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Analysis of Second-order Kernel Response Components of Multifocal Electroretinograms Elicited from Normal Subjects

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Purpose: It has been reported that the second-order kernel response components of multifocal electroretinograms (mERGs) reflect the electrical activity of the inner retinal layers. In this study, we have investigated whether the amplitudes of the second-order kernel response components correlate with the spatial distribution of human retinal ganglion cells.

Methods: Multifocal electroretinograms were recorded using the Veris III™ system from 5 healthy subjects with different stimulus and recording parameters. The mERGs were analyzed using the Veris Science™ software programs. The stimuli consisted of densely arranged arrays of 103, 61, 37 or 19 hexagonal elements. Four minutes were required to record one set of mERG responses using 8 sessions, and 8 minutes using 16 sessions. The second-order kernel response components were extracted and analyzed using the Veris Science™ program.

Results: The signal-to-noise ratio of the first-order kernel response components was improved considerably by the summation of the nine reproducible responses from the same subject but the second-order kernel response components were not. The summation of the nine reproducible responses was insufficient to identify an array of the second-order kernel response components. Both the first- and second-order kernel response components were larger when fewer hexagonal elements were used. There was no significant difference in the individual responses between the 4-minute and the 8-minute recordings. A response density analysis revealed a weak correlation between the amplitude distribution of the second-order kernel response components and the spatial distribution of human retinal ganglion cells.

Conclusions: The distribution of the amplitudes of the second-order kernel response components of the mERGs elicited from normal subjects did not correlate with the distribution of human ganglion cells. This suggests that the theory that second-order kernel response components arise from the activity of retinal ganglion cells should be reconsidered. **Jpn J Ophthalmol 2001; 45:247–251**

Key Words: Human ganglion cell distribution, multifocal electroretinograms, second-order kernel response components.

Introduction

Pattern electroretinograms have played an important role in evaluating the function of the inner retinal layers.[1–3] It has been recently suggested that the second-order kernel response components of the multifocal electroretinogram (mERG) also originate in the inner retina.[4] Because the second-order kernel response components are very small, these local responses are usually spatially summated to obtain a better signal-to-noise ratio.[4] Because such a summation procedure[4–6] can be used only when the corresponding regions show spatial linearity, it should not be applied unless the spatially summated second-order kernel response components can be shown to agree

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with the nonlinear component elicited by stimulating the whole corresponding summated regions evenly.

In this study, we recorded mERGs from normal subjects and analyzed the second-order kernel response components in relation to the spatial distribution of human retinal ganglion cells. The optimal stimulus and recording parameters for eliciting the mERGs were used.

Materials and Methods

The mERGs were recorded with the Veris III™ system (Mayo, Inazawa, Aichi). The standard stimuli were displayed on a CRT monitor (MD-B1700; Chuomusen, Tokyo), and consisted of a densely arranged array of 103 hexagonal elements (in an 103 hexagon pattern). The CRT monitor subtended an angle of 42° high by 45° wide at the subject's eye level. Five well-trained healthy men (between 26 and 36 years of age), whose refractive errors were between −0.5 and −2.5 diopters, participated after informed consent was obtained.

Each hexagonal element was independently alternated between brightness and darkness according to a pseudo-random sequence mode (binary m-sequence) at a frequency of 75 Hz. The mean luminance was 91 cd/m^2 (L_{max} = 178 cd/m^2; L_{min} = 4 cd/m^2), and the contrast was 96%. The pupils were fully dilated by topical 0.5% tropicamide and 0.5% phenylephrine hydrochloride.

The mERGs were recorded using a bipolar contact lens electrode after corneal anesthesia was induced by a drop of oxybuprocaine chlorhydrate. One or more drops of artificial tears (sodium hyaluronate) were added before electrode insertion. A ground electrode was placed on the ipsilateral earlobe. Each subject was seated comfortably with his chin and forehead tightly fixed. He was asked to look intently at the fixation point in the center of the CRT monitor. The fixation was monocular by the tested eye during stimulation. The distance between the tested eye and the CRT monitor was 32 cm.

The signals were amplified with a model 12-4 Neurodata Acquisition System™ (Astro-Med, West Warwick, RI, USA) and bandpass filtered from 10 to 300 Hz. Four minutes were required to record one set of mERG responses using 8 sessions, or 8 minutes using 16 sessions. In order to study the Veris Science™ built-in Combination Program, mERGs that were recorded on 3 separate days were analyzed.

The data relating to the first-order kernel response components were reported in our previous paper.[7] Because the second-order kernel response

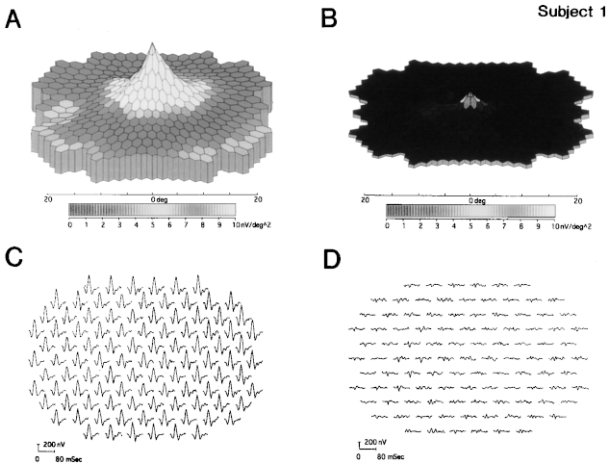

Figure 1. Subject 1. First-order kernel response components (**A**) and second-order kernel response components (**B**) of multifocal electroretinogram (mERG). Nine mERGs recorded from well-trained healthy subject were summated using Combination procedure. Trace arrays of first-order kernel response components (**C**) and second-order kernel response components (**D**) are depicted. Neither Artifact Removal nor Spatial Averaging procedure was used.

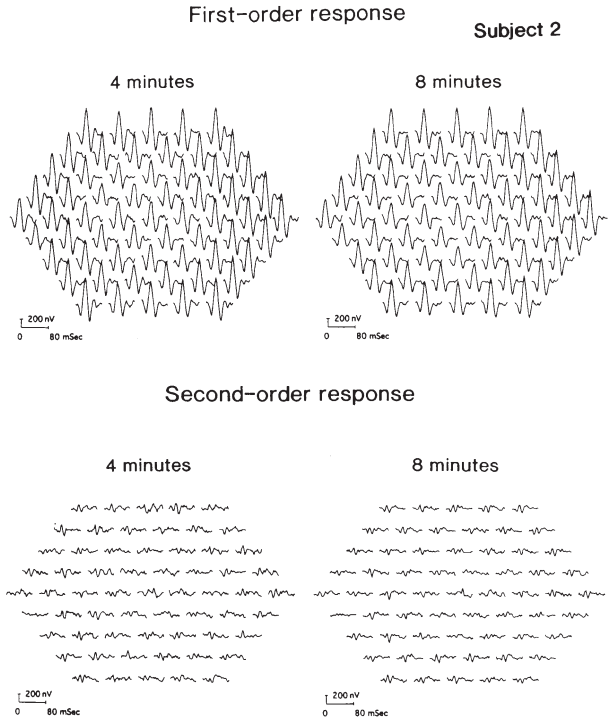

Figure 2. Subject 2. Comparison of 8-minute recording (right column) with 4-minute recording (left column). Upper: first-order kernel response components (first-order response); lower: second-order kernel response components (second-order response). Neither Artifact Removal nor Spatial Averaging procedure was used.

components were generally much smaller than the first-order kernel response components, it was necessary to determine the optimal stimulus parameters and recording conditions to obtain larger second-order kernel response components. The number of hexagonal elements in the stimulus was 19, 37, or 61 with a recording duration of 8 minutes for each set of hexagonal elements. The Artifact Removal and Spatial Averaging procedures were purposely not used in this study.

Results

As reported previously, the nine sets of mERGs recorded from a well-trained healthy subject on 3 separate days using the 103 hexagonal pattern had good reproducibility.[7] The nine individual responses were summated using the Combination software program of the Veris Science™ software package. However, the second-order kernel response components (Figures 1B and 1D), extracted using the Veris Science™ software program from the combined responses, were much smaller than the first-order components (Figures 1A and 1C). Thus, while the Combination procedure seems to be effective in improving the signal-to-noise ratio for the first-order kernel response components, little improvement was observed for the second-order kernel response components.

The first-order kernel response components obtained by a 4-minute recording were comparable to those obtained by an 8-minute recording (Figure 2). The second-order kernel response components were also very similar for these two conditions.

With a decrease in the number of elements, the amplitude of the first-order kernel response components increased (Figure 3, upper panel; note the decrease in the sensitivity in the 19 hexagonal pattern). The second-order kernel response components were also increased with a decrease in the number of elements. However, as noted in Figure 1, the second-order kernel response components were much

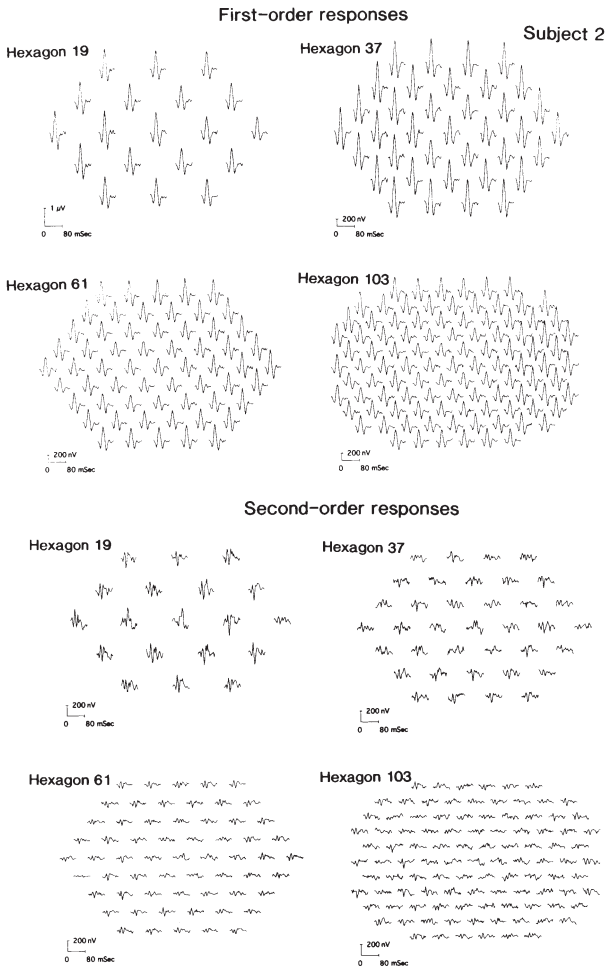

Figure 3. Subject 2. Multifocal electroretinogram recorded using 19, 37, 61, and 103 hexagon patterns. Upper: first-order kernel response components (first-order responses); lower: second-order kernel response components (second-order responses). Neither Artifact Removal nor Spatial Averaging procedure was used.

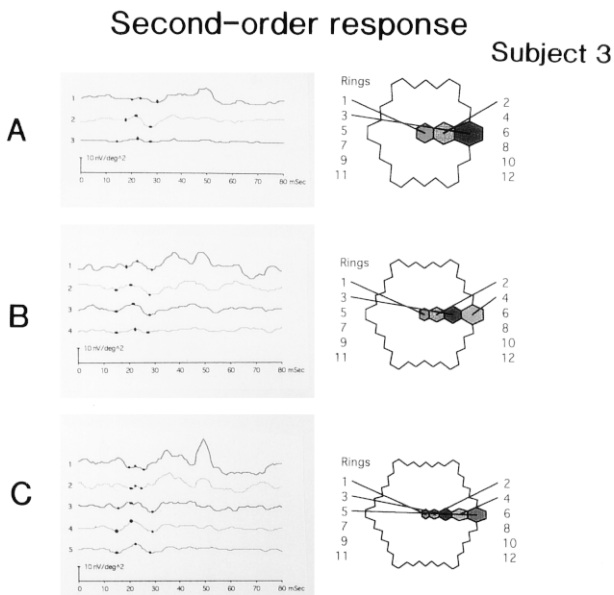

Figure 4. Response density in each element aligned horizontally from center to temporal retinal region. Three components are depicted with three dots on each wave. Response density between initial negative peak and subsequent positive peak was measured.

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smaller than the first-order kernel response components (Figure 3, lower panel).

The correlation between the amplitude of the second-order kernel response components and the spatial distribution of human ganglion cells was investigated using the 19, 37, and 61 hexagon patterns (Figure 4). The response density scale analysis was used to display the mERG responses per unit area, and this demonstrated a positive peak at around 23 milliseconds for each wave (Figure 4). Three peaks were observed, which are marked by three dots on each wave (Figure 4).

The response densities obtained from the 5 subjects between the initial negative peaks and the subsequent positive peaks were measured and these are plotted in Figure 5. Because the size of each hexagonal element is different for each pattern (Figure 5, right-hand column), the location of each hexagonal element was calculated at a visual angle and depicted in contrast with the retinal eccentricity. The human ganglion cell distribution reported by Curcio and Allen[8] is presented in the right-hand column of Figure 5. The response density study of the second-order kernel response component revealed that the largest amplitude was at element 4 for the 61 hexagons (Figure 5, upper panel), and at element 3 for the 37 hexagons (Figure 5, middle panel). Elements 2 or 3 tended to produce the largest response density for the 19 hexagons (Figure 5, lower panel).

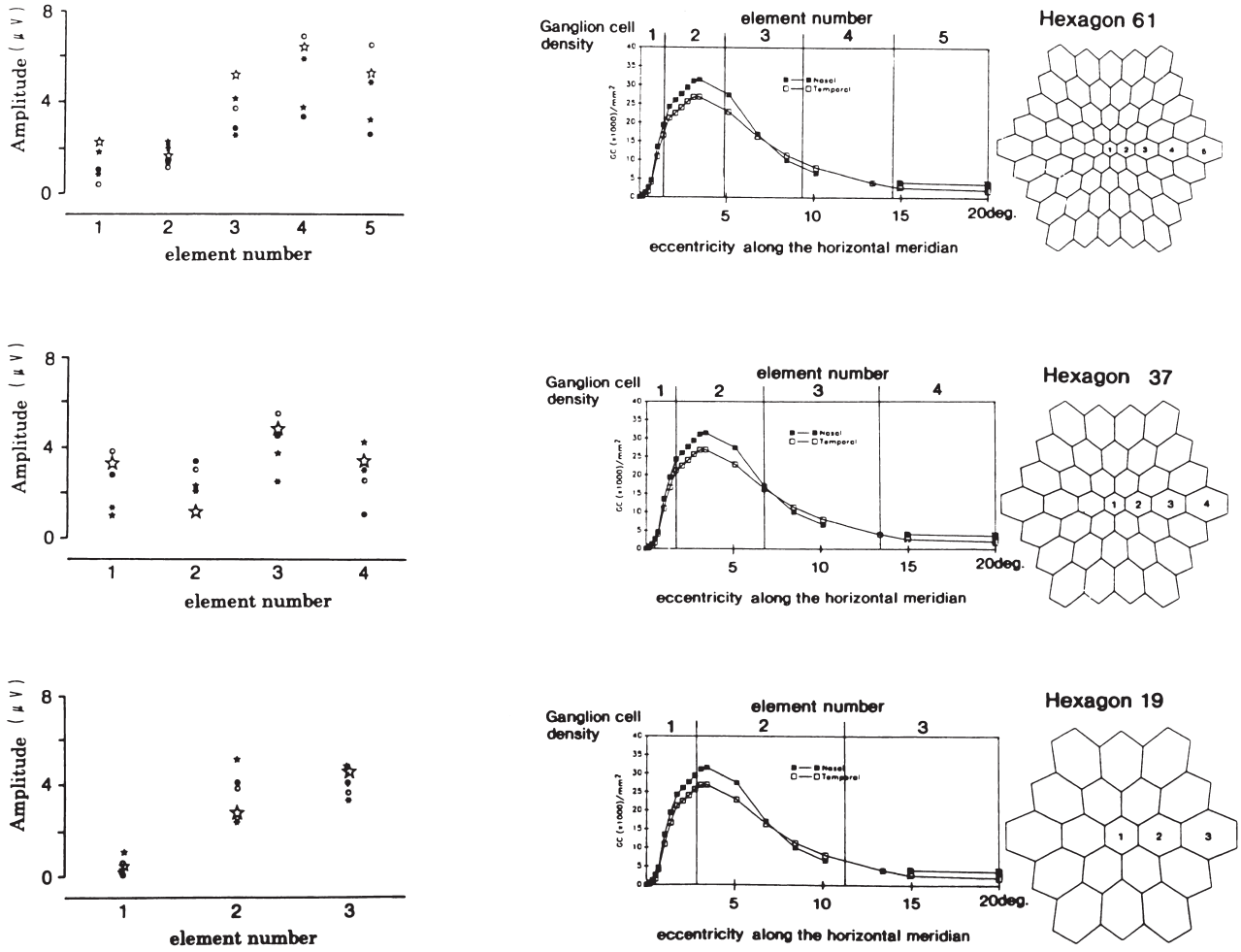

Figure 5. Response density values obtained from 5 normal subjects are plotted for each stimulus element number in left column. Upper, 61 hexagon; middle, 37 hexagon; and lower, 19 hexagon patterns. Location of each element was calculated as visual angle at nearest and farthest points within each element from fixation point on CRT display. Human ganglion cell distribution (right column) was compared with response density values for each hexagon pattern. Human ganglion cell distribution chart (right) from Curcio and Allen.[8] Translated by permission of John Wiley & Sons, Inc. All rights reserved.

Discussion

The Combination software program has an effect similar to that of the averaging or summation technique and enables us to recognize more clearly a small evoked signal in a noisy background. The first-order kernel response components of the mERGs became less noisy using the Combination program, while the signal-to-noise ratio for the second-order kernel response components was improved only slightly even after all nine reproducible mERGs were combined. This suggests that the existence of a second-order kernel response component produced by the Veris III™ system using the 103 hexagon pattern is questionable.

Our results demonstrated that increasing the recording duration from 4 to 8 minutes did not significantly change the amplitude of the first- or second-order kernel response components when the 61 hexagon pattern was used. It would thus appear difficult to detect the second-order kernel response components when the 103 and 61 hexagon patterns are used.

With a decrease in the number of hexagonal elements, both the first- and second-order kernel response components increased for all of the stimulus patterns.

We compared the amplitude of the second-order kernel response components with the human, retinal ganglion cell distribution under conditions in which larger second-order kernel response components were obtained. However, the distribution of the amplitude of the second-order kernel response components obtained from normal subjects using stimulus elements larger than the standard 103 elements did not correlate with the human ganglion cell distribution in terms of response density of the mERGs.

Recently, Sutter and Bearse[9] reported that the second-order kernel response component consisted of a retinal component and an optic nerve head component. Their study, however, had two limitations.

First, the details of the algorithm used to detect both components were not described. After careful examination, we have found a "bug"[10] in the Veris III™ system software used by Sutter and Bearse. Although they used the Artifact Removal procedure to eliminate artifacts in the mERGs, the details of its algorithm were not provided, and the Veris Science™ program for calculating the second-order kernel response component also was not described in detail. In addition, there is no precise explanation regarding these issues in the Veris Science™ instruction manual. Thus, the results obtained using unknown software programs and a software program that includes a "bug" are questionable.

Second, Sutter and Bearse also used a Spatial Averaging procedure and analyzed the response obtained from each element in an annular ring. Using the Spatial Averaging procedure, a part of the responses from the surrounding region are summed with the response obtained from the central element. Thus, the response obtained from the stimulus element does not reflect the response generated from only that element.

Our results show that the second-order kernel response components do not show the effects of the Combination procedure using the 103 hexagon pattern or the effects of the recording duration using the 61 hexagon pattern. When larger hexagonal element stimuli were used, ie, the 61, 37, and 19 hexagon patterns, larger first-order as well as larger second-order kernel response components were obtained. However, the response density did not correlate with the human, retinal ganglion cell distribution when the 61, 37, or 19 hexagon patterns were used. These findings strongly suggest that the second-order kernel response components of the mERGs do not reflect the activity of the ganglion cell layer. Thus, the recent theory that the origin of the second-order kernel response component is related to the activity of the retinal ganglion cells should be reconsidered.

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