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Guidelines for basic multifocal electroretinography (mfERG)

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Abbreviations: CRT – cathode ray tube; ERG – electroretinogram; ISCEV – International Society for Clinical Electrophysiology of Vision; mfERG – multifocal electroretinogram; PERG – pattern electroretinogram

Introduction

Full-field electroretinography (ERG) is a standard clinical test for evaluating the function of the retina as a whole [1]. Multifocal electroretinography (mfERG)* is a new technique that allows analysis of local retinal function. While the technology of these recordings and the knowledge about the physiology of these responses are evolving, there is sufficient experience to propose basic guidelines for the usage of this procedure. The intention of this document, is not to mandate a 'standard' of care or to fix a particular test protocol. Rather, the intention is to offer guidelines for recording the mfERG that will aid in obtaining stable and interpretable records, while minimizing artifacts. These guidelines should be especially helpful for those new to this technique, while informing the experienced user about procedures that colleagues find effective. However, we emphasize that these are guidelines and not standards. More research is needed on the applications and technology of this new technique before many aspects of these guidelines can be resolved. We anticipate that exploration of different recording protocols and their interpretation will continue, but users who are not specifically studying alternative techniques are encouraged to follow the guidelines as current 'best practice'. These guidelines will be re-examined in 4 years, consistent with all ISCEV practice recommendations, to make revisions as necessary and consider whether an ISCEV Standard for the mfERG should be established.

Description of multifocal electroretinography

The mfERG is a technique for assessing the local ERG from different regions of the posterior retina. Electrical responses from the eye are recorded with a corneal electrode just as in conventional ERG recording, but the special nature of the stimulus and analysis produce a topographic map of ERG responses. For the routine mfERG, the retina is stimulated with a computer monitor or other device that generates a pattern of elements (typically hexagons), each of which has a 50% chance of being illuminated every time the frame changes (Figure 1). The pattern seems to flicker randomly, but each element follows a fixed, predetermined sequence (presently an 'm sequence') so that the overall luminance of the screen over time is relatively stable (equiluminant). By correlating the continuous ERG signal with the on or off phases of each stimulus element, the focal ERG signal associated with each element is calculated. Data can be

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*Multifocal electroretinography has been abbreviated in various ways in the literature, including mfERG, MERG, and MFERG. Since MERG causes confusion with other procedures in some languages, we recommend mfERG as most universally distinct and recognizable abbreviation.

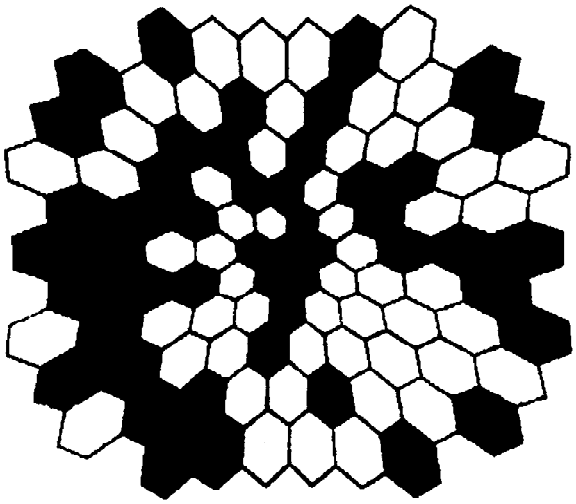


Figure 1. Representative hexagonal mfERG stimulus array with 103 elements, of which roughly half are illuminated at any one time.

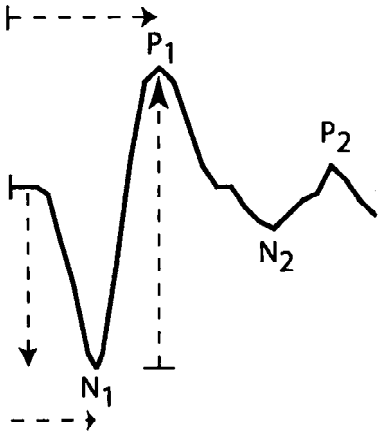


Figure 2. Diagram of an mfERG response to show designation of the major waveforms, and the recommended method for measuring amplitude and implicit time (time-to-peak).

displayed in various ways such as a topographic array or a three-dimensional plot. Interactions between responses as a result of adaptation or non-linear response properties can also be analyzed. Different stimulus patterns and flicker sequences can be used for specialized applications.

It is important to keep in mind that the tracings of the mfERG are not 'responses' in the sense of direct electrical responses from a local region of retina. The mfERG waveforms are a mathematical extraction of signals that correlate with the time that one portion of the stimulus screen is illuminated. Thus, mfERG signals may be influenced by adaptation effects (from preceding stimuli) and by the effects of scattered light on other fundus areas.

Waveforms

Nomenclature of peaks

The typical waveform of the primary mfERG response (also called the first order response or first order kernel K_1) is a biphasic wave with an initial negative deflection followed by a positive peak (Figure 2). There may be a second negative deflection after the peak. The preferred designation is to label these three peaks respectively N1, P1 and N2. There is some homology between this waveform and the conventional ERG, but they are probably not identical (see below). Thus the designations 'a wave' and 'b wave' are not recommended.

Cellular origin

Studies in humans have shown that the N1 response includes cellular contributions from the same components as the a-wave of the full-field cone ERG, and the P1 response includes contributions from the components of the cone b-wave and oscillatory potentials. However, this body of knowledge is still incomplete, and it would be premature to assume any simple correlation between the mfERG waveform and particular classes of retinal cells.

Basic technology

Electrodes

Recording electrodes

Electrodes that contact the cornea or nearby bulbar conjunctiva are strongly recommended for mfERG recording just as for the full-field ERG. The same recommendations as in the ERG Standard suffice, with the proviso that the optical opening or corneal lens must be clear to allow good visual acuity and refraction.

Reference and ground electrodes

Proper application of suitably conductive electrodes is essential for stable mfERG recording. Follow the recommendations in the ISCEV full-field ERG and/or PERG Standards [1,2].

Electrode characteristics, stability and cleaning
Follow the recommendations in the ISCEV full-field ERG and/or pattern ERG (PERG) Standards [1,2].

Stimulation

Stimulus source
The stimulus is generally delivered by a cathode ray tube (CRT), i.e. a monitor. Other devices are also used such as LCD projectors, LED arrays and scanning laser ophthalmoscopes. These alternative modes of stimulation may produce different waveforms and will not be discussed in these guidelines, but many of the principles of stimulation outlined below would apply to them as well.

Screen properties
Frame frequency. A CRT frame frequency of 75 Hz, has been used most widely. Users should be aware that use of a different frequency requires adjustment of the stimulus protocol and may alter the recorded signals. The frame frequency should never be line current frequency (50 or 60 Hz) which may cause interference artifacts.

Luminance. The luminance of the stimulus elements on the CRT screen should be 100–200 cd/m^2 in the lighted state and <1 cd/m^2 in the dark state. This means that the mean screen luminance during testing will be 50–100 cd/m^2.

Calibration. The luminance of dark and lighted stimulus elements should be measured with an appropriate calibrator or spot meter. Many monitor screens are not of uniform brightness over the entire screen, and variations of up to 15% are considered acceptable. If greater variation is present, stimulus size may need to be adjusted to insure equivalent effects in different regions of the retina. Techniques for calibrating stimulus and recording parameters are described in the ISCEV Guidelines for calibration [3]. We urge manufacturers of mfERG equipment to provide instruction for calibration of their devices.

Stimulus parameters
Stimulus pattern. The default hexagonal stimulus pattern was designed to compensate for local differences in signal density (and cone density) across the retina. Thus, the central hexagons are smaller than the more peripheral ones. Different patterns (e.g., equal size hexagons) can be generated and may be useful in special cases such as patients with eccentric fixation or when using specialized flicker sequences. However, these guidelines cover only the default stimulus pattern.

Flicker sequence. Commercial mfERG instruments use an m-sequence to control the order of flicker of the stimulus elements (between light and dark). This sequence is recommended for routine testing. Different sequences, or the inclusion of global light or dark frames, are possible for specialized applications, but will not be considered here.

Stimulus size. The overall stimulus pattern should subtend a visual angle of 20–30 degrees on either side of fixation. The stimulus region can be divided into different numbers of hexagons, and the choice depends on balancing the need for spatial resolution, signal-to-noise ratio, length of recording, etc. (see 'Discussion' below under Clinical Protocol). The standard patterns in most frequent use at present incorporate 61, 103 or 241 stimulus elements.

Contrast and background. Contrast between the lighted and darkened stimulus elements should be 90% or greater. The background region of the CRT (beyond the area of stimulus hexagons) should have a luminance equal to the mean luminance of the stimulus array.

Fixation targets. Stable fixation is essential to obtain reliable mfERG recordings. Central fixation dots or crosses are available with most stimulus programs. They should cover as little as possible of the central stimulus element to avoid diminishing the response (but may need enlargement for low vision patients).

Recording equipment

Amplifiers and filters
Amplifiers should be alternating current (AC) coupled and should be capable of gain and filter adjustment. A gain of 100,000 or 200,000 is most widely used; the gain should produce recognizable signals without saturating the amplifiers. The bandpass filter removes extraneous electrical noise while preserving waveforms of interest. For general use, a filter range of 3–300 Hz or 10–300 Hz is most suitable. Users should be aware that filter settings may influence waveforms that contain components near the ends of the frequency range. The filter settings should be the same for all

subjects studied by a given laboratory so that the waveforms are comparable. Line-frequency notch filters should be avoided. A masking cone (provided by some manufacturers) may reduce electrical interference.

Signal analysis

Artifact rejection. Because blinks and other movements can distort the recorded waveforms, there are 'artifact rejection' programs to eliminate some of the obvious peaks or drifts from being added to the cumulative recording. Artifact rejection is often used to 'clean up' a record, but should not in general be applied multiple times.

Averaging with neighbors. In order to smooth out waveforms and reduce noise, commercial programs can average the response from each stimulus element with a percentage of the signal from each of the adjacent elements. This can be useful with noisy records, but will blur the margins of small or critical regions of dysfunction. Thus, it should be used with care. With the VERIS system the percentage of neighboring responses to be averaged can be adjusted. A setting of 16% means that 50% of each trace comes from adjoining stimulus elements, and we advise using no more than 16% so that no more than 50% of each trace comes from adjoining areas. The Roland system digitally smoothes the stored data, and should be used with similar caution.

Display options

Trace arrays. All commercial programs can produce an array of the mfERG traces from different regions of the retina (Figure 3). This is the basic mfERG display and should be a part of all standard display protocols. It is useful for observing areas of variation and abnormality.

Group averages. Analysis programs can average together the responses from any designated number of traces. This can be helpful for comparing quadrants, hemiretinal areas, or successive rings from center to periphery. The latter can be useful for patients who have disease that is radially symmetric or diffuse. Responses from stimulus elements relating to a local area of interest can also be averaged together for comparison with a similar area in normals.

Topographic (3-D) response density plots. These plots show the overall signal strength per unit area of retina (combining N and P components) in a 3-dimensional figure. This is sometimes useful as an overview or demonstration of certain types of pathology, but there are major dangers which need to be understood. These 3-D plots typically incorporate both negative and positive deflections, so waveform information is lost and irrelevant components (noise) can be enhanced (see, Appendix: Artifact Recognition examples for electrical noise and weak signals). The generation of 3-D plots usually involves interpolation of the responses to create the appearance of a continuous surface, and as a result spatial resolution may be modified. Finally, the appearance of the 3D plot from a given recording is dependent on whether the scaling templates were derived using averaged data from the subject or from controls, and on the duration of the displayed waveforms. A comparison of scalar plots between patients can be misleading unless the parameters and reference data are consistent for all subjects. We recommend that 3-D plots not be used by themselves to display mfERG data; they should always be accompanied by a corresponding trace array.

Kernels. This document is aimed at the general mfERG user and only describes the measurement of the first order kernel.

Clinical protocol

Patient preparation

Pupils

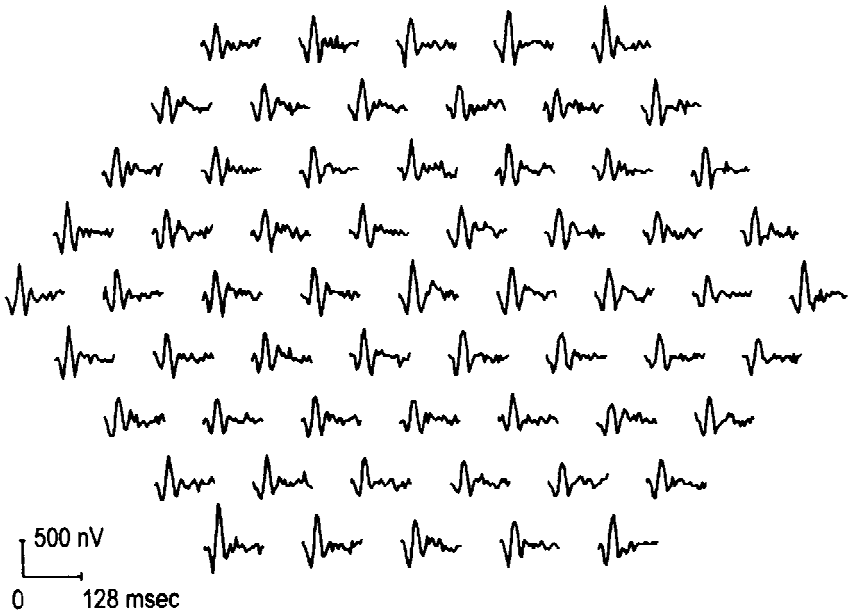
The pupils should be fully dilated.

Electrodes

These must be carefully applied according to instructions in the full-field ERG or PERG Standards [1,2]. Poor or unstable electrode contact is a major cause of poor quality records.

Patient positioning

Subjects should sit comfortably in front of the screen or instrument. The viewing distance will vary with screen size, in order to control the area (visual angle) of retina being stimulated. See the ISCEV Guidelines for calibration [3] for instructions on measuring visual angle and viewing distance.



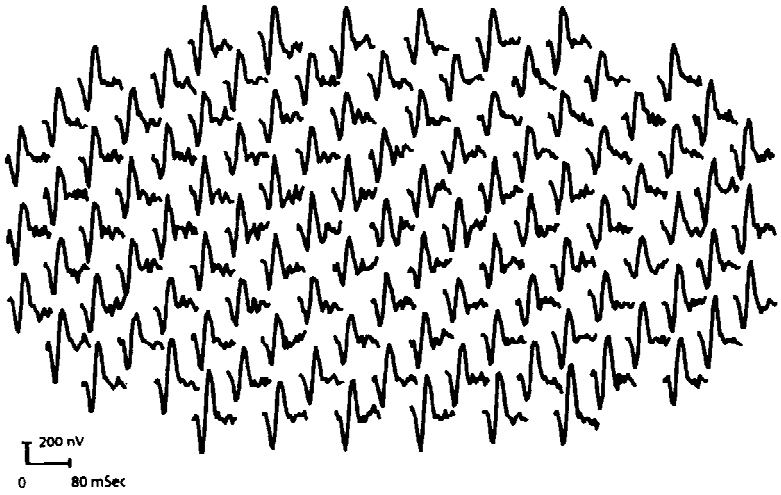


Figure 3. Sample mfERG trace arrays with 61 elements and 103 elements.

Fixation monitoring

Since good fixation is essential, fixation should be monitored in some fashion, either by direct observation of the patient or by the use of monitoring instrumentation available on some units.

Refraction

Manufacturers currently recommend refraction for optimal acuity. Lenses are typically placed in a holder positioned in front of the eye. Because lenses alter the relative magnification of the stimulus, the viewing distance must be adjusted to compensate, in accordance with the scale or guidelines provided by the manufacturer. Also care must be taken to avoid inducing a ring scotoma with a plus lens. There is some controversy about whether acuity is critical to the mfERG, at least within a range of $\pm$ 6D from emmetropia, so that some experts deem refraction unnecessary within these limits. It is not clear whether these data from normal eyes apply to all pathologic eyes since a small retinal lesion might be less defined in the mfERG if the stimulus cell boundaries were badly blurred.

Monocular vs. binocular recording
Either monocular or binocular recording is possible, but it is incumbent on those who record binocularly to be sure that signals are not altered by decentration of either eye or by asymmetric effects from the refractive or recording lenses.

Adaptation

Pre-adaptation (before test)
Subjects should be in ordinary room light for 15 min before testing, assuming no prior exposure to bright sun or fundus photography. Longer adaptation may be needed after such exposure. A previous full-field ERG with photopic recordings is acceptable as long as the exposure (especially flicker) was not unusually prolonged.

Room illumination
Room lights should be on, and ideally produce illumination at the subject close to that of the stimulus screen. A masking cone (provided by some manufacturers) can decrease stray light.

Recording sequence

Stimulus
Size. 20–30 degrees of visual angle on either side of fixation.

Number of elements. Most often 61 or 103 for routine use; 241 elements for more critical localization.

Duration of recording. Total time is typically about 4 min for 61 elements, or 8 min for 103 elements (although these times might be adjusted by experienced labs according to clinical needs). The overall recording time is divided into shorter segments (e.g. 15–30 s) so that subjects can rest between runs if necessary – and also so that a poor record (from noise, movement or other artifacts) can be discarded and run again without losing prior data. These recording times may be lengthened according to the stability of the patient and the amount of electrical interference (noise).

Choices
The choice of stimulus array and recording time is a trade-off between the stability of recording and the topographic resolution of the data. Large stimulus elements (e.g. 61) give signals with less noise, but are less sensitive to small areas of retinal dysfunction. Smaller stimulus elements (e.g. 103) will show more accurately the outline of dysfunctional areas, but require longer recording time to obtain an acceptable signal to noise ratio. Large elements with a short recording time are easier for patients and suitable for a general overview of macular function. Very small elements (such as a 241 hexagon array) may sometimes be needed for disease with small or irregular effects within the macula, or for accurate tracking of functional defects. To account for trial-to-trial variability, repeat recording is recommended to confirm small or subtle abnormalities.

Data reporting

Mode of display
Trace arrays. It is essential to show the trace array when reporting *on* the mfERG (see Figure 3). These arrays not only show topographic variations, but also demonstrate the quality of the records, which is important in judging the validity of any suspected variations from normal.

Group averages. Arranging responses by groups can be a useful way to summarize the data. Concentric rings of traces, from the center outward, are most commonly used. Regions with fundus pathology can be averaged together if desired.

Three-dimensional scalar plots. These are optional, and should be used with caution (see 'Discussion' above). Scalar plots should never be used as the sole method of display.

Measurements
Calibration marks. Must accompany all traces or graphs. It is also important for each laboratory to establish the typical range of values for the various modes of display, so that most data from the laboratory can be plotted at the same scale to facilitate comparisons among patients.

Responses. The N1 response amplitude is measured from the starting baseline to the base of the N1 trough; the P1 response amplitude is measured from the N1 trough to the P1 peak (see Figure 2). The peak latencies (implicit times) of N1 and P1 are measured from the stimulus onset. Measurements of group averages should routinely include the N1 and P1 amplitudes and peak latencies.

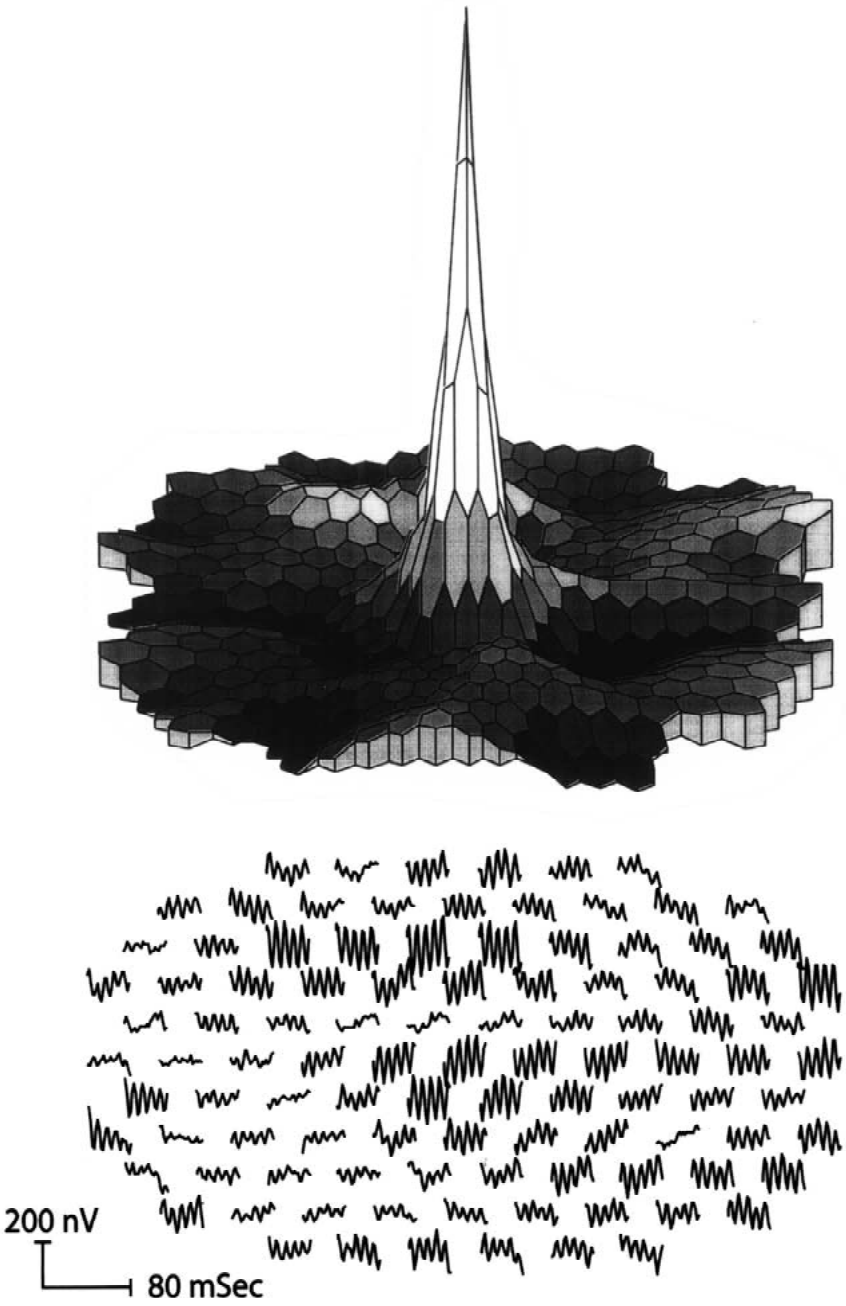


Figure A-1. Electrical noise. The trace array shows predominately 60 Hz signals, which vary in amplitude from hexagon to hexagon because the computer correlations are randomly in or out of phase. The topographic (3-D) density plot shows a misleading tall central peak which represents noise entirely, but which might be mistaken for a foveal signal if the trace array was not also displayed.

Color scales. Optional.

Normal values

Each laboratory needs to develop normative data, since variations in recording equipment and parameters makes the use of data from other sources inappropriate. Since electrophysiologic data do not necessarily follow a normal distribution about a mean, laboratories should report the median value rather than the mean, and determine 5 and 95% values as boundar-

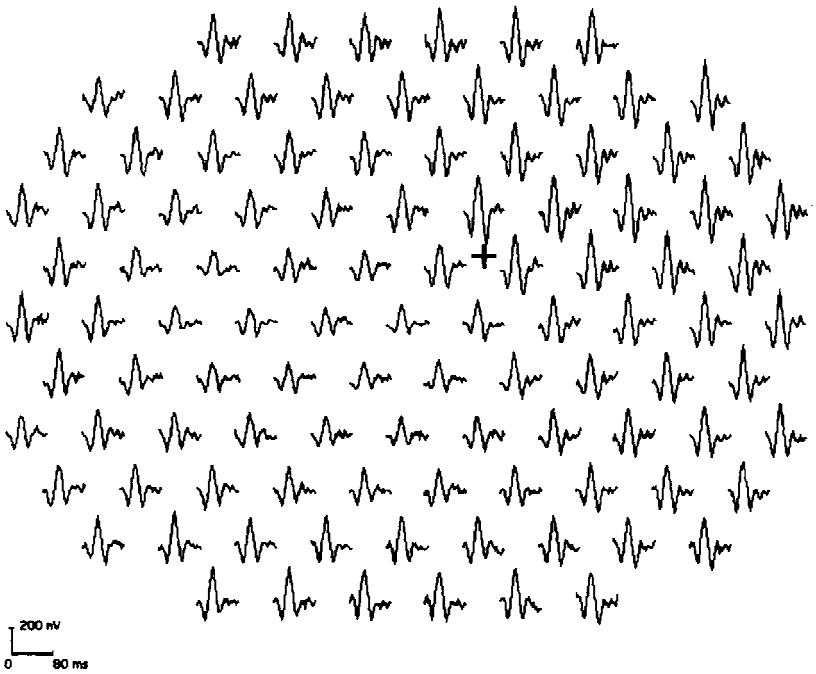


Figure A-2. Eccentric fixation. The subject fixated at the + instead of the center. As a result, the calculated response magnitudes are altered, and there is a false appearance of central retinal dysfunction.

ies of normality. The mfERG, like the full-field ERG, diminishes somewhat with age and shows lower values in myopic eyes. While these effects are generally not large they can be relevant in some patients.

Reporting of artifacts and their resolution

Reports should indicate explicitly any artifact reduction procedures or post-processing maneuvers used to prepare the data. This should include the type and number of artifact rejection steps, the averaging of results with neighbors (noting the extent and number of iterations), and any other smoothing or averaging procedures. Any unusual causes of artifact should be noted.

Acknowledgement

This document was approved by ISCEV in Montréal-Mont Orford, Canada, on June 21, 2001.

Appendix: Artifact recognition

There are a number of artifacts that can complicate the recording or interpretation of the mfERG. We list and illustrate some of the more common below, along with brief suggestions for avoidance or correction.

Common types of artifact

Electrical noise (Figure A-1)

Poor electrode contacts, poor grounding or ambient sources can cause line current (50/60 Hz) interference that alters the physiologic patterns of responses. Noise is usually evident in trace arrays but may produce topographic (3-D) plots that appear to be physiologic even when there is no retinal response. For this reason, 3-D plots are not recommended as the sole or primary means of mfERG display. Solution: Better electrode contact, grounding or shielding.

Movement errors

Inconsistent fixation and random eye movements can produce irregular signals with spikes, saturation of the amplifiers, and aberrant drifting or fluctuations in the waveforms. Milder degrees of eye movement, or unsteady fixation, cause smearing of the responses between different loci, and thus reduced resolution of small lesions. If the blind spot is not visible in a recording, this may be a clue to poor fixation. Solution: Observe the amount of noise during the recording.

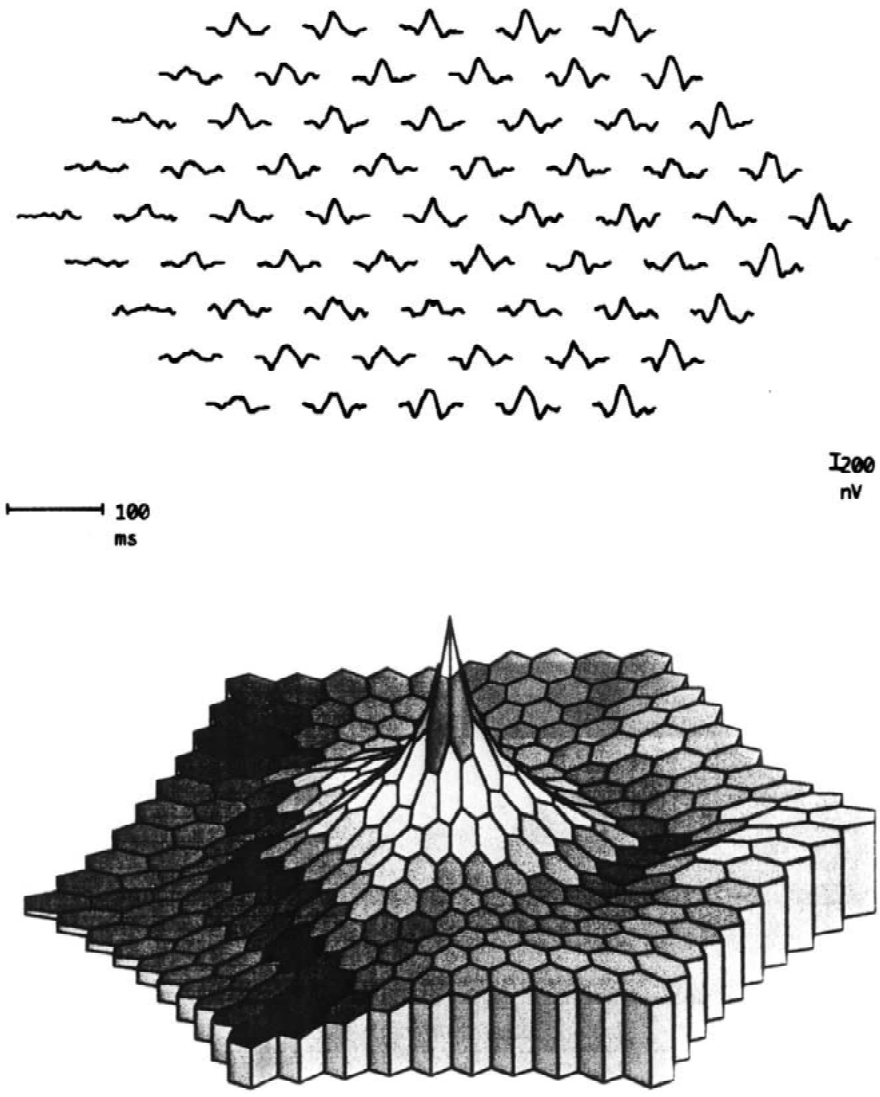


Figure A-3. Shadowing error. The subject's view was obscured on one side by the edge of a refracting lens. As a result, both the trace array and 3-D plot show a false reduction in amplitude on one side.

Contaminated runs or segments should be discarded and re-recorded. Improve fixation monitoring and fixation control.

Eccentric fixation (Figure A-2)

This can cause trace arrays and topographic scalar plots that are depressed centrally, or show a 'sloping' appearance with low signals on one side and high on the other. Solution: Check fixation, or use a special low vision target.

Orientation/shadowing errors (Figure A-3)

These appear when a subject is poorly centered or there is shadowing from the edge of either the refraction lens or the recording contact lens. The trace arrays and topographic plots show depression in one part of the array and sometimes elevation on the opposite side. These errors must be distinguished from patterns of disease, and from the small normal nasal-temporal variation. Solution: Center the lenses and subject, and monitor eye position.

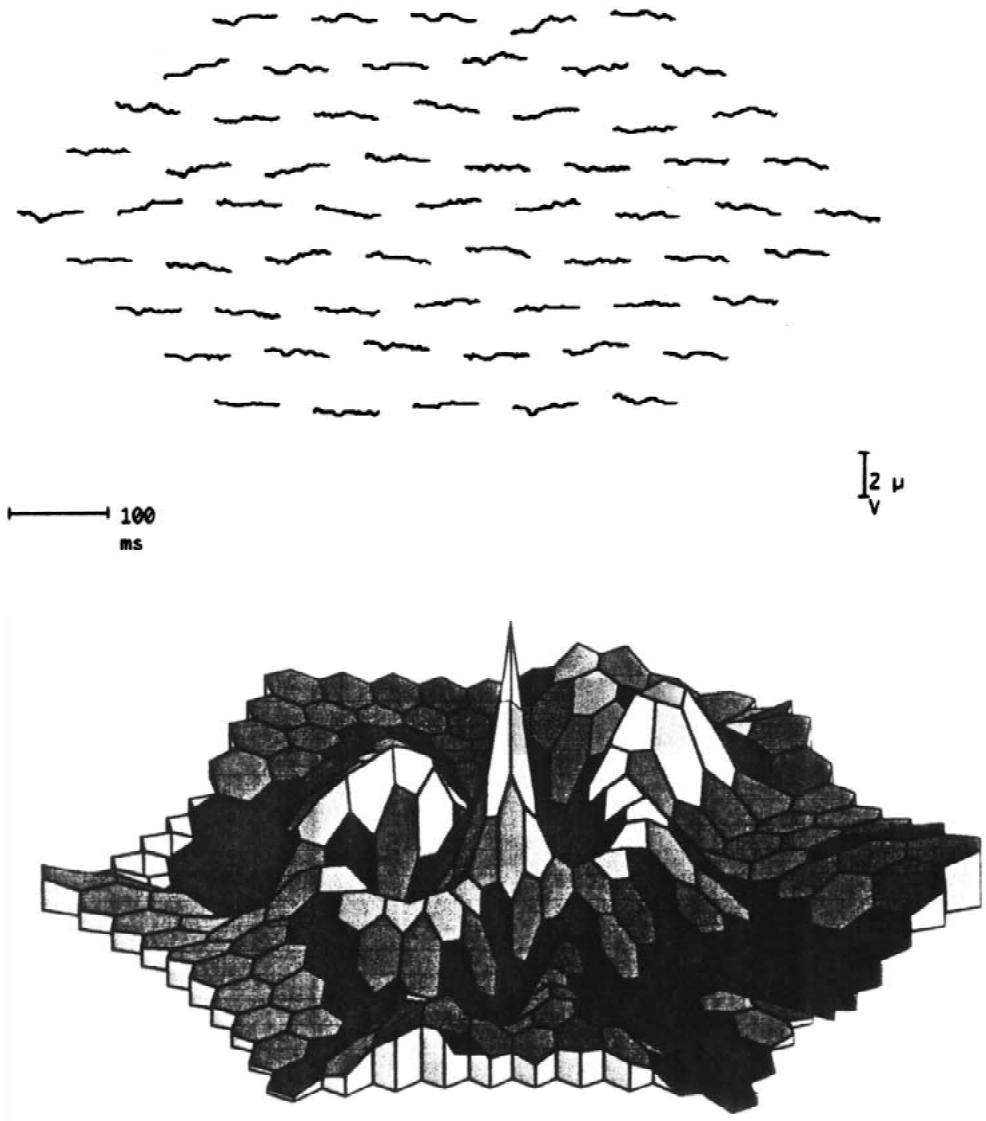


Figure A-4. Weak signals and erroneous central peak. This patient with cone dystrophy had minimal mfERG responses, as evident from the trace array. However, the 3-D plot shows an artifactual central peak that is generated by background noise (similar to the example in Figure A-1) and does not represent a physiologic signal.

Erroneous central peak (Weak signal artifact) (Figure A-4)

Artifactually large 'responses' can appear in the center of ring averages if there is an aberrant or spurious signal that would be averaged out in more peripheral areas. Scalar topographic plots often show an artifactual central peak, even when signals are weak, because they record the strength of noise as well as physiologic signals. The effects of noise are smoothed out in peripheral areas, but become amplified in the center where the overall amplitude of noise is divided by a small area. Solution: Look at the trace array to determine whether any recognizable waveform is present in areas of interest.

Averaging and smoothing artifacts

Excessive averaging or smoothing of signals can artifactually reduce spatial resolution. Severely smoothed records may not reveal small lesions, or show sharp lesion borders. Solution: Avoid unnecessary smoothing, and avoid excessive averaging with neighboring responses.

Blind spot

It is not an artifact that the blind spot is less sharply defined in the mfERG than one might expect. The optic nerve may not completely cover any one stimulus patch, so that some response is always obtained. Also, it has been hypothesized that because the nerve head reflects light more than other areas of retina, there is a response to this scatter from other parts of the retina (which is attributed in mfERG plots to the blind spot).

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