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Sensitivity and response kinetics alter during suppression-recovery in cone photoreceptors[1]

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Summary. Threshold and response amplitude of cone photoreceptors were measured during progression from flicker-induced suppression to recovery. Increases in sensitivity closely paralleled increases in amplitude. Recovered responses exhibited faster kinetics than suppressed responses. The idea that recovery from suppression is a manifestation of the shift in the stimulus-response relationship which occurs with light adaptation is supported.

Key words. Bullfrog; light adaptation; flicker; electroretinogram; photoreceptors.

The visual system of at least some vertebrates undergoes a somewhat peculiar form of light adaptation when the retina is stimulated by a light flickering at a frequency less than the fusion frequency. Thus, the *b*-wave of the electroretinogram (the electrical deflection due probably to activity of the retinal Müller cells[3,4]) elicited by the second light flash of a train of flashes is considerably smaller than that elicited by the first flash, but responses to subsequent flashes show an exponential recovery of amplitude to some steady level. This suppression-recovery effect[5], as it has come to be known, has been demonstrated in the electroretinogram of several different species including frog[6,7], turtle[7], cat[6,8-11], dog[7], guinea pig[7] and man[7]. A similar pattern of events has been observed in recordings from the optic tract of the cat[5,11-13]. The phenomenon as measured in the *b*-wave or along the optic tract undoubtedly reflects alterations in the activity of the photoreceptors in response to a flickering stimulus. Indeed, in a study on the superfused, isolated bullfrog retina, Owen and Sillman showed that the mass photoreceptor response is suppressed immediately after the initial stimulus, and then increases to a stable plateau value with continued flashing[14]. The recovered response was shown to be due to cone photoreceptors[14].

Intuitively, it would seem likely that recovery from suppression is a reflection of the shift in the cone stimulus-response curve, which is known to occur during light adaptation[15,16]. However, no experiments have been reported which test this contention. This paper provides support for the idea that recovery from suppression represents the dynamics of light adaptation in cones by showing that the criterion sensitivity increases as response amplitude increases, and that the kinetics of the individual responses change in such a way that the speed of the response becomes faster.

Materials and methods. The procedures used in this study have been described in detail elsewhere[14,17,18]. A bullfrog was dark-adapted overnight and then sacrificed by decapitation followed immediately by double pithing. The retina was dissected out, separated from its pigment epithelium, and then mounted in fixed position in a glass-enclosed, sealed chamber. There the retina was superfused with a Ringer solution consisting of 100.0 mM NaCl, 2.0 mM KCl, 0.4 mM $MgCl_2$, 0.4 mM $CaCl_2$, 5.0 mM glucose, and 20.0 mM Tris buffer (pH 7.8) in double distilled water. Sodium aspartate (10.0 mM) was also present to suppress the activity of bipolar and horizontal cells and thereby effectively isolate the photoreceptor response to light[19-21]. The mass photoreceptor response – the sum of the electrical activity of all photoreceptors – was recorded transretinally by two annular silver-silver chloride electrodes in the recording chamber. The signal was then amplified without filtration and displayed on a Nicolet Explorer III digital oscilloscope for immediate analysis and for recording on a floppy disk. Simultaneous film records were made by a Grass kymograph camera from a Tektronix Model 5112 oscilloscope. The flickering stimulus was, in all cases, a square, 28-msec pulse of white light derived from a tungsten-halogen lamp calibrated to deliver an unattenuated intensity of 47 $\mu W/cm^2$ to the retina. The test stimulus, derived from a second tungsten-halogen source, was also a 28-msec square pulse of white light, but was calibrated to deliver an unattenuated intensity of 4700 $\mu W/cm^2$ to the retina. This light source could be attenuated in steps of 0.1 log units by calibrated neutral density filters.

In each experiment, data collection began 60 min after dissection, allowing the retina to come to a steady state in total darkness. For a given trial, an initial pair of 47 $\mu W/cm^2$ flashes separated by 10 sec was administered. Previous studies have shown that the first stimulus of the pair elicits responses from both rod and cone photoreceptors, but the second stimulus generates a pure and maximal cone response[17,18]. This is so because the rods remain completely unresponsive for about 25 sec following the first flash, whereas the cones return to entirely normal responsiveness within 6 sec[17].

The maximal cone response determined by this two-flash technique then served as the basis for our criterion threshold during flicker. Thus, threshold was defined as that light intensity required to elicit a cone response equal in amplitude to 10% that of the maximal cone response due to the second of the initial pair of flashes. Following the initial pair of flashes the retina was allowed to dark adapt for 3 min, after which time a series of 28-msec flashes was delivered from the flicker light source at 1.75 Hz. The first flash elicited a large response, presumably again containing both rod and cone contributions[14]. For the purpose of these experiments, this response was not germane and was therefore ignored. The next stimulus in the flickering train elicited the first suppressed response. The flicker was terminated after 1, 5, 10, 15, 20 or 50 flashes, and a test flash of variable intensity administered within 1 sec. If the amplitude of the response to the test flash did not equal the predetermined criterion value, then the entire process was repeated 10 min later using a different test flash intensity. Thus the use of the maximal cone response amplitude from the initial flash pair of each trial allowed the determination of a criterion value for that trial, and eliminated the influence of decay in the isolated retina over time. Furthermore, the order of the trials was varied at random to confound any possible effect that unregenerated photoproducts might have on the sensitivity. Separate experiments were done for analysis of response kinetics. The initial (suppressed) and final (recovered) responses of a 71 response suppression-recov-

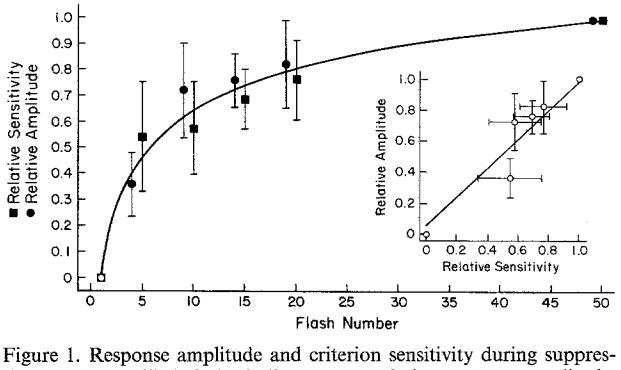

Figure 1. Response amplitude and criterion sensitivity during suppression-recovery. Filled circles indicate mean relative response amplitude, while filled squares indicate mean relative sensitivity for 5 experiments. Error bars show standard deviations. Curve is eye fit. Inset: Circles indicate the mean relative amplitude as a function of the mean relative sensitivity. Error bars indicate standard deviations for both variables. Linear regression line is $y = 0.922 \times +0.061$, and has a correlation coefficient of 0.864.

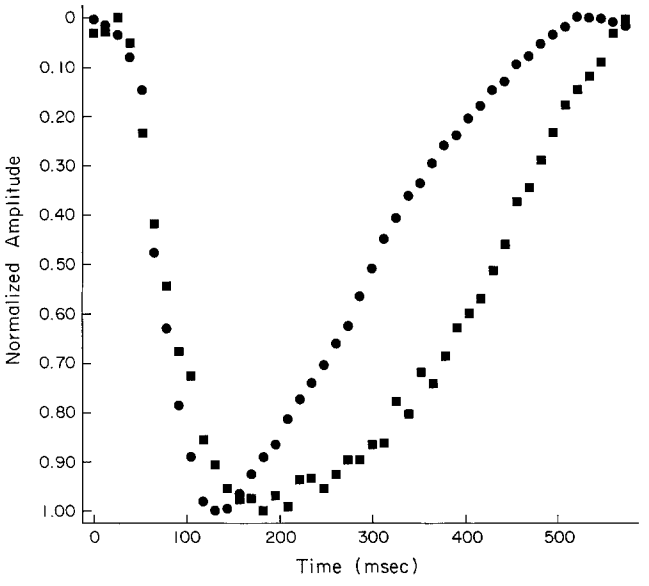

Figure 2. Kinetics of typical suppressed and recovered responses. Each response was sampled at 13-msec intervals and normalized so that the peak amplitudes were equal. Squares show the shape of the suppressed response; circles, the recovered response. The suppressed response peaked 169 msec after stimulation; recovered response, 130 msec after. The suppressed response decayed by 50% 260 msec after the peak; recovered response, 169 msec after. Qualitatively similar results were found for 6 other experiments.

ery series were manually digitized from enlarged film records using a digitizer connected to a microcomputer. The responses were then normalized, plotted, and the time to peak and rate of response onset and decay determined.

Results. The results of 5 criterion threshold/amplitude experiments are shown in figure 1. In this figure, both the sensitivity and the amplitude have been arbitrarily set to 0.0 for the initial suppressed response, and to 1.0 for the last response in order to facilitate comparison between the shapes of the respective curves. All other amplitudes and sensitivities then fell within these limits. The filled squares represent the mean relative sensitivity, and filled circles represent the mean relative response amplitude. In both cases the error bars represent standard deviations. It is clear from these data that both sensitivity and amplitude increased in tandem with successive flashes. Indeed, both sets of points could be adequately fitted with a single eye-fitted curve. When amplitude was plotted against sensitivity (fig. 1, inset), the relationship was described by a single linear relationship with a correlation coefficient of 0.86. Considering that response amplitude was measured on the response just prior to the one for which sensitivity was measured, the correlation was quite good.

When a suppressed response and a recovered response were normalized to the same peak amplitude and plotted together as in figure 2, there were clear differences in the response kinetics. In figure 2, squares represent a typical suppressed response whose waveform was sampled at approximately 13-msec intervals. Circles represent a typical recovered response which was sampled in the same fashion. Sampling started with the onset of the stimulus and ended with the onset of the next stimulus. In this typical experiment, the suppressed response peaked after about 169 msec while the recovered response peaked after about 130 msec. The rate of decay was also much faster in the recovered response than in the suppressed response, 169 msec and 260 msec being the respective half-times of decay. Qualitatively similar results were obtained in 6 other trials.

Discussion. After absorbing a single flash of the same intensity as that used in these experiments (47 $\mu W/cm^2$), a cone photoreceptor does not completely recover its ability to respond fully until 6 sec after stimulation[17]. Because the frequency of stimulation used to elicit the suppression-recovery phenomenon (1.75 Hz) does not allow sufficient time for the completion of rapid dark adaptation, the cones must perforce undergo light adaptation. During light adaptation, the cone's stimulus-response curve shifts toward higher light intensities[15,16], and an increase in the speed of the response occurs[20]. Alterations both in sensitivity and in response kinetics occur during recovery from suppression, and are consistent with what would be expected from light adapting cones. Both observations support the idea that recovery from suppression is a manifestation of the progressive light adaptation of the cone photoreceptors. The suppression-recovery phenomenon, therefore, represents a method by which the responsiveness of cones can be measured during the course of light adaptation. Individual responses can be viewed as being elicited by oscillations of light intensity around a background, that is, as responses to successive increments and decrements of light. It is possible, then, to examine the process of light adaptation, rather than merely its end result.

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