Retinal Sensitivity as Measured by Flicker Suppression

Flickering electroretinograms evoked by trains of intermittent stimuli were used to measure retinal sensitivity in midbrain-lesioned cats. Flicker suppression (defined as the decrease in the amplitude of the flickering b-wave relative to the amplitude of the first b-wave in the train) is an exponential function of the dark time separating the pulses in the train when the parametric manipulation is limited to dark time alone. The time constant of the function is increased when the light pulse duration of the intermittent stimulus is increased. If the light pulse duration of the intermittent stimulus is altered together with the dark time, however, flicker suppression is not easily described by a simple exponential function.

Among the various experimental methods which have been used to measure retinal recovery following prior stimulation there has been the amplitude of the flickering b-wave evoked by trains of brief light pulses. In rod-dominated eyes, the b-wave of the electroretinogram (ERG) evoked by the first pulse in a train is larger than the b-waves evoked by subsequent pulses (flicker suppression).

Arden et al. considered flicker suppression to be an index of retinal sensitivity and quantified it in terms of the ratio of the amplitude of the flickering b-wave to the amplitude of the b-wave evoked by the first pulse in the train. They concluded, on the basis of their experiments on decerebrate cats, that flicker suppression is an exponential function of the dark time separating the pulses in the train, with a time constant (the time at which 63% recovery occurred) of about 300 msec.

These authors also concluded that flicker suppression is primarily dependent upon the dark time separating the pulses in an intermittent stimulus and that increasing light pulse duration from 16 to 500 msec has no effect upon b-wave recovery. They arrived at this conclusion by comparing the amplitude of flickering b-waves evoked by light pulses of different durations. As a result of this finding, they concluded that the decrement in and recovery of the flickering b-wave is not related to the bleaching and regeneration of rhodopsin, but rather to

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some neural set of events (neural adaptation).

Elenius studied retinal recovery in humans by measuring the ratio of the b-wave evoked by the second member of a pair of pulses to the b-wave evoked by the first member of the pair and concluded that this ratio, plotted as a function of the dark time, is also exponential, with a time constant of 400-500 msec. In contraduction to the conclusions of Arden et al, Elenius found that changes in light pulse duration lengthen b-wave recovery in the human ERG. Hamasaki and Bridges also concluded on the basis of their experiments on the elasmobranch that b-wave recovery after prior stimulation is affected by pulse duration.

Elenius also investigated flicker suppression by using an intermittent stimulus with a constant light to dark ratio (LDR) of 1:5. The results of this investigation contradict the conclusions of Arden et al in yet another respect. Flicker suppression (the ratio of the flickering b-wave to the b-wave evoked by the first pulse in the train), plotted as a function of dark time, deviates from a straight line and, therefore, cannot be described as an exponential function. Elenius attributed this finding to the fact that with a constant LDR, every change in dark interval is accompanied by a change in light duration, resulting in a complex interaction of light duration and dark interval.

In summary, the form of the function relating flicker suppression to the dark interval of an intermittent stimulus and the determination of whether light duration affects flicker suppression remain as contested issues. Furthermore, as Elenius implies, these issues may be resolved by using intermittent stimuli whose light pulse durations are altered independently of dark interval. The experiments presented in this paper were designed as a preliminary investigation of these questions.

**Method**

The light stimuli were generated by a fluorescent tube (Sylvania model F14T12-CWX) driven by 500-v DC pulses. The duration of the light pulses and the dark time separating the pulses were controlled by Tektronix equipment. Luminance was varied by inserting metallic filters (Tiffen Photar) in the light path.

One end of a randomized fiber optic bundle was fixed in the light path of the fluorescent tube (after filtering) while the other end was fitted into the back of a plastic hemisphere (cup) fitted snugly over the cornea. The corneal cup served as an electrode holder and as a diffusing plate to produce a "ganzfeld" stimulus. This arrangement provided a relatively homogeneous stimulus field.

Light pulse durations, relative pulse luminance, and dark intervals were calibrated and monitored by a phototube (RCA model 929), the amplified output of which was displayed on an oscilloscope.

The luminance of the unfiltered light transmitted through the corneal cup was measured with a photometer (Spectra Pritchard) with a 1962 P.D. Spectra lens and found to be 13.4 millilamberts.

Midbrain-lesioned cats were used as subjects to facilitate comparison with reported data from unanesthetized animals. Initial injection of sodium methohexitol prepared the animal for stereotaxic placement. Six electrodes positioned at the stereotaxic coordinates, F + 2, H - 5.5, and extending laterally 4.5 mm on each side of the midline (for the total range of 9 mm) were used to lesion the midbrain. Topical application of 2% cyclopentolate hydrochloride was used to obtain maximal pupillary dilation.

The ERGs were recorded by means of a platinum-chloride electrode fixed into the side of the plastic corneal cup. Contact with the electrode was assured by a saline bath provided by a tube attached to the corneal cup. The indifferent electrode was inserted into the skull. The ERG was amplified by a low level preamplifier (Tektronix type 122) with a bandwidth of 0.2-1000 cycles. The output was amplified by a differential amplifier, viewed on the oscilloscope, and fed into a (Mnemotron) Computer of Average Transients (CAT). The number of ERGs averaged for a given stimulus condition depended upon the noise level of the recording and ranged from 5 to 20. The averaged ERG was written out by an x-y recorder.
The electrodes were attached to the corneal cup approximately 1-1½ hr after the midbrain lesion was made. The preparation was then dark-adapted for 30 min before recording began. Every subsequent filter change was followed by 15 min of dark adaptation. Intertrial intervals were 20 sec for single stimuli and 1 min for trains of flickering lights. The duration of a train of flickering lights was 5 sec. Flickering b-wave amplitude was determined by averaging across the flickering waves from the fourth to the last b-wave evoked by the intermittent light stimulus.

Results

Input-Output Function. Since midbrain-lesioned cats are not usually used in studies of electroretinography, it may be of interest to illustrate the effect of altering light intensity on the ERG amplitude. Fig 1A is an illustration of an input-output function for one dark-adapted animal, relating b-wave amplitude (measured from the trough of the corneal negative, the a-wave, to the peak of the corneal positive) to the log luminance of a 20-msec pulse. Maximum luminance, marked as 0.0 on the abscissa, is 13.4 millilamberts. b-Wave amplitude increases approximately 500 µv over a luminance range of 3.0 log units. Fig 1B illustrates the relationship of b-wave latency to log luminance. Latency decreases approximately 30 msec over a luminance range of 3.0 log units. Both b-wave amplitude and latency data for the midbrain-lesioned cat are quite similar to those reported for the decerebrate cat.14

Flicker Suppression. Flicker suppression was measured in terms of the ratio of the amplitude of the flickering b-wave (b,) to the amplitude of the b-wave evoked by the first pulse in the train (b). If b/b,
is exponentially related to the dark interval \( t \), then:

\[
\frac{b_t}{b_{t_0}} = 1 - ce^{-a\tau}
\]

\[
\log (1 - \frac{b_t}{b_{t_0}}) = K - at
\]

where \( \log c = K \). Therefore, when \( \log (1 - \frac{b_t}{b_{t_0}}) \) is plotted as a function of the dark interval \( t \) the function should be linear with a negative slope.

Fig 2 is an illustration of such flicker suppression data for three stimulation conditions for one animal. The luminance of the intermittent light pulses was 1.34 millilamberts. Curve A, a linear function with a time constant of 200 msec, represents data for the condition in which the duration of the light pulse was 20 msec at all dark intervals. Curve B represents data for the condition in which the duration of light pulse was 50 msec at all dark intervals. Curve B is also a linear function, having a slope which is less steep than curve A and a time constant of 250 msec. Curve C represents data for the condition in which the light pulse duration was altered together with dark time so as to produce a constant LDR of 1:4 (for example, a train of 20-msec light pulses separated by 80-msec dark intervals and a train of 100-msec light pulses separated by 400-msec dark intervals). The shortest light pulse duration was 16 msec; the longest light pulse duration was 160 msec. Note that curve C, which appears to have the same intercept as curve A, crosses curve B at a dark interval of 200 msec and continues to the right of curve B as light duration and dark interval increase.

In summary, these data indicate that flicker suppression, defined as the ratio \( \frac{b_t}{b_{t_0}} \), resembles an exponential function of dark interval if the light duration is unaltered as dark interval is increased. When light pulse duration is altered together with dark interval, so as to maintain LDR constant, the resulting function is complex—not as easily described by a simple exponential function.

**Discussion**

The effect of altering light pulse duration on the flickering electroretinogram was studied by comparing flicker suppression functions evoked by intermittent stimuli having different light pulse durations rather than by a direct comparison of the amplitude of the flickering b-waves. Several comments may be in order concerning the preference for such a technique.

A direct comparison of the amplitude of flickering b-waves evoked by different light pulse durations may be difficult to interpret for the following reasons. Increasing the duration of the light period of an intermittent stimulus may in fact be
equivalent to manipulating two stimulus parameters which lead to opposing effects. For several years, it has been known that increasing the duration of a single light pulse up to some limit leads to an increase in the amplitude of the b-wave, both in the dark-adapted and in the light-adapted retina.  

Increasing the light energy within each pulse should therefore lead to b-waves of larger amplitude evoked by each pulse. However, increasing the duration of the immediately preceding light pulses and, thus, the total preceding light flux on the retina should lead to decreased sensitivity and to smaller b-waves. The opposition of these two mechanisms, one integrating light energy within each pulse leading to larger b-waves, one integrating the total energy over the preceding light pulses and, by the process of light adaptation, reducing the gain of the system and thus leading to smaller b-waves may interact in a complex manner making it quite difficult to interpret the measure of flickering b-wave “amplitude.” This problem may be avoided if the amplitude of the flickering b-waves evoked by intermittent stimuli having different light pulse durations are not compared directly but rather with reference to the b-wave evoked by the first pulse in each respective train (a comparison of b/b₀, flicker suppression). The tendency of an increase in light pulse duration to increase b-wave amplitude will thus be cancelled since each b₀ is referred to a b₀ which is evoked by a light pulse of equivalent duration. Therefore, a comparison of such ratios obtained from intermittent stimuli having the same dark intervals but different light durations may be the preferred way of examining the effect of pulse duration on retinal sensitivity.

When such comparisons are made, the results reported in this paper indicate that altering light pulse durations independently of dark interval does affect flicker suppression. These data thus support Elenius’ claim that light duration interacts with dark interval in determining the slope and the time constant of flicker suppression. This fact is further emphasized by the finding that, when light pulse duration is altered together with dark interval, flicker suppression cannot be described by a simple exponential function of dark interval (Fig 2).

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