Rod and cone contributions to the off-effect of the human ERG

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From previous research, it has been unclear as to whether the off-effect is a purely cone response. Spectral sensitivity was measured here both in dark and light adaptation with a response-averaging computer. A large stimulus field and a slow frequency of flickering light stimuli with equal light and dark times were used. Curves of measured amplitudes of both on- and off-effects against stimulus luminance were plotted for each of 12 test wavelengths. The b-wave rapidly and the off-effect only slowly increased in amplitude with stimulus luminance. In dark adaptation, the spectral sensitivity (amplitude criterion) for both b-wave and off-effect was predominantly scotopic (rod-dominated). It was found that spectral sensitivity curves for the off-effect could also be based on a constant latency criterion. This spectral sensitivity also was scotopic. In light adaptation (2 FL) spectral sensitivity based on an amplitude criterion was a fair fit to the parafoveal cone sensitivity curve (Wald) for both b-wave and off-effect. It was concluded that rods mainly contributed to the off-effect in dark adaptation, while in the light-adapted condition cones became dominant.

The correlation between the off-effect and cone activity in flicker stimulation of the human ERG was shown by Dodt,1 and by Bornschein and Schubert.2 Further work by Best and Bohnen3 showed that the off-effect was minimal or absent after dark adaptation of the eye in their recordings. Thus it was thought by some investigators that the off-effect was a purely photopic or cone activity. On the other hand, an off-effect has been obtained by repetitive stimulation of the eye in the absence of a light adaptation field.4,5 In order to determine whether both cones and rods could contribute to the human off-effect, the present experiment was undertaken to measure the spectral sensitivity of the off-effect in dark and light adaptation. Only brief attempts previously have been made to stimulate the off-effect with colored lights.6-7 In both experiments, red light gave larger responses than blue light. Because of the very small magnitude of the human off-effect in comparison to the much larger on-response (a-wave, b-wave, etc.), a response-averaging computer technique has been employed in the present experiment.

Method

Apparatus. The apparatus was similar to that previously described.8 The subject was positioned inside a lighttight electrically shielded room, through the wall of which protruded the visual stimulating system. A 900 W D.C. xenon arc lamp operated from a well-regulated power supply was the source for the test flashes. The flashes were presented in Maxwellian view to the eye with a circular field of 28 degrees' visual angle. Balzer interference filters provided narrow wave-length bands of chromatic test stimulation while variations in luminance were obtained with Wrat-
ten neutral density filters. Calibration of the source energy transmitted through each interference filter was obtained from a Farrand thermocouple. In calculation of the spectral sensitivity, appropriate correction was made for each wavelength of the small deviations from neutrality of the Wratten filters in use.

Square-wave test flashes were delivered at the rate of one cycle per 1.26 seconds with equal on and off times obtained from a rotating sector disc shutter. Amplification of the electroretinograms (ERG) was by capacitance-coupled amplifiers, band pass 0.8-160 c.p.s., and the responses were processed by an average-response computer (CAT). The computer sweep was of 1 second duration and triggered by the light offset to include one off- and one on-effect in each sweep. The responses to 50 flashes were summed for each stimulation condition. Permanent records of the computer output were obtained from an x-y plotter.

Full-field light adaptation was obtained with a large white cardboard screen 2 inches before the subject's eyes. The screen was illuminated with white light from small D.C. tungsten lamps. The hole in the screen through which the test beam was delivered was also light-adapted by means of a beam splitter reflecting light from an illuminated milk glass surface. Light adaptation was at the level of 2 FL as calibrated with a Macbeth Illuminometer. For the dark-adapted condition the adaptation field was not illuminated. The subject's position was maintained with a biteboard and forehead rest. A thin glass plate in front of the final lens reflected a small fixation light at the center of the test area.

**Procedure.** For the dark-adapted runs, the subject spent 15 minutes inside the dark recording room before stimuli were presented. As the subject was stimulated with repetitive illumination for each test series, he was in a relatively constant state of adaptation short of complete dark adaptation. Visual inspection on a monitor oscilloscope of the ERG's from the higher luminance test flashes showed no changes in amplitude over the time course of the computer summations. For the light-adapted runs, 3 minutes of light adaptation on the biteboard were provided before stimulation was presented. For each of the test wavelengths, stimuli were administered at luminance levels differing in 0.3 or 0.4 neutral density. An interval of 1 minute between the test stimuli was used to keep adaptation constant.

The primary data of this experiment were gathered on 2 females, ages 20 and 21. Supplementary data were obtained from 2 additional female sub-

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**Fig. 1.** Off-effects under dark adaptation (DA) and light adaptation (LA). Each record begins with termination of stimulus light. Neutral filtering is given by number at left, wavelength 555 nanometers. Calibration is in microvolts and milliseconds.

**Fig. 2.** Amplitudes of dark-adapted b-wave (upper) and off-effect (lower) for two wavelengths, 595 (×) and 495 nanometers (•). Abscissa is neutral filtering in test beam. Note the larger amplitudes of the b-wave (different ordinate scale).
jects, ages 24 and 26. Each subject wore a Biggs contact lens electrode on the right eye for recording. The other active electrode was attached to the opposite cheek over the bone and the ground electrode was attached to the right mastoid.

**Results**

In Fig. 1 are presented examples of off-effects at various test luminance levels under both levels of adaptation. The waveform of the responses under these experimental conditions was primarily single-peaked although there is a suggestion of double positive peaks at the middle luminance levels. Where it was possible to measure two separate peaks, their peak latencies occurred near 20 to 25 and 40 to 50 milliseconds. There was little or no change in waveform of the off-effect with test wavelength.

The peak latencies of the off-effects under dark adaptation ranged from 80 milliseconds at dim test luminances to 30 milliseconds at high luminances for this subject, while the second subject varied from about 60 to 30 milliseconds. Light adaptation reduced the amplitudes and shortened the peak latencies of the responses from those in dark adaptation. The latencies for both subjects varied from 50 to 25 milliseconds in light adaptation.

The off-effect amplitudes were measured from the immediately preceding baseline while the amplitude of the on-response was measured from negative peak to positive peak. Fig. 2 illustrates amplitudes of off-effect and b-wave (on-effect) at two wavelengths in the dark-adapted condition. The b-wave rapidly increased in amplitude with increased stimulus luminance while the off-effect amplitude increased very slowly over the same range. The off-effect is relatively more responsive to long wavelengths than the b-wave.

From luminance amplitude curves like those of Fig. 2, the luminance required to produce a constant amplitude response at each wavelength was derived. The resulting luminance value was corrected for the spectral transmission of the neutral filters and the energy of the source to obtain the sensitivity for each wavelength. The spectral sensitivity curves thus obtained are illustrated in Fig. 3 for 2 subjects in dark adaptation. For the first subject (L) a 5 μV off-effect and a 10 μV b-wave are approximately equally sensitive. The same amplitude criterion for the second subject (M) produces an equally sensitive b-wave. On the other hand, the same criterion off-effect results in a sensitivity about 2 log units less sensitive for the second subject. (All curves in the figures are plotted at their obtained ordinate positions.) The three more sensitive curves all approximately fit the modified CIE scotopic sensitivity curve, evidencing rod response, although Subject L's sensitivity in the red is somewhat higher for her off-effect than for the b-wave. The off-effect for Subject M, however, is fitted by an appreciable component of photopic activity as well as scotopic response. This increased photopic responsiveness is consistent with the average test flash luminance required for Subject M's off-effect being nearly 2 log units higher. When a higher amplitude off-effect spectral sensitivity curve was plotted on Subject L (10 μV), it matched Subject M's 5 μV off-effect closely both in average height and in relatively increased photopic sensitivity. One limitation of the average response technique is the fact that repetitive test stimulation is required, keeping the eye above a state of complete dark adaptation.

Supplementary dark-adapted data were gathered on two other subjects. The average difference in threshold in the shorter wavelengths between a 5 μV off-effect and a 10 μV b-wave was 1 log unit for one subject and 2 log units for the other. In both cases the spectral sensitivity for the off-effect was primarily scotopic with some increased sensitivity in the red region.

In Fig. 4 are illustrated spectral sensitivity curves for Subjects L and M at the 2 FL light adaptation. For Subject L, a 10
Fig. 3. Dark-adapted spectral sensitivity curves for Subjects L (●) and M (▲). Solid lines are 5 μv off-effects and dashed lines are 10 μv b-waves. Ordinate positions of curves represent obtained relative sensitivities. Smooth line is CIE scotopic sensitivity.

Fig. 4. Light adapted spectral sensitivity curves for Subjects L (●) and M (▲). Solid lines are 7 μv off-effects and dashed lines are 10 μv b-waves. Smooth line is peripheral cone sensitivity.10
μV b-wave is about equally sensitive as a 7 μV off-effect in this condition. A 10 μV b-wave for Subject M also falls at about the same level. The comparable off-effect (7 μV) for Subject M is again less sensitive, as was the case in dark adaptation, although for light adaptation the difference is reduced to about one log unit. The shape of all four spectral sensitivity curves is comparable to that for parafoveal cone sensitivity (Wald) although all curves are slightly too high at the longer wavelengths.

For one subject (M), the two positive off-effects were measured separately under the light-adapted condition, and spectral sensitivity curves derived for each of the positive peaks. Both spectral sensitivity curves were very similar and both were a fair fit for the Wald parafoveal cone curve. There was a tendency, however, for the second peak to be less sensitive to the longer wavelengths at high criteria, and more sensitive to the shorter wavelengths at low criteria.

It was found that spectral sensitivity curves for the off-effect could also be based on the curves of peak latency vs. luminance (neutral density filtering) by taking a criterion latency. This criterion was corrected for the neutral filtering used and the energy of the source to provide a "sensitivity" value at each wavelength. Because of electrical noise and the broad peak of the response, in some cases alternation between two peaks of the off-effect,

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**Fig. 5.** Spectral sensitivity based upon a 45 milliseconds’ peak latency off-effect. Average of two subjects (L and M). Smooth line is CIE scotopic sensitivity. Note the greater sensitivity (shorter latency) of the light-adapted curve (LA) over the dark-adapted (DA).
these curves were more variable than spectral sensitivity based on an amplitude criterion. Both Subjects L and M, however, showed similar shifts between dark and light adaptation, and in Fig. 5 these spectral sensitivity curves are averaged for the two subjects. In dark adaptation, the latency-criterion spectral sensitivity is a fair fit for the CIE scotopic sensitivity curve, as was true for the amplitude spectral sensitivity for Subject L. Although Subject M showed a mixed photopic-scotopic curve for the amplitude criterion, she showed a relatively clear fit to the scotopic function alone for the latency criterion.

Light adaptation shortened the latency of the off-effect and required less stimulus energy to produce the same latency than a dark adaptation. Fig. 5 illustrates this increased “sensitivity” of the off-effect (based on latency) by a higher curve in light adaptation. The shape of the light-adapted curve shows an enhancement in the longer wavelengths and can be fitted by a mixture of rod and cone functions. This differs from the shape of the amplitude-criterion spectral sensitivity for light adaptation where a pure photopic function was found to fit the data.

**Discussion**

In dark adaptation, the off-effect appears primarily a rod function. Although there were differences between subjects on the degree of rod functioning involved, spectral sensitivity based on one or both of an amplitude or latency criterion showed a relatively pure match to the CIE scotopic sensitivity curve. Some subjects requiring more light energy to reach the amplitude criterion produced a mixed scotopic-photopic amplitude curve.

In light adaptation, the off-effect became primarily a cone function, as based on a fit to the Wald parafoveal cone sensitivity curve. This curve has been previously found to fit ERG spectral sensitivity curves derived from stimulation of large retinal areas. Cone functioning was based on a fit of one or both amplitude and latency criterion spectral sensitivity curves to the Wald curve. At this moderate level of light adaptation (2 FL) there was not pure cone functioning as judged from the latency spectral curve, but still a contribution from rod functioning.

Only slight evidence under the present experimental conditions was found for the concept that the spectral sensitivities of the two peaks of the off-effect differ significantly. There was a slight tendency for the second peak to be more blue-sensitive and the first peak to be more red-sensitive in light adaptation, although both peaks approximately fitted the Wald cone curve. Close temporal proximity of the two peaks causes summation of the separate sensitivities so that amplitude measures of each of the peaks represent a mixture of sensitivities (as is the case with the x- and b-waves on the on-effect). A better separation of the sensitivity of the two peaks might be achieved by basing spectral sensitivity on a latency criterion, although the present data do not suggest a clear separation on this basis either. Heck has suggested that the two peaks of the off-effect represent cones and cone-like rods, respectively. The present data represent but slight support for this concept in that the first peak has a somewhat more conelike spectral sensitivity while the second peak is somewhat more rodlike. The effects of light and dark adaptation suggest that cones and rods of the usual type (duplicity theory) contribute to the off-effect. In dark adaptation the off-effect has a mainly rod spectral sensitivity, while in light adaptation the off-effect changes to primarily cone functioning.

The author wishes to thank Harold F. Lawson for many hours spent in the gathering and analysis of the data.

**REFERENCES**