot quanta/deg^2 per second. The wave was plotted for 45-minute duration. A specific cycle of fluctuation was not observed.

range of those reported previously in vivo.[16–18] Figure 5 schematically illustrates a comparison of sensitivity presented here for the in-vitro perfused mammalian eye with corresponding in vivo data, compiling selected previously reported assessments of threshold data in the human eye compared to recordings from *feline* eyes.[9,19]

The ONR threshold was observed at a near psychophysical threshold and thus lower than that of the ERG components. The exquisite sensitivity of cat retinal ganglion cells[20] thus appears to be expressed also in the field potential ONR in vitro. Studies in vitro, ie, in a perfused mammalian eye preparation, can contribute to the in-depth understanding of the STR.[21] The clinically recorded STR potentially could prove useful in detection of subtle changes in the sensitivity of the rod system in early stages of hereditary degeneration of the retina.

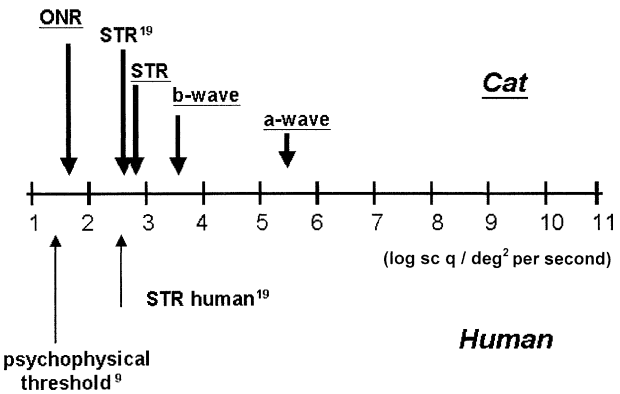

Figure 5. Presentation of threshold data recorded in vitro (thick arrows and underlined letters) and in vivo (thin arrows). The psychophysical threshold value is taken from Finkelstein, Gouras and Hoff (1969)[9] and the STR data from Robson and Frishman (1999).[19] The intensity scale is relative to the maximal output of the xenon source in our laboratory, the maximum unattenuated output being 11.5 log scotopic quanta [507 nm]/deg^2 per second measured at the position of the cornea of the isolated eye.

The STR originates in the proximal retina based on microelectrode recordings at various depths in the cat retina,[18] and is the ERG response to the dimmest stimuli occurring in the inner plexiform and inner nuclear layers. The STR[17,18] provides a very sensitive measure of retinal function near the rod threshold and it has also been observed in a variety of animals, including the toad, rat, cat, and sheep.

Fluctuation of *ONR* around threshold

Fluctuation in amplitude of the ONR ON- and OFF-components was observed in the low stimulus intensity range. At low light levels, the number of isomerizations per flash varies considerably even when the retina is stimulated by a series of identical flashes. This is referred to as photon noise[14] or spontaneous photolike event.[22] Fluctuation is also caused by thermal breakdown of photopigment in photoreceptors, spontaneous random release of neurotransmitters, and fluctuation in the physiological state of the retina and the brain, as well as in the neural response to random release of neurotransmitters. Actually in the toad, the mean rate of the spontaneous photolike events is one every 50 seconds, in the primate it is supposed to be *one event per rod every 160 seconds*. This rate increases with temperature in a manner that suggests that they are due to photoisomerization of the chromophore of a rhodopsin molecule as a result of thermal agitation. However, our results did not reveal a specific rhythm of fluctuation in ONR ON- and OFF-component amplitude at threshold.

Fluctuation of the absolute psychophysical threshold in man resembling a slow sine wave pattern was reported

by Ripps and Weale.[23] Such slow variation was not observed in the in vitro preparation used here.

In summary, we recorded in an in vitro perfused mammalian eye preparation a remarkably high absolute sensitivity of the ERG, and an even higher sensitivity of the optic nerve. The threshold intensity for the feline ONR was found to be near the intensity of the human psychophysical threshold. The STR and the ONR, reflecting network adaptation of the inner retina, can thus be investigated under controlled in vitro conditions, for example in pharmacological or metabolic studies.

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