Rod function evaluation by the use of synchronous detector techniques for electroretinographic analysis

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The synchronous detector technique is described for recording electroretinograms (ERG) with particular reference to separation of rod and cone function. Thus rod function is emphasized by using blue stimuli at rates of 5 to 17 cycles per second, with a darkened background. The basic principles of signal detection are the same as have been described for measurement of cone function, when white flash stimuli are used at frequencies of 17 to 60 cycles per second, with normal background illumination. The results are of markedly different character, however, with an average rod delay time which is longer (average 61 msec.) than the average cone delay time (24 msec.). The results are compared with the conventional averaged waveform ERG presentation in normal subjects and in subjects with achromatopsia and retinitis pigmentosa.

Key words: electroretinogram, synchronous detector, amplitude, phase, frequency, delay time, rod function

Conventional ERG measurements are used to give an indication of receptor cell function, and various methods have been developed for separating rod and cone function, usually by making changes in the stimulus and background illumination parameters. It is apparent that the use of bright stimuli in the light-adapted state tends to facilitate cone function, while a change to relatively low level stimuli in the dark emphasizes rod function. An additional parameter to be considered is that of the frequency of stimulation since the rods function at low stimulation frequencies, whereas mainly cone response is obtained at higher frequencies. This fact often is utilized to gain a measure of cone function by recording the “flicker” response at 30 cycles per second. It is the purpose of this paper to illustrate how a different method of measuring ERG responses utilizes these parameters in order to give direct numerical information re-
Synchronous detector techniques

Regarding rod function. The actual detection system has been described as "synchronous detection." Basically it is an adaptation of a technique which has been used for many years in other fields. The use of relatively simple equipment gives a major improvement in the signal-to-noise ratio. The signal output is a measure of the average amplitude and phase angle at a particular stimulation frequency, and the time delay is determined from the rate of change of phase with frequency, without consideration of the details of the waveform characteristics. The system also has a high immunity to interference.

Measurements of cone function have been described for subjects in a normal state of light adaptation, with white flash stimuli at frequencies from approximately 20 cycles per second up to 60 or 80 cycles per second. In order to record rod function with this technique, the background illumination is changed from normal room illumination level to a completely darkened background. Blue stimuli are used, and the stimulus frequency range is decreased to 5 to 20 cycles per second. Under these conditions, with normal subjects, a retinal delay time is obtained which is longer than that given by the previously described white flash (cone) test. In most subjects, at a frequency of approximately 15 to 20 cycles per second, there are marked changes in the amplitudes of the signals and the phase increments for given frequency increments. In an over-all sense this is an indication that the waveform characteristics of the response are changing at this point, reflecting the dominance of cone function over rod function at these higher frequencies.

Equipment and test procedure

The equipment for processing the signals is the same as that described previously, with the output obtained on an XY Plotter for direct measurements of average amplitudes and phase angles. An integration time of ten seconds is used most commonly. The subject is in his normal state of light adaptation at the beginning of the test. When possible the subject's pupils are dilated, and he is seated at two feet from a Grass PS-2 Photo Stimulator, at No. 2 setting, with a Grass Blue Filter placed over the face of the lamp. Initial adjustments and calibration are carried out under normal illumination, following which the room lights are turned off to achieve essentially total darkness and the test is commenced immediately. The period of dark adaptation may be used, but the procedure outlined above gives adequate signals and saves considerable time, a point of some clinical importance when children or restless patients are being examined. The lowest frequency of stimulation, usually 5 cycles per second, is used first and then increased in increments of 2 cycles per second up to 17 or 19 cycles per second, with a total recording time of approximately three minutes. The signals from both eyes are recorded simultaneously, with one signal being processed immediately and the other recorded on magnetic tape for later processing by the synchronous detector system. For comparison with the usual waveform presentation, some of the signals have been processed by the usual averaging techniques, using a Hewlett Packard No. 3721A in the signal recovery mode.

Results

Typical results obtained from a patient with normal rod function are shown in Fig. 1. This presentation is similar to that obtained with the use of the white flash stimulation at higher frequencies, and the same calibration level is used, namely a three-microvolt signal at 27 cycles per second, integrated for ten seconds. Phase angles obtained from the XY display are shown plotted against frequency in Fig. 2 for frequencies up to 17 cycles per second, starting at 5 cycles per second with an angle of approximately 165 degrees. (It should be noted that the amplifying system is AC coupled for stability reasons, and consequently there is a significant phase shift introduced at lower frequencies. At 5 cycles per second this amounts to 20 degrees when the signal is processed directly and to 30 degrees when the signal is recorded before being processed. At frequencies above 20 cycles per second the phase shift is negligible. Thus the actual phase angles plotted, as shown in Fig. 2, are slightly greater than the angles obtained from the XY plot.
Fig. 1. Patient R. S., No. 1021. Synchronous detector outputs for right eye retina, blue stimuli in the dark.

The same phase and frequency scales are used as for the white flash tests, in this case yielding a delay time of 60 msec. Fig. 2 also shows the results which were obtained at higher frequencies, using white flash stimuli and normal light adaptation conditions. It is evident that the phase angles define a different delay time (25 msec.) than that given by the lower frequency blue flash test.
In order to be able to compare these results with the usual ERG presentation, Fig. 3A (upper and lower) shows the average waveforms obtained by summing 128 consecutive waveforms at a repetition rate of 1 cycle per second. Fig. 3A (upper) is for the blue flash stimulus in the dark, as outlined above, whereas Fig. 3A (lower) is for a white flash stimulus in the normal light-adapted state. Note that the amplitude and time scales are the same for these two figures. The signals recorded during the actual Synchronous Detector test also have been processed to yield the average waveforms, and these are shown for frequencies of 5, 9, 13, and 17 cycles per second in Fig. 3B. Note that the amplitude scales are different than in Fig. 3A (upper and lower), but the time scale is the same. It is evident that the waveform changes significantly at the higher frequencies, and this is reflected in Fig. 2 by the change in slope of the phase-frequency curve at 15 to 17 cycles per second.

In general both rod and cone function may contribute to the usual ERG presentation, and as various authors have emphasized,2-4,6,7 it is desirable to be able to separate these functions. The waveform shown in Fig. 3A (lower), for the white flash stimuli in the light-adapted state, evidently is quite different from that in Fig. 3A (upper) for the blue flash stimuli in the dark. The differences between these waveforms may be described in terms of both amplitude and timing. It is possible that the cones provide some contribution to the response at lower frequencies with the blue stimuli in the dark, even though it is most unlikely that the rods contribute significantly at the higher frequencies in the light-adapted state with normal background illumination. Patients with well-documented achromatopsia provide useful information here, as described by other authors.11,12 The following results were obtained from two identical twin girls, age 11, with a well-established diagnosis of achromatopsia. Their clinical history was the usual one of nystagmus, photophobia, poor vision in the light (20/200 under normal illumination), essentially normal scotopic vision, absent color vision, and a normal fundus appearance. The family history as far as it was known was entirely unremarkable. These children were tested with the synchronous detector system as described above, and in addition conventional ERGs were obtained at a stimulation rate of 1 cycle per second and averaged for 128 stimuli. The results of these tests were very similar for each child, apart from minor variations in amplitudes of the signals. Responses obtained from one child are shown in Fig. 4A for blue flash stimuli in the dark, while the signals obtained with white flash stimulation in the light are shown in Fig. 4B. The signals due to the white flash stimuli are very small, and their phase angles are best seen as the signals actually are produced on the XY plot, rather than from the composite plot shown in Fig. 4B. Because of their low signal-to-noise ratio the angles are not well defined, but their average value can be approximated, and these values are plotted versus frequency in Fig. 5 within the range of 180 to 200 degrees. This gives the approximately horizontal phase-frequency plot shown, indicating in fact a minimum delay time whose actual value of course is not precisely defined due to the low signal-to-noise ratio. It is clear from Fig. 5 that the signals from the blue flash stimuli define a delay time which is markedly different than is given by the higher frequency white flash results. The ERG waveforms obtained from each of the twin girls also were very similar, with the waveforms for one of the children being shown in Fig. 6 (upper and lower). Fig. 6 (lower) shows the signals obtained with blue flash stimuli in the dark, while Fig. 6 (upper) illustrates the change which occurs when white flash stimuli are used with the room lights on and the eye in its normal light-adapted state. Clearly the results are quite different in the upper and lower por-
Fig. 3A. Upper, Patient R. S., No. 1021. Averaged ERG responses (128 sweeps), blue stimuli in the dark. Vertical scale 100 microvolts, horizontal scale 10 msec. Lower, Patient R. S., No. 1021. Averaged ERG responses (128 sweeps), white stimuli in the light. Vertical scale 100 microvolts, horizontal scale 10 msec.

Contrary to the upper figures, with no detectable signal present by this means in the light-adapted state for the white flash stimuli. This corresponds to the very small signals (with a flat phase-frequency curve) obtained with the synchronous detector, and the results can be regarded as illustrating the loss of the main bulk of the ERG response for white stimuli in the light. The small signals indicated by the synchronous detector technique are due to electrical responses.
occurring very soon after the flash. Until they are more precisely defined they should be regarded as a combination of small electrical responses and possible artifacts. The results provide a clear example of the manner in which relatively normal rod function can be measured in the presence of grossly abnormal cone function. The children were aware that a light was flashing at the lower frequencies for the blue flash test in the dark, and with the higher frequency white flashes in the light they were aware that a light was on but could not describe it precisely.

Normal subjects

In order to establish the range of clinically normal subjects with this method, a similar procedure was followed as in the case of the white flash (cone) tests. The
Fig. 4A. Patient L. C., No. 990. Synchronous detector output for left retina, blue stimuli in the dark.

Fig. 4B. Patient L. C., No. 990. Synchronous detector output for left retina, white stimuli in the light.
subjects used had normal rod function and over-all normal eye function. Some of the patients had two normal eyes, whereas others were being evaluated for problems in one eye, but with the second eye described unequivocally as normal. It was not possible to obtain dark adaptation curves on most of these patients, but if there was any question at all about difficulty with night vision the patient was not accepted in the group. Fig. 7A shows the amplitude distribution at a frequency of 9 cycles per second, obtained from tests with 101 normal eyes. The delay times shown in Fig. 7B have an average value of 60.7 msec, while the standard deviation is 9.6 msec. The range of these delay times is larger than that in the case of the white flash (cone) delays, in which the average delay time was approximately 24 msec, with a standard deviation of approximately 2 msec. There has been no gross indication of any significant variation in delay time with age, but the numbers tested so far have not allowed a significant test for this possibility.

Abnormal results

Patients with well-documented retinitis pigmentosa are appropriate for demonstrating gross abnormalities in rod response. The cone response for such patients has been described as showing two straight line segments for the phase-frequency plot, with the lower frequency portion indicating an increased delay time, with respect to the normal average of 24 msec, while the higher frequency portion indicates a decreased delay time. Because of the frequency range concerned, the higher frequency portion often is the only one measured satisfactorily with white flash stimulation parameters. In these cases such patients have been described as having an abnormally low delay time, but it must be remembered that this refers to cone function. Associated with this is a reduced visual field. The situation with the blue flash stimulation in the dark has certain similarities, modified by the different parameters involved. In normal subjects, if the frequency range for the blue flash stimulation in the dark is continued beyond approximately 17 cycles per sec-
ond, the nature of the response changes significantly as the rod function diminishes at the higher frequencies, and presumably some cone response becomes apparent. The phase-frequency curve undergoes a change in slope and usually begins to approximate that obtained with the white flash test in most cases. In patients with moderately abnormal rod function the upper frequencies at which decreased responses are obtained with blue flash stimulation is often approximately 10 to 12 cycles per second. Above this frequency the signals become small and irregular. The rod response is decreasing at these higher frequencies, while the cones are operating under non-optimum conditions of stimulus and background illumination.

Fig. 6. Upper, Patient L. C., No. 990. Averaged ERG responses (128 sweeps), white stimuli in the light. Vertical scale 10 microvolts, horizontal scale 100 msec. Lower, Patient L. C., No. 990. Averaged ERG responses (128 sweeps), blue stimuli in the dark. Vertical scale 40 microvolts, horizontal scale 100 msec.
An increased delay time often is indicated at the lower frequency ranges, approximately 5 to 10 cycles per second, while for the higher frequencies either the time delay is not well defined or it has a smaller value. When the abnormality is more marked, the signal-to-noise ratio is such that the accuracy with which the delay time can be specified is markedly reduced, consequently it becomes difficult to give an accurate assessment of the delay time at these lower frequencies.

Examples of the signals obtained from a patient with moderately advanced retinitis pigmentosa are shown in Fig. 8A. This patient was Case 1 in a previous report, a 16-year-old boy with a significant family history of retinitis pigmentosa. There was little pigmentation present in the fundi, but the arterioles were slightly thin. Fields were constricted in normal illumination, and dark adaptation curves had been reported as typical for retinitis pigmentosa of medium severity. He displayed abnormal cone function on testing, consistent with his field difficulties. The phase-frequency plot of the rod function test is shown in Fig. 8B, with points which are scattered but which indicate a longer delay time for the lower frequencies and then a reduced delay time for the higher frequencies. Fig. 8C shows the waveform obtained by averaging 60 sweeps (100 msec, base-line duration) at stimulus frequency of 11 cycles per second.

**Discussion**

In general, similar remarks may be made about this application of the synchronous detector technique as were made for cone function testing with white flash stimulation in the light-adapted state. The signal outputs are the averaged results of many individual responses, without individual responses being considered in detail. The delay time is obtained in an indirect manner, without reference to specific points on a waveform. In the presence of media opacities there is obviously some question regarding the color and intensity of transmitted stimulus light, but the most important parameter is the frequency of stimulation, which of course is invariant. Differentiation of rod and cone
response is obtained by the use of different stimulation rates, colors and intensities, and background illumination. When discussing the results obtained with the white flash stimulation test it was pointed out that the phase information, and consequently the derived time delay information, was not critically dependent upon the amplitudes of the signals, and therefore significant data could be obtained even with opaque media. The same considerations apply to the rod function test.
Similar remarks apply when there is some lack of cooperation on the part of the patient, for example when testing children. Under these circumstances the amplitudes of the signals depend to a large extent upon the child's behavior, and unless one wishes to introduce the hazards of anesthesia the results must be interpreted with some caution when amplitude is regarded as a primary factor.

When the usual waveform presentation is used to give timing information one must be careful how the implicit time is defined, namely to which specific point on the waveform the time delay is measured. This in turn implies that such a specific point on the waveform is well defined, which unfortunately is not the case when the signal-to-noise ratio is low and the signal waveform has a long duration compared with the delay time. Consequently if relatively small changes in implicit times are regarded as significant, this can only be valid statistically when the signal-to-noise ratio is high, and even then the shape of the response introduces a large degree of uncertainty. These points sometimes appear to be overlooked, particularly when other information indicates that signals should be present. In cases in which the signal and the noise statistics are known in a mathematical sense, it is possible to derive formulas relating the signal-to-noise ratio to the probable errors in various parameters of the signal. Obviously this is not possible with these electrophysiologic signals, but the same basic principles apply. The synchronous detector system has some advantages here, as the signal-to-noise ratio is increased in a
manner which is close to the optimum. The phase angles are average values, and obtaining the delay from the phase-frequency curve introduces more averaging. Even with this the scatter of points about the straight line approximation to the phase-frequency curve is an indication of the effect of a finite signal-to-noise ratio, as well as indicating possible changes in the time characteristics of the signal and of the noise.

The relationship between the usual waveform output and the synchronous detector output may be described mathematically, as for the case of the cone function tests. In effect one is dealing with a Fourier series representation of a repetitive waveform, with the output depending upon the size and shape of the waveform. It should be recalled that the waveshape at stimulation rates of 5 to 15 cycles per second is not necessarily the same as that obtained at 1 cycle per second. Changes taking place in the waveform with increasing frequency are represented in the synchronous detector output by changes in the signal amplitudes and relative phases measured at these frequencies. This is illustrated by the average waveforms in Figs. 3A (upper) and 3B, where there is evident similarity of the waveforms at 1, 5, and 9 cycles per second, but at 13 and 17 cycles per second the waveform is changing. It is a measure of this change which is given automatically by the synchronous detector system.

Patients with achromatopsia illustrate that in these particular cases results are obtained for the rod test parameters which appear to be the same as those obtained from subjects with normal retinas. Although it is not known absolutely what residual cone function (if any) may be present in achromatopsia, the usual clinical tests indicate that it must have been minimal to zero in the two patients discussed. Hence the similarity of their rod responses to those obtained from normal subjects is a strong indication that mainly rod function is being tested in normal subjects. Just how much cone function is included when normal subjects are tested is debatable. By decreasing the intensity of the stimulus it should be possible to effectively eliminate any cone response, while maintaining some rod response, provided of course that such differences are observable in the output. Tests have been carried out with a wide variety of patients, some of whom have had their pupils dilated and others not, others who have had opaque media of varying degrees. In these patients, when good grounds existed for thinking that their rod function was normal, the results fell within the normal limits given above. Hence changes of stimulus intensity must have a relatively limited effect on the actual responses obtained.

If the stimulus frequency is increased beyond 17 to 20 cycles per second, some subjects give outputs whose phase-frequency curves bend over and approximate the slope of the curve obtained with the usual cone function test. Obviously the rod response is dropping out and the cone response is becoming evident in such subjects. When the transition is made smoothly there must obviously be a region in which both rod and cone responses are present. In other normal subjects the transition is made evident by a marked decrease in signal amplitude, and a more irregular type of signal output. It is not clear what distinguishes these two broad groups of normal subjects.

Patients with retinitis pigmentosa provide examples of abnormal rod response, usually with signals of diminished amplitude and an abnormal phase-frequency curve. Their cone response usually is affected too, and can range from mildly to grossly abnormal. Rod function tests in such patients usually show abnormal signals regardless of the state of their cone function. Thus in these patients any cone response to the rod test must in general be at levels which do not affect the rod response in any significant manner.

This method of measuring rod response
can be regarded as complimentary to its use for recording cone response. As such it offers an alternative to the usual ERG methods, with some advantages and disadvantages as discussed above. The derivation and use of the average rod time delay as a function of frequency has no direct parallel in the conventional ERG measurements. Routine single-flash ERG techniques obviously have limitations when the signal-to-noise ratio is low. The conventional waveform averaging techniques work well in general, but at the expense of considerably greater equipment complexity and susceptibility to interference. The ability of the synchronous detector system to work with moderate degrees of interference is often a significant clinical advantage.

Questions may arise as to whether signal averaging methods need to be used at all for these measurements, particularly in clinical situations. Two main points deserve to be emphasized here. When the signal is relatively small compared with the noise, some type of averaging is needed in order to distinguish the signal from the noise in a relatively unambiguous manner, and in order to make meaningful measurements of the signal parameters. In addition, whatever the signal-to-noise ratio is, successive responses may differ from each other, and the process of averaging involves a statistical recognition of the existence of these differences.

The authors wish to thank Dr. David G. Cogan for his continued support and advice during the course of this work.

REFERENCES