The vector voltmeter as a tool to measure electroretinogram spectral sensitivity and dark adaptation

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The vector voltmeter gives on line both phase and amplitude of the ERG (electroretinogram) response to a flickering stimulus. This makes the apparatus useful in determining spectral sensitivity functions and dark adaptation curves of man and animals, in particular since it enables automatic measurement of these functions. The limitations and applications are briefly discussed.

Key words: electroretinography, synchronous rectification, spectral sensitivity, dark adaptation, adaptometry, cone function, rod function, phase, vector voltmeter.

In electroretinography (ERG) the use of the averaging computer has become standard practice for extracting the often low responses from the background electrical noise. If, besides the response magnitude the waveform of the response is of interest to the experimenter, the averaging technique indeed is a powerful tool.

There are situations however, in which the waveform of the ERG is not of primary interest, for instance for the determination of spectral sensitivity functions, or the recovery of sensitivity after exposure to a very bright light (dark adaptation curve). The usual way to measure, e.g., spectral sensitivity, is to obtain a set of response vs. intensity curves and then, for each wavelength, to determine the intensity necessary to evoke a certain criterion response. In this particular application the averaging technique is an inefficient and time consuming way of data reduction: One measures by hand the amplitude of the waveform from the obtained records while the waveform itself is disregarded in the final sensitivity curves. Besides being time consuming the averaging technique constitutes more problems. Because of uncontrollable nonstationarities (e.g., eye movements, blinks) the accuracy is considerably limited, as the averaged response is only available off-line after, e.g., plotting on a recorder. These drawbacks cumulate if one wants to measure dark adaptation curves, as they represent a change of sensitivity in time.

Recently a so called "vector voltmeter"
has become commercially available, which operates essentially with a flickering stimulus. The apparatus selectively amplifies the stimulus-locked part of the ERG, canceling at the same time the nonstimulus-locked background noise. In this respect its action is comparable to an averaging computer, but with the enormous advantage that it gives on line, the response magnitude as a DC (direct current) voltage. This opens the possibility of adjusting the light intensity such that a criterion response is obtained, which is analogous to what is done in psychophysical sensitivity measurements. With an extension of the basic system even automatic measurement of the spectral sensitivity functions and the dark adaptation curve becomes possible.

In this paper, a short description of the operation of the vector voltmeter in ERGs will be given, together with examples of its performance in the measurement of spectral sensitivity and dark adaptation.

Methods

Short description of the vector voltmeter. The vector voltmeter uses the lock-in principle* in order to extract a repetitive signal from its background noise. In a lock-in amplifier the amplification goes up and down in synchrony with the signal of interest, which results in canceling of the nonstimulus-related parts of the signal and retaining the amplitude of the desired signal as a DC voltage at its output. This output is integrated with an adjustable time constant, which determines the effective bandwidth of the instrument: The longer the response time the better the noise-rejecting capability. The necessary synchronization of the amplifier with the input signal is achieved by means of a reference signal which is time-locked to the stimulus.

To apply the lock-in principle in electroretinography one uses a light chopper or modulator which provides both a flickering light stimulus and the reference signal (Fig. 1). The ERG response, which contains a fundamental frequency equal to the reference signal, is then fed into the lock-in amplifier. In general the phase of the ERG response will not have a fixed relationship to the phase of the stimulus, in particular because the phase of the response depends on the intensity of the stimulus. Thus, if one would use a single lock-in amplifier for ERG the phase of the reference signal had to be tuned for maximum signal output with every change in stimulus intensity. In the vector voltmeter this difficulty is overcome by the use of two lock-in amplifiers, where the phase setting of one amplifier is shifted 90 degrees with respect to that of the other amplifier. Through this arrangement the vector voltmeter is able to translate the output of the two lock-in amplifiers into two wanted variables: The full amplitude and the phase of the input ERG signal, without the need for tuning the phase.*

One property of the instrument should be mentioned here. In the above it is stated that the

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*Other terms are synchronous rectification or phase-sensitive detection.

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*In the experiments described below we used one lock-in amplifier, the Brower model 131 (Brower Laboratories Inc., Waltham, Mass.) extended with a home-built lock-in amplifier plus modules to provide the amplitude and phase output. Recently complete vector voltmeter systems have come commercially available, examples of which are the Ithaco 353 CQ system (Ithaco Inc., Ithaca, N. Y.) and the PAR model 129 (Princeton Applied Research Corp., Princeton, N. J.).
vector voltmeter measures the amplitude of a repetitive signal. This is only strictly true if the signal is a pure sine wave. If the signal deviates from a sine wave, which in ERG occurs often if one uses stimulus frequencies which are lower than 30 Hz., then the vector voltmeter measures only the fundamental Fourier component of the response (and to a much lesser extent also the third harmonic). This fundamental will generally have a lower amplitude than the peak amplitude of the response. To evaluate this effect an averaging computer may be of help in the piloting stage of the experiment.

A useful extension of the vector voltmeter is the addition of a motor-driven neutral density wedge in the stimulus path (Fig. 1). The wedge position is controlled by a servo module in which the amplitude output of the vector voltmeter is compared with a previously set criterion voltage. The difference signal is fed into the motor of the wedge. Thus, the light intensity is changed until the criterion response is reached. In order to prevent oscillations in the so constructed feedback loop, the maximum wedge speed has to be limited to a certain value. As this value depends on various factors such as density increment of the wedge and integration time of the lock-in voltmeter this maximum wedge speed was made adjustable. The wedge position, which is linear with density, is recorded on the Y axis of a Y-T plotter. As density is defined as the logarithm of the ratio, between incident light and transmitted light, the Y axis of the plotter likewise represents log sensitivity, sensitivity being inversely proportional to the energy of the light necessary for a criterion response.

**Optical equipment.** The light source was a Xenon arc (Osram XBO450W) powered from a light-current stabilized power supply (Heinzinger, stability better than 1 per cent), which provided both the stimulus and the background. The wavelength of the stimulus was controlled by a set of interference filters (Schott DAL, half bandpass 13-18 nm.). The intensities of stimulus and background were controlled by neutral density filters (Agfa, gelatin). The stimulus path contained the servo-controlled circular neutral density wedge and the rotating sectored disk which was driven by the vector voltmeter. The wedge was from Agfa, the linearity and neutrality were better than 0.15 log unit, over a total span of 2 log units. The servo-drive of the wedge was constructed at our laboratory. Stimulus and background light were combined by a beamsplitter and entered the subject's eye either in maxwellian view or, in the dark adaptation experiment, via a diffusor on the contact lens (Ganzfeld). The energy calibration occurred with a thermocouple (Hilger and Schwarz FT 17) operated with the vector voltmeter for high sensitivity. The luminance of the white light of the Ganzfeld was determined by comparing the psychophysical flicker fusion threshold at 40 Hz. with the flicker fusion threshold in the maxwellian view setup. In the latter situation the luminance was calibrated using a luxmeter in the output beam. For the spectral sensitivity experiment neutral density filters were added to each interference filter to provide an equal energy spectrum with 0.1 log unit accuracy.

**Results**

The performance of the vector voltmeter is demonstrated by a recording of the output of phase and amplitude of the ERG signal at various stimulus intensities (Fig. 2). The neutral density wedge is used here in the "open loop mode" which means that its position is not guided by
the vector voltmeter output but by manual control. The stimulus frequency was 40 Hz. At this frequency the rod response is completely suppressed. The stimulus field was 40 degrees of arc and the rise time of the integrator on the vector voltmeter was set at 1 second. A clear difference in response amplitude can be noticed for stimulus intensities which differ by only 0.1 log unit. With increasing stimulus intensity the phase lag of the response gradually decreases.

**Spectral sensitivity.** A cone spectral sensitivity curve as shown in Fig. 3 is easily obtained in the following way. The Xenon arc, combined with a set of 19 interference filters, provides the wavelength-adjustable stimulus, each interference filter being tuned for equal energy output. The wedge and light chopper further control the stimulus as indicated in Fig. 1. The chopper is set at a frequency of 40 Hz in order to isolate the cone function. The stimulus enters the subject’s eye in maxwellian view, with a field of 40° subtense. The wedge control unit was set to a criterion voltage of 3 μV. The risetime of the integrator was set at 0.1 second. If the response amplitude is higher than the criterion, the wedge is automatically driven to a less transmitting position until the criterion response is reached, and vice versa (without the wedge control unit the experimenter can perform this task manually). Every 15 seconds a new interference filter is inserted. Fig. 3 gives a recording of the wedge position at each wavelength. The horizontal scale is the time axis which represents in this case the parameter wavelength (by the regular insertion of the interference filters). The vertical scale represents the wedge setting and thus sensitivity (see before). In this way a photopic spectral sensitivity curve consisting of 19 wavelength points was obtained within 5 minutes. The photopic spectral sensitivity of the extra-foveal retina, as determined by Wald, is indicated by dots in the same figure. In this experiment the intensity necessary to reach the 3 μV criterion at 552 nm. was 630 trolands. With a lower criterion a longer integration time would be needed to get sufficient resolution and hence the complete procedure would take more time. With an equal response criterion the output of the phase module (not shown) remains constant within 15 degrees.

**Objective adaptometer.** Fig. 4 demonstrates how the system can be used to measure the dark-adaptation function. After the offset of the background (white light, 6 x 10^6 trolands), the amplitude of the ERG response to a flickering test stimulus starts to grow. As soon as the signal rises above the previously determined criterion response (1 μV for the photopic branch of the curve) the intensity of the stimulus is reduced by turning the neutral density wedge to a less light-transmitting position (which can be done manually or automatically by means of the wedge control unit). This process continues to take place during the course of the dark adaptation.
Vector voltmeter in ERG measurement

Fig. 4. Dark adaptation curve recorded with the vector voltmeter. The vertical scale gives the wedge position, calibrated as log threshold intensity. Both stimulus and adaptation were white and offered as a Ganzfeld. Preadaptation was $6 \times 10^5$ trolands during 3 minutes. The inset shows the expanded cone branch, recorded automatically with the wedge control unit. Stimulus frequency 40 Hz., response criterion 1 $\mu$V, integration time 0.1 second, wedge speed limit 0.2 density units per second. During the rod branch the stimulus was 8 Hz., the integration time was 10 seconds. As at this integration time the motor speed limit could not be set low enough to prevent oscillations, the rod branch was measured by manually controlling the wedge position in order to reach a criterion response of 2.5 $\mu$V. The cone branch was shifted about 1 log unit downwards to obtain a fit at $t = 5$ minutes. The threshold at 25 minutes approaches $8 \times 10^{-2}$ trolands.

The wedge position is recorded as a function of time and is calibrated as log threshold intensity. To show the cone-adaptation optimally we used a 40 Hz. stimulus for the first 4 minutes of dark adaptation. The automatically controlled wedge follows the rapidly changing threshold accurately, which makes it superior to manual control (Fig. 4, inset).

In the experiment of Fig. 4 we used the time interval between 4 and 5 minutes to change towards a stimulus frequency of 8 Hz., which enabled measurement of the rod response. One might want to facilitate operation by studying both rod and cone branch at the same low frequency. This is not advisable because at the transition from cone to rod branch unpredictable interactions may occur between rod and cone signals (because of different latencies the responses from the two systems may even cancel each other in this period). The threshold after 25 minutes of dark adaptation approaches $8 \times 10^{-2}$ trolands, which is about 30 times higher than the average psychophysical threshold as measured in conventional adaptometry. This discrepancy is partially caused by a residual light adaptation due to the 8 Hz. flickering stimulus, and partially because the criterion response had to be rather high (2.5 $\mu$V) in order to get an acceptable signal to noise ratio. To what extent this may influence the diagnostic power of the vector voltmeter has to be established in clinical practice.

Although the change of phase may give additional information about the process of dark adaptation we did not include this function in the figure as we are not yet sure how its course depends on the various stimulus parameters.
Discussion

The foregoing demonstrates the use of the vector-voltmeter in ERG. The use of phase vs. frequency plots in the diagnosis of certain diseases was already implied by the study of Berson, Gouras, and Hoff, and the application of the lock-in technique for these clinical problems was demonstrated by Fricker. The system may also prove its value in model studies of the ERG, where the amplitude and phase characteristics of the response to a sinusoidal-modulated stimulus are measured. We added to these applications the measurement of the sensitivity functions. Especially for the determination of the rapid time course of the cone adaptation this instrument seems to be superior to other methods.

A word of caution, however, should be added. Since only the fundamental Fourier component of the signal is measured, the waveform of the ERG is lost. At frequencies above 25 Hz. this can hardly be called a disadvantage since the waveform deviates only slightly from a sine wave. But at lower frequencies the different components of the ERG (a-wave, b-wave, etc.) may contribute in a somewhat hidden way to this fundamental Fourier component. Moreover, much of the noise due to eye movements is in the lower frequency band which necessitates the use of relatively high response criteria.

That at these low frequencies one still may extract meaningful data is already indicated by the dark adaptation curve of Fig. 4. Like in conventional adaptometers, the proportion of rod branch to cone branch will depend on many parameters. But, if one keeps frequency, wavelength, and field width at standardized values it is clear that this method will provide valuable information about the relative functioning of rods and cones. A further illustration of the use of the vector voltmeter at low frequencies is, that the spectral sensitivity recorded at 5 Hz. in dark-adapted monkeys reflects with high precision the scotopic luminosity curve. From the latter experiments appeared that in anesthetized subjects the noise problem due to eye movements is less serious so that reliable recording of rod function at a 1 μV criterion is possible.

The conclusion, therefore, is that although the vector voltmeter may give valuable information about rod sensitivity, its optimal use is in studying, at relative high frequencies, the sensitivity functions of the cone mechanisms.

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REFERENCES