PERGAMON

Vision Research 39 (1999) 2285–2291

Vision Research

Identifying inner retinal contributions to the human multifocal ERG

Donald C. Hood [a,*], Vivienne Greenstein [b], Laura Frishman [c], Karen Holopigian [b], Suresh Viswanathan [c], William Seiple [b], Jameel Ahmed [c], John G. Robson [c]

[a] *Department of Psychology, Columbia University, 406 S Hall, New York, NY 10027, USA*
[b] *Department of Ophthalmology, NYU Medical Center, New York, NY 10016, USA*
[c] *College of Optometry, University of Houston, Houston, TX 77204, USA*

Received 6 May 1998; received in revised form 14 September 1998

Abstract

Contributions to the multifocal electroretinogram (ERG) from the inner retina (i.e. ganglion and amacrine cells) were identified by recording from monkeys before and after intravitreal injections of n-methyl DL aspartate (NMDLA) and/or tetrodotoxin (TTX). Components similar in waveform to those removed by the drugs were identified in the human multifocal ERG if the stimulus contrast was set at 50% rather than the typically employed 100% contrast. These components were found to be missing or diminished in the records from some patients with glaucoma and diabetes, diseases which affect the inner retina. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Electroretinogram; Multifocal ERG; Glaucoma; Diabetes

1. Introduction

The full-field flash electroretinogram (ERG) has had mixed success for detecting the early signs of retinal damage caused by glaucoma and diabetes. There are at least two reasons. Firstly, retinal damage can be restricted to relatively local regions while full-field ERG techniques record cellular activity averaged over a wide retinal area. Secondly, damage, especially in the case of glaucoma, is seen first in the inner retina, where amacrine and ganglion cells reside, and until recently these cells have been observed to make relatively small contributions to the flash ERG. A new technique may improve the situation. Retinal activity in the form of focal ERGs can be recorded simultaneously from 100 or more retinal regions employing the multifocal technique developed by Sutter and his colleagues (Sutter & Tran, 1992). Furthermore, Sutter and Bearse have reported that ganglion cells contribute to the human multifocal ERG (Sutter & Bearse, 1995, 1998) and have provided preliminary indications that glaucomatous damage can be identified (Bearse, Sutter, Smith & Stamper, 1995; Bearse, Sutter, Sim & Stamper, 1996). However, the existence of a ganglion cell contribution to the human multifocal ERG has been questioned as also has the effectiveness of the multifocal technique in detecting changes in patients with glaucoma (Vaegan & Buckland, 1996; Vaegan & Sanderson, 1997). While studies of the human multifocal ERG have produced conflicting results, animal studies have clearly demonstrated that the inner retina contributes to the multifocal and flash ERG. Tetrodotoxin (TTX), which blocks all sodium-based action potentials, substantially alters the multifocal ERG of monkeys (Hood, Frishman, Viswanathan, Robson & Ahmed, 1999), as well as the full-field ERG of cats and monkeys (Viswanathan, Frishman, Robson, Harwerth & Smith, 1996; Viswanathan & Frishman, 1997). Here we provide evidence that simply reducing the contrast of the display allows for the detection of inner retinal components in the human multifocal ERG.

* Corresponding author. Fax: +1-212-8543609; e-mail: don@psych.columbia.edu.

0042-6989/99/$ - see front matter
PII: S0042-6989(98)00296-X

2. Methods

2.1. Animal

Recordings were made from two adult monkeys (*Macaca mulatta*). Experimental and animal care procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were reviewed by the Institutional Animal Care Committee of the University of Houston. Pupils were fully dilated to about 9 mm and the eye to be studied was refracted retinoscopically and fitted with appropriate contact lenses. An ophthalmoscopic technique was used to locate the projection of the fovea on the center of the stimulus pattern and to determine the position of the optic disc. Both monkeys received injections of TTX. One of the monkeys also received injections of NMDLA and was studied in two sessions. In session one, the injection of TTX was preceded by injections of NMDLA. In session two, after 8 weeks of recovery and when the ERG was back to normal, the TTX injection was followed by injections of NMDLA. Intravitreal injections of 40–60 μl were made with a sterile 30 gauge needle inserted through the pars plana into the vitreal cavity. Intravitreal concentrations of the pharmacological agents were estimated by assuming that the vitreal volume is 2.1 ml. Recordings were made before and at least 90 min after injections. For other details see Frishman, Shen, Du, Robson, Harwerth, Smith, Carter-Dawson and Crawford (1996) and Hood et al. (1999).

2.2. Human subjects

Records were obtained from ten normal subjects (ages 35–64 years, median age of 52 years) and two patients, one with primary open-angle glaucoma (POAG) and one with diabetic retinopathy. The patient with POAG was 38 years of age. The visual acuity in the tested eye was 20/20, and the cup-to-disk ratio was 0.9. The mean deviation (MD) and corrected pattern deviations (CPSD) of the Humphrey Field Analyzer 30-2 program were -7.0 and 3.3 dB, respectively. The patient with moderate nonproliferative diabetic retinopathy who was 55 years of age, had clinically significant macular edema. The edema (zone of thickening) was located approximately 4° temporal to the fovea and covered a region 5° in diameter. Thus, in total it covered a small portion, equivalent to less than four hexagons, of the field stimulated. The visual acuity in the tested eye was 20/30-2. For all subjects, the pupil of the tested eye was dilated (1% cyclopentolate hydrochloride and 2.5% phenylephrine hydrochloride) and kept light-adapted at room illumination until the experiment began. All subjects signed informed consent forms after the experimental procedures were described to them. Tenets of the Declaration of Helsinki were followed and institutional human experimentation committee approval was obtained.

2.3. Stimulation

The stimulus used to obtain multifocal ERGs has been described in detail elsewhere (Sutter & Tran, 1992; Hood, Seiple, Holopigian & Greenstein, 1997). For the monkey experiments, the stimulus array consisted of 103 equal sized hexagons, each about 3.3° wide, in a field of about $35 \times 33°$ (see Fig. 1B and Hood et al. (1999)). For the human experiments, the array consisted of the more typically employed 103 scaled hexagons in a field of about $47 \times 39°$ (Fig. 3B). In all cases, the surround region and the space average luminance were 100 cd m^{-2}. The contrast between the bright and dark hexagons was either 50%, or the maximum possible, about 88% for the monkey experiments and about 98% for the human experiments. An experimental run consisted of a m-sequence with $2^{15}-1$ steps. The elements of this sequence were 13.33 ms in duration (corresponding to a screen rate of 75 Hz). However, the actual duration of the incremental light producing a white hexagon was about 1 ms. Each run required about 7 min total recording time, broken into two equal segments for the monkeys, and 16 segments for the human subjects. Analyses were based on the average of two runs. Only first-order responses were analyzed using the VERIS software from EDI (Electro-Diagnostic Imaging).

2.4. Recording

For the monkey, ERGs were recorded differentially between DTL electrodes that were placed under the corneal contact lenses of both eyes (see Frishman et al. (1996) for details); one eye was covered. For the human subjects, ERGs were recorded using Burian–Allen bipolar, contact lens electrodes (Hood et al., 1997). The records shown here were recorded with low and high frequency cut-offs of 10 and 300 Hz for the human subjects and 1 and 300 Hz for the monkey. Control recordings demonstrated that the type of electrodes and cut-off frequencies employed had little effect on the results.

3. Results

Fig. 1A shows the multifocal responses from one of the monkeys. As previously reported, the waveform of the monkeys multifocal ERG varies widely across the field (Hood et al., 1999). Fig. 1D shows the averaged responses for groups of hexagons falling at approximately equal distances from the fovea but different

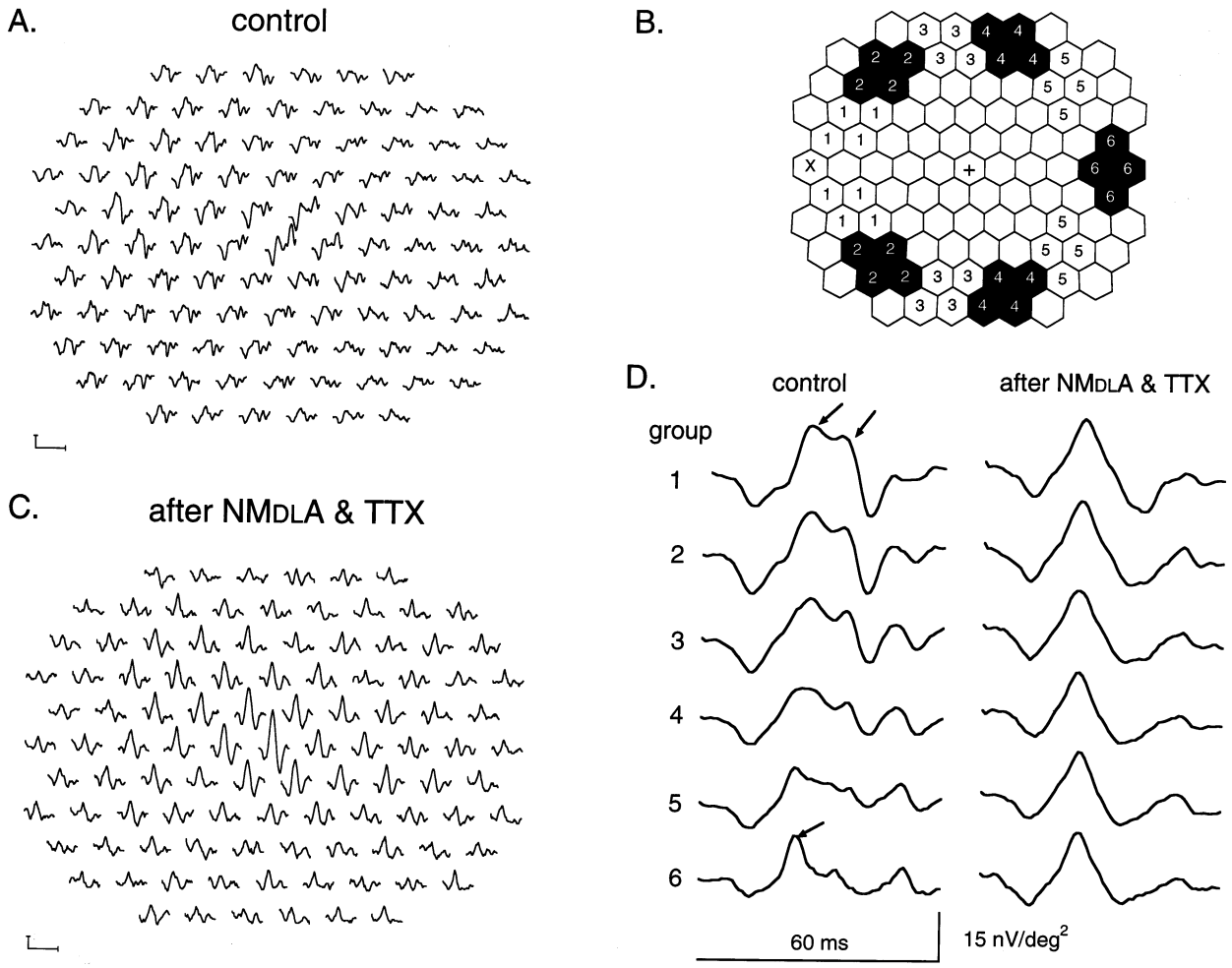

Fig. 1. (A) Multifocal records are shown for the control condition for the monkey. The calibration markers indicate 200 nV and 60 ms. (B) The pattern employed in the multifocal recordings from the macaque. (C) Multifocal records after intravitreal injection of NMDLA (7.7 mM) and TTX (7.6 μM). The responses are larger in the center because the display (see panel B) has equal size hexagons unlike the display (see Fig. 3B) used for most of the human experimentation. (D) Responses from panels A and C averaged over the groups shown in panel B. Stimulus contrast was nearly 100%.

distances from the optic nerve head (ONH) (Sutter & Bearse, 1995; Hood et al., 1999; Sutter & Bearse, 1998). The ONH was about 16.5° from the fovea in the monkey (Cowey, 1967); the closest hexagon to this location is the one marked with the 'x' in Fig. 1B. The left hand records in Fig. 1D are control responses averaged over the groups shown in Fig. 1B. With increasing distance of the hexagons from the ONH, the response waveform changes from having two positive peaks to a single peak (see arrows in Fig. 1D). Similar results are seen for the second monkey in Fig. 2A (first column).

Inner retinal activity was blocked with intravitreal injections of NMDLA and/or TTX. TTX blocks voltage-gated sodium channels and prevents spike generation in the ganglion cells and their axons as well as in amacrine cells. NMDLA depolarizes ganglion and at least some types of amacrine cells (see Massey, 1990; Massey & Maguire, 1995 for reviews). After treatment with NMDLA and TTX, the responses appeared to be smoother (Fig. 1C). The responses from different retinal regions are now essentially identical in shape and have a single positive peak (see second column in Fig. 1D). Similarly, as previously reported (Hood et al., 1999), preventing spike generation by injecting only TTX is sufficient for removing the regional variations in waveform (see Fig. 2A–second column). The addition of NMDLA, however, further simplifies the waveform. Note for example, the region marked with the arrow in Fig. 2A after TTX alone, and the change in that region that occurred when NMDLA was injected after TTX (Fig. 2B). We have also observed a smoothing of the waveform of the flash ERG after NMDLA in this monkey as well as in other monkeys (Viswanathan et al., unpublished observations).

Although variations in response waveforms with retinal location can be seen in the second-order responses of the human multifocal ERG (Wu & Sutter, 1995),

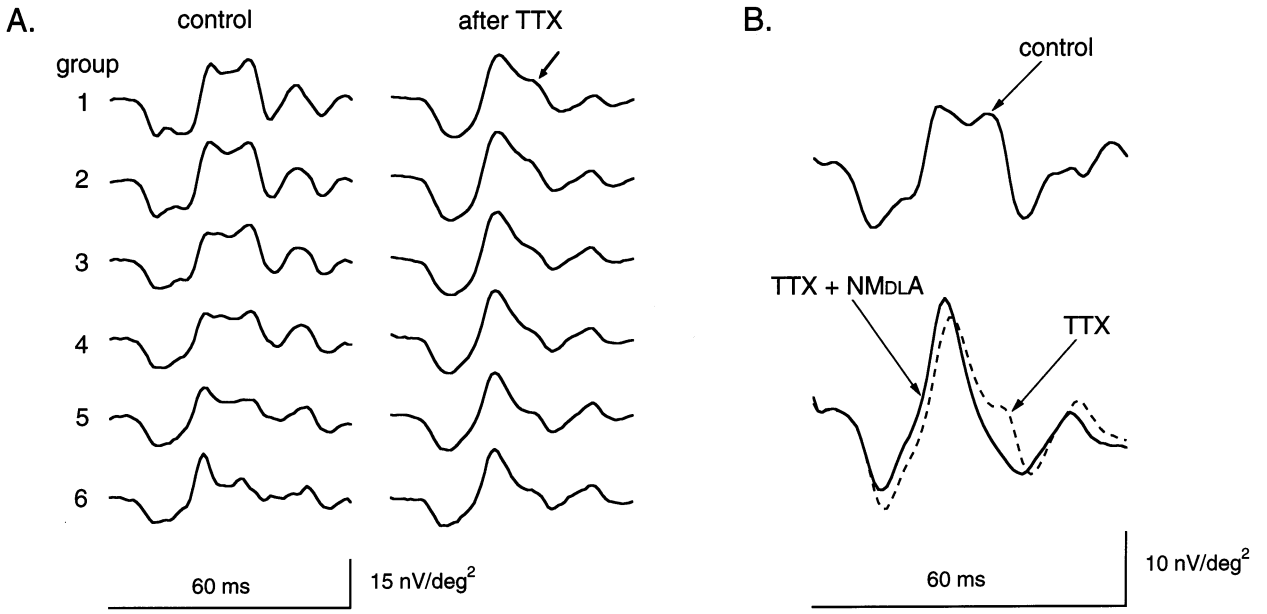

Fig. 2. (A) For a second monkey treated with TTX (6.6 μM) alone, responses before and after TTX were averaged over the groups shown in Fig. 1B. (B) For the first monkey 8 weeks after the initial injections, when the ERG had recovery to normal (top), TTX (6.6 μM) was injected first, and then NMDLA (2.7 mM) was injected. All responses were averaged together. Stimulus contrast was nearly 100%.

previously published first-order responses of the human multifocal ERGs do not show the wide variations seen in the monkey's control records. The dashed curves in Fig. 3D (first column) show averaged records from a human subject for a stimulus with the near 100% contrast that is commonly employed. As in Fig. 1D, these records are for groups of hexagons falling on retinal areas that are at approximately equal distances from the ONH[1]. The differences in waveforms with distance from the ONH are subtle. However, on re-examination of the unpublished data (Hood, Holopigian, Seiple, Greenstein, Li, Sutter & Carr, 1996), we discovered that waveforms more closely resembling those seen in the monkey were present in the records from humans if the stimulus contrast was less than 75%. The solid curves in Fig. 3D (first column) show the averaged responses for a stimulus contrast of 50%; the full set of records is shown in Fig. 3A. With 50% contrast, there is a qualitative similarity between the records in Fig. 3D and the control records from the monkeys in Fig. 1D and Fig. 2A. The arrows show that when the stimulus is close to the ONH there are two positive peaks, while with more remote stimuli, the responses show a single peak. This qualitative finding has been replicated in three additional human subjects. Responses from one of these subjects are included in Fig. 4B (first column).

[1] Because of the geometry of the displays it is not possible to obtain responses that are exactly equidistant from the ONH. Based upon the visual fields from the human subjects, the OHN probably falls just below and to the right of the 'x' in Fig. 3B.

Glaucomatous damage can produce qualitatively similar changes to those seen with TTX and NMDLA. The records from a patient with POAG measured at 50% contrast are shown in Fig. 3C. This patient's responses are large, and 'smoother' than the responses from the normal observer in Fig. 3A. Likewise the waveform of the averaged responses at increasing distances from the ONH (second column in Fig. 3D) are more similar to each other than in the case of the normal observer. (See Bearse et al., 1996 for a similar observation based upon second-order responses.) Although the records from the control subject show variation in the extent to which they vary across the field, none of the ten control subjects have records that resemble those of the patient in Fig. 3.

Fig. 4A shows records measured at 50% contrast from the patient with nonproliferative diabetic retinopathy. Diabetic retinopathy in addition to affecting the outer and mid-retinal layers also affects the inner retina. In fact, inner retinal changes can occur before signs of proliferative changes (e.g. Yonemura, Aoki & Tsuzuki, 1962; Simonsen, 1980). The averaged responses in Fig. 4B (second column) from this patient, like the results in the monkey after TTX and NMDLA, show large, smooth waveforms that are extremely similar across the retina. Unlike the records from the patient with glaucoma, however, positive peaks of the response are slightly delayed. Again, the changes seen in this patient were not reproduced in any of our ten controls.

For now, we merely use the patient's records to support our claim that the human multifocal ERG has

a sizeable contribution from the inner retina. Before we can conclude that our findings can be generalized to other patients, a comprehensive study must be completed, one in which local ERG changes are compared with local visual field changes in a patient population with either glaucomatous damage or diabetic retinopathy. However, based upon a preliminary analysis of data from a sample of 11 glaucoma patients with field losses equal to or greater than those for the patient shown here, we can say that there are other patients (two in this sample) with dramatic changes that fall outside our range of normal controls. On the other hand, there are glaucoma patients with clear field losses that do not appear to fall outside the normal range, at least not with the measures thus far devised. With regard to our findings for the patient with diabetic retinopathy we have found similar changes in two more patients with nonproliferative retinopathy and macular edema. On the other hand, relatively normal responses were observed in a patient with minimal background retinopathy.

4. Discussion

The monkey's multifocal responses vary in waveform depending upon the retinal region stimulated. Previous work (Hood et al., 1999), confirmed in the present study, has shown that these differences in response waveform are eliminated by TTX, an agent that blocks sodium-dependent action potentials in ganglion cells, their axons, and amacrine cells. In other words, it is the spiking activity of inner retinal neurons that appears to be the cause of the waveform variations across the retina. Thus, it seemed important to find conditions that produce similar location-dependent waveform variations in humans. When a 50% contrast pattern rather than a 100% contrast pattern was used, this variation in waveform could be identified.

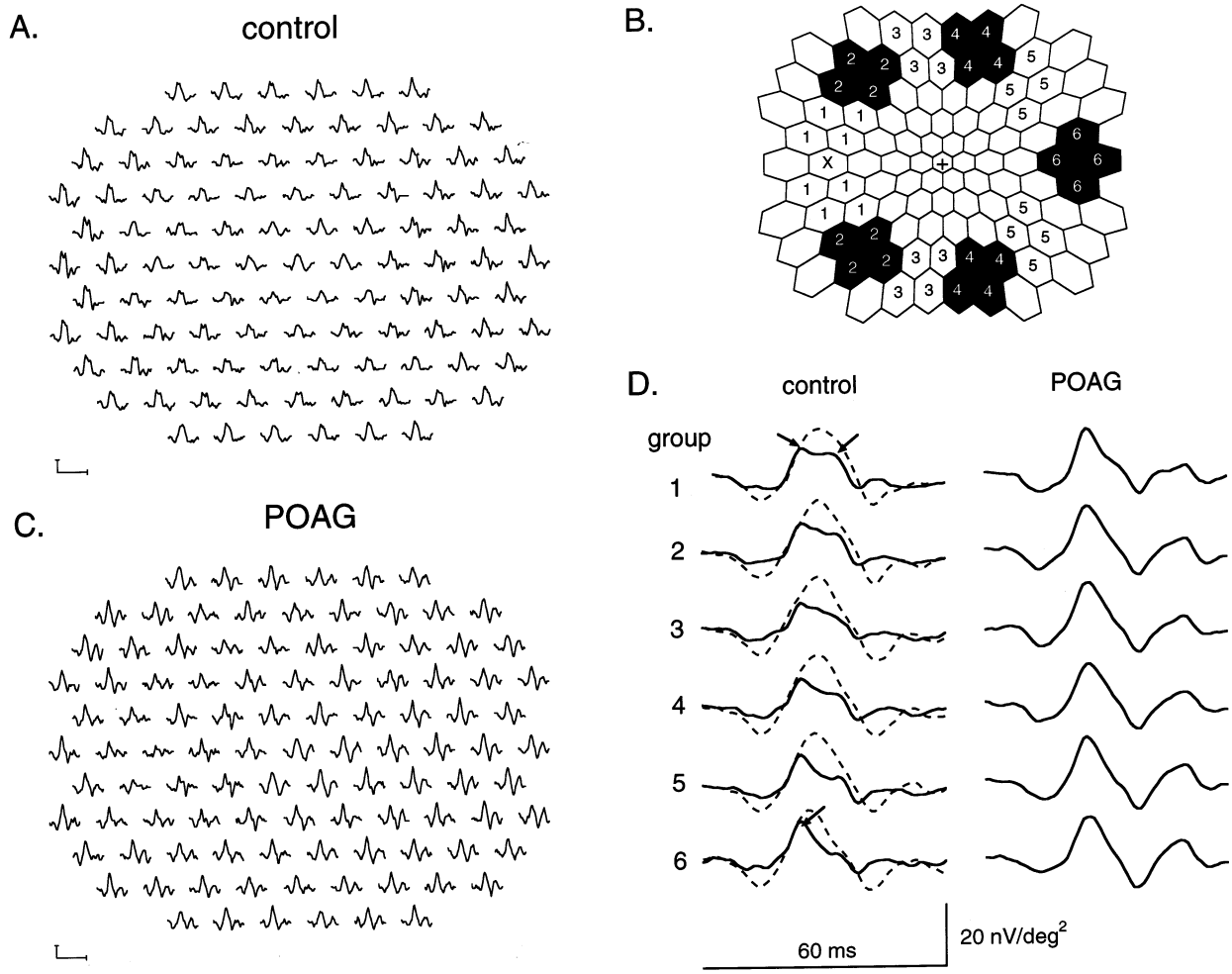

Fig. 3. (A) Multifocal records, measured using a stimulus contrast of 50%, are shown for a normal human subject. The calibration markers indicate 120 nV and 60 ms. (B) The pattern employed in the multifocal recordings for human subjects. (C) As in panel A for the records from a patient with POAG. (D) The solid curves are the responses from panels A and C averaged over the groups shown in panel B. The dashed lines are the same group averages for a nearly 100% contrast stimulus.

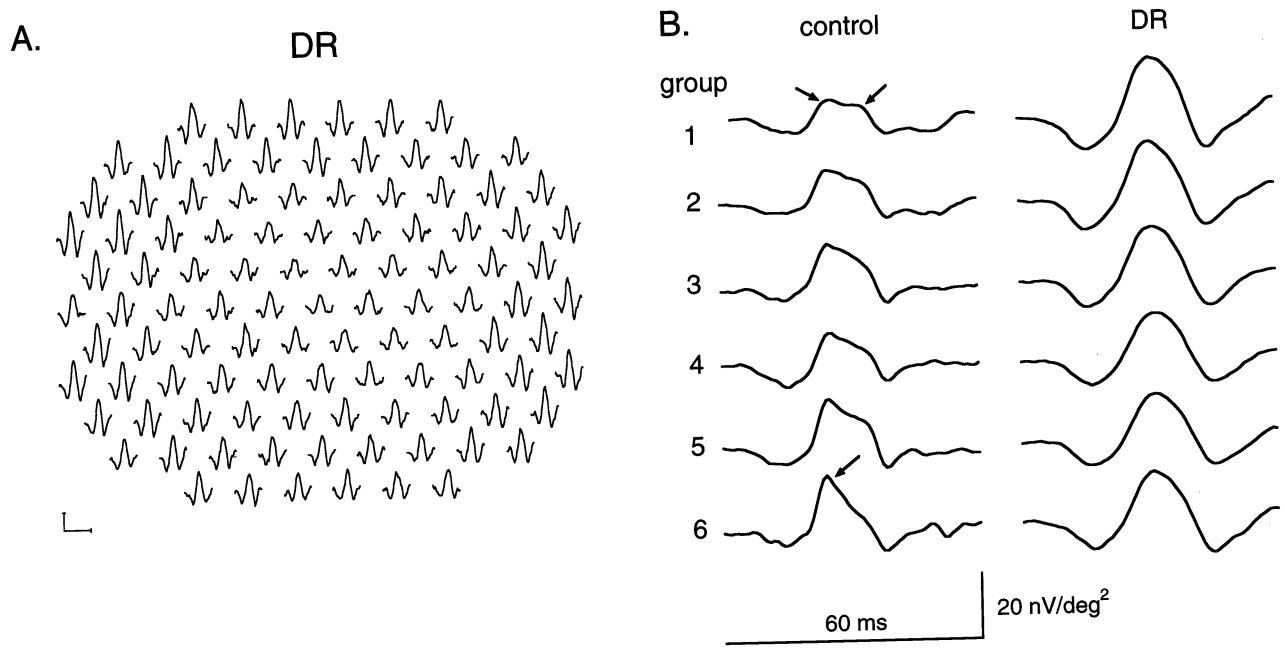

Fig. 4. (A) Multifocal records measured using a stimulus contrast of 50% are shown for a patient with diabetic retinopathy. The calibration markers indicate 120 nV and 60 ms. (B) Responses averaged over the groups are shown in Fig. 3B for a control subject and for the patient whose records are in panel A.

There are three reasons for believing that there is a discernible inner retinal contribution to the human multifocal ERG in response to the 50% contrast stimulus. Firstly, the response waveforms from normal subjects resemble those from the monkey and appear to have components (see arrows in Fig. 1D and Fig. 3D) that correspond to those removed by TTX and NMDLA in the monkey. Secondly, as with the monkey's records, the waveforms vary systematically with distance from the ONH suggesting that the spiking activity of the axons of the ganglion cells plays an important part in shaping the waveforms. And, finally, the records from the patients with POAG and diabetic retinopathy (diseases which affect the inner retina) have two characteristics in common with the records from the monkey treated with TTX and NMDLA. Firstly, the waveform of the patient's responses is approximately the same across the stimulated retinal areas. And, secondly, the actual waveforms resemble those from the treated monkey.

Interestingly, while the patient's records show a close resemblance to the records of the treated monkey (Fig. 1C and Fig. 3C), the human control records exhibit some clear differences when compared to those of the control monkey (Fig. 1A and Fig. 3A). The differences are most apparent in the central responses where the monkey's records show a greater variation among responses. Although it is possible that these differences reflect a relatively larger contribution from the inner retina in the monkey, an explanation must await a fuller understanding of the cause of the regional variations.

Our findings support Sutter and Bearses conclusion that the multifocal ERG technique can detect local damage to the inner retina. Sutter and Bearse (1995; 1998) developed an algorithm to extract a component of the multifocal ERG that they attributed to action potentials passing through the region of the optic nerve head. They called this the optic nerve head component (ONHC) and provided preliminary indications that glaucomatous damage eliminated it (Bearse et al., 1995, 1996). Recently, Bearse and Sutter (1998) reported that their ONHC saturates at 60% contrast while the other components of the multifocal ERG continue to grow and to dominate the response. This may account for our finding that a 50% contrast stimulus is superior to a high contrast stimulus in revealing inner retinal activity. Increasing the contrast to 100% may also affect the relative timing of the underlying components (Hood et al., 1997).

The exact relationship of our findings to Sutter and Bearse's ONHC remain to be determined. The patients records in Fig. 3D and Fig. 4B appear to be more similar to the monkey's records after TTX plus NMDLA than to the monkey's records after TTX alone (see Fig. 2 and records in Hood et al. (1999)). NMDLA depolarizes retinal neurons with NMDA receptors, in general believed to be both ganglion and at least some types of amacrine cells (e.g. see reviews of Massey (1990) and Massey & Maguire (1995)). The post-synap-

tic currents generated by one or both of these cell classes seem to be affected in the patients we studied here.

Previous multifocal studies of patients with diabetes and glaucoma, using 100% contrast stimuli, have reported more subtle changes compared with those shown here (Bearse et al., 1995, 1996; Greenstein, Holopigian, Seiple, Kahanowicz & Katz, 1997; Palmowski, Sutter, Bearse & Fung, 1997). Although it is likely that relatively larger effects will be seen when multifocal ERGs are obtained with a 50% contrast stimulus, whether the multifocal ERGs will prove more effective than existing techniques in detecting early diabetic retinopathy and glaucomatous damage remains to be determined.

Acknowledgements

Supported in part by the National Eye Institute Grants R01-EY-02115, R01-EY-09076, R01-EY06671, P30-EY07751, T31 EY07024 and a William C. Ezell fellowship to SV.

References

Bearse, M. A. & Sutter, E. E. (1998). Contrast dependence of multifocal ERG components. *Visual Science and its Application*, *OSA Technical Digest Series*, 24–27.

Bearse, M. A., Sutter, E. E., Sim, D. & Stamper, R. (1996). Glaucomatous dysfunction revealed in higher order components of the electroretinogram. *Visual Science and its Application*, *OSA Technical Digest Series*, 104–107.

Bearse, M. A., Sutter, E. E., Smith, D. N., & Stamper, R. (1995). Ganglion cell components of the human multi-focal ERG are abnormal in optic nerve atrophy and glaucoma (ARVO abstract). *Investigative Ophthalmology and Visual Science*, *36*, 445.

Cowey, A. (1967). Perimetric study of field defects in monkeys after cortical and retinal ablations. *Quarterly Journal of Experimental Psychology*, *19*, 232–245.

Frishman, L. J., Shen, F. F., Du, L., Robson, J. G., Harwerth, R. S., Smith, E. L., Carter-Dawson, L., & Crawford, M. L. J. (1996). The scotopic electroretinogram of macaque after retinal ganglion cell loss from experimental glaucoma. *Investigative Ophthalmology and Visual Science*, *37*, 25–141.

Greenstein, V. G., Holopigian, K., Seiple, W., Kahanowicz, R., & Katz, A. (1997). Multi-focal ERGs and visual fields in diabetic patients with macular edema. (ARVO abstract). *Investigative Ophthalmology and Visual Science*, *38*, 767.

Hood, D. C., Seiple, W., Holopigian, K., & Greenstein, V. (1997). A comparison of the components of the multi-focal and full-field ERGs. *Visual Neuroscience*, *14*, 533–544.

Hood, D. C., Holopigian, K., Seiple, W., Greenstein, V., Li, J., Sutter, E. E., & Carr, R. E. (1996). Do the delays in the cone ERG from patients with RP indicate global retinal damage? (ARVO abstract). *Investigative Ophthalmology and Visual Science*, *37*, 341.

Hood, D. C., Frishman, L. J., Viswanathan, S., Robson, J. G. & Ahmed, J. (1999). Evidence for a substantial ganglion cell contribution to the primate electroretinogram (ERG): effects of TTX on the multifocal ERG in macaque. *Visual Neuroscience* (in press).

Massey, S. C. (1990). Cell types using glutamate as a neurotransmitter in the vertebrate retina. In N. N. Osborne, & G. J. Chader, *Progress in retinal research*, vol. 9 (pp. 339–425). Oxford, UK: Pergamon Press.

Massey, S. C., & Maguire, G. (1995). The role of glutamate in retinal circuitry. In H. V. Wheal, & A. M. Thomson, *Excitatory amino acids and synaptic transmission* (pp. 201–221). San Diego, CA: Academic Press.

Palmowski, A. M., Sutter, E. E., Bearse, M., & Fung, W. (1997). Mapping of retinal function in diabetic retinopathy using the multifocal electroretinogram. *Investigative Ophthalmology and Visual Science*, *38*, 2589–2596.

Simonsen, S. E. (1980). The value of the oscillatory potential in selecting juvenile diabetics at risk of developing proliferative retinopathy. *Acta Ophthalmology*, *58*, 865–878.

Sutter, E. E., & Bearse, M. A. (1995). Extraction of a ganglion cell component from the corneal response. *Visual Science and its Application OSA Technical Digest Series*, 1, 310–313.

Sutter, E. E., & Bearse, M. A. (1998). The optic nerve head component of the human ERG. *Vision Research*, *39*, 419–436.

Sutter, E. E., & Tran, D. (1992). The field topography of ERG components in man-I. The photopic luminance response. *Vision Research*, *2*, 433–466.

Vaegan, & Buckland, L. (1996). The spatial distribution of ERG losses across the posterior pole of glaucomatous eyes in multifocal recordings. *Australian and New Zealand Journal of Ophthalmology*, *24*, 28–31.

Vaegan, & Sanderson, G. (1997). Absence of ganglion cell subcomponents in multifocal luminance electroretinograms. *Australian and New Zealand Journal of Ophthalmology*, *25*, S87–S90.

Viswanathan, S., Frishman, L. J., Robson, J. G., Harwerth, R. S., & Smith, E. L III (1996). Inner retinal contributions to the photopic ERG in macaque monkey: suppression of responses by pharmacological agents and experimental glaucoma (ARVO abstract). *Investigative Ophthalmology and Visual Science*, *37*, 348.

Viswanathan, S., & Frishman, L. J. (1997). Evidence that negative potentials in the photopic electroretinograms of cats and primates depend upon spiking activity of retinal ganglion cell axons. *Society for Neuroscience*, Abstract, 23.

Wu, S., & Sutter, E. E. (1995). A topographic study of oscillatory potentials in man. *Visual Neuroscience*, *12*, 1013–1025.

Yonemura, D., Aoki, T., & Tsuzuki, K. (1962). Electroretinogram in diabetic retinopathy. *Archives of Ophthalmology*, *68*, 19–24.