Effects of luminance combinations on the characteristics of the global flash multifocal electroretinogram (mfERG)

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Abstract

Purpose: This study aims to ascertain the characteristics of the response triggered by the global flash multifocal electroretinogram (MOFO mfERG) under various combinations of global and focal flash luminance, and to determine the optimal conditions for this measurement.

Methods: Ten normal subjects with mean age 23.2 yrs (± 1.14 yrs) were recruited for the MOFO mfERG measurement. The visual stimulation consisted of four video frames (stimulus frame with 103 scaled hexagonal focal flashes, followed by a dark frame, a global flash and then another dark frame). The focal and global flash intensities were varied independently for four levels (50, 100, 200 and 400cd/m^2). The subjects then underwent measurements with sixteen combinations of focal and global flash luminance. The direct component (DC) and induced component (IC) of the MOFO mfERG were grouped into central and peripheral regions for analysis.

Results: The central and peripheral DC amplitude increased with the focal flash luminance under constant global flash luminance. Moreover, the proportion of the global flash and focal flash intensity was shown to be important to achieve an optimal IC response. When the ratio of global flash luminance to focal flash luminance (g/f ratio) was kept at about 2:1, the central and peripheral IC amplitude reached the peak value, and further increasing the global flash luminance would not enhance the IC response magnitude. The implicit time of both central and peripheral DC generally decreased with the increase of g/f ratio. However, the implicit time of central and peripheral IC increased with the g/f ratio.

Conclusion: The g/f ratio is important in the MOFO mfERG paradigm since the DC and IC responses change with this ratio. In order to obtain both optimal DC and IC responses, a g/f ratio of 1:1 with focal flash luminance between 100cd/m^2 and 200cd/m^2 would be recommended. As the global flash mfERG paradigm is studying the

interaction triggered by both flashes, the g/f ratio is a vital parameter for measurement in future studies.

Keywords: Multifocal electroretinogram, global flash, luminance, adaptation

Introduction

The multifocal electroretinogram (mfERG) provides a tool for the assessment of topographic retinal responses. It helps in examining local functional losses in various retinal diseases such as glaucoma, diabetic retinopathy, age-related macular degeneration and retinitis pigmentosa [1-11]. The conventional mfERG signals were reported to mainly originate from bipolar cells [12, 13]. With a modified mfERG protocol suggested by Sutter and his co-workers, the retinal responses from the inner retinal layer, especially ganglion cell activity, were also studied [14].

This modified multifocal stimulation was used to study the retinal adaptive mechanism by inserting interleaved global flashes between the successive frames of the multifocal stimulus [14]. A large non-linear inner retinal response could be triggered. This “global flash” protocol has been further applied to study ocular diseases that involve the inner retina. Shimada et al. [15] used the global flash paradigm for the early detection of functional changes in diabetic retinopathy. Chu et al. [16, 17] further modified the global flash protocol to facilitate the early detection of glaucoma.

In the global flash mfERG response, there are two main components: the direct component (DC) and the induced component (IC). DC is the mean response of the focal flash and IC is the adaptive response due to the interaction of the focal and the global flash [18]. The DC was proposed to be composed of outer retinal responses and inner retinal oscillation-like wavelets; while for the IC, its origin was proposed to be predominant from the inner retinal layer [19, 20]. This modified protocol demonstrates its capability in diagnosing inner retinal dysfunction. Since the luminance intensities of the focal and global flashes can influence the retinal physiology that alters the

characteristics (i.e. amplitude, implicit time) of the DC and IC, apart from applying it as a clinical tool, the effect of luminance on this protocol should have more understanding.

Shimada and co-workers firstly studied the effect of different combinations of focal and global flash luminance [18] but only three subjects with a wide age range (23-63 yrs) completed all experimental conditions. It was not adequate to determine MOFO mfERG response characteristics. Moreover, a thorough understanding of the relationship between the global and focal flash luminance and the DC and IC performance help in enhancing the mfERG measurement to achieve different purposes. Hence, the optimal setting of the focal and global flash luminance would help in maximizing the measurement of outer and inner retinal responses. It is necessary in achieving the most effective paradigm, especially in the clinical assessment of retinal diseases. In this study, we investigated the characteristics of the DC and IC in different retinal regions under various luminance combinations (both global and focal flashes) in the global flash (MOFO) mfERG paradigm. We attempted to suggest the optimal luminance setting for this particular mfERG measurement to obtain the good retinal signals.

Methods:

Subjects

Ten normal subjects (age range 21-24 yrs, mean age 23.2 ± 1.14 yrs) were recruited for this study. The subjects had a visual acuity of 6/6 without any ocular or systemic disorders. Their refractive errors were within +3 to -6 DS and less than -1.25 DC. One eye was randomly selected for the measurement. Pupil dilatation was carried out on the tested eye of the subjects who would be light-adapted at room illumination (~100 lux) throughout the whole experiment.

All procedures of the study followed the tenets of the Declaration of Hesinki. The study was approved by the Ethics Committee of The Hong Kong Polytechnic University. Informed consent was obtained from each subject after the experimental procedures were described.

Stimulus conditions

The VERIS Science 5.1 system (Electro-Diagnostic-Imaging; San Mateo, CA, USA) was used for the mfERG measurement. The visual stimuli contained 103 scaled hexagons subtending a visual field of about 45°. The stimulus was displayed on a high luminance CRT monitor (FIMI, Medical Electr. Equipm., Italy). The hexagonal stimulation followed a pseudo-random binary m-sequence (2^{13}-1) with a video frame rate of 75Hz. There were four video frames in the stimulation: focal flash, followed by a full screen dark frame, a full screen global flash and another full screen dark frame. The total duration of these four video frames was 53.2ms. The luminance of the focal flash and the global flash was varied independently with four different luminance levels (50, 100, 200 and 400cd/m^2); while the dark frame was set to a luminance of 2cd/m^2. This would have sixteen combinations of global (g) and focal (f) flash luminance for this study. The background luminance was set at 100cd/m^2. A central cross on the stimulus pattern was used for fixation.

Recording conditions

Before testing, the pupil of the tested eye was fully dilated with 1% tropicamide (Alcon, Fort Worth, TX). The untested eye was occluded by an eye patch. A Dawson-Trick-Litzkow (DTL) electrode was used as the active electrode. Gold-cup electrodes were placed at the ipsilateral temporal side and forehead respectively as reference and ground electrodes. The refractive error of the tested eye was corrected by the spherical

equivalent power for a viewing distance of 33 cm. The signals were amplified by 100,000 with a band-pass filter from 3 to 300 Hz (Grass Instrument Co., Quincy, MA, U.S.A.). The recording time for each luminance combination was about eight minutes. There were a total of sixteen recordings for each subject. The sequence of the sixteen recordings was randomized. The sixteen recordings took place in two days to avoid subjects being fatigue. Each recording was divided into thirty-two segments of approximately 14 seconds and a break was allowed between each segment. Any segment contaminated by poor fixation, eye movements, or blinks was rejected and re-recorded immediately.

Analysis

The mfERG responses were grouped into two regions: central (Ring 1-2, ~ central 7° visual field) and peripheral (Ring 4-6, ~ 17.2° to 44.5° visual field). Ring 3 was the transitional region between the central and peripheral regions [16], so it was excluded in the analysis to avoid a confounding factor in either region. The first-order kernel of the mfERG response was extracted and analysed. The DC and IC peak-to-peak amplitudes of the mfERG were measured and compared among different combinations of the global and focal flash luminance as shown in Figure 1a and Figure 1b. The implicit times of the DC and IC were also measured for analysis. All the comparisons were performed by repeated measures ANOVA and Bonferroni post-hoc test. The ratio of global flash luminance to focal flash luminance (g/f ratio) was also applied to correlate with changes in the DC and IC responses.

Results

DC Amplitude

In the central retinal region, the DC amplitude increased with focal flash luminance

under a fixed global flash luminance. Figure 2a showed the change in amplitude of DC with different g/f ratios (i.e. ratio of global flash to focal flash luminance) for four focal flash luminance levels. When the focal flash luminance was within the range of 100 to 400cd/m^2, the DC amplitude achieved the maximum value when the g/f ratio was minimal (with the global flash set at the experimental dimmest value, i.e. 50cd/m^2). Further increasing the g/f ratio would decrease the DC amplitude and the central DC amplitude was significantly affected by the luminance of focal flash (f) ($p<0.001$), global flash (g) ($p<0.001$) and was also affected by their interaction ($p<0.05$).

In the peripheral retinal region, the DC amplitude increased with the focal flash luminance but decreased with the global flash luminance. When the focal flash luminance was within the range of 100 to 400cd/m^2, the DC response reached the maximum value while the g/f ratio was the smallest (Figure 2b). It was affected by the luminance of focal flash ($p<0.001$), global flash ($p<0.005$) and their interaction ($p<0.025$).

When the global flash intensity was less than the focal flash intensity (i.e. g/f ratio< 1), the DC responses for the focal flash intensity from 100 to 400cd/m^2 were in a similar trend. From the post-hoc test, the combination of g/f at 50/400, the DC amplitude achieved the maximum value in both the central and peripheral regions; while the combination of g/f at 400/50, it achieved the minimum value of the amplitude. From our findings, a focal flash luminance greater than or equal to 100cd/m^2, together with a global flash of not more than 400cd/m^2 would give rise to a better DC signal in the measurement. Under constant focal flash luminance, the global flash dimmer than or equal to the focal flash luminance (i.e. g/f ratio≤ 1) would achieve a reasonable DC signal.

IC Amplitude

In the central retinal region, when the focal flash luminance ranged from 50 to 200cd/m^2, the IC amplitude increased with the global flash luminance until the g/f ratio reached 2 (i.e. the global flash luminance was twice the focal flash luminance). Afterwards, the IC amplitude began to drop even with further increasing the global flash intensity (Figure 3a). The central IC signal was significantly affected by the luminance of the focal flash ($p<0.005$) and its interaction with the global flash luminance ($p<0.025$).

The IC amplitude in the peripheral region showed the same characteristic as in the central region. When the focal flash luminance was within the range of 50 to 200cd/m^2, the IC response increased with the global flash luminance until the g/f ratio was approximating to 2 (Figure 3b). Beyond this point, the IC amplitude would drop. The peripheral IC response was significantly affected by the focal flash ($p<0.025$), global flash ($p<0.001$) and their interaction ($p<0.005$).

From the post-hoc test results, the g/f combinations of 100/50 and 200/100 led to the maximum IC amplitude in the central and peripheral regions respectively, while the combinations of 50/200 and 50/100 led to the minimum IC amplitude in the central and peripheral regions respectively. A focal flash luminance dimmer than or equal to 400cd/m^2 and a global flash greater than or equal to 100cd/m^2 gave rise to a better IC response. For a focal flash within 50 to 200cd/m^2, the global flash luminance should be greater than or equal to the focal flash in order to achieve a reasonable IC signal (i.e. g/f ratio$\geq$ 1), except the combination with focal flash of 200cd/m^2 and global flash of 100cd/m^2. The IC signal generated by this combination did not show significant difference compared with other combinations.

DC Implicit time

The change in the central DC implicit time against the g/f ratio is shown in Figure 4a. When the global flash was dimmer than the focal flash (g/f ratio< 1), the higher the

focal flash intensity, the longer the delay in the DC implicit time, under the same g/f ratio. Initially, the implicit time increased with the g/f ratio. After increasing to a certain level, it would then shorten. It was significantly affected by changes in the focal flash luminance ($p<0.001$). The scattered points of the DC implicit time in the central region converged with the increase in g/f ratio (Figure 4a).

The peripheral DC implicit time was shortened with an increasing g/f ratio. In other words, a brighter global flash would trigger the DC to occur earlier (Figure 4b). It was affected by the intensity of the focal flash ($p<0.001$), global flash ($p<0.001$) and their interaction ($p<0.001$). The peripheral DC implicit time seemed to reach the maximum as the g/f ratio was less than 1. Afterwards, the implicit time was shortened.

IC Implicit time

The IC implicit time in the central retinal region was generally delayed with the g/f ratio (Figure 5a), and the changes were variable at different focal flash luminance levels. It was significantly affected by the global flash luminance ($p<0.01$).

However in the peripheral retinal region, the IC implicit time was delayed with an increase in the g/f ratio, that is, the IC implicit time was lengthened with a brighter global flash. The delay of response then seemed not to increase when the g/f ratio exceeded 1 (Figure 5b). It was significantly affected by both the intensities of the focal ($p<0.001$) and global flashes ($p<0.01$).

Discussion

In the periodic global flash mfERG measurement, the resultant waveform contains two sharp peaks: the direct component (DC) and the induced component (IC). Our findings clearly demonstrated that the amplitudes and implicit times of both responses were influenced by the intensity of global and focal flashes as well as the combination of

these two flashes (i.e. g/f ratio). The DC contains the response predominantly from the ON- and OFF-bipolar cells with three oscillatory wavelets from the inner retina hidden underneath; while for the IC, it is generated from the inner retina, mainly the ganglion cells and amacrine cells [19].

The mfERG waveforms and amplitudes change with retinal eccentricity [14, 16, 20, 21]; therefore, in this study, we divided retinal eccentricity into central (~7°) and peripheral regions (~17.2° to 44.5°) for analysing the variations of DC and IC responses under different luminance intensity conditions. A new parameter, the g/f ratio for this MOFO measurement, is introduced for understanding how the interaction of the global and focal flashes influences both DC and IC in terms of amplitude and implicit time.

In a previous study [18], the DC amplitude was found to grow approximately linearly with increasing log units of focal flash intensity. Our results were consistent with their findings and both the central and peripheral DC amplitudes increased with the focal flash intensity. The DC amplitude seemed to achieve its largest value when the ratio was kept at the minimum. Different proportions of global flash intensity and focal flash intensity could obtain a larger DC response instead of increasing the focal flash intensity alone. A stronger flash intensity could improve the signal-to-noise ratio, but the greater luminance would cause irritation to the subjects during measurements. By applying this finding, the discomfort of the subjects could be minimized.

The IC is the response change to the global flash from the preceding focal flash and it was reported to be related to the inner retina [19]. After the IC amplitude peaked at a g/f ratio of 2:1, the retina seemed to be subtle and thus did not react to further increase in the global flash intensity. This demonstrated the non-linear characteristics of the inner retinal adaptive mechanisms. Shimada et al [18] found a point of inflexion for the individual data when both intensities of focal flash and global flash were equal to 200cd/m^2. This point was absent in our study. There are two possible explanations.

Firstly, ring 3, regarded as a transition zone, was discarded in the retinal area grouping for analysis in the current study. Secondly, the adaptive mechanisms of the central and peripheral IC may not be similar. Figure 1a and 1b illustrate the waveforms from the central and peripheral regions. Due to the shift of implicit time the waveform at the central and peripheral regions showed different patterns. This may be why the waveforms from our findings differed from the summated one by grouping all the responses as a whole in their study. The separated analysis of the central and peripheral responses may make the point of inflexion less obvious. This may also explain why the IC property at g/f ratio =2:1 was not obvious in Shimada's study.

Under constant focal flash intensity, a shortened DC implicit time was reported with increasing global flash intensity [18]. Their results were only similar to our findings when g/f ratio was less than or equal to 1. The performance of the central DC implicit time was opposite to that reported when g/f ratio was greater than 1. For the peripheral DC implicit time, it was initially stable with the g/f ratio and then was shortened when the ratio was greater than 1. This showed that the implicit time of the DC in different retinal regions behaved differently according to the g/f ratio. The IC implicit time in the central region was firstly delayed with an increase in the g/f ratio until a saturated level with the ratio more than 1. Its variation was more obvious at the peripheral region. It was also very similar to the case reported in Shimada's study [18] which showed a shorter IC implicit time existed when the focal flash intensity was greater than the global flash intensity. When comparing their reported implicit times in both central and peripheral regions, different behaviors under different g/f ratios suggested that the adaptive mechanism had different characteristics across the retina. In terms of the implicit times between DC and IC, the trends of the variations in both DC and IC implicit times were totally different. The DC became less delayed but the IC became more delayed as the g/f ratio increased in value. This clearly demonstrated different

physiological characteristics of the DC and IC in response to the combinations of the global and focal flash intensities.

Considering with the stray light problem suggested by the ISCEV guideline (2007) [22] and the patients' discomfort, the focal flash luminance, according to our findings, should be between 100 and 200cd/m^2. Together with a global flash dimmer than the focal flash, a considerable DC amplitude with good signal-to-noise ratio can be obtained (i.e. 100cd/m^2<f ≤200cd/m^2, f≥ g). Our recommended focal flash luminance is higher than the range suggested by Shimada et al. (i.e. 50cd/m^2<f <100cd/m^2) [18]. For a reasonable IC amplitude, it is recommended to have the focal flash less than or equal to 200cd/m^2 and a global flash higher than or equal to 100cd/m^2. The g/f ratio should be kept at greater than or equal to 1 (i.e. f≤200cd/m^2; g≥100cd/m^2; f≤ g). The focal flash luminance suggested in this study is higher than that of Shimada et al. [18]. Thus, in order to obtain both optimal DC and IC responses, a g/f ratio of 1:1 and with focal flash luminance greater than 100cd/m^2 and smaller than 200cd/m^2 would be recommended.

Conclusion

By studying different combinations of global and focal flash luminance, it was found that the amplitude and implicit time of the central and peripheral DC and IC were affected by different factors (focal flash intensity, global flash intensity and their combinations) respectively. Their characteristics should be studied with respect to the retinal eccentricity. The ratio of the global flash intensity to focal flash intensity (g/f ratio) is a useful parameter in designing a protocol for MOFO mfERG measurement to study linear and non-linear retinal properties. For the different behaviors between the DC and IC implicit time with the g/f ratio in different retinal regions, further studies are required to understand the underlying mechanism. Similar studies should also be carried out in an older population group to study the effect of age on the MOFO

paradigm and the adaptive mechanism.

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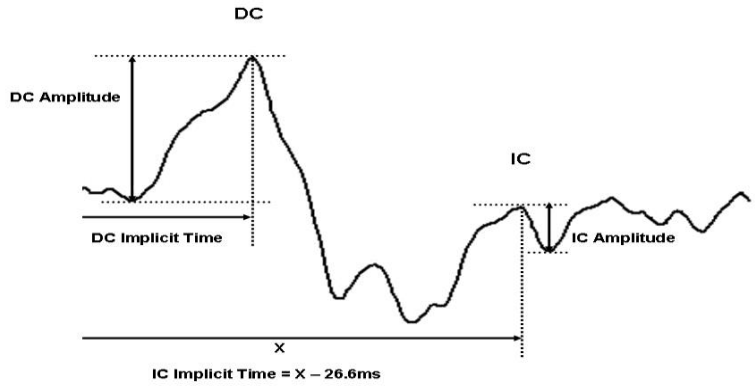

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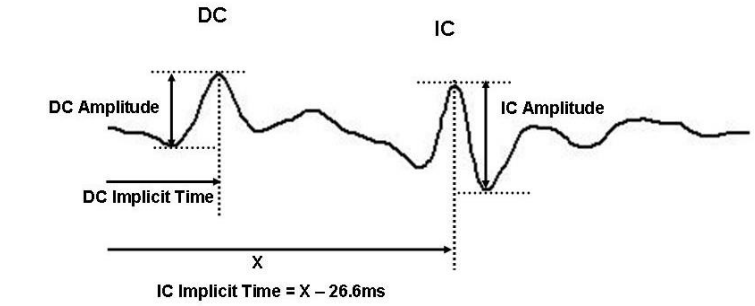

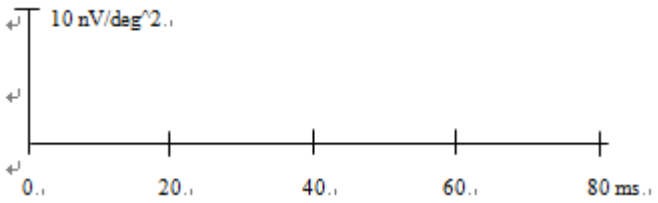

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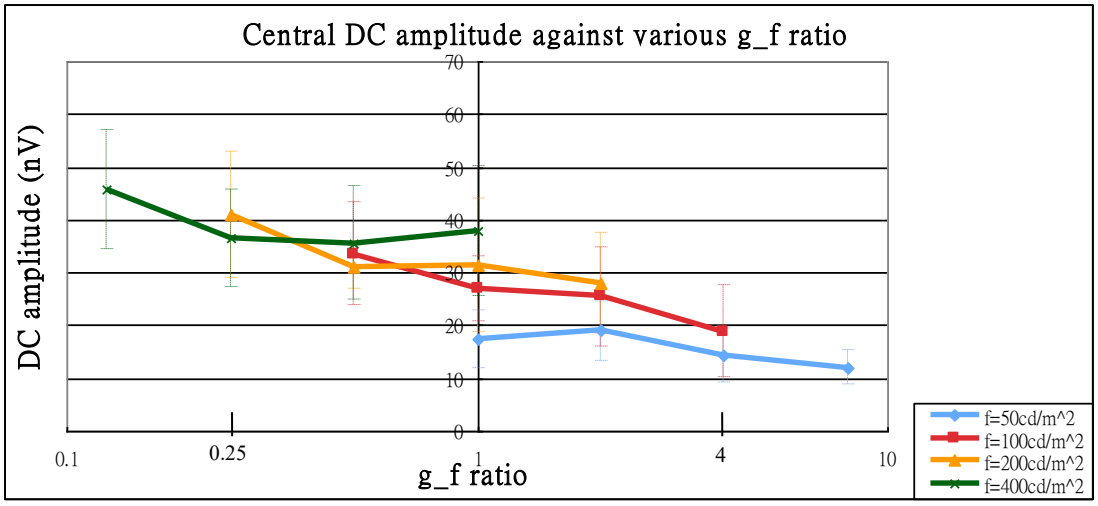

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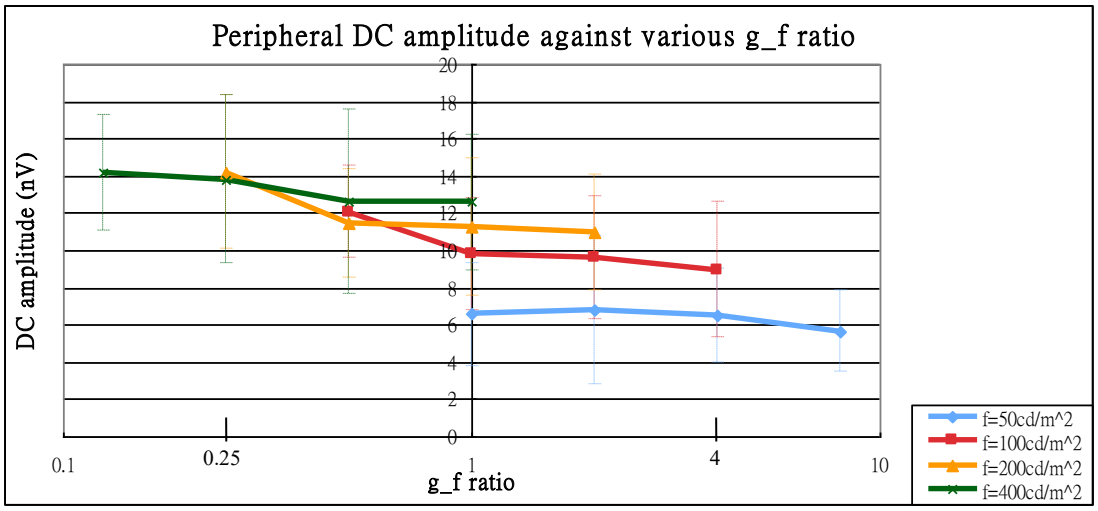

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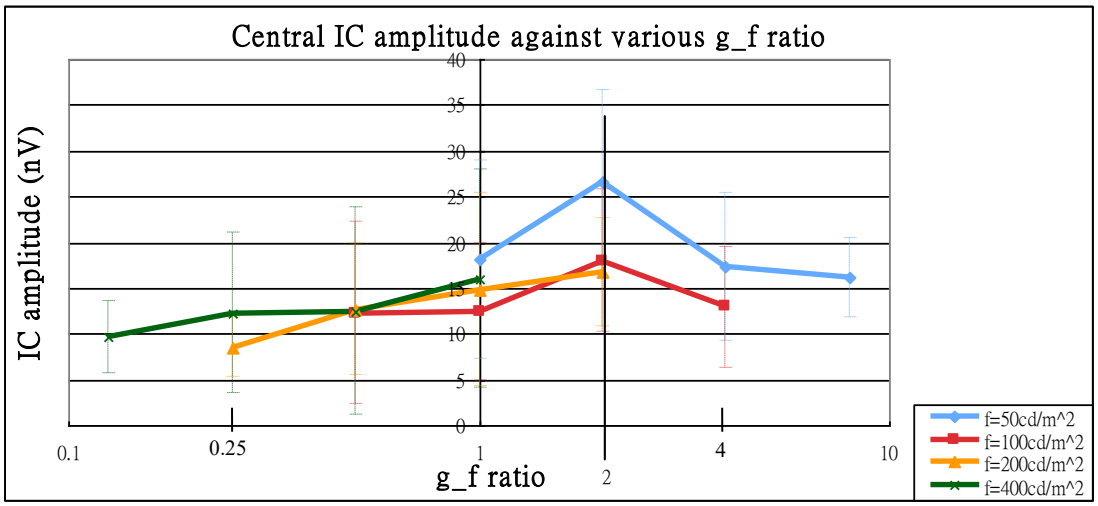

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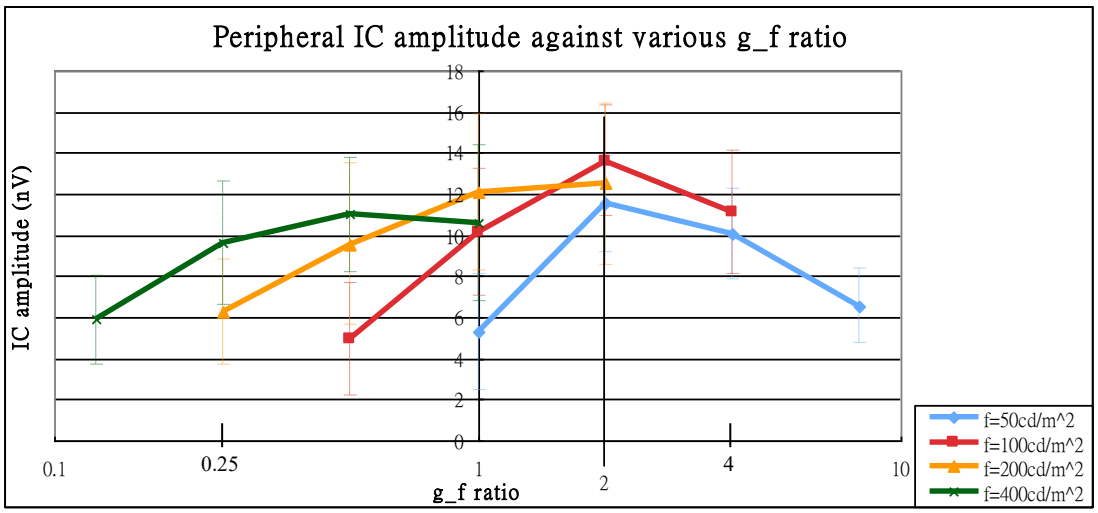

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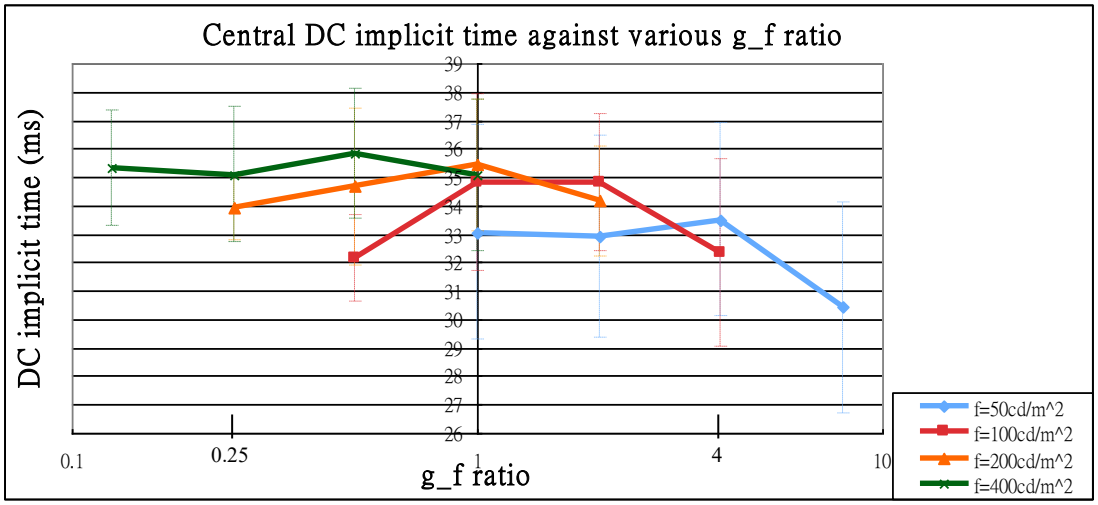

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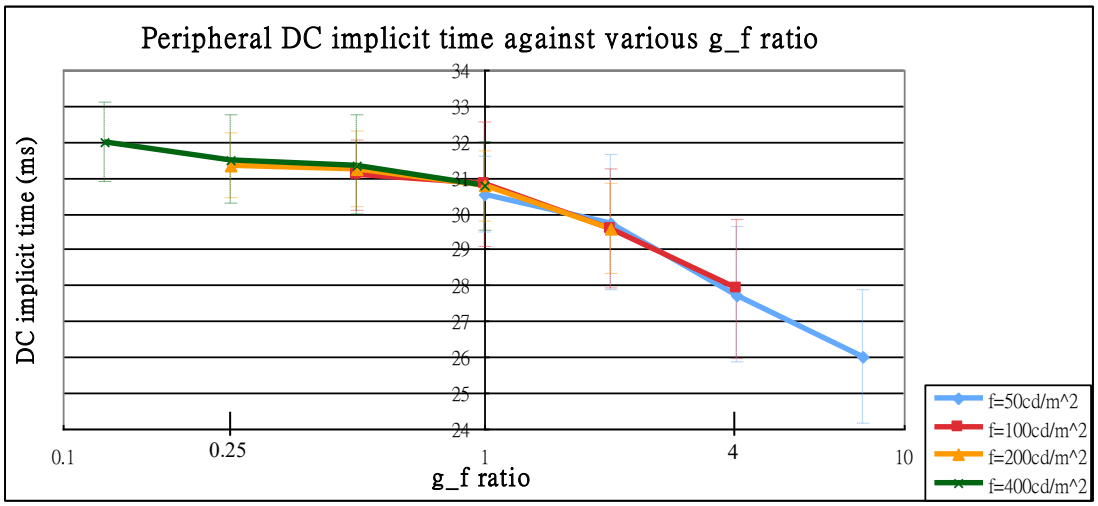

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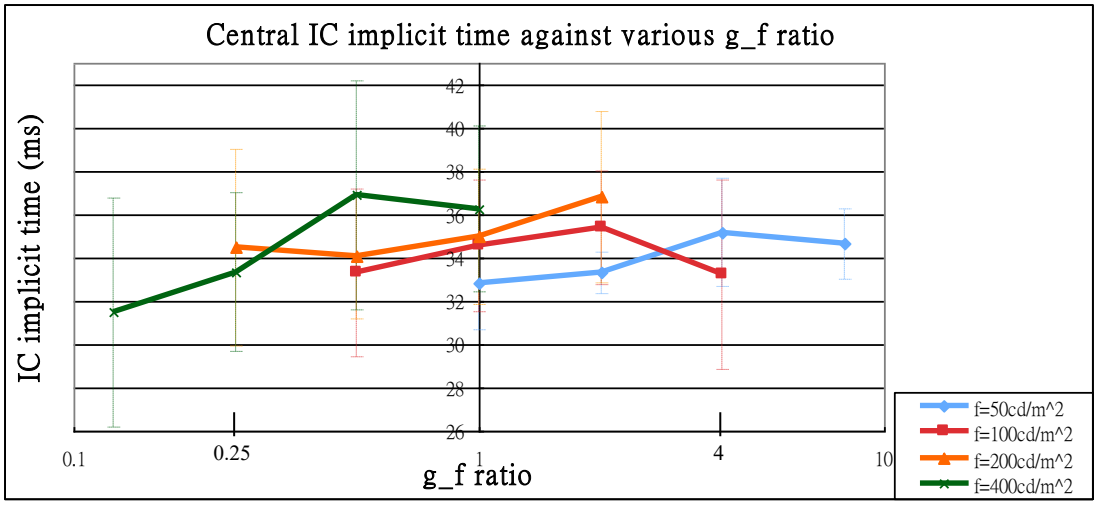

Figure 5a.

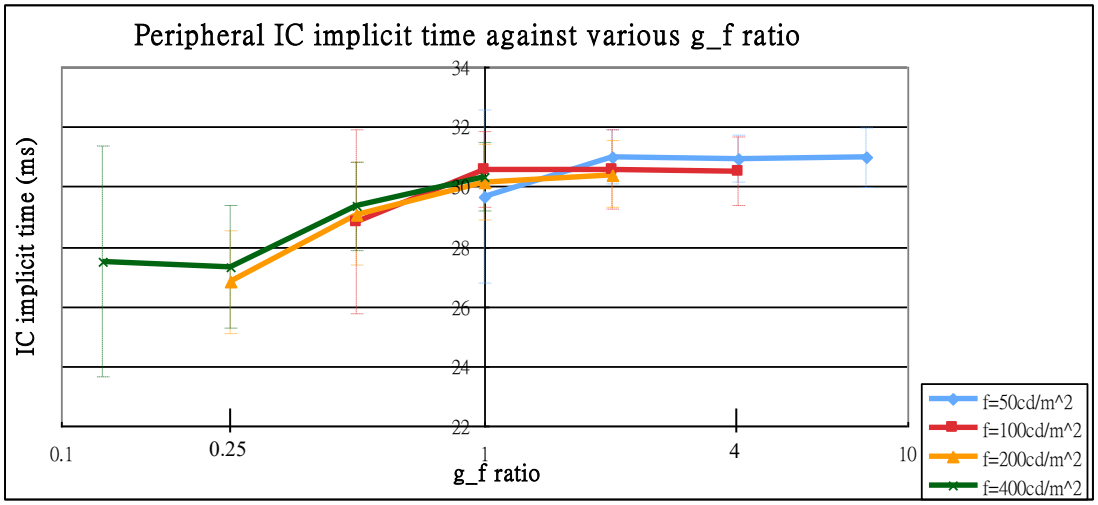

Figure 5b.

Figure 1a. The central DC and IC amplitudes were measured by the peak-to-peak method and their implicit times were the time of the response peak after the onset of the stimulus.

Figure 1b. The peripheral DC and IC amplitudes were measure by the peak-to-peak method and their implicit times were the time of the response peak after the onset of the stimulus.

Figure 2a. The central DC amplitude at various g/f ratios.

Figure 2b. The peripheral DC amplitude at various g/f ratios.

Figure 3a. The central IC amplitude at various g/f ratios.

Figure 3b. The peripheral IC amplitude at various g/f ratios.

Figure 4a. The central DC implicit time at various g/f ratios.

Figure 4b. The peripheral DC implicit time at various g/f ratios.

Figure 5a. The central IC implicit time at various g/f ratios.

Figure 5b. The peripheral IC implicit time at various g/f ratios.