Functional Assessment of the Regional Distribution of Disease in a Cat Model of Hereditary Retinal Degeneration

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PURPOSE. To establish a method for the recording of multifocal electroretinograms (MF–ERGs) in animals under fundus control using a scanning-laser ophthalmoscope (SLO) and to analyze the spatial distribution of disease in a strain of Abyssinian cats with a recessively inherited rod-cone degeneration (ARCD).

METHODS. Four normal and 12 Abyssinian cats at four different clinical stages of ARCD were examined with the RETIscan MF–ERG system using 61 hexagonal elements within a visual field of approximately 30° radius. The stimulus pattern was generated by the green laser beam (515 nm) of a Heidelberg Engineering HRA SLO, whose power was reduced with a Schott long-pass filter allowing for simultaneous infrared fundus imaging.

RESULTS. Topographical recordings could be obtained in all animals except one in stage 4. Amplitudes were minimal at the optic disc and had a slight maximum at the area centralis. Implicit times had a tendency to lower values in the central region, most pronounced in progressed stages of ARCD. The clinical stages of ARCD correlated with a successive generalized loss of amplitude and a rise in implicit time. Without a decrease in retinal illuminance, topographical landmarks like the optic disc were no longer detectable, pointing to stray light as a possible cause.

CONCLUSIONS. It was demonstrated that topographical MF–ERG recordings can be obtained in an animal model under fundus control using SLO stimulation. The appearance of retinal landmarks was found to be dependent on sufficient attenuation of laser power. Because the changes in ARCD are more patchy than in human retinitis pigmentosa (RP), a generalized loss of function was detected. However, like in RP, the central area was found to retain a better function than the periphery, especially in later stages of the disease. In summary, fundus controlled methods like the one presented will greatly improve the reliability of MF–ERG in future research on glaucoma, transplantation studies, and evaluation of gene therapy. (Invest Ophthalmol Vis Sci. 2000;41:1998–2005)

Multifocal electroretinography (MF-ERG) has been demonstrated to be very useful in the detection of the topographical distribution of disease in many inherited retinal degenerations.¹⁻⁵ The method introduced by Sutter and Tran⁴ is based on the m-sequence stimulation technique and allows for the simultaneous measurement of the ERG activity of many retinal locations.

A comparison of the components of multifocal and photopic Ganzfeld–ERG has shown that the waveform of the primary response (the first-order kernel) is shaped by both the b-wave and the oscillatory potentials.⁵

The stimulus, a set of several thousand subsequent pseudo-random patterns consisting of commonly 61 or more hexagons of either black or white color, is usually presented on a CRT screen. Experience has shown that fixation is usually not a problem in human clinical recordings. However, reliable positioning of the stimulus is a problem in animal studies. Depending on the type and depth of anesthesia, there is also a considerable degree of slow eye movements and/or rotation of the globe that is hard to detect without simultaneous fundus visualization.

Recently, a method was developed that uses a scanning-laser ophthalmoscope (SLO) for a combined stimulation and imaging of the retina.⁶ In many animals, the use of the green laser (515 nm) is important to obtain satisfactory results due to the lack of long-wavelength cones.

The purpose of this study was to assess the feasibility of this method to detect the retinal distribution of disease in a strain of Abyssinian cats with a recessively inherited rod-cone degeneration (ARCD).⁷

METHODS

Animals

Four normal (ages, 1 to 6 years) and 12 Abyssinian cats at four different clinical stages of ARCD were studied. Five cats were at stage 1 (ages, 1 to 2.5 years), 3 at stage 2 (ages, 2 to 3 years), 3 at stage 3 (ages, 3 to 4 years), and 1 at stage 4 (age, 6 years).
2 at stage 3 (ages, 2 to 3 years), and 2 at stage 4 (ages, 4 to 6 years). The cats were anesthetized using a single intramuscular dose of medetomidine hydrochloride (0.25 mg Domitor vet.; Orion Pharma AB, Animal Health, Sollentuna, Sweden). After 15 minutes an injection of ketamine (15 mg Ketalar; Parke-Davis, a division of Warner Lambert Nordic AB, Solna, Sweden) was similarly given. Ketamine was iterated after 30 minutes of anesthesia using half the initial dose. Eyes were dilated using tropicamide (Mydriacyl; Alcon, Alcon Nordiska AB, Stockholm, Sweden). The research was performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Methodology

Cats were kept in normal room light at least 1 hour before the experiments. Multifocal ERG responses of the right eye of every cat were obtained with the RETIscan system (Roland Consult, Wiesbaden, Germany) using 61 hexagonal elements within a visual field of approximately 30° radius. Evoked field potentials were recorded from the cornea using JET electrodes (Roland Consult, Wiesbaden, Germany) after dilatation of the pupil, and subsequently amplified (×250,000) and filtered (10–100 Hz). The spacing between samples was 0.98 msec. Each data set was generated as an average of 8 subsequent runs of the stimulation sequence. The stimulus pattern was generated by the green laser beam (515 nm) of a HRA confocal SLO (Heidelberg Engineering, Heidelberg, Germany) at a frame rate of 20 Hz whereas the infrared beam (835 nm) was used for simultaneous imaging of the stimulated area (Fig. 1A). A view of the stimulus in relation to the fundus is provided in Figure 1B. Because the trace array (i.e., the compilation of the traces of all local responses) is provided by the software in a way that
marked by a supplied by the filter manufacturer. The laser wavelength of the SLO is very low power values. nonlinearity is presumably due to technical constraints in recording (see Fig. 1A). All data shown are averages over a 10-second period. The filter material used in this study, measured at the outlet after filtering (see Fig. 1A). All data shown are averages over a 10-second period. The nonlinearity is presumably due to technical constraints in recording very low power values.

reverses up and down, but preserves left and right, all further ERG topographies follow that orientation. Figure 1C is an example of the relation between fundus position and trace array data.

Laser power was reduced with Schott long-pass filters OG 515 and OG 530 (Schott, Mainz, Germany) of different thickness, which were added at the aperture of the HRA (see arrow in Fig. 1A) to obtain the desired attenuation without reduction of the quality of the retinal image. Figure 2A shows the filter characteristics as supplied by the manufacturer. The actual power output using these filters (Fig. 2B) was determined at the level of the cornea with a Nova laser power meter connected to a PD300 head (Ophir Optronics, Peabody, MA). In this study, a combination of an OG 515 and OG 530 filter, both 3-mm-thick, was used. In some animals, another recording session followed after the removal of filters.

For the calculation of group statistics, a part of the fundus was chosen so that the stimulated area included important retinal landmarks such as the optic disc, the major vessels, the area centralis, and the visual streak. These structures were marked on the computer monitor to ensure the same position of the stimulating pattern on the retina of every animal. After calculation of first-order kernels, all traces were exported and further processed on an IBM-compatible personal computer. The group medians from each of the 61 locations were used to obtain three-dimensional plots of the functional topography separately for amplitude and implicit time as previously described.8

**RESULTS**

**Fundus Imaging**

The HRA allowed high-contrast images of the fundus in all cats to be obtained. The configuration and distribution of vessels (Figs. 3A through 3E) correlated well with the clinical staging9 previously to this study. This staging is also based on color changes during direct visualization or in standard fundus phography. Furthermore, the SLO allowed an assessment of the nerve fiber layer, which appeared to be well preserved even in late stages of ARCD (Fig. 3F).

**Local Origin of Responses**

It was found that in the normal cat there was no substantial gradient in MF–ERG amplitude between areas with morphologically determined high receptor density, such as the visual streak and the area centralis, and adjacent regions with lower cone density. Because the blind spot is commonly used as a retinal landmark in human recordings, the presence of low amplitudes at the position of the optic disc was used in this study as a sign that the respective signal was generated in the stimulated region. By the use of appropriate filters (see below), the blind spot (i.e., a strongly reduced amplitude at the position of the optic disc) was detected in all cats except one in stage 4. Changes in the position of the stimulus resulted in a corresponding change of the blind spot (Fig. 4A), also indicating the local character of the responses.

**Reduction of Laser Power**

The importance of a reduction in laser power became apparent when some or all of the filters were removed. Compared with the previous recordings, a clear response appeared in the blind spot region, indicating a loss of the local nature of the signal (Fig. 4B). This phenomenon was found in all cats, but was more pronounced in later stages of ARCD. As the progression of the retinal degeneration causes a successive thinning of the neuroretina, there is a change in reflectivity of the cat tapetal fundus. The hyperreflectivity of the fundus in cases with severe retinal atrophy may give rise to increased effects of stray light. Best results in terms of both signal-to-noise ratio and local origin were obtained in the cats studied with 3-mm thicknesses of both OG 515 and OG 530.

**Retinal Topography in ARCD**

Clear responses across the stimulated area were present in normal cats and in affected animals up to stage 3 (Fig. 4B). In the most advanced stage (stage 4), more intense stimulation (i.e., a different filter setting) was needed to evoke substantial responses, presumably at the cost of a higher degree of stray light. To avoid problems of comparability induced by the use of weaker filters and the subsequent failure to detect the blind spot, the results of stage 4 animals were excluded from the topographical analysis (Fig. 5). Amplitudes and implicit times in normal and affected cats (up to stage 2) did not show major regional differences, with the exception of the blind spot. Amplitudes were minimal at the optic disc and in advanced stages had a slight maximum at the area centralis (Fig. 5, right column). Implicit times had a tendency to lower values in the central region, again most pronounced in advanced stages (Fig. 5, left column). Overall, the clinical stages of ARCD correlated with a successive generalized loss of amplitude and a rise in implicit time.

**DISCUSSION**

Despite the growing number of applications in human diseases, there are few reports on multifocal electroretinography in animal models.9–11 The main problem appears to be that because of the lack of fixation, methods to control the position
of the stimulus on the retina are needed to interpret the results. Two methods are most commonly used in this regard. Back projection uses a bright light that, when applied to the eye, leads to a projection of retinal structures onto the stimulating screen. Repetitive short MF-ERG recording sequences before the final measurements can also be used to determine and adjust the position of the stimulus relative to functional retinal landmarks like the macula (if existent in the respective model) and the blind spot. However, both methods do not allow for the continuous control of stimulus position during the measurements. Depending on the type and depth of anesthesia, there is a considerable degree of slow eye movements and/or rotation of the globe. Because the eye often slowly returns to its starting position, these movements are hard to detect without simultaneous fundus visualization. Other issues are the adaptation caused by the bright light in back projection, which further prolongs the time without fundus control due to a mandatory recovery period before the start of recordings, and
the much smaller macular peak (compared with that in humans) even in monkeys that is a problem for the correct positioning with the functional method. Only a few groups have thus successfully obtained meaningful records with these techniques. It is common to both methods that an exactly reproducible placement of the stimulus is not possible, which prevents an element-by-element statistical evaluation in a larger collective of animals.

In this study, a MF–ERG setup that uses a SLO for a combined stimulation and imaging of the retina was evaluated. It was demonstrated that topographical MF–ERG recordings can be obtained with such a setup in an animal model under real-time fundus control. The use of a short-wavelength laser is in many animals the only way to obtain satisfactory results due to the lack of long-wavelength cones. However, a reduction of the power of the stimulating laser was found to be necessary to preserve the local character of the responses. A direct reduction of power output of the laser source turned out not to be as reproducible and exact as it was felt to be needed for this study. Neutral density filters at the level of the aperture led to good results but did also strongly reduce the quality of fundus images. For technical reasons, the insertion of neutral density filters just in the path of the stimulating laser was not possible, so long-pass filters were used to selectively reduce the stimulating laser power without interference with the infrared beam, which gave quite good results. Because of the sharp edge in the transmission characteristic close to the laser wavelength (Fig. 2A), slight manufacturing tolerances have a big impact on the degree of attenuation, so that each filter had to be calibrated separately (Fig. 2B).

Previous full-field cone ERGs in the group of cats in this study, using a rod-desensitizing background, have yielded ERG waveforms similar to those obtained in the present MF–ERG study (K. Narfström, unpublished observations, May 1998). However, it is evident that these results are not directly comparable, because the procedures and, especially, the stimulus characteristics (wavelength, intensity) vary between the two setups. The HRA proved also to be a very useful tool for fundus imaging in cats. Although discoloration may not be observable so well in the gray-scale pictures, the high-contrast images clearly showed the changes to retinal structures like the loss of small and the thinning of main vessels with increasing stage (Figs. 3A through 3E). The confocal laser allows also to focus on the nerve fiber layer, which appeared relatively intact even in late stages of ARCD (Fig. 3F). This finding is important for therapeutical approaches like the transplantation of pigment epithelial tissue because it suggests that it may be possible to evoke central responses after successful therapy.

The reason for the increased response at the position of the optic disc in the case of higher laser power (Fig. 4B) is very probably stray light. The exact mechanism is not known yet, but it is believed that aberrant light from the stimulated region reaches photoreceptors in other parts of the retina, thereby causing a contribution to the sum response. Because the method cannot determine where a signal originates from, the evoked response is attributed to the stimulated area. Further work is needed to determine whether this phenomenon is restricted to the optic disc, and whether the effect is more of a general, a widespread, or a local, restricted character (i.e., an increased spot size).

A major advantage of the exact alignment of the stimulated area with retinal structures in each animal is the possibility to obtain group statistics. For each stage of ARCD and the control group, medians of amplitude and implicit time were calculated for each single element of the multifocal stimulus as described previously in humans. An evaluation of these stage group medians revealed that the topography of normal and diseased cats is relatively “flat” in comparison to human (i.e., the regions of histologically determined higher cone density like the visual streak and the area centralis were not functionally discernible except for the later stages of the disease; Fig. 5). It appears that the differences between these and the adjacent regions, in contrast to the changes induced by ARCD, are not big enough to be clearly detectable with the current setup. This is supported by the fact that even in the monkey the differences in MF–ERG amplitude between the macula and the surrounding retina are less distinct compared with those in humans (data not shown), which is a problem for the correct positioning of the stimulus with the functional method as described above. There is work in progress that aims at an improvement of the signal-to-noise ratio with different modes of stimulation, which may eventually lead to a better detection of the central structures in normals and early stages of ARCD. The photopic conditions guaranteed by the ambient light previous to and during recording, and the relatively fast stimulus sequence, assured that the obtained responses were cone-driven. We have confirmed that assumption by recording MF–ERGs with the same setup from young Rho−/− (which lack any rod function due to the absence of rhodopsin)12 and CNG3−/− (a model for achromatopsia that lacks cone function)13 mice. The latter do have a normal rod Ganzfeld ERG but no MF response, whereas the first have no rod ERG but almost a full MF response.14

In human retinitis pigmentosa (RP), the central area is usually damaged relatively little, whereas the peripheral regions are strongly abnormal. In contrast, feline ARCD is morphologically evenly distributed during early stages, so that normal-looking and diseased photoreceptors are often found side-by-side. However, like in RP, the central region is histologically relatively better preserved in late stages. In accordance with these facts, a clear generalized loss of function was detected in the early stages of the disease (Fig. 5), whereas in later stages the central area was found to retain a better function than the periphery.

In summary, it was demonstrated that topographical MF–ERG recordings can be obtained in an animal model under

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**Figure 4.** Local character of MF–ERG responses. (A) Trace arrays of recordings obtained in a stage 1 cat with the blind spot in the center of the recording area (left) and after movement to standard position (right). The corresponding retinal images are shown in the central column. (B) Effect of filtering on the trace array data (shown in a stage 3 cat). Left trace array: Normal filter setting. Right trace array: After removal of all filters. Lower central trace array: Control cat with normal filter setting. Removal of filters increases signal amplitudes (here by an approximate factor of 2), but the blind spot is no longer detectable, indicating a loss of local character. The responses of a control cat with filters have approximately the same amplitude as those of a stage 3 cat without filters, but implicit times are close to the one of the stage 3 cat with filters.
fundus control using SLO stimulation. The appearance of retinal landmarks was found to be dependent on sufficient attenuation of laser power. Because the changes in early ARCD are more evenly distributed than in human RP, a generalized loss of function was detected. However, as in RP, the central area was found to retain a better function than the periphery, especially in later stages of the disease. Fundus-controlled methods like the one presented will greatly improve the reliability of MF-ERG in future research on glaucoma, transplantation studies, and evaluation of gene therapy.

References
