Principles and Practice of Clinical Electrophysiology of Vision

Editors

JOHN R. HECKENLIVELY, M.D.
Professor of Ophthalmology
Jules Stein Eye Institute
Los Angeles, California

GEOFFREY B. ARDEN, M.D., PH.D.
Professor of Ophthalmology and Neurophysiology
Institute of Ophthalmology
Moorfields Eye Hospital
London, England

Associate Editors

EMIKO ADACHI-USAMI, M.D.
Professor of Ophthalmology
Chiba University School of Medicine
Chiba, Japan

G.F.A. HARDING, PH.D.
Professor of Neurosciences
Department of Vision Sciences
Aston University
Birmingham, England

SVEN ERIK NILSSON, M.D., PH.D.
Professor of Ophthalmology
University of Linköping
Linköping, Sweden

RICHARD G. WELEBER, M.D.
Professor of Ophthalmology
University of Oregon Health Science Center
Portland, Oregon

Mosby Year Book

St. Louis Baltimore Boston Chicago London Philadelphia Sydney Toronto

Mosby
Year Book

Dedicated to Publishing Excellence

Sponsoring Editor: David K. Marshall
Assistant Director, Manuscript Services: Frances M. Perveiler
Production Project Coordinator: Karen E. Halm
Proofroom Manager: Barbara Kelly

A Year Book Medical Publishers imprint of Mosby-Year Book, Inc.

Mosby-Year Book, Inc.
11830 Westline Industrial Drive
St. Louis, MO 63146

1 2 3 4 5 6 7 8 9 0 CL CL MV 95 94 93 92 91

Library of Congress Cataloging-in-Publication Data
Principles and practice of visual electrophysiology / [edited by] John R. Heckenlively, Geoffrey B. Arden.
p. cm.
Includes bibliographical references.
Includes index.
ISBN 0-8151-4290-0
1. Electroretinography. 2. Electrooculography. 3. Visual evoked response. I. Heckenlively, John R. II. Arden, Geoffrey B. (Geoffrey Bernard)
[DNLM: 1. Electrooculography. 2. Electrophysiology. 3. Electroretinography. 4. Evoked Potentials, Visual. 5. Vision Disorders—physiopathology. WW 270 P957]
RE79.E4P75 1991 91-13378
617.7 1547—dc20 CIP
DNLM/DLC
for Library of Congress

PART III

Origin of Electroretinographic Components

8

Introduction to the Origins of Electroretinographic Components

Chester Karwoski

It is not yet possible to examine all the different components of an electroretinogram (ERG) and decide which retinal cells are abnormal, but advances in methodology, physiology, and clinical observation are leading toward this goal. It is therefore important to understand the cellular origins of the ERG as well as the methods used to identify the sites and modes of generation of the ERG currents.

GENERAL PRINCIPLES

Extracellular responses like the ERG (and visual evoked cortical potential [VECP]) arise during neural activity because localized regions of cell membrane become depolarized or hyperpolarized and thus become sinks or sources of current. This current flows through the resistance of the extracellular fluid compartment to relatively inactive sites on the cell. These currents spread out through the tissue, somewhat like lines of magnetic force around a magnet. If the neurons are arranged in an orderly fashion, the resulting "field potential" may be large enough to record at a distance. In the retina, all cells generate light-evoked responses, and in principle, all must contribute to the field potential. However, depending on various factors, the contribution from a certain cell could be undetectable or could dominate the recording.

One such factor is cellular orientation. It is the radial component of a cellular field potential that is sensed in the commonly recorded transretinal ERG. Radially oriented cells (photoreceptors, pigmented epithelium, bipolar cells, and Müller cells) should generate fields with stronger radial components than should cells oriented more irregularly or tangentially (horizontal, amacrine, interplexiform, and ganglion cells).

Another factor derives from lateral inhibitory interactions being best developed at and proximal to the bipolar cells. If a stimulating light flash illuminates a small retinal area, fields from cells of the proximal retina (bipolar, amacrine, ganglion, and the proximal half of the Müller cells) will make a proportionately greater contribution to the ERG. On the other hand, cells of the distal retina (photoreceptors, pigmented epithelium, horizontal cells, and the distal half of the Müller cells) are best excited by large homogeneous stimuli.

Other factors that will receive mention in ~~this chapter~~ Part III are flash intensity, background illumination, and whether the retina contains predominately rods or cones. Mammalian species will be discussed where possible, but the emphasis in most sections is on the intensively studied retinas of lower vertebrates.

K^+ SPATIAL BUFFERING

Field potentials generated by *neurons* are well known, and their origin constitutes a classic topic area in neurophysiology.[4] However, in the retina, most of the ERG components that have been identified are believed to arise from Müller *(glial)* cells and pigmented *epithelial* cells rather than from neurons. There are several ways by which glial and epithelial cells can generate field potentials, and some of these

are described later in conjunction with the specific ERG components. One mechanism, the spatial buffering of potassium ions (K^+), is described here in detail because it is known to occur in the retina[6] and is thought to be involved in the generation of most ERG components.

The K^+ spatial buffer process is schematized for an amphibian glial cell in Figure 8–1.[11] In the resting condition (Fig 8–1,A), the extracellular K^+ concentration ($[K^+]_o$) is constant at 3mM and the intracellular K^+ concentration ($[K^+]_i$) at 108mM. The K^+ equilibrium potential (E_K) is given by the Nernst equation as −90 mV, and since glial cell membrane permeability is dominated by K^+, the membrane potential (V_m) equals E_K. In Figure 8–1,B, neural activity has locally increased $[K^+]_o$ to 12mM, and this shifts E_K to a less negative value (see also Fig 8–1,C). But V_m does not reach the new E_K because the membrane is electrically connected to regions of membrane where $[K^+]_o$ is not raised. Where $[K^+]_o$ is raised, there is a driving force ($V_m - E_K$) for K^+ to enter the cell, and at other cell regions (where ΔV_m has spread), the driving force is for K^+ to leave the cell. Thus, a current loop is set up (Fig 8–1, B): K^+ enters the cell where $[K^+]_o$ is raised, and it leaves the cell at other regions. Inside the cell, there must also be a current that is carried primarily by K^+, and to complete the circuit, an extracellular return current is carried by the dominant extracellular ions Cl^- (not shown in Fig 8–1, B) and Na^+. As described here, this process reduces local *increases* in $[K^+]_o$, but the model works equally well "backwards," i.e., to reduce local *decreases* in $[K^+]_o$.

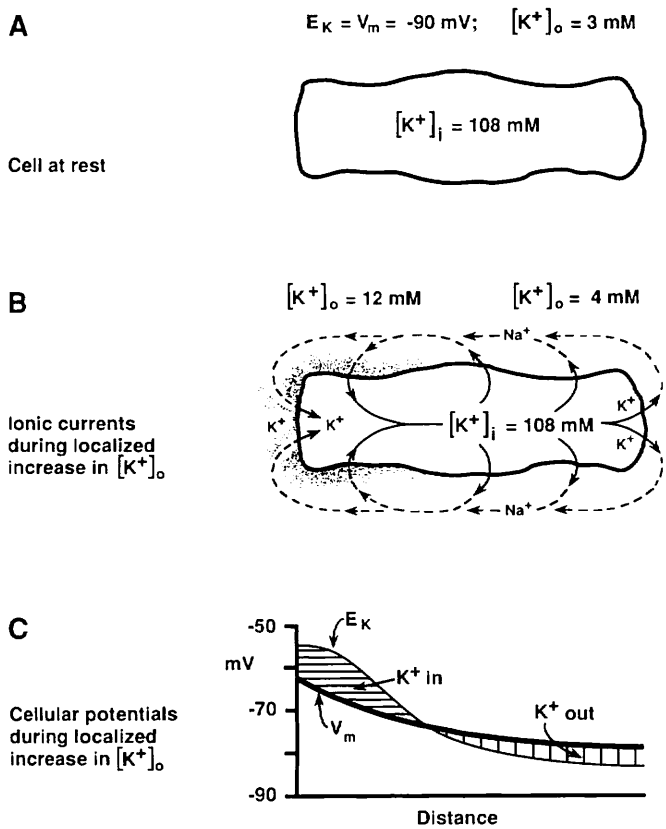

FIG 8–1.
Schematic of the K^+ spatial buffer process in a glial cell. **A,** the cell at rest. Since glial cell membranes have relatively high K^+ conductance, the resting membrane potential (V_m) is given by the Nernst equation ($E_K = 58 \log_{10}([K^+]_o)/([K^+]_i)$), where E_K is the K^+ equilibrium potential. E_K = −90 mV since $[K^+]_i$ is much higher than $[K^+]_o$. **B,** activity in a group of neurons has produced a local increase in $[K^+]_o$ to 12mM *(stippled area).* This K^+ increase produces a local intracellular depolarization that spreads passively to the rest of the cell. As explained in the text, differences between V_m and E_K produce transmembrane K^+ currents that are associated with intracellular and extracellular current flow, the latter giving rise to field potentials. **C,** graph showing the distribution of E_K and V_m as a function of distance along the glial cell. Where $V_m < E_K$, K^+ flows into the cell; where $E_K < V_m$, K^+ flows out. (Adapted from Orkland RK: *Ann NY Acad Sci* 1987; 481:269–272.)

The function of K^+ spatial buffering is to minimize $\Delta[K^+]_o$, which could be harmful to normal neural activity, and to redistribute it to regions where it should cause fewer problems. But more important to electroretinography is the return current carried by Na^+ and Cl^- through the extracellular space. This current generates field potentials, several of which are major components of the ERG. Because the buffer process will work for any cell whose membrane permeability is dominated by K^+, it is applicable to retinal pigmented epithelial cells as well as glial cells.

METHODS FOR DETERMINING ELECTRORETINOGRAM ORIGINS

The following methods are straightforward, and interpretation of results hinges primarily on how well the targeted ERG component can be isolated from other components.

1. *Intracellular recordings.*—Cell types are identified that have intracellular responses that best match the waveform of an ERG component. A caution is that the exact relationship between field potential waveform and intracellular responses may be complex.[4]

2. *Ion recordings.*—Where an ERG component is thought to arise through a mechanism such as K^+ spatial buffering, the waveform can be compared

with recordings of light-evoked $\Delta[K^+]_o$.[2, 9] This technique has proved quite useful, although the relation of field potential waveform to ion signals may also be complex.[7]

3. *Pharmacology.*—Particularly valuable are pharmacological agents that have defined actions on retinal cells.[1, 15] A correlation of these actions with drug effects on the ERG permits ERG components to be localized to specific cells or neural circuits.

4. *Anatomy.*—Some studies find correlations between retinal anatomy and ERG components. For example, anatomy may be studied developmentally[12] or by induction of cellular degeneration.[9]

Other methods for determining ERG origins require an understanding of the electrical current pathways in the retina and eye. A particularly useful equivalent electrical circuit described by Rodieck[13] is reproduced in Figure 8–2. Here, the extracellular circuit is represented by five resistors, with the length of each being proportional to its ohmic value. A cellular current generator (I) within the retina is shown to the left. Current enters the extracellular space at one retinal depth (a current source), and it returns into the cell at another depth (a current sink). Most of the current traverses the extracellular space within the retina, but a small fraction travels extraretinally—through the vitreous humor, the extraocular tissues, the sclera and choroid, and the high resistance of the pigmented epithelium (R membrane in Fig 8–2), and back into the neural retina. ERG waveform, polarity, and amplitude are critically dependent on where the electrodes are placed in this circuit. Figure 8–2 shows electrode placements for a typical (and small-amplitude) ERG recorded from the cornea (ERG), for the (larger) ERG recorded with an electrode in the vitreous (VITREAL ERG), and for the ERG recorded with an electrode within the retina (local ERG, LERG).

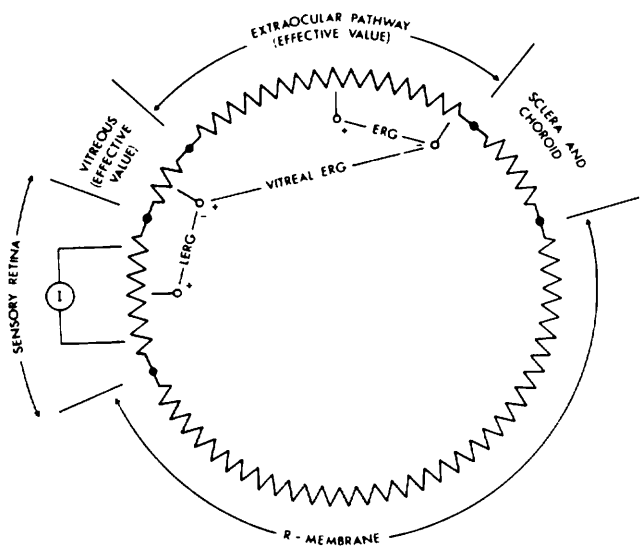

FIG 8–2.
Electrical equivalent circuit for current pathways in the eye. The lengths of the resistors are proportional to typical resistance values of the structures they represent. The current source *(I)* represents the intracellular current through a retinal cell or cells. (From Rodieck RW: *The Vertebrate Retina.* San Francisco, WH Freeman & Co, 1969. Used by permission.)

Because cell types are localized within retinal layers, the contribution of each cell type to the ERG depends on the depth within the retina at which a recording is made. Recordings at different depths (laminar analysis) have been used in numerous studies to localize ERG origins. These analyses are useful if the electrode depth is specified, and this can be done by micromanipulator readings, by deposition of extracellular stain, or by various physiological criteria. Laminar analyses have shown, for example, that the c-wave is generated by the pigmented epithelium and the proximal negative response by neurons in the proximal retina.

There are two difficulties with laminar analysis: (1) field potentials may spread far, and they superimpose in space and time, which makes it difficult to determine whether a change in amplitude of one ERG component is intrinsic to that component or is due to interference by another component. (2) Retinal resistance differs from the resistance of adjacent structures, and it varies even within retinal layers.[5, 10, 14] Thus, currents passing through regions of different resistance will set up complex voltage distributions.

Current source density (CSD) analysis[3] provides a solution to these difficulties, because resistance is included in the analysis. The result is a spatiotemporal profile of relatively well-localized current sources and sinks, and this profile can be compared with known features of retinal anatomy and physiology. When properly performed, CSD analysis can provide powerful evidence regarding the origins of ERG components. However, this technique, particularly its application to the retina, is fraught with difficulties, and results from published CSD analyses need careful scrutiny.

OVERVIEW

The following chapters describe retinal extracellular potentials, and focus on their origins. ERG components initiated by events in the distal retina (i.e.,

the subretinal space and adjacent structures) are discussed in Chapter 9. ERG components initiated by events in the mid- and proximal-retina (i.e., by cells post-synaptic to the photoreceptors) are discussed in Chapters 10 through 16. Finally, some retinal extracellular potentials not generated by light are discussed in Chapter 17.

BIBLIOGRAPHY

1. Dick E, Miller RF: Extracellular K^+ activity changes related to electroretinogram components. I. Amphibian (I-type) retinas. *J Gen Physiol* 1985; 85:885–909.
2. Dick E, Miller RF: Light-evoked potassium activity in mudpuppy retina: Its relationship to the b-wave of the electroretinogram. *Brain Res* 1978; 154:388–394.
3. Freeman JA, Nicholson C: Experimental optimization of current source-density technique for anuran cerebellum. *J Neurophysiol* 1975; 38:369–382.
4. Hubbard JI, Llinas R, Quastel DMJ: *Electrophysiological Analysis of Synaptic Transmission*. Baltimore, Williams & Wilkins, 1969.
5. Karwoski CJ, Frambach DA, Proenza LM: Laminar profile of resistivity in frog retina. *J Neurophysiol* 1985; 54:1607–1619.
6. Karwoski CJ, Lu H-K, Newman EA: Spatial buffering of light-evoked potassium increases by retinal Müller (glial) cell. *Science* 1989; 244:578–580.
7. Kline RP, Ripps H, Dowling JE: Generation of b-wave currents in the skate retina. *Proc Natl Acad Sci USA* 1978; 75:5727–5731.
8. Maffei L, Fiorentini A: Electroretinographic responses to alternating gratings before and after section of the optic nerve. *Science* 1981; 211:953–955.
9. Oakley B: Potassium and the photoreceptor-dependent pigment epithelial hyperpolarization. *J Gen Physiol* 1977; 70:405–425.
10. Ogden TE, Ito H: Avian retina. II. An evaluation of retinal electrical anisotropy. *J Neurophysiol* 1971; 34:367–373.
11. Orkand RK: Glial-interstitial fluid exchange. *Ann N Y Acad Sci* 1987; 481:269–272.
12. Rager G: The cellular origin of the b-wave in the electroretinogram—a developmental approach. *J Comp Neurol* 1979; 188:225–244.
13. Rodieck RW: *The Vertebrate Retina*. San Francisco, WH Freeman & Co, 1973.
14. Sieving PA, Wakabayashi K, Lemon WJ: Current source density analysis of cat scotopic threshold response. *Invest Ophthalmol Vis Sci* 1988; 29(suppl):103.
15. Sillman AJ, Ito H, Tomita T: Studies on the mass receptor potential of the isolated frog retina. I. General properties of the response. *Vision Res* 1969; 9:1435–1447.