Rod Multifocal Electroretinograms in Mice

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PURPOSE. To test the feasibility of recording rod multifocal electroretinograms (ERGs) from the mouse eye.

METHODS. Multifocal ERGs were recorded from normal mice (C57BL/6J) using an array of equal-sized hexagons. Local stimuli were blue (W47A), and the number of blank frames between successive flashes at the same location was fixed at 14 (minimum 200 msec between flashes). Flash and surround intensity, and the number of hexagons, were varied to optimize the stimulus conditions for the mouse, and alterations in adaptation level were used to assess cone intrusion. Local response isolation was evaluated by comparing multifocal responses to full-field ERGs and by mapping local defects in laser-treated mice.

RESULTS. Rod multifocal ERGs, although small, were clearly recordable and well formed under many conditions. Decreasing flash intensity or the size of stimulus elements, and/or increasing the surround intensity or adaptation level, decreased local response amplitudes. At the dimmest flash intensity (−0.70 log scotopic trolands [scot td]/s) and the smallest stimulus element (2.9° × 3.5°), local responses were nondetectable. Comparisons with full-field ERGs supported the hypothesis that the local responses were not contaminated by contributions from dark-adapted retinal areas surrounding the multifocal display. With sufficiently bright (0.30 log scot td-s) and relatively large (5.6° × 6.9°) stimulus elements, multifocal responses clearly revealed local retinal defects created with laser treatment.

CONCLUSIONS. Rod multifocal ERGs can be recorded from the mouse eye to provide topographical maps of retinal function that have sufficient spatial resolution to be of practical use. The technique will be useful in characterizing the natural history of regional loss in mouse models of human retinal disease and in evaluating some forms of interventional therapy. (Invest Ophthalmol Vis Sci. 1999; 40:2848–2858)

A technique for recording multifocal electroretinograms (ERGs) has been described by Sutter and his colleagues. In the multifocal recording technique, small areas of the retina are stimulated simultaneously, and local contributions to a massed electrical potential are extracted from a continuously recorded ERG. Under photopic conditions, the local waveforms appear biphasic, with negative and positive deflections that appear to have the same origins as the a- and b- waves of the full-field ERG.

The power of the multifocal recording technique lies in the ability to detect local retinal abnormalities that would not be detected by conventional full-field ERGs, which reflect the massed response of the retina. For example, full-field cone ERGs from patients with macular disease are generally within normal limits. However, the multifocal ERG can reveal localized abnormalities by mapping regions in which ERG amplitudes are reduced. As a result, the multifocal ERG has become an increasingly popular research tool to investigate local cone-mediated function in a variety of retinal disorders.

Recently, the multifocal stimulus was modified to assess the feasibility of obtaining reliable rod-mediated responses in humans. Under conditions that increased the probability of detection by rods (e.g., blue flashes of moderate intensity with a surround and a slowed presentation rate), rod multifocal ERGs could be recorded, but the local responses were relatively small and noisy compared with those from cones. Nevertheless, with optimal recording conditions, the rod multifocal ERGs were sufficiently localized to be clinically useful, as demonstrated with recordings from a patient with retinitis pigmentosa.

In this study, we assessed the feasibility of extending the multifocal recording technique to the study of rod-mediated function in the mouse. The motivation for this application was twofold. First, the mouse is used extensively to model human retinal disease, and an objective measure of the topography of retinal function in the mouse will be useful in understanding the natural history of some forms of retinal degeneration and the mechanism of action of specific gene mutations. Second, a more sensitive tool is needed in the mouse to demonstrate recovery of retinal function after interventional therapies. For example, a recent study has shown that delivery of the β-subunit of cGMP phosphodiesterase (PDE) via a novel gene delivery system, known as encapsulated adenovirus minichromosomes, allows rescue of the rd1 phenotype. However, the rescued regions are currently relatively small. Conventional full-field ERGs have not demonstrated convincing evidence of functional recovery because as a massed response the ERG averages the rescued area with much larger areas of nonfunc-
tional retina. In contrast, a multifocal ERG would have the potential to detect and evaluate the function in local regions of the retina.

METHODS

The experiments described here were carried out on 14- to 16-week-old C57BL/6j mice weighing 23 to 25 grams. Mice were dark-adapted overnight and anesthetized before testing with an intraperitoneal injection of normal saline solution containing ketamine (15 mg/g body weight) and xylazine (7 mg/g body weight). The right eye of each animal was dilated (0.5% cyclopentolate hydrochloride and 2.5% phenylephrine hydrochloride) to a maximum diameter of 2.0 mm. Animals were placed on a heated water pad maintained at a constant temperature of 38°C throughout the experiment. All experimental procedures were carried out in compliance with the guidelines on animal experimentation set forth by the National Institutes of Health and the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.

Recording Technique

Multifocal ERGs were recorded using a gold loop electrode placed on the corneal surface at the limbus and referenced to a gold wire in the mouth. When fixed in place the gold loop minimized eye movements and held the eyelids open. The corneal surface was anesthetized with a drop of proparacaine hydrochloride (0.5%) solution. A needle electrode inserted into the tail served as ground. A drop of hydroxypropyl methylcellulose (2.5%) was placed on the corneal surface to ensure corneal integrity. Signals were sampled every 0.833 msec (1,200 Hz) with the A/D board supplied with the VERIS system (Electro-Diagnostics Imaging, San Mateo, CA). Low- and high-frequency cutoffs were set at 1 and 300 Hz, respectively.

Each mouse was secured to a moveable platform positioned in front of the stimulus monitor at a distance of 33.0 cm. The display screen, including the surround, measured 38.0 cm horizontally and 29.0 cm vertically, giving an angular subtense of approximately 60° by 47° (see Fig. 1A). The array of hexagons, with a radius of approximately 15.0 cm, subtended 50° horizontally and 47° vertically. The head of the mouse was adjusted to align the mouse’s line of sight with the center of the display screen. Although we cannot specify precisely the image location on the retina, securing the head and body of the anesthetized mouse to the platform ensured stability of the retinal image during a test session. (Attempts were made to back-project local retinal landmarks, such as the optic disc, onto a screen to more precisely identify where the mouse was looking and what region of the retina was being stimulated. However, it was not practical to locate and map retinal landmarks during a recording session because the light required to back-project the retina would have compromised dark-adaptation. Other techniques to specify image location are currently being investigated.)

Focusing lenses were not used. Several studies have shown that the depth of field for the mouse eye is roughly 10 D and that the refractive error of the rat eye, and likely the mouse eye, is believed to be emmetropic or slightly myopic.16–18 A 10-D depth of field in an emmetropic eye results in clear retinal images for objects placed from 10 cm to infinity from the eye. Because our monitor was placed at 33 cm from the mouse eye, a clear image of the stimulus was produced on the retina.

In many of the experiments to be described, repeated measures were obtained on the same mouse. Unless stated otherwise, the mouse was exposed only once to the conditions of a particular experiment and only one m-sequence was run for each condition. A testing session was discontinued if movement of the eye, head, or body was detected, usually as the mouse emerged from anesthesia, which occurred infrequently. An experiment was replicated with a different mouse that was given additional anesthetic when necessary to ensure immobility throughout the entire course of an experiment.

Multifocal Stimulus

The multifocal stimulus consisted of an array of hexagons displayed on a high-resolution video monitor (Nortech Imaging Systems, Plymouth, MN). For most of the experiments described in this article, the stimulus was an array of 61 equal-sized hexagons (see Fig. 1A). Each hexagon in the array was luminance-modulated according to a binary m-sequence, and the same m-sequence was used to modulate all elements of the display. Local responses were computed as the cross-correlation between the m-sequence and a continuously recorded massed ERG (see Refs. 1 and 2 for more detailed explanation). A blue filter (Kodak W47A; \( \lambda_{\text{max}} = 470 \text{ nm} \), half bandwidth = 55 nm), placed 2.0 cm in front of the mouse eye, was used to generate an array of blue flashes. Flash intensity was varied with neutral density filters sandwiched with the blue filter.

All stimuli were calibrated with a Radiometer/Photometer System (model 550; EG & G, Gamma Scientific, San Diego, CA) and are expressed in scotopic trolands (td). To measure the intensity of the multifocal flashes, a single hexagon was enlarged to cover the entire multifocal display area. The detector (silicone multipoole with flat radiometric filter) was placed at the plane of the mouse pupil, and the total radiant energy (\( F_v \); in watts) falling on the detector’s surface was measured through the combination of blue and neutral density filters. Measurements were integrated over time. The luminous flux (\( F_v \), scotopic lumens) was obtained from the following relation:

\[
F_v (\text{scotopic lumens}) = K_m V'_{\lambda = 470 \text{ nm}} F_v (\text{watts}) = 1180 F_v (\text{watts})
\]

where \( K_m = 1745 \) scotopic lumens/W and \( V'_{\lambda = 470 \text{ mm}} = 0.676 \). Scotopic luminance (\( L_v \)) was given by the relation shown below:

\[
L_v (\text{scotopic cd/m}^2) = F_v (\text{scotopic lumens})/2\pi A_{\text{detector}} (\text{m}^2)
\]

and retinal illuminance (in trolands) was calculated using the method of Wyszecki and Stiles19 assuming a pupil of 2.0 mm diameter. The same technique was used to calibrate the full-field stimuli (described below). (Although the meaning of the troland is the same in the mouse and human eyes, the effective retinal illuminance of a particular stimulus will be significantly higher in the mouse for the same pupil area because of the smaller size of the mouse eye. Measures of retinal illuminance that are given have not been adjusted for this difference. For a detailed discussion of this issue, the reader is referred to Ref. 20.)
Figure 1. (A) Spatial configuration of the multifocal display. At a distance of 33.0 cm, the display screen, including the surround, measured 38.0 cm horizontally and 29.0 cm vertically, giving an angular subtense of approximately $60^\circ \times 47^\circ$. The array of hexagons, with a radius of approximately 15.0 cm, subtended $50^\circ$ horizontally and $47^\circ$ vertically. The standard display was composed of 61 hexagons, each $5.6^\circ \times 6.9^\circ$, with blue (W47A) flashes set to 0.30 log scot td/s. Intervening blank frames and the surround were set to $-2.14$ log scot td and $-0.53$ log scot td, respectively. The number of blank frames between successive blue flashes of the m-sequence was fixed at 14. (B) Rod multifocal ERGs from a representative normal mouse (C57BL/6J) using the standard display. Similar responses were obtained from two additional normal mice.
The rate of stimulation was slowed by introducing blank frames between successive flashes in the m-sequence. For all the experiments described in this article, the number of blank frames was fixed at 14. For a frame rate of 75 Hz, the time between two successive blue flashes at the same location was a minimum of 199.5 msec (15 × 13.3 msec). The m-sequence length was $2^{12} - 1$ and required 13 minutes and 39 seconds to complete. Each m-sequence was divided into four overlapping segments, each 3 minutes and 40 seconds in duration, so that the mouse status and position could be evaluated. Whenever possible, the m-sequence was replicated to ensure reliability. Data collection and analysis were performed by the VERIS software.

Independent variations were made in adaptation level, flash and surround intensity, and angular size of stimulus elements. These various conditions will be described below.

Full-field ERGs
Full-field ERGs were obtained using the same blue filter (W47A) and a range of flash intensities. Except for the shift to full-field stimulation, the recording technique was identical with that for the multifocal ERGs.

Laser Application
To test whether regional defects could be detected with the multifocal ERG, confluent laser spots could be placed in two normal C57BL/6J mice. Pupils were dilated with 1% topical atropine. Using the indirect Argon laser delivery system, one mouse had a total of 21 laser spots placed in two rows around the optic nerve with power settings of 150 mW and 0.1 s duration. A second mouse had 44 laser spots distributed in pockets throughout the retina. The intent was to simulate a heterogeneous retina with multiple local defects.

RESULTS
A representative array of multifocal ERGs recorded to blue flashes is shown in Figure 1B. For this experiment, flash intensity was set to 0.30 log scot td/s. Blank frames between successive flashes were set to −2.14 log scot td, and the surround of the multifocal display measured −0.53 log scot td. Hereafter, this will be referred to as the standard condition. (For all the experimental conditions described in this article, a replication of the standard condition was included to allow comparisons between conditions.) The multifocal responses are approximately uniform across the field except for the lower right where responses are smaller and less well-defined, likely the result of minor clipping of the pupil by the loop electrode. Implicit times of individual multifocal responses are on the order of 95 to 100 msec with response amplitudes of 1.0 to 1.5 µV. Similar responses were obtained from two additional normal mice (not shown).

Cone Intrusion
Because only 3% to 5% of mouse photoreceptors are cones, we assumed that cones would contribute minimally to a local response to blue flashes. Nevertheless, an important control condition is to test for cone intrusion. In humans, the contribution from long-wavelength sensitive cones can be evaluated by comparing the responses to blue and red flashes. Although this technique works well with human subjects, it cannot work with the mouse because the mouse lacks a long-wavelength-sensitive cone. In the mouse, the spectral sensitivity of rods and their longer-wavelength cone are virtually identical.

To explore the contribution from mouse cones, multifocal responses were recorded using the standard display (described above) under different levels of light adaptation. These conditions were designed to differentially suppress the contribution from rods. Adaptation level was modified by exposing the mouse to different ambient light levels in the experimental chamber. Measured from the viewing position, the different illumination levels produced equivalent retinal illuminance of approximately 0.17, 1.64, and 1.97 log scot td, respectively. Five minutes of preadaptation was allowed to adjust to each of the lighting conditions.

Multifocal responses obtained from a single mouse are shown in Figure 2. Shown are the summed multifocal responses (the sum of 61 individual local responses) recorded in the dark-adapted state (solid line) and for three light-adapted conditions. With increasing light adaptation, the summed multifocal responses are smaller and have slightly shortened implicit times. At the highest level of ambient illumination, which was just sufficient to saturate rods, multifocal responses were nondetectable. This pattern of results supports the hypothesis that the relatively large responses to blue flashes in the dark are rod-dominated.

In the following experiments, stimulus variations were introduced to assess the optimal conditions for local response isolation and to evaluate the contributions from stray light.

Flash Intensity
At relatively high retinal illuminances, stray light may contaminate local rod responses by including a response from the surround and/or adjacent local regions. Thus, one method of increasing the probability that responses are locally determined is to decrease flash intensity. The summed multifocal responses for the standard condition and the two lower flash intensities are shown in Figure 3 for a normal mouse. In general, multifocal responses were smaller and noisier with reduced flash intensity. At the lowest intensity (−0.70 log scot td/s), local responses were poorly defined in many locations, although when summed together a clear signal was identifiable. Local responses at the next higher intensity level were significantly better but were still relatively noisy, although spatial averaging improved the appearance of the signals. With only a single replication of the m-sequence, as in this experiment, the best array of local responses was generated by the conditions of the standard display. (It is possible, however, that better signal-to-noise ratios can be obtained even at the dimmest flash intensities with longer m-sequences, replications of m-sequences, or both.) A similar pattern of results was observed in a second normal mouse.

Surround Intensity
Stray light stimulating dark-adapted retina surrounding the multifocal display can produce a sizable response that may obscure a local response. In this experiment, surround intensity was varied to examine this potential source of contamination. Surround intensity was varied over four levels covering a range of approximately 2.44 log units. Except for the change in surround, conditions were as in the standard display described above.
The summed multifocal responses recorded from the same mouse are shown in Figure 4. With increasing surround intensity, the summed multifocal response decreased in amplitude, and implicit times were shortened, especially for the highest surround intensity (0.30 log scot td). Again, although the summed responses gave well-defined waveforms at all
surround intensities, individual local responses for the two highest intensities were not well formed and difficult to distinguish from noise.

These results can be interpreted to mean that stray light stimulating surrounding dark-adapted retina contributes significantly to a local response and that this source of contamination can be suppressed with the addition of a surround. However, this experiment cannot be interpreted unambiguously, because the addition of a steady surround increasingly light-adapted the mouse retina. As shown by the data of Figure 2, light-adapting the mouse retina to progressively higher levels produces smaller and slightly faster responses. Thus, in this experiment, we could not separate the effects of an increase in overall adaptation level from the effects of stray light stimulating the surround. In the following experiment, we examined the contribution from global stray light and local response isolation while avoiding changes in overall adaptation level.

Hexagon Size

Isolating a local retinal landmark, such as the optic nerve depression, in the multifocal response array, would be a powerful test of local response isolation. In the response arrays obtained to the standard display (see for example Fig. 1B), evidence of an optic nerve head depression is not seen. There are a number of possible explanations for this. First, the position of the stimulus on the retina is not precisely known, and it is possible that the optic nerve head fell outside the stimulated area. However, given the angular dimensions of the display (50° × 47°) and the alignment of the mouse eye with respect to the screen, we are reasonably confident that the optic nerve head region of the retina was captured within our response array. (Altering the line of gaze did not help reveal the nerve head depression.)

A more likely explanation for the inability to identify the site of the nerve head derives from a rod study in humans.14 In that study, the relatively large size of an individual hexagon (approximately 5° across), combined with the effects of image blur and local stray light, was used to explain why the blind spot and fovea were not seen in the response arrays. In this study, individual hexagons of the standard display were also relatively large (approximately 5.6° × 6.9°), and this factor alone would have made it difficult to identify the site of the nerve head in the mouse.

In this experiment, the angular size of a hexagon was varied to assess the possibility of detecting the site of the optic nerve head. Hexagon size was varied over three levels. The angular size of each hexagon (and the number of elements in the display) were 5.6° × 6.9° (61 hexagons), 4.3° × 5.2° (103 hexagons), and 2.9° × 3.5° (241 hexagons), respectively. Except for the variation in element size, flash and surround intensities were as in the standard display.

Response arrays obtained from a normal mouse are shown in Figure 5. Response amplitudes are smaller and noisier with progressively smaller stimulus elements. Decreasing the angular size of each hexagon (increasing resolution) did not improve the ability to detect the optic nerve head, primarily because the local responses were exceedingly small at all retinal locations (Fig. 5C). This experiment was replicated on a second mouse with similar results.

A notable feature of this experiment is shown in Figure 6. Across the three element sizes (including a replication of the 241 hexagon condition), the summed multifocal responses are...
remarkably similar in waveform, amplitude, and timing (Fig. 6A). In contrast, the average multifocal response for an individual stimulus element (Fig. 6B) decreases systematically as hexagon size is reduced. Thus, although a local response is smaller with reduced element size, the local responses are additive, giving the same summed response. This additivity of local responses is consistent with the hypothesis that the multifocal responses represent relatively local function and that stray light is not a significant factor.

Comparison to Full-Field ERGs

Full-field and multifocal ERGs were recorded from the same mouse under comparable stimulus conditions. For the multifocal recordings, the standard display of 61 hexagons was used, and the intensity of the blue flashes was set to 0.30 log scot td/s. Full-field ERGs were recorded with a conventional Ganzfeld dome and the same blue filter (W47A), with flash intensity set to 0.32 log scot td/s. (For both full-field and focal stimuli, flash intensities were in the linear range of the inten-
rod multifocal ERGs in mice in which regions of the retina had been destroyed with laser treatment.

Examples of ERG topographies before and after laser treatment are shown in Figure 8 (top and middle panels). This mouse had laser burns delivered around the optic nerve to produce a confluent area extending approximately five to seven disc diameters across. The response arrays are shown on the left, and, for comparison, three-dimensional response density plots are shown on the right. Local responses were essentially nondetectable in the region of local laser scarring (lower right quadrant of the trace array). The local defect encapsulating the optic nerve head is shown more vividly as a deep depression on the three-dimensional density plot shown in the middle panel. (For this recording session, the mouse eye was aligned so that more of the inferior temporal retina was captured.)

For the mouse data shown in the bottom panels of Figure 8, laser burns were delivered throughout the retina to create several pockets of nonfunctional retina, the intent being to simulate a diseased retina with a number of regions of normal and abnormal function. The ERG topography after laser treatment shows depressed local function over many relatively large and confluent areas that reflect the regions of the retina treated with the laser.

**DISCUSSION**

The purpose of this study was to assess the feasibility of obtaining rod multifocal ERGs from the mouse eye. This discussion, therefore, will focus on two questions. First, are the multifocal responses rod-mediated? Second, do the responses reflect 'local' function?

The first question is relatively easy to answer. To maximize the probability of rod-mediation, a combination of dim blue flashes and a slowed presentation rate was used. Multi focal responses that were obtained under these conditions appeared qualitatively similar to rod responses that are obtained routinely with standard full-field ERGs in both humans and mice, and appear similar to those obtained from rats. Cone intrusion was expected to be minimal because the mouse retina is rod-dominated, and cones are sparsely distributed. Nevertheless, cone contributions to the responses to blue flashes were evaluated by altering the adaptation level of the mouse eye. Under illumination conditions that significantly light-adapted the mouse retina and suppressed rod function, multifocal responses were nondetectable. This pattern of results supports the conclusion that dark-adapted multifocal responses to dim blue flashes are rod-dominated.

A more difficult question to answer is the extent to which the multifocal responses reflect local function. The mouse eye is small compared with human and other mammalian eyes, and even relatively dim light levels can produce a significant amount of internally reflected (or stray) light. Global stray light can mask entirely a local response, especially if the number of cells generating a local response is small and stimulus intensity is high.

In the multifocal recordings from humans, local rod responses appear bimodal, with separate early and late components. In 1998, Hood et al. interpreted the small and early component of the waveforms to be the local response and the relatively large and late component to be the response to...
global stray light. This interpretation derives from the finding that the addition of a surround reduces the amplitude of the late component while leaving the early component relatively unchanged. The potential influence of global stray light was anticipated in this study, and a moderate surround intensity and a relatively dim flash were used as part of the standard display. Nevertheless, the multifocal records from mice were clearly not bimodal. Rather, a single peak was observed in approximately the same location as the late component found in the human multifocal records.

An attempt was made to reproduce a bimodal waveform like that found in humans by varying surround intensity (see Fig. 4). Increasing surround intensity decreased the amplitude and shortened implicit times of the local responses, but a well-defined bimodal waveform was not observed under any of the conditions. A similar pattern of results was observed when overall adaptation level was altered (see Fig. 2). In contrast to the reduced amplitudes, which can be explained by rod suppression, the shortened implicit times with increasing light adaptation are not easy to explain. Increasing light adaptation may have desensitized rods so that the cone response became more evident. However, cone responses have a characteristic appearance, typically with faster implicit times, and responses were nondetectable under rod-saturating conditions (see Fig. 2). These factors would seem to rule out the possibility that the smaller and faster responses are cone-mediated. Alternatively, the kinetics of the rod-driven response could have been altered due to light adaptation.

**Figure 8.** Rod multifocal response arrays (left panel) and three-dimensional response density plots (right panel) from normal mice in which areas of the region were destroyed with laser photocoagulation. (A) Representative normal mouse. (B) The mouse shown in (A) after laser treatment. A total of 21 laser burns was administered in two rows around the optic nerve head. (C) Forty-four laser burns were delivered throughout the retina to create several pockets of nonfunctional retina. See text for details.
The failure to see a bimodal waveform produces a problem. A tempting interpretation of these results, based on the human data, is that local responses in the mouse are extremely small, perhaps nondetectable, and that only the response to global stray light was observed. Consider the evidence against this interpretation.

Comparisons of multifocal and full-field ERGs were made as a first step in determining whether the multifocal responses are dominated by global stray light. A full-field and a multifocal ERG were recorded from the same mouse and for the same flash intensity and chromaticity. The full-field ERG and the summed multifocal responses are shown in Figure 7. The area of the mouse retina stimulated by the multifocal display was calculated, and based on this calculation it was estimated that 20% of the response of the entire retina was contributed by the region stimulated by the multifocal display. The full-field ERG recorded from the same mouse in a conventional Ganzfeld dome gave the total response of the retina. This response was divided by a factor of five to predict the response given by the area of the retina occupied by the multifocal stimulus, assuming that all retinal regions contribute approximately equally to the full-field response. This predicted response was compared with the sum of the actual multifocal recordings (dashed and dotted lines in Fig. 7), and the agreement was remarkable despite the differences in methodology and the computations required to arrive at the prediction. This agreement supports the hypothesis that responses from retinal areas not stimulated directly by the multifocal stimulus (global stray light) play a minor role, if any, in determining a local response. In addition, the agreement implies that the local response amplitudes were appropriate for the angular extent of a stimulus element.

In a second experiment (Fig. 5), local response isolation was examined by reducing the angular size of stimulus elements. The goal was to identify the site of the optic nerve. Isolating the nerve head within the response array would have provided a powerful test of local response isolation. However, a local depression associated with the optic nerve head could not be identified in the response arrays for the smaller elements because responses were smaller and noisier at all retinal locations, presumably because fewer retinal cells were contributing to a local response. However, the reduced amplitudes observed with smaller element sizes are consistent with a local basis for the multifocal responses. Nevertheless, they do not conclusively establish a local basis for the response because stray light thrown from individual hexagons to other hexagons, and to the surround, may also have been scaled as the element size was reduced, thereby contributing to the reduction of a local response.

To investigate the issue of local response isolation more directly, a different approach was used. We created local retinal defects with laser treatment in the retinas of normal mice. The intent was to show that the areas of the retina destroyed by the laser would be represented in the response arrays recorded with the multifocal ERG. Two mice were described, one in which the optic nerve head was surrounded by a large confluent patch of laser-treated retina, thereby enlarging the site of the nerve head, and a second mouse in which patches of tissue were ablated throughout the retina. Although the laser-treated areas of the retina were relatively large, the multifocal ERG clearly revealed these regions as nonrecordable local responses (see Fig. 8). We are currently evaluating spatial resolution by mapping retinal abnormalities in laser-treated mice in which scotomas are relatively circumscribed and systematically reduced in areal extent.

Overall, the results of the experiments reported here suggest that rod multifocal ERGs can be recorded from the mouse, that the multifocal responses reflect relatively local function, and that the multifocal technique can be used to map heterogeneous retinal function. However, an important technical issue that has not yet been resolved concerns the placement of the multifocal stimulus on the mouse retina. As stated previously, we cannot specify precisely the image location on the retina. We took special care in securing the head and body of the anesthetized mouse to ensure immobility during a testing session so that the same retinal tissue was being stimulated as stimulus conditions were changed. This procedure allowed a test of the main experimental questions driving this study. However, the site corresponding to a particular response is an important technical problem. In fact, when searching for the regions of abnormal retina in mice treated with lasers, several recordings were required to locate the lasered region, each with a slightly different line of gaze. This procedure proved time consuming. Nevertheless, a primary goal of this study was to develop a sensitive test for the mouse, to assess local retinal function after some forms of interventional therapy in which the treatment effects are expected to be relatively local. Sequential recordings with different lines of gaze, although time consuming, will likely reveal the regions of the retina that are relatively healthy.

In conclusion, the multifocal ERG can be extended to the study of local rod-mediated function in the mouse. As a first approximation, however, it appears that the multifocal technique will have the greatest success in cases in which the local regions of normal and abnormal function are relatively large and relatively homogeneous, and in which the stimulus conditions are selected appropriately for the particular experimental question or questions.

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