Detection of early functional changes in diabetic retina using slow double-stimulation mfERG paradigm

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Keywords

Retina, Electrophysiology, Physiology, Diagnostic tests/Investigation

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ABSTRACT

Aim

Diabetes mellitus (DM) is a systemic disease with insufficient secretion of insulin or poor response to insulin. This typically causes poor control of blood glucose level leading to a range of complications. Early detection of the retinal function alteration in DM is needed.

Methods

A newly modified paradigm – slow double-stimulation mfERG – was introduced to measure early changes of retinal function in DM and to investigate changes in the adaptation mechanisms in the diabetic retina. The mfERG was measured by using a slow double-stimulation mfERG paradigm (M^1M^2OOO).

Results

The m1 amplitude of M^1 stimulation from diabetic subjects was significantly reduced in ring 1 in contrast to that of a control group. The m2 amplitude of M^2 stimulation from diabetic subjects was also significantly reduced in ring 1 and 2 as compared with those of the control group. The m1/m2 ratio which minimizes inter-subject variation shows reasonable differentiation between the control and diabetic groups. There was a

significant increase of the amplitude ratio from diabetic subjects in ring 2 and 3 as compared with those of the control group.

Conclusions

The present findings suggest that the new mfERG paradigm is a fast and sensitive test for the detection of early functional changes in the diabetic retina.

INTRODUCTION

Diabetes mellitus (DM) is a systemic disease with insufficient secretion of insulin or poor response to insulin. This causes poor control of blood glucose level, leading to a wide range of complications. DM is a worldwide health problem of increasing concern. According to a report from the Hong Kong Society for Endocrinology, nearly 10% of the Hong Kong population suffers from DM[1] and the prevalence of early-onset type II diabetes continues to increase. Diabetic retinopathy (DR) which is a leading cause of blindness is one of the common complications of DM. In 2005, Tam and co-workers reported that about 28% of DM patients in Hong Kong had developed DR with levels ranging from minimal non-proliferative type to sight-threatening proliferative type[2]. Additionally, nearly all patients with type I DM and more than 60% of the patients with type II DM will develop DR within two decades of the onset of DM[3]. About 90% of the visual loss caused by DR can be prevented by early treatment[4]. Therefore, early detection of the alteration of retinal function caused by DM is necessary, both to monitor the progress of the condition and to facilitate treatment.

Most clinical tests such as visual acuity[5], contrast sensitivity[6], color vision[7], visual field[8] and dark adaptation[9] can be used to investigate functional changes in DR but subjective variations in responses reduce their validity and their sensitivity for early

detection. Objective assessments based on dilated fundus photography, retinal nerve fiber layer thickness and the electroretinogram are often preferred. Dilated fundus photography is held to be effective and accurate for screening of DR[10], but it only detects observable morphological defects, and not early functional changes. Retinal nerve fiber thickness measurement may show retinal thinning in DR[11] but also cannot reflect the early functional loss. Yonemura et al[12] noted the relationship between altered electroretinogram and severity of DR. They reported that the amplitudes of oscillatory potentials were reduced in more than 50% of DM patients without ophthalmoscopic changes.

Since the lesions in DR do not have a fixed pattern or location, the multifocal electroretinogram (mfERG) would be a better tool to examine multiple retinal areas simultaneously to provide a clear indication of electrical responses from different retinal areas, for example, central and peri-central regions. The mfERG technique has been widely used in the investigation of pathological changes or functional variations in the retina[13-18] as well as the assessment of diabetic retinopathy[19-22]. The mfERG provides a fast flickering stimulation (75Hz frame rate), which allows examination of retinal temporal interactions. The mfERG response is generated by retinal cells which respond to the rapid stimulation sequence and an impaired recovery from the desensitization

produced by the preceding flash will result in impaired successive responses. Non-linear responses can be obtained as 'higher-order kernel' responses due to the effects of adaptive mechanisms[23], but these higher order kernel responses have relatively low signal to noise ratios, and can be difficult to measure.

In this study, we introduce a newly modified simplified paradigm – slow double-stimulation mfERG – to measure the early changes of retinal adaptation functions in DM.

METHODS

Subjects

Thirty insulin independent diabetic subjects without diabetic retinopathy, mean age of 54 (± 6.7) years, with mean diabetic duration of 4.7 (± 4.8) years were assessed; they were compared with 28 age-matched control subjects, mean age of 51 (± 6.2) years, who had no history of ocular or systemic disease. The range of refractive errors of all subjects was within ±2.00 diopter sphere with no more than 1.00 diopter cylinder. All research procedures adhered to the tenets of the Declaration of Helsinki and were approved by the Ethics Committee of The Hong Kong Polytechnic University. All subjects were fully informed of the possible risks and gave written voluntary consent.

Stimulation

The mfERG stimulus pattern was presented on a 19-inch LCD (Liquid Crystal Display) monitor (model MWC1230F; Philips, Japan), and the mfERG stimulation was driven by VERIS (version 5.01) from EDI (Electro-Diagnostic-Imaging; San Mateo, CA, USA). The mfERG was measured by using a slow double-stimulation mfERG paradigm (sequence of video frames: M^1M^2OOO), as shown in Figure 1A. In this paradigm, each stimulation cycle consisted of five video frames (each frame lasts 13.3 ms, with a 75-Hz frame rate). In the first two video frames (ie., M^1 and M^2), there were

multifocal patterns with 103 hexagons, scaled with eccentricity (scale factor, 10.46), and each hexagon was either bright or dark according to two independent pseudorandom binary m-sequences (2^{12}). After the two independent frames of multifocal flashes, there were three dark frames (ie., OOO) (0.04 cd · s/m^2, i.e., 3 cd/m^2 per frame) before the next cycle of stimulation. The average luminance of the multifocal flashes was approximately 1.11 cd · s/m^2 (i.e., 83 cd/m^2 per frame) and the background was also set to this luminance. The stimulus contrast was 94%. Recordings were divided into 16 slightly overlapping recording segments approximately 17 seconds in length. The recording time for the measurement was about 4 minutes and 32 seconds.

Multifocal ERG recording

A Dawson-Trick-Litzkow (DTL) electrode was used, as active and gold-cup surface electrodes were used for both the reference and the ground. Before testing, the pupil of the tested eye was fully dilated to at least 7 mm in diameter with 1% tropicamide (Alcon, Ltd.). During the mfERG recording, the untested eye was occluded. The refractive error of the tested eye was fully corrected for the viewing distance of 30 cm. The signal was amplified (Grass P511K amplifier; band-pass: 10–300 Hz; gain: x100,000). The recording was monitored using the real time signals shown by the VERIS mfERG program. Any recording segments contaminated with blinks or small

eye movements were rejected and immediately re-recorded.

Data analysis

The responses were processed separately at two 'time slices' for the m-sequence frames by using the mfERG system. The peak-to-peak response amplitudes and implicit times of the two m-sequence frames (m1 and m2) from DM and control groups were measured and compared. Ratios of the response amplitude (m1/m2) from the two independent multifocal frames in DM group and control group were also compared. Figure 1B shows the grouping strategy for averaging of concentric ring responses. Two-way ANOVA with Bonferroni post-hoc test was applied. The level of significance (α) was set at 0.05.

RESULTS

Figures 1C and 1D show typical response waveforms triggered by the first (m1) and second (m2) m-sequence stimuli respectively. The m1 and m2 responses were reduced in diabetic subjects. The m1 amplitude from diabetic subjects was significantly reduced for ring 1 in contrast to that of control group (*fig 2A*). However, there was no significant difference in m1 amplitude for ring 2 and 3 between diabetic subjects and normal subjects. The m2 amplitude from diabetic subjects was significantly reduced for

ring 1 and 2 when compared to the control group (*fig 2B*). There was no significant difference in m1 amplitude for ring 3 between diabetic subjects and normal subjects. The m1/m2 ratio shows a reasonable differentiation between the normal and diabetic groups. Figure 2C shows the m1/m2 ratio of normal subjects and diabetic subjects. There was a significant increase of the amplitude ratio from diabetic subjects in ring 2 and 3.

ROC curves were obtained using different cut-off values of the m1 and m2 amplitudes and the m1/m2 ratio (*fig 3*). The ROC curves illustrate the balance between sensitivity and specificity for discrimination of diabetics from normal subjects. The area under the ROC curve (AUC) provides an index for quantifying the accuracy of the M^1M^2OOO test. Table 1 shows the AUC and the best cutoff points of m1 amplitude, m2 amplitude and m1/m2 ratio for different eccentricities. The m2 amplitude for ring 2 shows the largest AUC and the best cutoff point for m2 amplitude is 9.7 nV with 90% sensitivity and 79% specificity. The m1/m2 amplitude ratio for ring 2 also shows a relatively large AUC. The best cutoff point for the amplitude ratio for this ring is 1.93 with 83% sensitivity and 60% specificity. The ROC curves of m1 amplitude show very similar AUC for different rings.

There was no significant difference between diabetic subjects and normal subjects in implicit time for m1 and m2 responses (*fig 4A and B*). In addition, there was no

significant difference in the m1/m2 ratio for implicit time for any rings between diabetic subjects and normal subjects (*fig 4C*).

DISCUSSION

This study examined retinal adaptation in diabetics, to demonstrate a new and effective protocol for early detection of functional retinal changes. Some researchers have suggested that retinal physiology alters before the presence of clinically manifested diabetic retinopathy. Psychophysical tests may reveal reduced contrast sensitivity[6] and dark adaptation[9,24] of diabetics with or without clinical retinopathy. However, these tests involve subjective responses from patients and the high inter-subject variation may reduce effectiveness in diagnosis. In objective clinical measurement, Palmowski and colleagues[19] demonstrated alterations of the mfERG response in diabetic patients in advance of retinopathy; Klemp and colleagues[22] showed changes in the mfERG in short-term hyperglycemia.

In this study, we adopted a slow double-stimulation mfERG paradigm (M^1M^2OOO) as a screening tool. In this paradigm, each stimulation cycle consisted of five video frames including two independent frames with multifocal pattern followed by three dark frames. Each hexagon in the pattern was either bright or dark according to two

independent pseudorandom binary m-sequences. Conceptually, the two multifocal flashes act as two succeeding stimulations similar to the idea of double-flash stimulation in conventional full field flash ERG. The three dark frames provided a short period for the retina to recover from the prior stimulations. Unlike the conventional double-flash full field flash ERG, this new mfERG paradigm provides topographical information of retinal function. Moreover, the chance for a specific retinal locus to be stimulated twice in one cycle of m-sequence was only 0.25 although this paradigm was comprised of two successive m-frames, Hence, this paradigm does not totally mimic the conventional double-flash full field ERG.

The advantage of this paradigm is the ability to gain topographical information from separate analyses of the m1 and m2 responses. Hence, the conventional mfERG result can be extracted from the m1 findings and adaptive effects on the mfERG can be extracted from the m2 findings. This can provide the clinician with different perspectives. While the variation in the mfERG response amplitude among subjects may cause a problem in clinical interpretation, the m1/m2 ratio uses the m1 as an internal reference to minimize inter-subject variation, and this would be expected to improve the efficacy of clinical diagnosis. However, the m1/m2 ratio does not confer any greater diagnostic value than m2 amplitude alone.

The results in this study suggest that the retinal functional integrity of diabetic patients must be compromised to a certain degree before the occurrence of clinical signs of diabetic retinopathy. There was a general reduction of response amplitude in diabetic subjects in both the m1 and m2 responses. The enlarged m1/m2 ratio in diabetic subjects suggested a more severe reduction in the m2 response which is caused by altered retinal adaptation. This finding is consistent with the reduced nyctometry[241] and reduced recovery of the conventional double flash ERG[25] in diabetic patients. The recovery of photoreceptor function after glare may be altered in diabetics.

In conclusion, the present findings suggest that the new mfERG paradigm is a fast and sensitive test for the detection of early functional changes before diabetic retinopathy is manifest. The use of m2 amplitude and m1/m2 ratio at ring 2 provides good sensitivity and specificity for detection of functional deterioration in diabetic retina. The adaptation or functional recovery of the diabetic retina has been altered in our diabetic subjects despite the absence of clinically manifested retinopathy. For the monitoring of retinopathy progress, future work is needed to investigate the correlation between mfERG responses and the severity of diabetic retinopathy.

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Competing interests None.

Patient consent Obtained.

Ethics approval Ethics approval was provided by the Ethics Committee of The Hong Kong Polytechnic University.

REFERENCES

1. Diabetes Division, Hong Kong Society for Endocrinology, Metabolism, and Reproduction. A statement for health care professionals on type 2 diabetes mellitus in Hong Kong. *HK Med J* 2000; **6**:105-7.
2. Tam TK, Lau CM, Tsang LC et al. Epidemiological study of diabetic retinopathy in a primary care setting in Hong Kong. *HK Med J* 2005; **11**: 438-44.
3. Fong DS, Aiello L, Gardner TW et al. Diabetic retinopathy. *Diabetes Care* 2003; **26**: 226-9.
4. Li PSH, Wong TH, Tang WW et al. Diabetic retinopathy. *HK Pract* 2004; **26**: 346-53.
5. Cho WB, Moon JW, Kim HC. Intravitreal triamcinolone and bevacizumab as adjunctive treatments to panretinal photocoagulation in diabetic retinopathy. *Br J Ophthalmol* 2010; **94**: 858-63.
6. Sukha AY, Rubin A. High, medium, and low contrast visual acuities in diabetic retinal disease. *Optom Vis Sci* 2009; **86**: 1086-95.
7. Feitosa-Santana C, Paramei GV, Nishi M et al. Color vision impairment in type 2 diabetes assessed by the D-15d test and the Cambridge Colour Test. *Ophthalmic Physiol Opt* 2010; **30**: 717-23.

8. Parravano M, Oddone F, Mineo D et al. The role of Humphrey Matrix testing in the early diagnosis of retinopathy in type 1 diabetes. *Br J Ophthalmol* 2008; **92**: 1656-60.
9. Kiser AK, Mladenovich D, Eshraghi F et al. Reliability and consistency of dark-adapted psychophysical measures in advanced eye disease. *Invest Ophthalmol Vis Sci* 2006; **47**: 444-52.
10. Carmichael TR, Carp GI, Welsh ND et al. Effective and accurate screening for diabetic retinopathy using a 60 degree mydriatic fundus camera. *S Afr Med J* 2005; **95**: 57-61.
11. Oshitari T, Hanawa K, Adachi-Usami E. Changes of macular and RNFL thicknesses measured by Stratus OCT in patients with early stage diabetes. *Eye (Lond)* 2009; **23**: 884-9.
12. Yonemura D, Aoki T, Tsuzuki K. Electroretinogram in diabetic retinopathy. *Arch Ophthalmol* 1962; **68**: 19-24.
13. Chan HL, Brown B. Investigation of retinitis pigmentosa using the multifocal electroretinogram. *Ophthalmic Physiol Opt* 1998; **18**: 335-50.
14. Chan HL, Brown B. Multifocal ERG changes in glaucoma. *Ophthalmic Physiol Opt* 1999; **19**: 306-16.
15. Hood DC, Greenstein V, Frishman L et al. Identifying inner retinal contributions to the human multifocal ERG. *Vision Res* 1999; **39**: 2285-91.
16. Chan HH, Brown B. Pilot study of the multifocal electroretinogram in ocular hypertension. *Br J Ophthalmol* 2000; **84**: 1147-53.
17. Chu PH, Chan HH, Brown B. Luminance-modulated adaptation of global flash mfERG: fellow eye losses in asymmetric glaucoma. *Invest Ophthalmol Vis Sci* 2007; **48**: 2626-33.
18. Ng YF, Chan HH, Chu PH et al. Multifocal Electroretinogram in Rhodopsin P347L Transgenic Pigs. *Invest Ophthalmol Vis Sci* 2008; **49**: 2208-15.
19. Palmowski AM, Sutter EE, Bearse MA, Jr. et al. Mapping of retinal function in diabetic retinopathy using the multifocal electroretinogram. *Invest Ophthalmol Vis Sci* 1997; **38**: 2586-96.
20. Shimada Y, Li Y, Bearse MA et al. Assessment of early retinal changes in diabetes using a new multifocal ERG protocol. *Br J Ophthalmol* 2001; **85**: 414-9
21. Bearse MA, Han Y, Schneck ME et al. Local multifocal oscillatory potential abnormalities in diabetes and early diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2004; **45**: 3259-65
22. Klemp K, Larsen M, Sander B et al. Effect of short-term hyperglycemia on multifocal electroretinogram in diabetic patients without retinopathy. *Invest Ophthalmol Vis Sci* 2004; **45**: 3812-9.

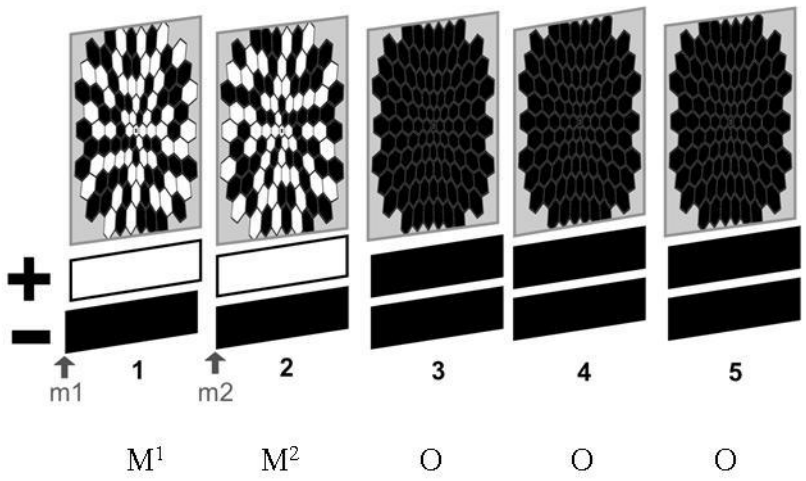
23. Hood DC. Assessing retinal function with the multifocal technique. *Prog Retin Eye Res* 2000;**19**:607-46.
24. Midena E, Segato T, Giuliano M et al. Macular recovery function (nyctometry) in diabetics without and with early retinopathy. *Br J Ophthalmol* 1990; **74**: 106-8.
25. Gliem H, Moller DE, Kietzmann G. [The double flash ERG in diabetic retinopathy]. *Acta Ophthalmol (Copenh)* 1973; **51**: 85-94.

Table 1 The area under the ROC curves (AUC) and different parameters obtained using the M^1M^2OOO mfERG stimulation paradigm (see text).

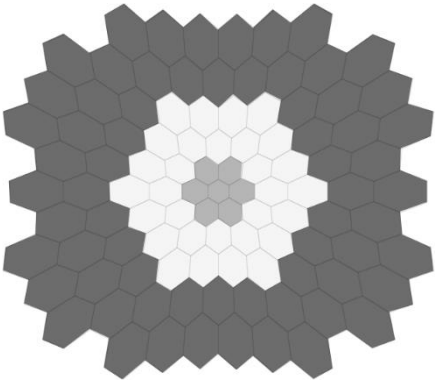
* p<0.05, ** p<0.01, ***p<0.001.

		Ring 1	Ring 2	Ring 3
m1 amplitude	AUC	0.68*	0.69*	0.64*
	Best cutoff point	47.8nV	15.2nV	9.9nV
	Sensitivity	90%	57%	73%
	Specificity	50%	79%	60%
m2 amplitude	AUC	0.75***	0.85***	0.78***
	Best cutoff point	20.5nV	9.7nV	4.3nV
	Sensitivity	77%	90%	73%
	Specificity	64%	79%	79%
m1/m2 ratio	AUC	0.56	0.77***	0.74**
	Best cutoff point	2.34	1.93	2.18
	Sensitivity	43%	83%	47%
	Specificity	86%	60%	90%

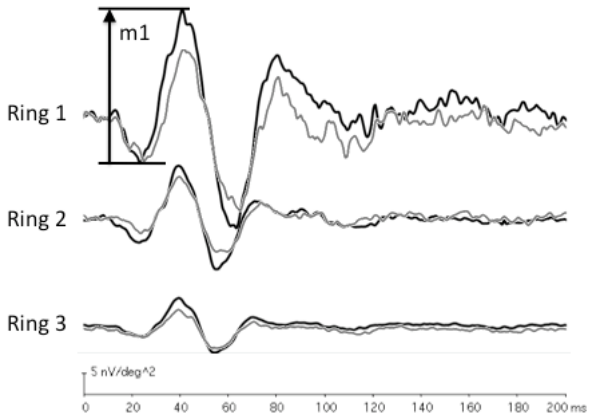
(1A)


m1
1
m2
2
3
4
5
M1
M2
O
O
O

(1B)



(1C)


m1
Ring 1
Ring 2
Ring 3
5 nV/deg^2
0
20
40
60
80
100
120
140
160
180
200 ms

(1D)

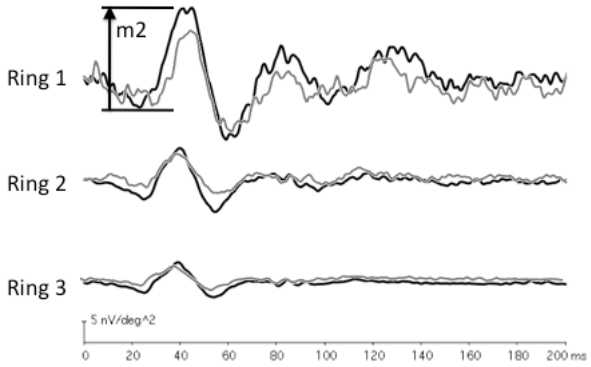
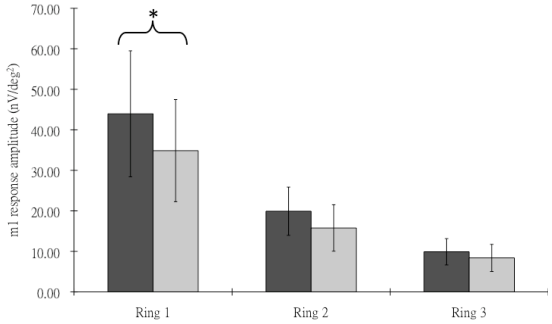
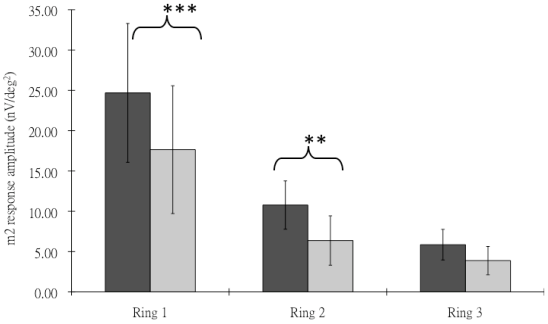


Figure 1 (A) The slow double-stimulation mfERG paradigm (M^1M^2OOO) contained five frames. Hexagons in two initial frames were alternated between bright and dark according to two independent pseudorandom binary m-sequences (m1 and m2). After the M^1 frame and M^2 frames, three dark frames were presented. (B) Schematic diagram shows the grouping strategy for concentric ring responses. The central hexagons (ring 1, light grey), the mid peripheral hexagons (ring 2, white) and the peripheral hexagons (ring 3, dark grey) covered 10˚, 10-29˚ and 29-30˚ field of view respectively. (C) An example of the averaged concentric ring responses of the first m-sequence. Dark traces show the responses obtained from a normal subject while grey traces show responses from a diabetic; m1 shows the measurement of peak-to-peak amplitude. (D) An example of the averaged concentric ring responses of the second m-sequence. Dark traces show the responses obtained from a normal subject while grey traces were obtained from a diabetic; m2 shows the measurement of peak-to-peak amplitude.

(2A)



(2B)



(2C)

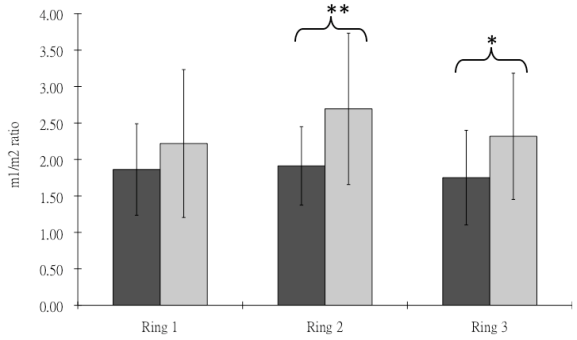


Figure 2 Averaged peak-to-peak amplitudes for ring 1, 2 and 3. (A) The averaged m1 amplitude of the first m-sequence stimulation. (B) The averaged m2 amplitude of the second m-sequence stimulation. (C) Averaged m1/m2 amplitude ratios for ring 1, 2 and 3. Each pair of histograms shows the results for the controls (dark) and the diabetics (light). Error bars are ±SD. * $p<0.05$, ** $p<0.01$, ***$p<0.001$.

(3A) (3B) (3C)

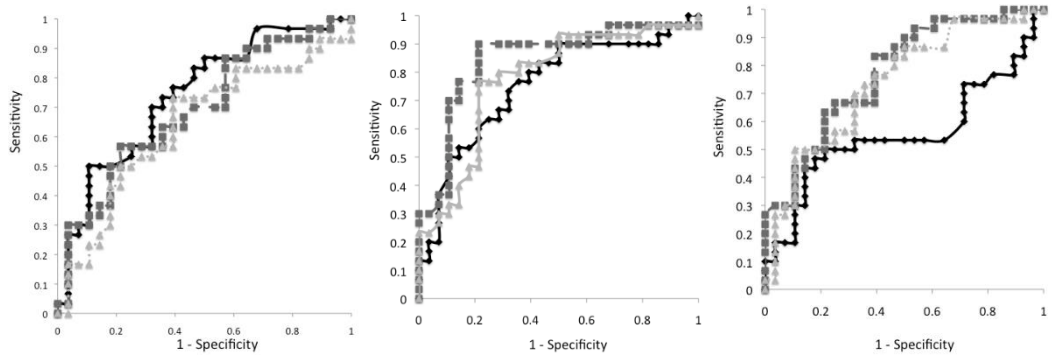
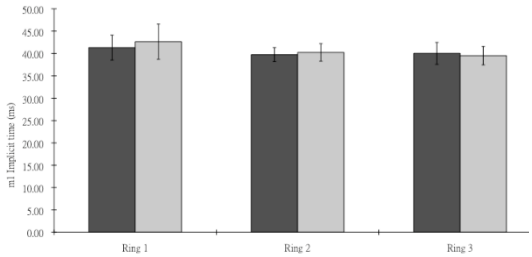
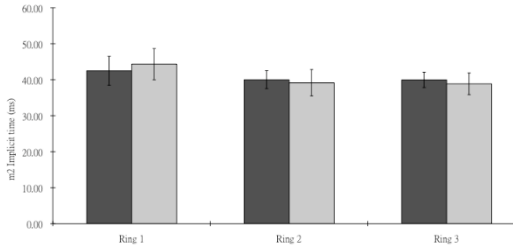


Figure 3 ROC curves for m1 amplitude (A), m2 amplitude (B) and m1/m2 ratio (C). Each group of ROC curves shows the results for ring 1 (black), ring 2 (dark grey) and ring 3 (light grey).

(4A)



(4B)



(4C)

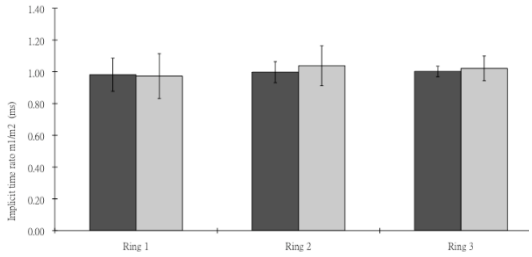


Figure 4 Averaged implicit time for ring 1, 2 and 3. (A) The averaged m1 implicit time of the first m-sequence stimulation. (B) The averaged m2 implicit time of the second m-sequence stimulation. (C) Averaged m1/m2 implicit time ratios for ring 1, 2 and 3. Each pair of histograms shows the results for the controls (dark) and the diabetics (light). Error bars are ±SD.