Development of a technique for Objective Perimetry using the multifocal visual evoked potential (mVEP)

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The publications and patents have been shared in terms of first authorship since we could not separate our contributions for each. I have tended to be designated first author on the major glaucoma clinical trials, with Dr Klistorner first author on the more electrophysiologically oriented papers. However we both contributed significantly to all.

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Publications involved in thesis

Preliminary studies

1. Graham, S.L, Klistorner, A. *Electrophysiology - a review of signal origins and applications to investigating glaucoma* **Aust NZ J Ophthalmol** - 1998, 26: 71-85
2. Klistorner, A, Graham, S.L. *Early magnocellular losses in glaucoma demonstrated with the pseudorandom multifocal flash VEP* **Journal of Glaucoma** 1999;8:140-148
3. Klistorner, A, Graham, S.L, Martins, A. *Multifocal pattern electroretinogram does not demonstrate localised field defects in glaucoma.* **Doc Ophthalmol** 2000, 100:155-165
4. Klistorner, A.I., Graham, S.L., Grigg, J.R., Billson, F.A., *Multifocal topographic visual evoked potential: improving objective detection of local visual field defects.* **Invest Ophthalmol Vis Sci**, 1998. 39(6): p. 937-950
5. Klistorner, A.I., Graham, S.L., Grigg, J.R., Billson, F.A. *Electrode position and the multi-focal visual-evoked potential: Role in objective visual field assessment.* **Aust N Z J Ohthalmol**, 1998. 26: p. S91-94
6. Klistorner, A.I., Graham, S.L., *Multifocal pattern VEP perimetry: analysis of sectoral waveforms.* **Doc Ophthalmol** 1999, 98,183-96
7. Graham, S.L, Klistorner, A. *The diagnostic significance of the multifocal pattern VEP in Glaucoma* **Curr Opin Ophthalmol** 1999;10:140-146

Major studies – main body of thesis

1. Graham, S.L, Klistorner, A. *Objective perimetry in glaucoma - recent advances using multifocal stimuli* - **Surv Ophthalmol** 1999;43,Suppl1:s199-209
2. Graham, S.L., A. Klistorner, J.R. Grigg, and F.A. Billson, *Objective VEP perimetry in glaucoma - Asymmetry analysis to identify early deficits.* **J Glaucoma**, 2000. 9(1): p. 10-19
3. Klistorner, A, Graham, S.L, *Objective perimetry in glaucoma* **Ophthalmology** 2000, 107:2283-99
4. Klistorner, A, Graham, S.L., *Electroencephalogram-based scaling of multifocal VEP: effect on inter-subject amplitude variability.* **Invest Ophthalmol Vis Sci**, 2001. 42(9): p.2145-2152
5. Goldberg, I., Graham, S.L. and Klistorner A. *Multifocal objective perimetry in the detection of glaucomatous field loss.* **Am J Ophthalmol.,** 2001, 133, p:29-39
6. Graham S.L, Klistorner A.I. and Goldberg I., *Clinical Application of Multifocal VEP Objective Perimetry in Glaucoma Practice,* **Archives Ophthalmol** 2005,123,729-793
7. Klistorner, AI, Graham,SL, Grigg, J., Balachandran, C *Objective perimetry using the multifocal VEP in central visual pathway lesions* **Br J Ophthalmol** 2005 89,739-744

8. Klistorner, AI, Graham,SL *Effect of eccentricity on pattern-pulse multifocal VEP.* **Doc Ophthalmol** (in press)

Other publications (see Appendices Chapt 11)

1. Balachandran, C, Klistorner, AI, Graham, SL. *Effect of stimulus check size on multifocal visual evoked potentials.* **Doc Ophthalmol** 2003, 106: 183-188
2. Martins A, Balachandran C, Klistorner AI & Graham SL *Effect of pupil size on multifocal pattern visual evoked potentials* **Clinical Exp Ophthalmol**, 2003. 31(4): p. 354-356
3. Martins, A., Klistorner, AI, Graham, SL, Billson, FC. *Effect of fixation tasks on multifocal visual evoked potentials* **Br J Ophthalmol** (Submitted)
4. Martins' A, Klistorner, AI, and Graham,SL. Effect of check size and frame rate on blue-yellow multifocal visual evoked potentials **Clinical Exp Ophthalmol** (in press)
5. Balachandran, C, Graham, SL, Klistorner, AI, Goldberg, I Comparison of objective methods in glaucoma – Multifocal Objective Perimetry and Heidelberg Retinal Tomography. **J Glaucoma** (resubmitted)

Patents

1. Klistorner A, Graham,SL, "Electrophysiological visual field measurement" 1999, PCT/AU99/00340/US#6477407

2. Graham SL, Malov I, Kozlovski A, Klistorner A, "Method and apparatus for objective electrophysiological assessment of visual function" 2001, PCT/AU01/00423

Rationale

To assess the human visual system we typically rely on subjective responses from the patient, which introduces uncertainty into the result. To record the actual electrical signals that the visual cortex generates in response to stimuli in the visual field, potentially removes this uncertainty. The visually evoked cortical potential (VEP) is such a signal and represents the final common pathway of the visual system from retina to brain. Any pathology that affects visual processing will reduce the measured response. In glaucoma this represents an opportunity to map blind spots objectively if it can be refined to show focal responses. The fact that glaucoma affects ganglion cells predominantly means that recording signals directly from the eye would only be useful if those signals were ganglion cell specific. Conventional electrophysiology (including pattern electroretinography) has not been able to topographically map visual responses. This project sought to determine if the multifocal VEP could provide accurate focal response data that could be interpreted as reflecting local ganglion cell loss. While the signals themselves are not ganglion cell specific the pattern of loss would provide a clue as to whether the loss was retinal, optic nerve or post-chiasmal. In addition the VEP signals provide information not only about the strength of the signal, but its conductivity (latency) which would be delayed in demyelinating conditions.

Aims

The main purpose of this project was to determine if the visual evoked potential (VEP) could be used to measure focal visual responses and thus provide an objective measure of the human visual field.

The test would utilize a multifocal visual evoked potential which would record signals from multiple areas of the visual field simultaneously. The responses would need to reflect localized losses, with good topographical correlation.

The technique would then be modified to develop a reliable objective test of the human visual field, suitable for use by the clinician, with particular relevance to glaucoma.

The technique would provide an alternative to subjective perimetry, and would need to produce interpretable results in subjects who could not perform subjective tests reliably.

The usefulness of the technique would then be investigated in other forms of visual loss such as cortical lesions, optic nerve compression and optic neuritis.

The test stimulus and recording methods would be modified to maximize signal to noise ratios and optimize reproducibility.

The concept of using a head-mounted display would be investigated.

Thesis outline

Chapter 1 - Introduction
The introduction describes the various conventional electrophysiological tests that can be used to assess visual function and their origin. Their potential as an objective marker of glaucomatous damage is reviewed, and their limitations. The background to multifocal technology is described and its application to recording different multifocal signal types.

The potential of each of these multifocal responses to be used as an objective topographic test for glaucoma is discussed, with reference to two initial studies on multifocal ERGs and multifocal flash VEPs. The multifocal pattern VEP has emerged as the most promising technique.

Chapter 2 – General Methods and Preliminary Studies on mVEP
The General Methods section outlines the methods used in all subsequent chapters/publications using the VERIS recording system, unless otherwise specified. The AccuMap multifocal VEP system was developed later as a result of initial studies with the VERIS system and is described in more detail in chapters 6-11.

Several preliminary studies were undertaken to better define the multifocal pattern VEP and to maximise signal detection. The 3 papers summarised here were critical to defining the potential of the technique, and the importance of electrode position when recording the mVEP. These studies formed the basis on which the core project to develop an objective perimeter was designed, and confirmed the feasibility of the technique as a means of assessing the visual field.

Chapter 3 – Single channel bipolar mVEP in glaucoma
In this study the mVEP technique using a bipolar single channel was applied to a larger group of glaucoma patients. Correlations between the mVEP amplitudes and subjective perimetric thresholds were determined. The bipolar mVEP corresponded well with Humphrey visual field defects, showing loss of signal in the scotoma area. This study represented the first practical application of the multifocal pattern VEP to objective detection of visual field defects in glaucoma.

Chapter 4 – Asymmetry analysis to identify early glaucoma
Since the mVEP shows markedly symmetrical responses between the two eyes of normal subjects, in this study 101 patients with glaucoma or high risk suspects were examined to determine if inter-eye VEP asymmetry could be used to identify glaucoma damage. Asymmetry analysis correctly identified patients with field loss and showed abnormalities in many high risk suspects with still normal fields. It was therefore possible that it may be able to detect changes before subjective field loss occurs.

Chapter 5 - Multichannel bipolar mVEP in normals and glaucoma
This chapter introduces the concept of multichannel recording to cover all underlying signal origins more effectively and thus improve signal detection in some areas of the field not adequately sampled with the single channel technique. The multichannel

technique was applied to glaucoma detection in both suspects (30) and established glaucoma (30), and was successful at identifying all cases of established glaucoma. The concept of an electrode cross to hold the recording electrodes was introduced.

Chapter 6 - Development of a new multifocal stimulation system
This chapter describes the development of the ObjectiVision multifocal system (AccuMap) using spread spectrum sequences as a means of driving the stimulus. The new technique gave several advantages in recording and was flexible to our changing design parameters. The concept of scaling individual signal amplitudes by using underlying electroencephalogram amplitudes was developed as a means of reducing inter-individual variability

Chapter 7 – Ability of the AccuMap system to detect glaucoma
A clinical trial using patients with established glaucoma was conducted. 100 patients with confirmed glaucomatous visual field defects were tested and compared with the database of 100 normals subjects. In 95/100 glaucoma patients, Humphrey field defects were demonstrated by mVEP amplitude reductions in a cluster of ≥3 abnormal zones. In 2/5 remaining cases the defect was detected on the inter-eye asymmetry analysis. Topographic location was strongly correlated with Humphrey fields. This trial confirmed the ability of the new test technique to detect glaucoma.

Chapter 8 - Clinical Application of mVEP in Glaucoma Practice
This study involved a large retrospective case series of 436 consecutive subjects referred for glaucoma investigation who were tested with the AccuMap™V1.3 system within a defined 12 month period. The purpose was to evaluate the roles of objective perimetry in glaucoma practice, and to assess its utility in patients with inconclusive standard automated perimetry (SAP) performance. MVEP is an effective method for detecting visual field loss in glaucoma. The mVEP was found to provide a valuable aid to the clinician in categorising patients with unreliable, variable, unconfirmed or excessive subjective field loss.

Chapter 9 - Application of mVEP to cortical lesions
In this chapter the ability of the mVEP to detect field loss caused by more posterior neurological lesions affecting the visual pathway such as tumors and cortical infarcts was examined. The mVEP can detect hemianopic field loss from cortical lesions, but not in some cases of homonymous quadrantinopia where the lesion may have been in the extra-striate cortex. This supports the concept that the mVEP is generated in V1 striate cortex and that it may be able to distinguish striate from extra-striate lesions.

Chapter 10 - Effect of slow stimulation rates on mVEP
The sparse pattern-pulse stimulation has been suggested to produce better cortical evoked responses compared to pattern reversal stimulation. This study examines varying pattern-pulse states and the effect of eccentricity of the stimulated visual field on the response. While the pattern-pulse method offers some advantages for achieving larger mVEP signals from the central visual field, the more peripheral field where it is the most difficult to obtain signals, did not show any enhancement.

Chapter 11 - Appendices
This section includes abstracts from other relevant publications and presentations. Several other projects were undertaken by our research team. These projects looked at the effects of variables on the recording technique, or examined alternative stimuli such as blue/yellow check patterns. The relationship to structural change as seen on the scanning laser ophthalmoscope (HRT) was examined. Pilot studies into the application of mVEP to the diagnosis and monitoring of Optic Neuritis were conducted.

Chapter 12 – Conclusions and Future Directions
The final section provides an overview of the results of all the studies and a guide to the potential future directions of research in the area of objective perimetry. In particular the possibility of using head-mounted displays is discussed.

Chapter 1 - Background

Electrophysiology in Glaucoma Detection

Summary

Testing for functional (visual) loss in glaucoma has traditionally relied on subjective methods, such as automated static threshold perimetry. These tests have limitations due to their dependence on the performance of the individual. Therefore there is a need for an objective measure of visual function. To date, objective tests have not been accurate enough to quantify damage to the visual pathway. Conventional electrophysiological responses such as the electroretinogram (ERG) have had limited application to glaucoma.

The pattern ERG, and more recently described photopic negative response may be derived from ganglion cells and therefore have potential to reflect glaucomatous loss, but they have not been widely adopted as a means of clinical testing. The conventional visually evoked potential (VEP) is also reduced in glaucoma but it has been difficult to adapt it to show localised loss.

However, the recent development of multifocal stimulus methods has allowed a new dimension to electrophysiological recording. The techniques for multifocal ERG and VEP recording have allowed multiple simultaneous recordings to be made from many focal areas throughout the visual field.

This section describes what has been achieved using electrophysiological measures as an objective marker of glaucomatous damage, and outlines their limitations in investigating glaucoma. The background to multifocal technology is described and its application to recording different multifocal signal types. Finally the potential of each of these multifocal responses to be used as an objective topographic test for glaucoma is discussed. The multifocal pattern VEP has emerged as the most promising technique.

A. Limitations of subjective tests for glaucoma

In the assessment of glaucoma it is essential to evaluate visual function, in particular to look for visual field loss. Glaucoma is characterised by progressive structural damage to the optic disc (cupping) and as a result of this, nerve fibre loss produces characteristic visual field defects, which can eventually lead to blindness. The gold standard for detecting field loss is subjective automated static perimetry. There are however many patients who have difficulty providing reliable perimetric results, which complicates the clinician's task. Elderly patients find the tests difficult to understand and perform, and many report the experience as stressful. Even young healthy normal subjects can have difficulties performing the tests reliably and reproducibly. In fact, in a recent study of the performance of the gold standard (Humphrey SITA full threshold visual field testing) [1] the specificity of SITA in normals was only 38% at first test and 73.7% after two tests.

Clinical trials have shown large fluctuations in subjective defects from test to test and patients may be repeatedly poor performers. There is also a learning curve associated with subjective perimetry that complicates interpretation in new patients, such that 2-3 field tests need to be done before a reliable result is achieved. In addition it is acknowledged that up to 50% of nerve fibres may be lost before a filed defect is detected[2].

Therefore there is a need to develop an objective means of assessing visual function to address the above limitations related to patient performance and also to potentially detect the disease at an earlier stage. Such a test would also have potential application to testing children, and to investigating functional loss or suspected malingerers. It could also be applied to assessing optic neuritis patients and other neurological causes of vision loss.

The recording of electrophysiological responses has been the main area where potential objective assessment might be achieved. I will outline in the following section the various electrophysiological responses and how they have been applied to the investigation of glaucoma and detection of field loss.

B. Conventional electrophysiological components and their origin

The **electroretinogram (ERG)** signal is the sum of electrical responses to a visual stimulus (usually a flash), recorded from an electrode in contact with the eye. The **visually evoked response (VER)** or **visually evoked potential (VEP)** is the signal which is generated by the visual cortex in response to either flash or pattern stimuli.

The ERG was initially recognised as consisting of three components termed the **'a', 'b' and 'c' waves** [3]. The a wave was the initial negative deflection, the b wave followed as a larger positive wave and the c wave was the final prolonged positive wave. When light strikes the photoreceptor outer segments, it generates a hyperpolarisation of the photoreceptor via a reduction in sodium conductance of the plasma membrane. The phototransduction of visual pigments such as rhodopsin leads to closure of sodium channels which, in the dark, are normally open. The reduced sodium influx leads to an intracellular negativity - hyperpolarisation. This produces the **a wave** of the ERG. The hyperpolarisation reduces the release of neurotransmitter at the photoreceptor synaptic terminals. The adjoining bipolar or horizontal cells then become depolarised or hyperpolarised depending on their type.

An increase in extra cellular potassium occurs as a consequence of bipolar cell depolarisation [4]. The potassium enters and depolarises the Muller cells. The resulting current flow which is predominantly towards the inner retina along the radially oriented Muller cell, produces the corneal positive **b wave** [5, 6]. An increase in extra cellular potassium occurs at the level of both the inner and outer plexiform layer, but the predominant signal appears to be that from the outer plexiform [7, 8].

The **c-wave** is a monophasic, corneal positive deflection that follows the b-wave. The decrease in extracellular potassium around photoreceptor cell outer segments following light absorption alters a standing electrical potential that exists between the apical and basal surfaces of the retinal pigment epithelium (RPE). The c-wave represents the algebraic

summation of the positive component generated at the RPE, and a corneal negative component generated by hyperpolarization at the distal portion of the Muller cells, the so called slow P-III [9]. **The phoptopic negative response (PhNR)**, is a negative wave that can be identified following the b wave peak in photopic conditions, and seems to be generated in inner retina.

Cone responses can be seen in relative isolation by stimulating the eye in the presence of a background light sufficient to eliminate the rod contribution to the ERG. White light flashes presented at 25-30 flashes per second (**30 Hz flicker**) separate the red and green cone responses from the rod response, since the rods fuse to a stimulus above 20 Hz [10]. A **focal ERG** can be recorded from the cones in the perimacular region (only about 7% of the total cone population). This is done by either using a hand held device to project the stimulus onto the macula [11], or by and using various techniques to present a stimulus within a steady surround that saturates rods and reduces the effects of stray light [12].

A further component of the luminance ERG is the **scotopic threshold response (STR) [13, 14]**. It is a negative response to light at the depths of dark adaptation at intensities just above absolute threshold and has a latency of 100-140 msec. It occurs below the b-wave threshold, is dependent on rods at dim illumination and probably reflects inner retinal function [15-17]. It correlates well with psychophysical threshold to the same stimulus [18].

A series of **oscillatory wavelets** occur superimposed on the ascending limb of the ERG b-wave [19]. Yonemura termed these wavelets **oscillatory potentials (OPs)**. These potentials are high-frequency, low-amplitude components of the electroretinogram, with a frequency of about 100-160 Hz, to which both rod and cone systems contribute [20, 21]. The cellular origin of OPs within the retina is uncertain, although it is likely that they are generated by cellular elements other than those that generate the a- and b-waves. [20, 22]. The site of generation is thought to be prior to the ganglion cells and to involve several processes [23-27].

The **pattern ERG (PERG)** is a biphasic potential of much smaller amplitude than the ERG (4 μV compared to 400 μV) and is recorded to a pattern-reversal stimulus [28]. It is predominantly perimacular in origin and is sensitive to spatial characteristics. The PERG was found to be abolished in monkeys [29] with optic atrophy induced by sectioning the optic nerve, while the luminance ERG remained normal. Its origin was therefore thought to be retinal ganglion cells and neighbouring inner retinal structures [30, 31]. The PERG is either absent or reduced in humans with optic atrophy [32] and shows an age-related decline in amplitude in visually normal adults [33].

Studies of spatial and temporal tuning of the PERG [34, 35] and non-linear systems analysis [36-38] have supported a ganglion cell-derived component. Non-linearities associated with lateral interactions give rise to a major component of the PERG signal, and these are pattern specific and spatially selective [38, 39]. Clinical studies in various disease states are also in support of a predominantly macular ganglion cell origin [40, 41].

The PERG has an initial small negative component with an implicit time of about 25 ms (N1) followed by a large positive component of about 50 ms (P1 or P50). This is followed by a further negative wave at around 95 ms implicit time (N2 or N95). If the pattern reversal frequency is fast, with > 10 reversals per second, the N95 becomes obscured by the next P50, producing a steady state response.

PERGs are usually recorded using a 10-30° centrally fixated achromatic screen with a checkerboard or grating pattern. They can also be recorded to chromatic patterns (eg, red/green or blue/yellow) but they are even smaller in amplitude [42]. The PERG is sensitive to optical blur and retinal illuminance, so refractive error, media opacities or pupil size will affect the response [43, 44]. It requires thread, fibre or gold foil electrodes rather than standard ERG contact lens electrodes, since the latter can produce optical blurring [43-46].

The **visually evoked response (VER)** or **visually evoked potential (VEP)** is a signal which is generated by the visual cortex in response to both flash and pattern stimuli. The recording electrodes are placed on the scalp rather than in contact with the cornea, with the main signal detected over the occipital region. Since the occipital area of the cortex predominantly subserves macular function, the VEP signal is mainly derived from the macular region [47]. It has been estimated that the central 2° of visual field contributes 65% of the response, compared to the ERG where the fovea is thought to represent <2% of the total response. A pattern stimulus similar to that used for the PERG gives good responses that tend to be less affected by problems of stray light. A spatial frequency of 10-20 minutes of arc with a temporal frequency of 8 Hz gives an optimal response [48, 49]. A typical waveform derived from a slowly repetitive stimulus and recorded with a monopolar electrode contains a primary response, a secondary response, and rhythmic afterwaves. Its components reflect the activity of the striate cells, and possibly extrastriate areas [50-52]. Amplitude is measured from the major positive to the major negative deflection, latency as the time from stimulus onset to the positive peak (usually p100).

C. Conventional electrophysiological studies in glaucoma

(i) The Luminance ERG

The luminance or standard flash ERG has traditionally been found to be unhelpful in glaucoma [53-55] although some minor changes have been reported [56-58]. Vaegan et al [59], found mild amplitude reductions and latency delays but the changes tended to lose statistical significance in older patients however, and were not consistent enough to be used in the individual case. Graham et al, in a subsequent study found only slight changes in ERG parameters in 43 cases of early to moderate glaucoma (Humphrey MD <12dB). The ERG showed poor sensitivity in identifying glaucoma patients compared to the PERG [60]. The b wave latency was the most frequently affected of the ERG components, but the changes were not consistent.

(ii) Oscillatory Potentials

Most studies have found OPs are normal after optic nerve damage [61]. Wagner and Persson [62] found the OPs were normal while the PERG was reduced in unilateral glaucoma. Other studies have identified OP reductions in glaucoma [63-65]. Graham et al [60] found OP

amplitudes to have a poor sensitivity for glaucoma. Further analysis found no statistically significant changes in either photopic or scotopic OP individual wavelet peak times or amplitudes (unpublished data). However a recent report in the rat model suggests there may be some OP reductions[66].

(iii) The STR

Korth [67] examined the STR and scotopic PII in glaucoma patients and concluded that the amplitude of the STR was only mildly affected by glaucoma whereas the scotopic PII was significantly reduced. Frishman et al have shown that in the cat STR there are two components, one of which may be derived from the ganglion cells and one not [68]. The same group have reported that in the macaque monkey with experimental ocular hypertension and induced ganglion cell loss, there is loss of the sensitive negative component of the scotopic threshold ERG [69]. They feel that there may be interspecies differences and that the responses recorded by Korth et al in humans were not absolute threshold. [67] [70].

(iv) The photopic negative response

Recent papers suggest the phoptopic negative response (PhNR) , a component that follows the b wave peak, may be more promising than other ERG components described above [71]. In a small sample of 11 glaucoma patients, the PhNR was reported to correlate with PERG reductions. [72]. A further study with 18 glaucoma subjects found the s-cone PhNR to be more sensitive in glaucoma detection than the PERG.[73]. However one report suggested that the late negative ERG component of either scotopic or photopic ERGs did not distinguish between human controls and glaucoma. [74]

(v) The PERG

The Pattern ERG (PERG) has been of great interest in glaucoma as it is thought to reflect retinal ganglion cell activity along with activity in neighbouring retinal layers. [29, 75]. [76, 77] Many studies dating back to the 1980's have described abnormal PERGs in patients with glaucoma [65, 78-87]. Changes have been observed in the amplitudes and latencies of the P50, N95, their sum (P50+N95) and their relative reductions. It appears that both components are affected in glaucoma[60, 77]. Differences between relative reductions in PERGs, colour vision and contrast sensitivity have been reported in NTG and POAG[88]. In ocular hypertension the results have been less consistent, with some studies reporting no changes, while others confirm a delay with or without a reduction in amplitude. Ruben et al reported abnormal PERGs in 40% of 206 OHT eyes [89].

Topographic localisation of pattern ERG changes has been attempted in several studies with some success, for example using a ring stimulus [86] or hemifield PERGs [90] . A PERG stimulus has also been linked to an SLO to project the stimulus into quadrants, which gave a sensitivity of 82%, specificity 80%, based on 34 glaucomas. It is unlikely, however, that these techniques will be useful clinically to map glaucomatous defects.

Bach et al have attempted to improve PERG detection rates in glaucoma by introducing a ratio (the Freiburg PERG paradigm) for steady state PERGs recorded for 0.8 and 16 degree check sizes [77]. The use of a ratio helps overcome inter-individual differences in PERG amplitudes. The PERG recorded to the smaller check size tends to be differentially

reduced in glaucoma. However, reduced visual acuity such as cataract can also affect the response. They report abnormalities in OHT patients subsequently converting to glaucoma, although only 4 eyes of 67 patients converted to manifest glaucoma during the study, which limited the conclusions that could be drawn. The ROC analysis specificity for the test was 95%. A PERG recorded to frequency doubled stimuli has been described, with 9 test stimulus regions within the field. This multi-region FD PERG was able to detect 100% of moderate to severe glaucoma, and found abnormalities in 67% of high risk suspects [91].

The clinical utility of the PERG may be limited by the intersubject and intrasubject variability of the response [92]. However, through the use of standardized recording procedures the variability can be minimized [93, 94] adding to its potential for clinical application. Abnormal PERGs have been reported in diabetes, retinopathies, age-related macular degeneration, optic neuropathies, amblyopia and Alzheimer's disease. Pathologic processes in the outer retina also produce significant PERG deficits meaning that if retinal cells distal to the ganglion cells are disordered then the PERG is also reduced. Also, since it is acuity dependant, any cause of retinal image blurring from tear film problems to cornea, lens and vitreous opacities will confound its interpretation.

(vii) The pattern VEP

The conventional flash or pattern VEP is generally agreed to be abnormal in glaucoma, [95-98] but many cases are not detected and the sensitivity early in the disease is not high. It is widely recognised that P-VEP is predominantly generated by cortical elements receiving projections from the central retina (which is mainly due to it heavy cortical overrepresentation) [99-103]. This limits application of the method in detection of peripheral field defects which are crucial in diagnosis of such common diseases as glaucoma. A small unified check size, which is commonly used for stimulation, is another factor which tends to bias the central response [104, 105].

During the long history of visually evoked cortical recordings there have been numerous attempts to overcome these constraints. Different modes of stimulation have been used. The most common approach employed partial stimulation of the visual field including half-fields, quadrants, segments, annulus or peripheral field vs central field modes and also local stimulation using light emitting diodes (LED) which greatly improve detection of the peripheral visual field defects [95, 106-116]. However, significantly higher responses from stimulation of upper hemiretina (lower visual field) compared with lower hemiretina (upper visual field) has often been reported [95, 117-122]. It was interpreted by some researchers as a reflection of functional superiority of the lower visual field [118].

Due to the large inter-individual variability in VEP amplitude most studies have concentrated on VEP latency. In an early study with an age corrected cohort of patients with OAG and OHT, the full field pattern VEP showed about 50% and 25% of patients respectively to have a delay in latency compared to normals. The VEP latency was correlated with severity of field defects and the degree of cupping and pallor of the optic disc. [123]. Delayed VEP latency has also been reported by other groups [124]. Recently using black and white, grey/black and

red/black VEP, glaucoma suspects with OHT were found to have 15 to 20 ms delay in P100 latency compared with controls [125].

Flicker VEPs in POAG and suspects were studied retrospectively in 428 visual fields and amplitude was found to be reduced, correlating with optic cup size and visual field grade, but not with peak time IOP [126]. In monkeys with monocular laser induced glaucoma there was a reduction in amplitude, but no phase shift seen in the pattern VEP [127].

Flash VEP P1 amplitude has been found to be correlated with the size of optic disc cupping, and field loss [128] and to be reduced in glaucomatous eyes compared with normal or OHT eyes [129]. However, the luminance-contrast pattern VEP performed on age corrected patients with glaucoma (md8.9+/-5.3), showed little change in amplitude or latency, whereas abnormalities were detected with colour-contrast pattern VEP [130].

In a study of 73 patients with glaucoma it was reported that the blue on yellow pattern onset VEP gave a sensitivity of 85%, which when combined with results from PERG and temporal contrast testing could increase the overall sensitivity to 94%.

In terms of a topographic or structural relationship, hemifield defects were observed to cause a decrease in amplitude and showed topographical correlation in quadrantic analysis in 9 patients with glaucoma [111]. Also the pattern VEP generated by stimulating the midperipheral visual field (central 5 degrees blocked) detected 44% of POAG and 15.4% of OHT had a delay in latency ranging from 3-20ms [131]. However in a large multivariate comparison of psychophysical and electrophysiological techniques in 203 glaucoma patients, where glaucoma was assessed according to disc and nerve fibre layer changes rather than fields or IOP, the psychophysical tests performed better than PERG or blue/yellow VEP.[132]

Therefore the conventional VEP to either flash or pattern is reflecting the disease process but still does not perform as well as required for accurate diagnosis and monitoring of disease.

D. Multifocal recording techniques for ERG/VEP

A significant advancement in stimulus and recording technology was introduced by Sutter [133] which enables the presentation of a multifocal stimulus. This is now commercially available as the VERIS - Scientific™ system (Electro-Diagnostic Imaging, Inc., San Francisco) . The system provides the opportunity for topographical analysis of recordings, with the capability of examining the effects of sequential flashes. This adds a time domain to the analysis and allows examination for temporal non-linearities in the response.

Visual stimuli consist of preset numbers of hexagons in arrays (or segments in a dart board) with the possibility of flash or pattern stimuli within each area. The stimulus areas can be retinally or cortically scaled. This means that the area of each hexagon or dartboard segment increases with eccentricity, proportional to cone density for retinal stimulation, or cortical magnification (M-scale) for VEP recording. For the pattern stimulus it utilises a pseudo-random binary exchange of two opposite checkerboard pattern conditions at each of

the test sites of the visual field. According to this sequence, there is a 50% probability for the checkerboard pattern to reverse its polarity with every frame of the stimulating display (15 msec). Each input (stimulation site) is modulated in time according to the same pseudo-random binary m-sequence.

An important advantage of the m-sequences - to be orthogonal to all their cyclic shifts - is that it permits computation of the signal by cross-correlation of the response evoked by the m-sequence stimulation, with the m-sequence itself. This property allows one to obtain responses for hundreds of inputs from records of up to a million data points in a fraction of a minute and therefore makes m-sequence stimulation very effective for mapping purposes.

Analysis is based on the Wiener kernel expansion [134, 135]. This allows computation of cross-correlation kernels which can characterize non-linear interaction between visual events [133] . The first order "kernel" corresponds to a conventional impulse response. Second order kernels however, show the difference between the anticipated summation response in a linear system when consecutive flashes (or polarity reversals) are presented, and the response that is actually recorded. In other words it is a measure of the temporal non-linearity of the visual system. The "slices" of the second order kernel represent non-linearities observed when the consecutive flash is presented at 15ms, 30 ms, 45 ms etc. This technique of non-linear analysis is important since the human visual system demonstrates high temporal non-linearity.

With VERIS one can record a detailed multifocal photopic (and scotopic) flash ERG that shows a topographic distribution of signal amplitudes. The ERG amplitudes are very useful in delineating areas of outer retinal damage in many diseases (eg retinal dystrophies) [136, 137]. Figure 1 shows an example of a **normal multifocal ERG** response, with the 3-D plot below representing the response per area (density response function), showing larger responses proportionally from the fovea. The waveforms are all of similar shape and latency throughout the field, and can be averaged together over areas or rings within the field. Figure 2 shows a mERG from a subject with a cone dystrophy, demonstrating loss of the photopic cone response, particularly in the central field.

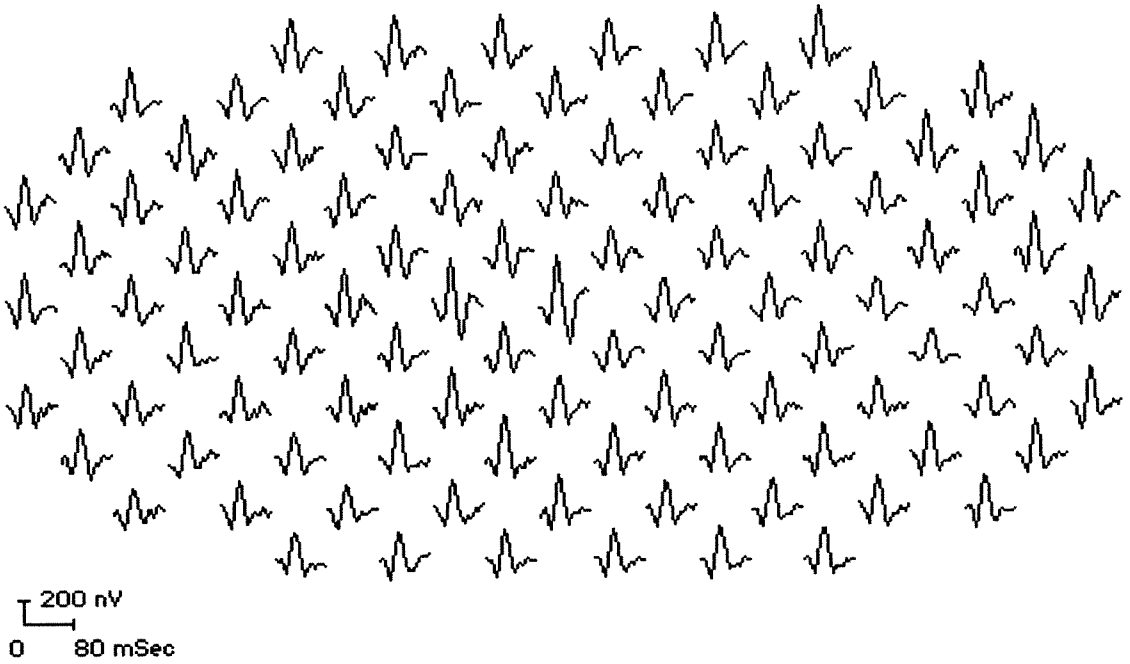


Figure 1a – Normal mERG with responses of similar waveform throughout the field

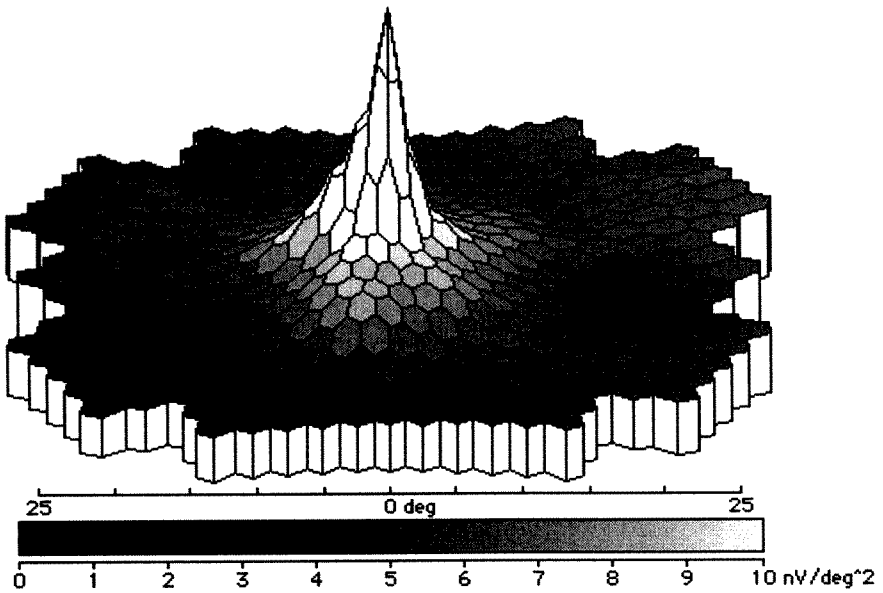


Figure 1b – 3D plot of relative amplitudes from each test segment of the field showing a "hill of vision" as seen on subjective testing, but with a more exaggerated peak at the fovea

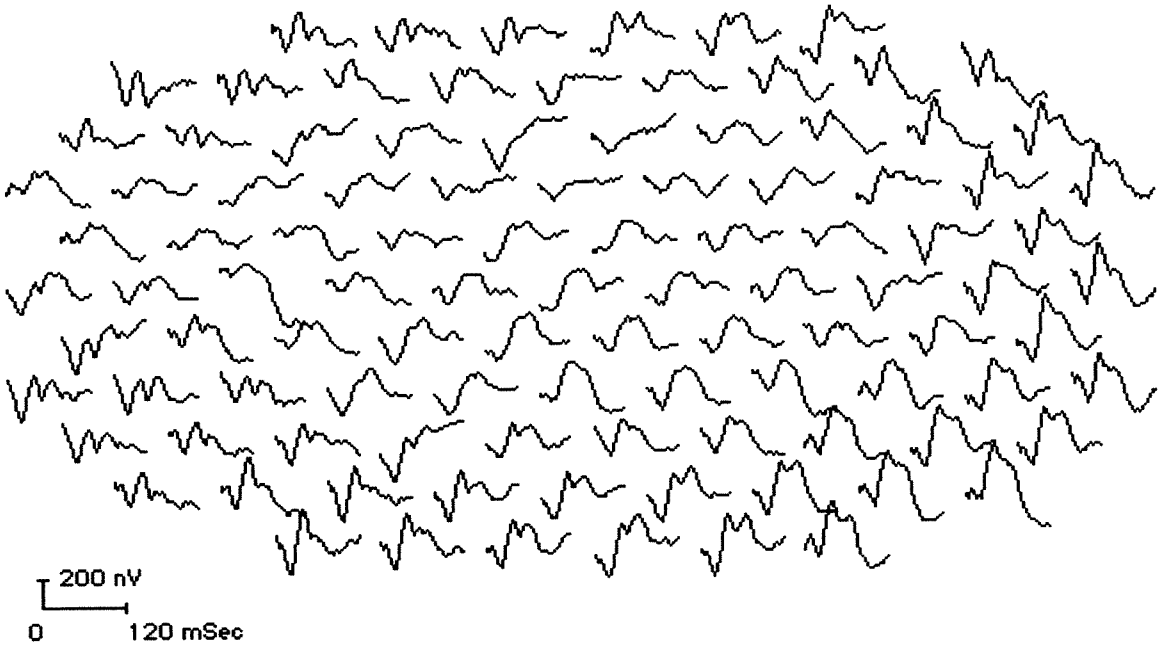


Figure 2 - mERG from a subject with a cone dystrophy, demonstrating loss of the photopic cone response, particularly in the central field.

By changing the stimulus design to an alternating pattern of black and white checks or triangles which reverse according to the pseudorandom sequence, it becomes possible to record the responses of a **pattern mERG**. Figure 3 below shows an example in a normal subject. The stimulus uses fewer test zones since the response is much smaller in amplitude than the flash mERG, with resulting poor signal to noise ratios when finer arrays are used.

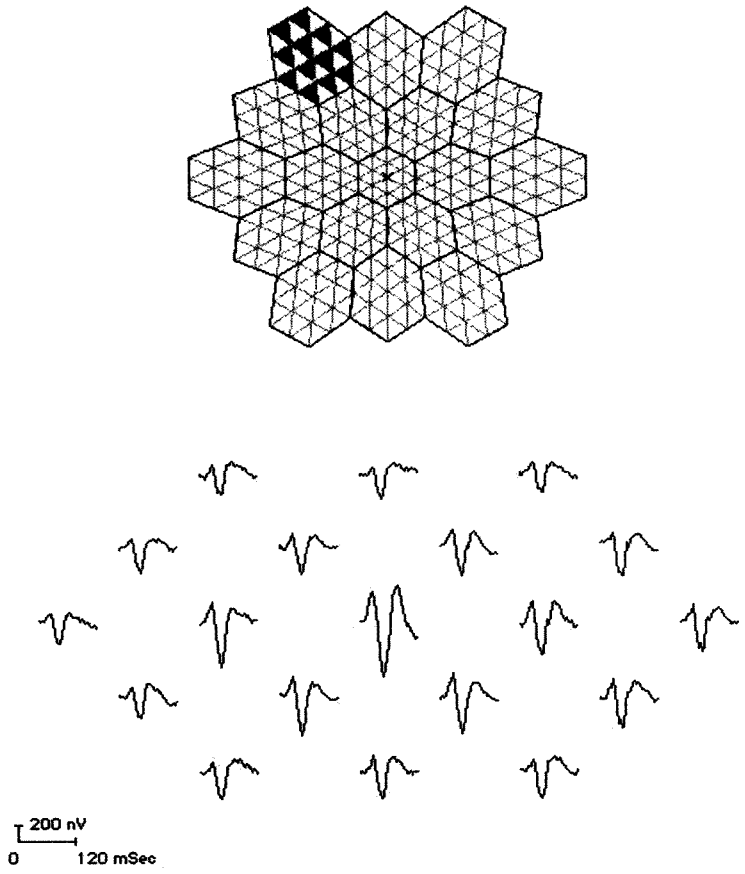


Figure 3 – Normal multifocal pattern ERG. Upper figure shows stimulus pattern – 19 test zones scaled with eccentricity. Lower figure shows responses.

In addition to mERGs, it possible to record the cortical responses to flash and pattern stimuli to produce a multifocal VEP. Figure 4 shows a **flash mVEP** recorded to 19 test zones with conventional fronto-occipital electrode placement. It can be seen that the signal is dominated by the central responses and lower field, with very poor signals in the periphery, despite the large stimulus zones. In contrast Figure 5 shows a **pattern mVEP** recorded to a cortically scaled stimulus pattern of checks. The signals although smaller than the central flash mVEP response are more uniform throughout the field.

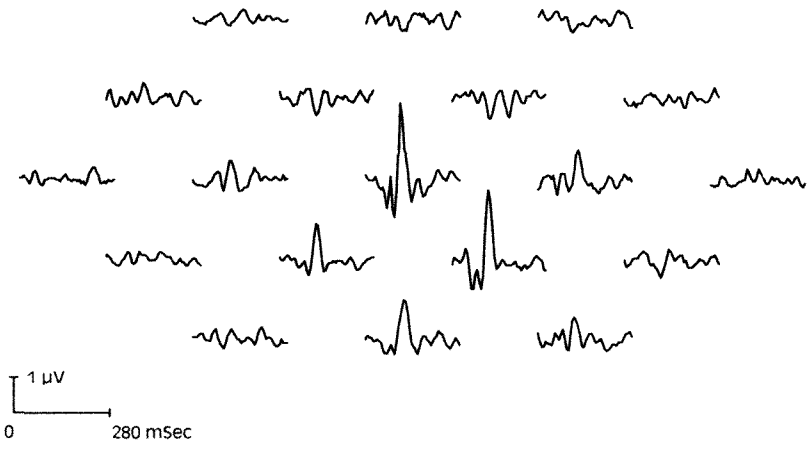


Figure 4 – Normal multifocal flash VEP

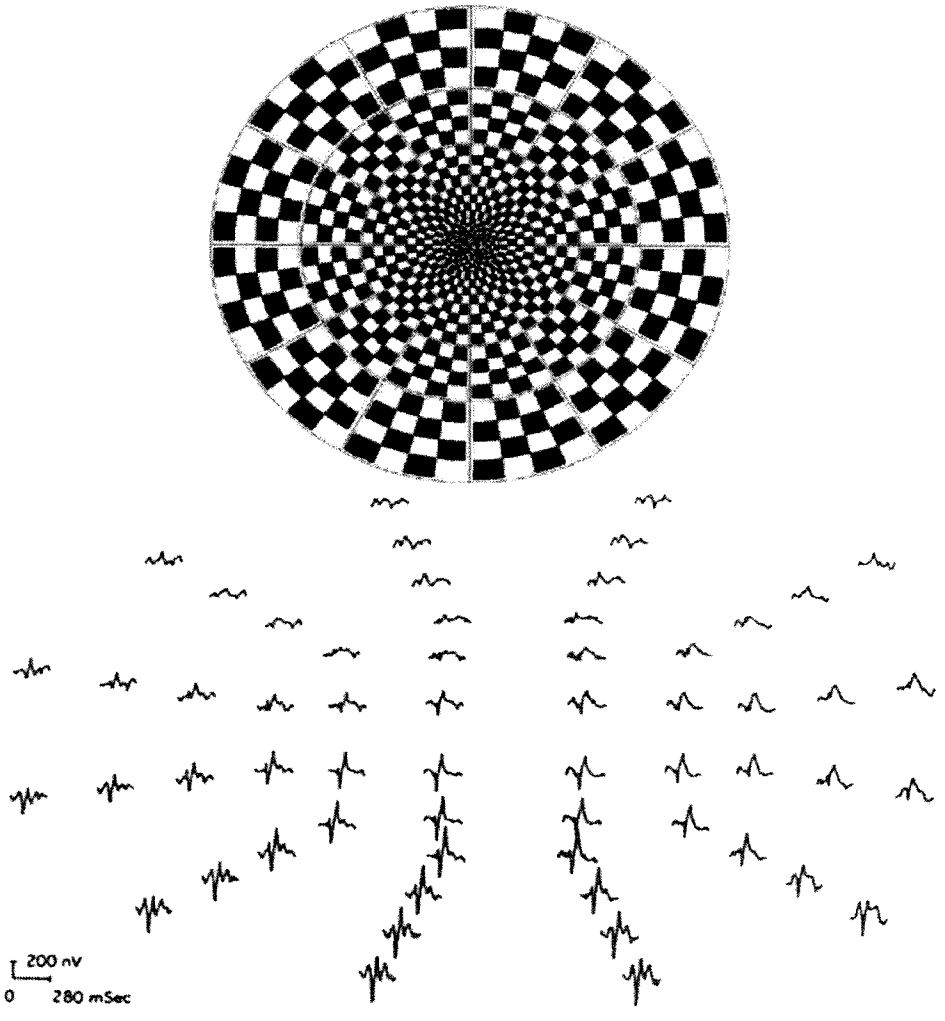


Figure 5 – Cortically scaled dartboard stimulus (above) and multifocal pattern VEP recorded with fronto-occipital monopolar electrodes.

E. Multifocal techniques in glaucoma

As described in Section D above, the conventional technique for recording the ERG or VEP can be modified to record a multifocal response. In pilot studies we have examined several different applications of multifocal stimuli to glaucoma testing. These include **multifocal flash VEP, multifocal flash ERG(mERG), multifocal pattern ERG, and multifocal pattern VEP (mVEP).** Of these the pattern VEP has been by far the most successful and is the principal focus of this thesis. The flash mERG showed no significant changes in glaucoma, so is only mentioned briefly. However 2 other studies on flash mVEP and pattern mERG will be summarised with the potential and limitations of these techniques discussed.

(i) The Multifocal flash VEP

Published as Klistorner, A, Graham, S.L. *Early magnocellular losses in glaucoma demonstrated with the pseudorandom multifocal flash VEP* **Journal of Glaucoma** 1999;8:140-148

Summary of publication:

Based on the notion that magnocellular pathways may be damaged earlier in glaucoma (see review by Graham)[138] we tried a multifocal flash VEP with different luminances to attempt to separate parvocellular and magnocellular responses. Retinal ganglion cells that have larger diameter fibres, larger receptive fields, and faster conduction velocities project to the magnocellular (M-cell) layers of the LGN. They represent only about 10% of the total ganglion cell population. The receptive fields of retinal nerve fibres representing the M-cell pathway tend to be particularly responsive to high temporal and low spatial frequency information and achromatic or luminance information. They are therefore thought to be involved in the processing of motion and high-frequency flicker perception. A second pathway includes retinal ganglion cells that have smaller diameter fibres, small receptive fields, slow conduction velocities and project to the parvocellular layers of the LGN. These fibres constitute the parvocellular (P-cell) pathway. They tend to be especially responsive to high spatial frequency information, low temporal frequency and chromatic information. The P cell system is thought to be involved in the processing of color, form perception and spatial acuity [139, 140].

The existence of parallel M-cell and P-cell pathways is of clinical interest because of the possibility that glaucoma may preferentially damage one of these visual pathways, especially at early stages of the disease process. In studies that were conducted by Quigley et al on chronic human glaucoma and experimentally induced glaucoma in monkeys , they reported that there is a selective loss of large-diameter optic nerve fibres throughout all portions of the optic nerve [141, 142]. Because large-diameter fibres are also more prevalent in the superior and inferior poles of the optic nerve where damage seems to occur earlier, they proposed proportionately greater losses of large fibres in those regions[143]. Since there is a high prevalence of large-diameter fibres present in the M-cell pathways, it was concluded that it

should be possible to develop psychophysical (or electrophysiological) test procedures that are able to detect the earliest functional deficits in glaucoma by targeting these pathways[144].

However there are conflicting reports in the literature as to whether M-Cell loss precedes that of P-Cells, including data from histological [145], and psychophysical [146] studies.

Klistorner et al [147] suggested that responses from the two pathways could be separated by using the contrast response function of the flash mVEP. The first slice of the second order kernel could be considered as an indicator of the relative activity of the M-Cell system – the more saturated the contrast/response function, the bigger the contribution of the M-Cell pathway to the second order kernel. The first order kernel represents the best approximation of the response elicited by the stimulus. The second order kernel represents the non-linear effect of the first flash has on the response to the second flash when two are presented in rapid sequence.

The methods are described in detail in the full publication, but are briefly summarised as follows: For recording we used the central hexagon response from a 19 hexagonal zone multifocal flash VEP on the VERIS 3 system (Electro-Diagnostic Imaging, Inc., San Francisco). Two levels of luminance contrast were used: 32% (M-Cell) and 99% (P-Cell). A saturation coefficient was calculated as the ratio of VEP amplitude recorded in response to low luminance compared to that recorded at high luminance. Single channel monopolar gold cup electrodes were positioned at the Oz and referenced to Fz, with the ear as ground. The m=14 stimulus sequence was used with 16 overlapping segments taking 4 minutes of recording time. The distance to the screen was 30cm, the central hexagon subtending around 8°. The study included 15 age and sex matched normal subjects and 28 patients with varying degrees of glaucoma ranging from ocular hypertension to advanced disease.

The results can be summarised in the figure below. In OHT to early glaucoma there was a statistically significant loss of the low luminance responses (early glaucoma –45%). However the high contrast responses remained normal in early disease, but were reduced in the moderate glaucoma group (-25%). The saturation coefficient (amplitude ratio between mfVEPs recorded at low and high contrast) for normal eyes was high (0.81+/-0.14), whereas for early glaucoma it was reduced to 0.47+/-0.13 ($P<0.0001$). In advanced disease the low contrast response was completely lost, so no coefficient could be calculated.

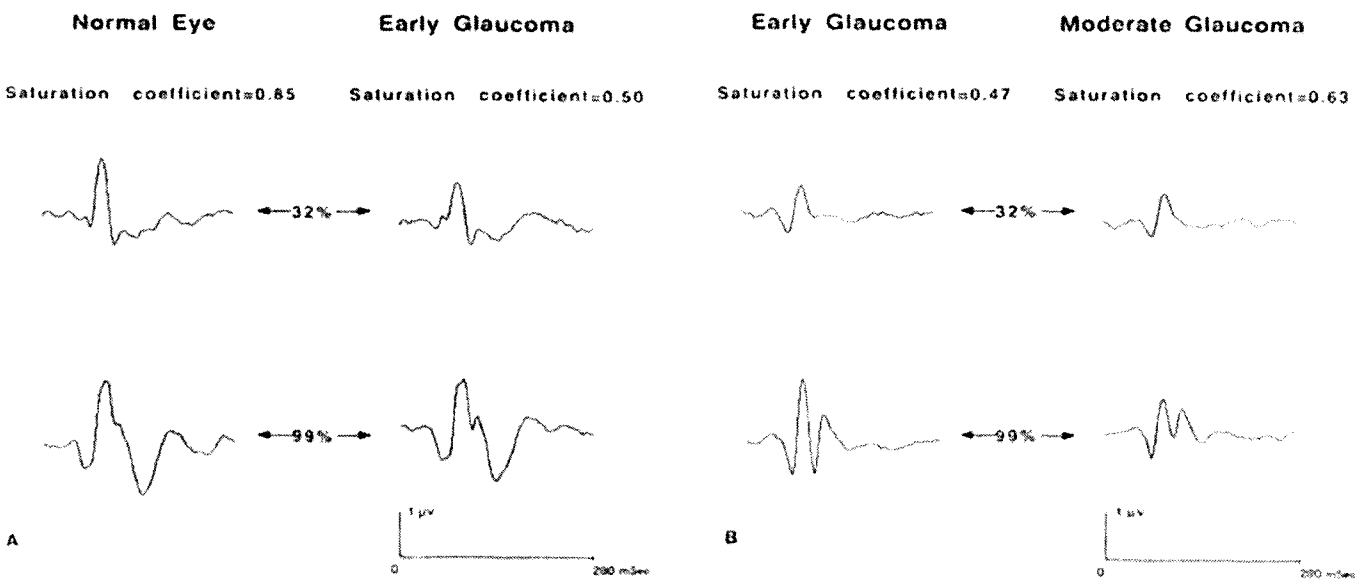


FIG. Traces of the first slice of the second order kernel from **(A)** a patient with early glaucoma in one eye and no glaucomatous changes in the other eye, and **(B)** a patient with early glaucomatous change in one eye and moderate glaucoma in the other, recorded at different levels of luminance contrast. Although in the first patient the value of the saturation coefficient is high in the normal eye and reduced significantly in the eye with early glaucoma, the data from the second patient demonstrate an increase in the coefficient value in the later stage of the disease due to more substantial decrease in amplitude of high-contrast visual evoked potential.

Conclusion

The results support the notion of early M-Cell losses in glaucoma, and are relevant to the design of future stimulation techniques. The technique is very limited however, by the fact that it is only analysing the central test zone – more peripheral flash VEP signals are too small. Therefore it has no value in topographic assessment of visual field damage in glaucoma, and could not be used for objective perimetry. There may be some value in testing central flash VEP responses in early glaucoma or suspects, but at this stage we have not undertaken a longitudinal study.

(ii) Multifocal flash ERG

Multifocal flash ERGs have been recorded in several studies in glaucoma, but have not been shown to correspond well topographically with areas of glaucomatous nerve fibre loss and visual field defects. [137, 148-155]. This was replicated in our studies, with little identifiable change in mERG amplitudes seen in glaucoma subjects. Non-linearities detectable in the second order kernels however, may have an inner retinal origin and have recently been suggested to be altered in areas of glaucomatous damage [150, 156].
A potentially useful change in the mERG associated with visual field defects are the loss of oscillatory potentials. However, the standard luminance flicker mERG responses contain a relatively small ganglion cell contribution, which limits the usefulness of such stimuli for assessment of function in glaucoma [151, 157].

A number of paradigms have been developed to attempt to isolate response components related to ganglion cell activity, with new stimulation protocols for the multifocal ERG. [158-163]. A group of these multifocal paradigms, the Global Flash techniques, which combine multifocal stimulation with periodic "global" (full-screen) are claimed to extract a larger ganglion cell contribution [164]. These techniques evoke a large nonlinear mERG component, termed the "Induced Component", which is an interaction between a focal flash and a periodic global flash that probably contains contributions from lateral interactions. The Induced Component represents the mean difference between successive bright, full-screen flash responses induced by the effect of prior local stimulation [162]. Abnormalities of these gain control mechanisms are believed to be a sensitive indication of early disease effects on the dynamics of inner retinal signal processing.

However there are difficulties in extracting the "optic nerve head component" (ONHC) from the "retinal component" in the analysis. Identifying the ONHC in raw traces is arbitrary, so most analysis is done on the combined Induced Component. It therefore requires significant further development before it is likely to be useful in the clinical setting. It also requires contact lens electrodes which will make it far less acceptable for patients and technicians than the mVEP.

(iii) **Multifocal pattern ERG**

Published in Klistorner, A, Graham, S.L,,, Martins, A. *Multifocal pattern electroretinogram does not demonstrate localised field defects in glaucoma.* Doc Ophthalmol 2000, 100:155-165

Summary of publication

This study performed pattern mERG (mPERG) and pattern mVEP tests on the same subjects. We recruited 20 normal subjects (mean age 42.2 +/- 15.2 years) and 15 glaucoma patients (mean age 67.3 +/- 5.3 years, not age matched) with abnormal optic discs and confirmed visual field loss on subjective testing. The latter required a cluster of 3 points $p<0.5\%$ and abnormal GHT result. For the mPERG a triangular pattern was generated within each hexagon with the size increasing with eccentricity (see figure 3 in section D). Luminance of the white and black checks produced a Michelson contrast of 99%. Surround luminance of the screen was maintained at a mean level of 73.5 cd/m2. The two opposite checkerboard pattern conditions exchanged at each of the 19 sites of the visual field according to the pseudorandom binary m-sequence. To establish optimal numbers of test sites, one individual was tested at 19, 37, and 103 hexagon patterns. The signal-to-noise ratio was best with only 19 zones so this was used for all subsequent tests. The m-15 sequence was used for both mPERG and mVEP and required 8 minutes of recording time per eye, divided into 16 short segments. Distance to the screen was 30cm with all recordings monocular using best corrected near refraction and undilated pupils.

For the mPERG gold foil electrodes were used (Specialised Laboratory Equipment Ltd, Sth Croydon, UK). The cornea was anaesthetised with proxymetacaine hydrochloride. The

ipsilateral ear was ground. The mVEP was recorded on the same individuals using the method described in the General Methods section and Study 1 in Chapter 2. It used a bipolar occipital electrode straddling the inion in the vertical, 2cm above and 2cm below, referenced to the ear, with the lower midline electrode negative. The pattern VEP stimulus was a cortically scaled dartboard (see figure 5 in section D above).

The first order kernel is flat for mPERG and mVEP which confirms that the alternating pattern is cancelling out the luminance component. The first slice of the second order kernel was calculated and analysed for both tests. For the mPERG the waveform in the first 120msec was analysed, and overall amplitudes were taken as the greatest peak to trough, usually P1-N1. In slices 2-4 the earlier P1 component disappeared and amplitude was measured for the later negative-positive wave complex. For the mVEP the maximal peak-trough in the interval 50-165 msec was chosen.

Results

The normal mPERG response decreased through the 5 slices of the second order kernel., averaged over the whole field, with gradually decreasing amplitude in successive slices. MPERG amplitude showed a large range between individuals and decreased with age. This meant a direct comparison could not be reliably performed between normals and glaucomas as they were not age matched. A comparison was done however using the normals >55 years of age (n=5) and Table 1 below shows results for the two groups. Latency did not show a clear age related trend.

Table 1

	Control	Glaucoma	P
Amplitude (μV)	5.2±1.9	1.9±1.0	<0.001
Latency (msec)	45.5±1.5	44.4±4.2	0.2

The mPERG appeared to show a generalised reduction in amplitude in the glaucomas, but did not show any localised losses in any of the cases. In contrast the mVEP performed on the same subjects showed localised reductions in amplitude that corresponded topographically with the reduction s seen on the Humphrey field. Figure 7 below shows an example of mPERG and mVEP performed on the same subject with an inferior arcuate scotoma on Humphrey visual field.

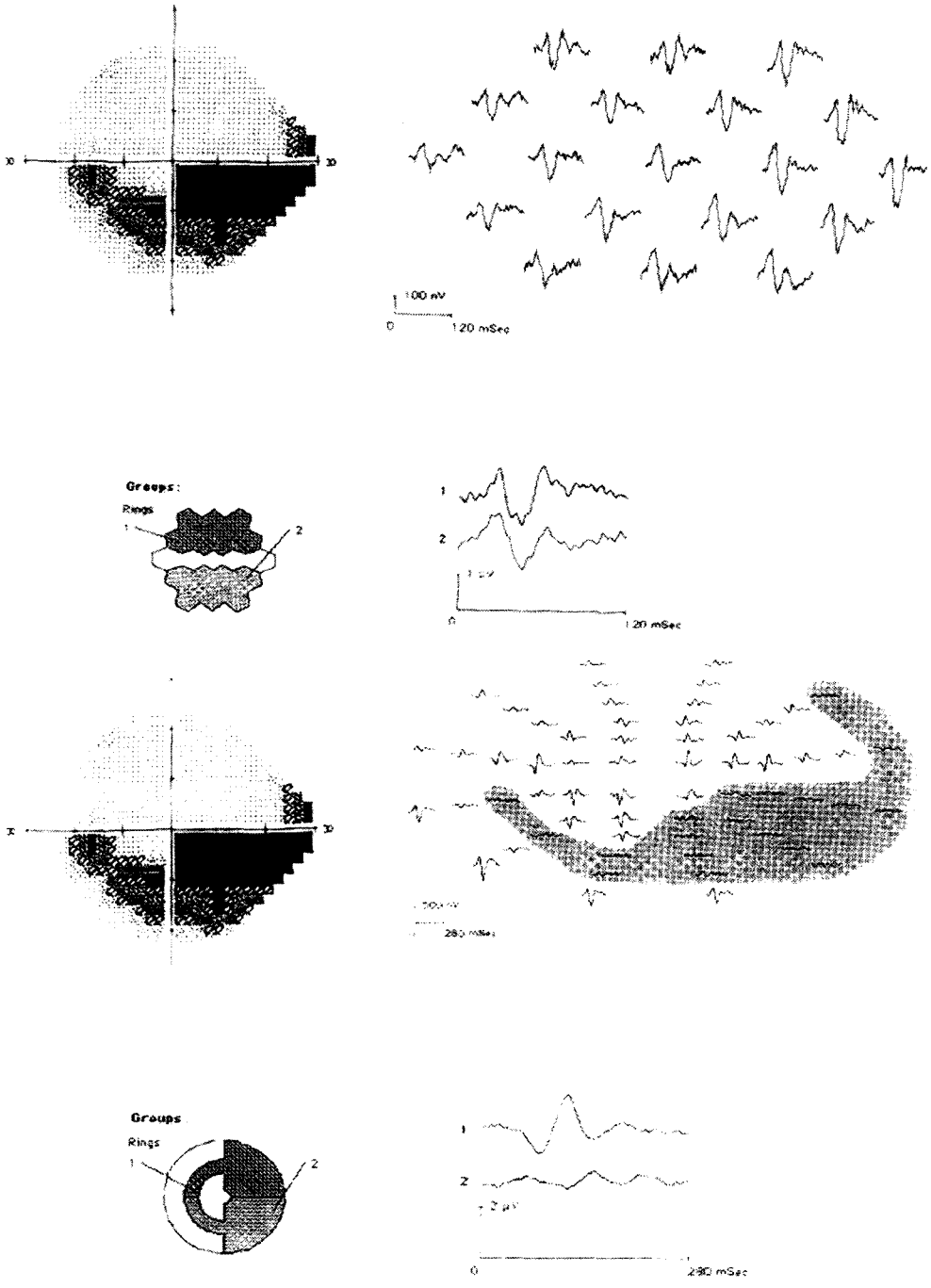


Figure 7 Glaucoma patient with inferior arcuate defect in left eye shown on Humphrey grayscale plot.. Upper figures show mPERG responses and hemifield signals. There is no focal loss of response in the mPERG. Lower figures show mVEP recorded in the same test session. There is good correspondence between Humphrey field and mVEP signals.

Conclusions

The conclusions from this study were that while there may be some generalised reductions in mPERG amplitudes, there was not a clear application to developing it as a form of objective perimetry. Possible explanations were that the mPERG does not solely reflect inner retinal

function, or that the rapidly reversing stimulus was not ideal for isolating ganglion cell responses. Further experiments were conducted using slower frame rates but this had the effect of reducing signal to noise ratios to levels where the interpretation became extremely difficult. However there was a definite role for the mVEP in mapping glaucomatous field loss and this is discussed below.

(iv) **Multifocal pattern VEP**

We first reported using a pattern mVEP to delineate a glaucomatous field defect in:
Graham, S.L, Klistorner, A. *Electrophysiology - a review of signal origins and applications to investigating glaucoma* Aust NZ J Ophthalmol - 1998, 26: 71-85.

The mVEP technique was further developed with attention to stimulus parameters, filter settings, electrode position and analysis of resulting waveforms (all summarised in Chapter 2). Briefly, by employing a cortically scaled pattern stimuli, and appropriate electrode positions we were able to record VEP responses from as far as 20-26° eccentricity [165]. A bipolar electrode, placed 2cm above and 2 cm below the inion, allowed recording of a response of similar magnitude but opposite polarity from upper and lower visual hemifields and an example of a normal recording is shown in Chapter 2 Figure 2. This is in contrast to the conventional fronto-occipital monopolar electrode placement where the upper hemifield responses are consistently smaller (as in Figure 5 above). We proposed that this is most likely a consequence of the cortical distribution of the visual field, with the upper hemifield representation being on the inferior surface of the occipital lobe, further away from the recording electrode on the occiput, and with its cells oriented differently from those of the lower visual field. This may explain why using a full field stimulus to record the VEP has previously shown variable results in glaucoma patients, depending on the distribution of the field loss relative to the surface electrodes. An upper field defect for example, may not produce a significant change in the signal.

The multifocal pattern VEP showed definite focal reductions in signal that corresponded to areas of visual field loss. Pilot studies showed that it was reproducible and it might therefore be feasible to design a clinical test that could be adapted to a form of objective perimetry. The example shown in Figure 5 above in section (iii) reveals its potential for topographic correspondence. Obvious limitations that needed to be addressed included standardising electrode positioning, accounting for inter-individual variability in signal amplitude, minimising noise artefacts, controlling fixation, determining how to analyse data and reject noise, collection of a normals database with statistical application to assess the data. The technique then required verification in the clinical setting to determine sensitivity in established as well as early disease, and to assess specificity and reproducibility. Its application to other forms of visual loss such as neurological disorders also needed to be addressed.

The evolution of the mVEP technique as a potential method of objective perimetry is outlined through the following chapters of this thesis, and these issues are examined and discussed.

Chapter 2

General Methods and Preliminary studies

Characterisation of a bipolar single channel mVEP for topographic recording

The General Methods section outlines the methods used in all subsequent chapters/publications using the VERIS recording system, unless otherwise specified. The AccuMap multifocal VEP system was developed later as a result of initial studies with the VERIS system and is described in more detail in later chapters.

Several preliminary studies were undertaken to better define the multifocal pattern VEP and to maximise signal detection. The following 3 papers were critical to defining the potential of the technique, and the importance of electrode position when recording the mVEP.

1. Klistorner, A.I., Graham, S.L., Grigg, J.R., Billson, F.A., *Multifocal topographic visual evoked potential: improving objective detection of local visual field defects.* Invest Ophthalmol Vis Sci, 1998. 39(6): p. 937-950.
2. Klistorner, A.I., Graham, S.L., Grigg, J.R., Billson, F.A. *Electrode position and the multi-focal visual-evoked potential: Role in objective visual field assessment.* Aust N Z J Ohthalmol, 1998. 26: p. S91-94.
3. Klistorner, A.I., Graham, S.L., *Multifocal pattern VEP perimetry: analysis of sectoral waveforms*. Doc Ophthalmol 1999, 98,183-96

These studies formed the basis on which the core project to develop an objective perimeter was designed, and confirmed the feasibility of the technique as a means of assessing the visual field. The 3 publications are summarised after the General Methods section. My role in each of these studies was: discussion and design of experiments together with Dr Klistorner, recruitment of suitable subjects – both normal and disease states, jointly analysing results and cowriting the manuscripts. Dr Klistorner performed the actual recordings.

A. General Methods

Description of Multifocal technique

The electrophysiological method used in all initial studies was the VERIS Scientific™ system for topographic and temporal analysis of evoked potentials (Electro-Diagnostic Imaging, Inc., San Francisco) which employs a special class of pseudo-random "white noise" stimulation called binary m-sequences. It is based on the Wiener kernel expansion and utilises a deterministic pseudo-random binary exchange of two opposite checkerboard pattern conditions at each of a number of sites (60 sites used in these studies) of the visual field. According to this sequence, there is a 50% probability for the checkerboard pattern to reverse its polarity with every frame of the stimulating display (15 msec). Each input (stimulation site) is modulated in time according to the same pseudo-random binary m-sequence.

An important advantage of the m-sequences - to be orthogonal to all their cyclic shifts - is that it permits computation of the signal by cross-correlation of the response evoked by the m-sequence stimulation, with the m-sequence itself. This property allows one to obtain responses for hundreds of inputs from records of up to a million data points in a fraction of a minute[133] and therefore makes m-sequence stimulation very effective for mapping purposes [133, 166-168].

The technique allows computation of first and higher order kernels, which characterize non-linear interaction between visual events [133, 169]. The first order response however, is zero under the condition of pattern stimulation when both pattern polarities are equal. Thus, only the second order kernel (first slice) which represents the interaction between two consecutive frames of the monitor and is considered to be analogous to the conventional pattern-VEP [166] was analysed in this study. A full theoretical exposé of the method can be found elsewhere [133, 170].

Stimulation and recording.

The visual stimulus was generated on a CRT screen (22" Mitsubishi high resolution display, stimulation rate 67 Hz). It consisted of 60 close-packed segments (Fig.1), the sizes of which were cortically (M-) scaled with eccentricity to stimulate approximately equal areas of cortical (striate) surface [103, 166, 171-174]. The M- scaling would therefore be expected to produce a signal of similar order of amplitude from each stimulating segment. The insert in Figure 1 demonstrates the relationship between size of the stimulus segments and visual field. Each segment included a checkerboard pattern (16 checks) with the size of individual checks being proportional to the size of the segment and therefore also dependent on eccentricity. Checks were alternated in pseudo-random sequences, from which the individual kernels were calculated using cross-correlation of the digitized output signal with the binary input sequences via a fast Walsh transform. The m-16 sequence used resulted in 2^{16}- 1 frames corresponding to 32,767 pattern reversals at each site in each 16 minutes recording.

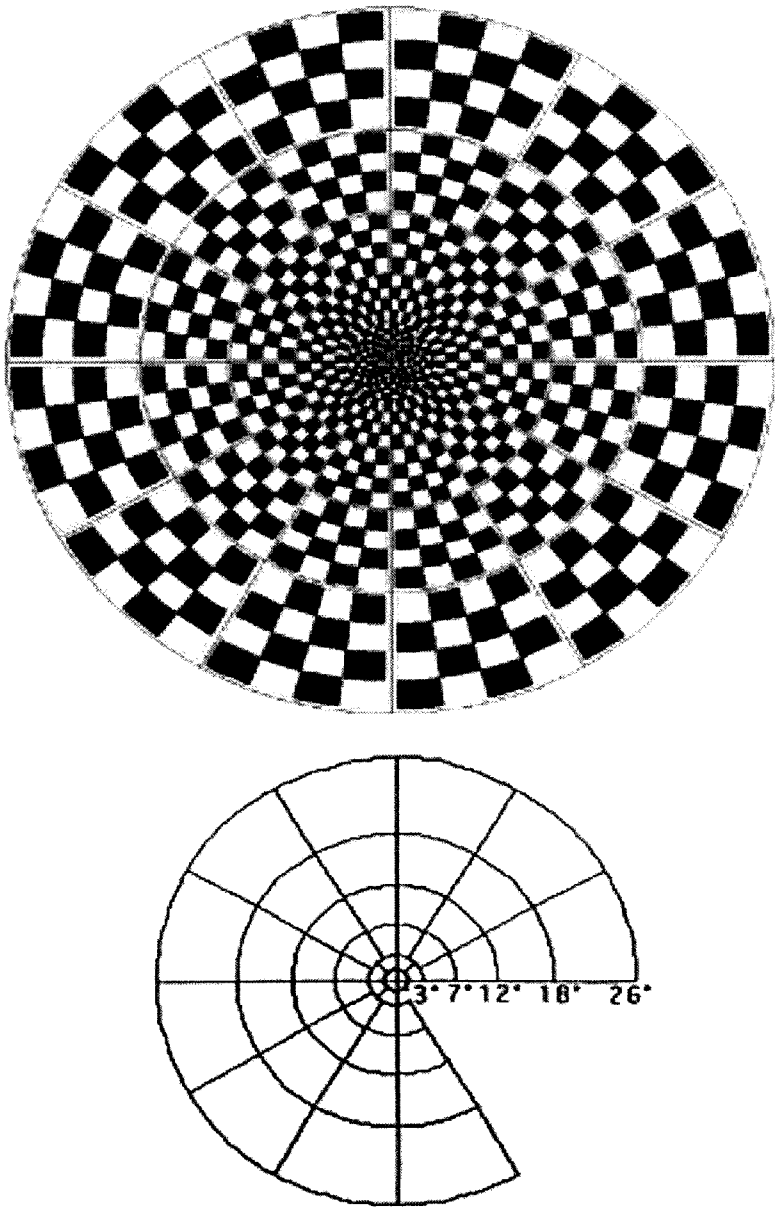


Fig 1 Stimulus for multifocal pattern VEP showing dartboard array or cortically scaled checks with 60 test zones each with 16 checks. Lower figure shows eccentricity of stimulus

The luminance characteristics of the screen were measured using a Minolta TV-Analyser. The C.I.E. coordinates for the monitor white colour were: x= 0.29, y= 0.30. Luminance of the white check was 146 cd/m^2 and luminance of the black check-1.1 cd/m^2 producing Michelson contrast of 99%. Background luminance of the screen was maintained at mean level of 73.5 cd/m^2. A dim room light was always on.

Subjects were comfortably sitting in chair and were asked to fixate on a red fixation point at the centre of the dartboard pattern. The distance to the screen was 30 cm, corresponding to a total subtense of the stimulus of 52°. All subjects were optimally

refracted. Pupils were not dilated. All recordings were collected using monocular stimulation.

Data acquisition.

All data presented in this study was recorded using a Neotrace amplifier (Digitimer Ltd, Hertfordshire). The signal was amplified 100,000 times and band-pass filtered between 3 and 100 Hz. The data sampling rate was 500 Hz and the m-16 binary stimulation sequence was divided into 32 slightly overlapping segments. Raw data was scanned in real time and segments contaminated by a high level of noise, eye movements or blinking were rejected.

B. Preliminary Studies on mVEP

(i) Multifocal topographic visual evoked potential: improving objective detection of local visual field defects

Published in: Klistorner, A.I., Graham, S.L., Grigg, J.R., Billson, F.A.. Invest Ophthalmol Vis Sci, 1998. 39(6): p. 937-950.

Purpose: To investigate the relationship between pattern stimulation of different parts of the visual field (up to 26° of eccentricity), electrode position and the cortical response in order to improve objective detection of local visual field defects.
Method: The human visual evoked potential (VEP) was assessed using multi-focal pseudo-randomly alternated pattern stimuli which were cortically scaled in size. Monopolar and bipolar electrode positions were employed. The visual field up to 26° of eccentricity was investigated. Twelve normal subjects and five subjects with visual field defects of different nature were studied.
Result: While the monopolar response is heavily biased towards the lower hemi-field, bipolar leads overlying the active occipital cortex (straddling the inion) demonstrate good signals from all areas of the visual field tested. The amplitude is almost equal for averaged upper and lower hemi-fields, but the polarity is opposite causing partial cancellation of the full-field VEP. The degree of cancellation depends mainly on latency differences between the vertical hemi-fields. The bipolar VEP corresponded well with Humphrey visual field defects, showing loss of signal in the scotoma area.
Conclusions: The multi-focal VEP demonstrates good correspondence with the topography of the visual field. Occipital bipolar (BOS) electrodes placement is superior to standard monopolar recording. In order to avoid a full-field cancellation effect, separate evaluation of upper and lower hemi-fields should be used for the best assessment of retino-cortical pathways. This technique represents a significant step towards the possible application of the multi-focal VEP to objective detection of local defects in the visual field.

Introduction.

It has been shown that varying the placement of electrodes can considerably affect the field topography of the VEP response. For instance, conventional (occipito-frontal)

electrode placement, used in the majority of studies and recommended by ISCEV as a standard for clinical use [175] favours the lower field response. Transferring the position of the reference electrode from mid-frontal to linked ears, on the other hand, significantly improves detection of the signals from upper field [113, 176]. The same tendency is observed when occipito-occipital bipolar electrode montage is used [166].

This variation with electrode position is most likely due to the complicated anatomy of retino-cortical projections from different parts of the visual field and the extreme convolution of the cortex [103, 177, 178]which lead to a variation of the underlying dipole sources [113]. The central part of the upper retina (up to 20-25 deg) projects to the external (posterior) surface of occipital cortex, just anterior to the inion. The central part of the lower retina projects to the inferior surface of occipital cortex. This makes the cortical dipoles from lower and upper central hemi-fields almost perpendicular. Therefore electrode position is critical when recording VEPs from the central visual field. Orientation of the electrodes relative to the generating dipoles may substantially favour the input from either upper or lower field. The projection of the more peripheral parts of the upper and lower hemiretinas are to the upper and lower banks of the fissure calcarinus. This leads to almost opposite orientation of the generating dipoles [103, 113, 177-179].

Previous attempts to record a type of multi-focal VEP by stimulating individual parts of the visual field have been limited by time constraints to using larger test areas such as hemi- or quadrantic fields [180]. A recent study attempting to detect visual field defects by means of traditional sequential (site by site) stimulation employed relatively large stimulus size (hemifields or quadrantic fields) which limited the resolution of the technique [111].

A different approach was suggested by Sutter's group [166, 169]. They used the method of pseudo-randomly presented multi-focal stimulation together with cortical scaling of the size of stimulated patches. They were able to stimulate a number of locations of the visual field simultaneously and extract individual responses from each of them. Signal-to-noise ratios were acceptable from as far as 7° of eccentricity. Bipolar (occipito-occipital) electrodes placement, employed in their study, permitted recording of reliable responses from many locations of the visual field including both upper and lower hemi-retina.

The aim of the present investigation was to extend the study of Sutter's group in establishing the relationship between topography of the expanded visual field (up to 26° of eccentricity) and the cortical response (VEP).

Methods.

Recording techniques were as described in the General Methods section above.
For electrode placement two different set-ups were used. Conventional monopolar (Occipito-Frontal) electrode placement used the Oz (active electrode) and Fz (reference electrode) positions according to the 10-20 design. Baseler et al have recently suggested a bipolar (Occipito-Occipital) electrode arrangement [166, 169]which requires electrodes

to be positioned 2cm and 4 cm above the inion in the midline. In a pilot study we discovered that shifting the electrodes downward (compared with the Baseler et al position) further improved detection of signals from the upper field. The best position of the electrodes in this respect (which produced approximately equal responses from upper and lower hemi-fields) was found when electrodes were placed at equal distance 2 cm inferiorly (negative electrode) and 2cm superiorly (positive electrode) straddling the inion[181]. Thus, this modified electrodes position will be referred in the current study as bipolar-occipital-straddle placement (BOS) to differentiate it from Baseler's position.

Subjects.

Two groups of subjects were used in this study. The first group consisted of 12 normal volunteers (six males and six females). They had an age range from 22 to 65 years. All subjects were given a routine visual examination, had corrected Snellen acuity of 20/20 or better, refractive error less than 2 dioptres and a normal Humphrey 24-2 visual field. There was no history of ophthalmological abnormality in any of the subjects, and no systemic illness such as diabetes that could affect visual function. Except for the authors none of the subjects were familiar with the experimental set-up. The study followed the tenets of the Declaration of Helsinki and for all subjects informed consent was obtained.

The second group included patients with well documented visual field defects. This included three subjects with extensive glaucomatous field changes confirmed on repeat automated perimetry, a patient with a pituitary adenoma causing chiasmal compression, and a patient with optic atrophy secondary to trauma. The visual acuity in these five patients was 20/30 or better. The glaucoma patients had primary open angle glaucoma (POAG) - intraocular pressure >20mmHg, typical optic disc cupping and confirmed visual field loss .

Results.
(i) Monopolar (Occipito-Frontal) and Bipolar electrode (BOS) placements.

In the first series of experiments the effectiveness of conventional monopolar (Occipito-Frontal) electrode placement and the bipolar (BOS) electrode placement were compared. Traces derived from stimulation of 60 cortically scaled individual segments of visual field using monopolar and BOS electrode placements are presented in Fig.2.

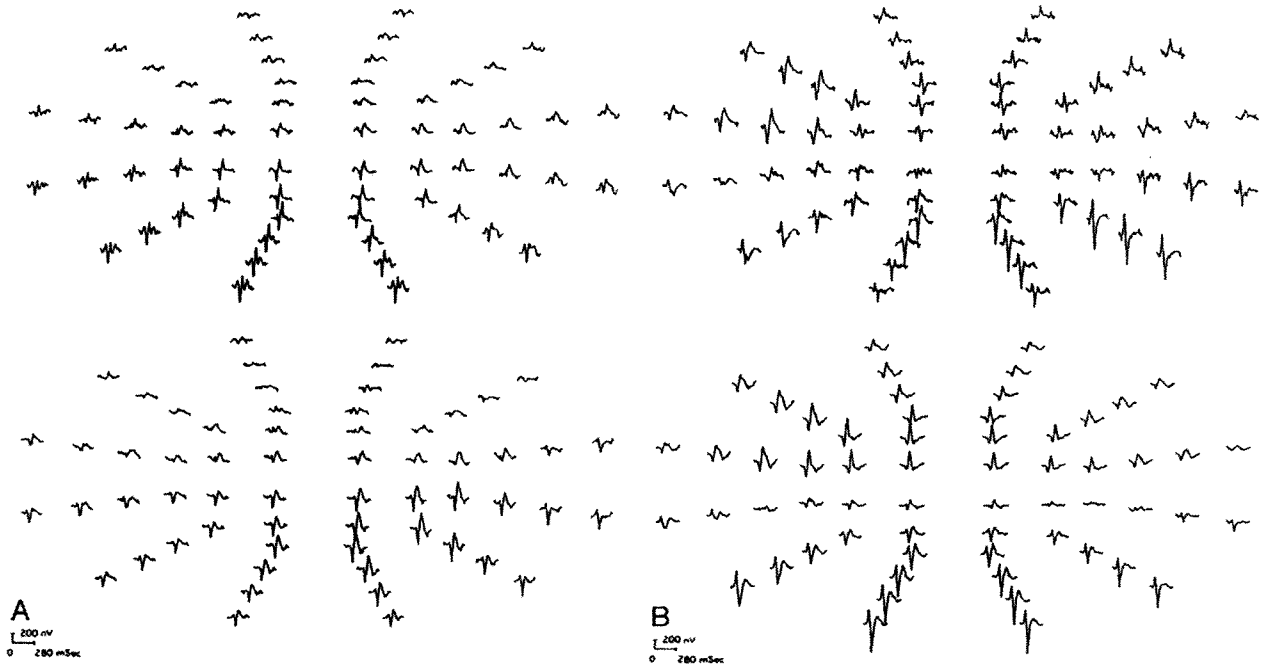


Figure 2 Comparison of monopolar and bipolar occipital [placement of electrodes. Column A shows 2 different subjects recorded with conventional fronto-occipital electrode positions. Note small signals in upper field. Column B shows respoinses from the same 2 subjects recorded at the same time using bipolar occipital electrodes. The signals are improved in the upper field, although smaller along the horizontal.

There are several points which are apparent. Firstly, the stimulation of the segments of the upper hemi-field using monopolar placement (Fig.2a) yields much smaller responses than the stimulation of the segments of the lower hemi-field. In fact, the waveforms of the monopolar potential in a number of upper-field locations do not exceed the level of noise. Two examples from different subjects are shown.

BOS electrode placement (Fig.2b, from the same subjects and the same recordings), however, produces a much more proportional distribution of the recorded potential throughout whole stimulated area. Responses from practically all locations of lower as well as upper parts of the visual field demonstrate good signal-to-noise ratios with well defined waveform and significant amplitude.

Secondly, practically all traces derived using monopolar electrode placement show the same polarity of the waveform with the major negativity at around 100 msec. As mentioned above, this is much more pronounced in the lower field. Traces produced using the BOS electrode placement however, demonstrate very much opposite polarity in upper and lower hemi-fields. The majority of the cortical signals derived from the stimulation of the different segments of the lower field exhibit well-defined negativity at around 100 msec while most of the upper field stimulating sites produce traces with significant positive deflection at about the same latency.

These differences become even more apparent when combined responses from upper and lower hemi-fields recorded using monopolar (Fig.3) and BOS (Fig.4) electrode

arrangement are compared. As can be clearly seen in Fig.3, the responses recorded from monopolar electrodes exhibit the same polarity when both upper and lower hemi-field are stimulated. The amplitude of the responses, however, differs dramatically. While the combined response from the lower hemi-field demonstrates a well-defined waveform of significant amplitude the upper field potential is notably smaller in amplitude and much noisier (fig.3a,b). In some cases the waveform of the response from the upper field is not even well-determined (see Fig.3c).

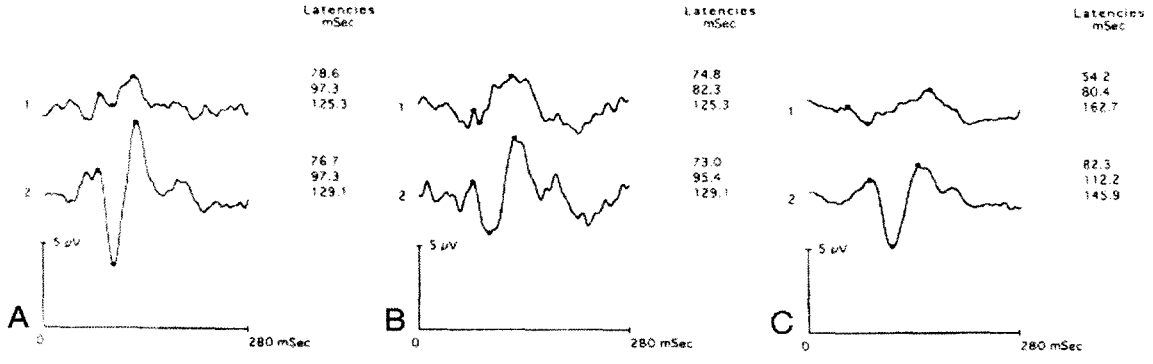


Figure 3 MVEP responses recorded from monopolar electrodes showing upper and lower hemi-field rersponses from 3 subjects. Signals are of the same polarity, but with much smaller responses from the upper field.

In contrast, the averaged hemi-field responses derived from BOS electrodes (Fig.4) yields well-defined waveforms of substantial amplitude from both upper and lower hemi-field. Fig. 4 demonstrates averaged hemi-field VEPs from nine normal subjects. The polarity of the major components in all tested subjects demonstrates a clearly opposite character. The lower hemi-field response exhibits positive-negative waveform with a latency of the first positivity of 75.0 (SD=3.4) msec and major negativity at 97.2 msec (SD=2.7). The upper hemi-field response shows almost opposite polarity of the peaks with negative deflection at 79.0 (SD=6.7) msec and major positive peak at 105.5 msec (SD=4.7).

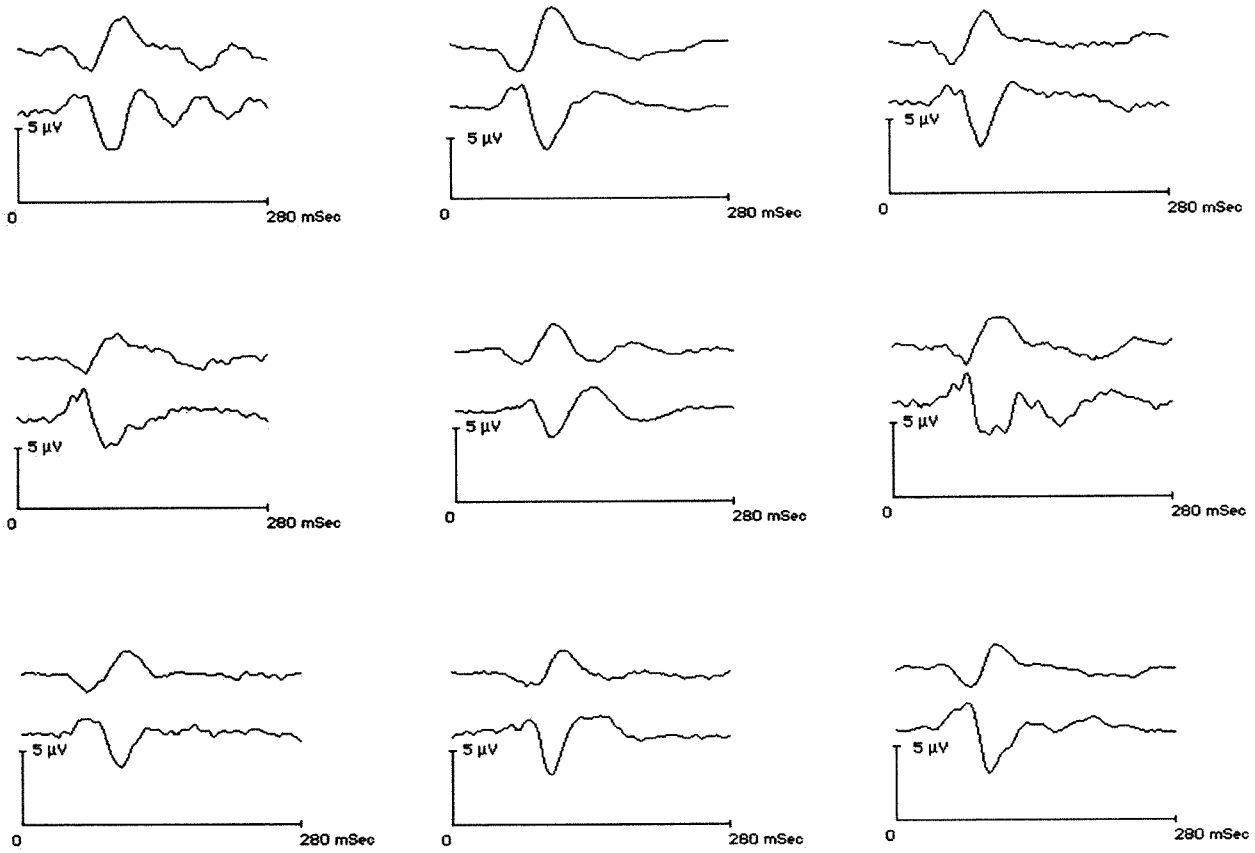


Fig 4 Hemifield mVEPs recorded using BOS electrode placement in nine normal subjects showing opposite polarity of responses from upper and lower hemifields in all cases.

Table 1 Amplitude (µV) and latency (msec) values for summed upper and lower hemifield response derived from normal subjects

	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
Sex	M	M	F	M	M	F	F	F	M	M	F	F		
Age	22	32	37	38	39	44	52	55	62	63	65	65	47.9	
Upper Hemifield latency	110	99	102	106	100	96	105	110	104	115	112	106	105.5	4.7
Lower Hemifield latency	97	97	97	97	94	92	93	99	100	102	102	99	97.2	2.7
Upper Hemifield amplitude	3.6	2.9	5.5	2.4	3.7	4.1	3.4	2.5	2.9	2.5	3.7	4.7	3.49	0.94
Lower Hemifield amplitude	4.4	2.7	5.5	3.3	4.2	4.4	4.5	3.6	3.4	3.8	3.8	4.1	3.99	0.68

The value of the latencies of the major peaks for all tested subjects, which are presented in Tab.1, clearly indicates that the lower field tends to respond faster. The difference of a few msec is detected in all subjects tested.

While there are some inter-individual variations, the data presented in Tab.1 illustrate that the magnitude of the VEP signals from the upper and lower parts of the tested visual field recorded using bipolar electrode placement tends to be similar. Calculated upper-to-lower hemi-field amplitude ratio shows mean value of 0.89 (SD=0.18), which is close to unity. The inter-individual variations in relative amplitude are possibly due to individual anatomical variations of brain-to-scalp relationship. Intra-individual variability, on the other hand, is quite small. Fig. 5 shows extremely small variations of amplitude, waveform and latency of the VEP recorded from one of the subject on four different occasions. The upper hemi-field amplitude had a mean value and SD of 4.3 $\mu V \pm 0.082$ (coefficient of variation=0.019) and the lower hemi-field had a mean value and SD of 4.16 $\mu V \pm 0.15$ (coefficient of variation=0.036).

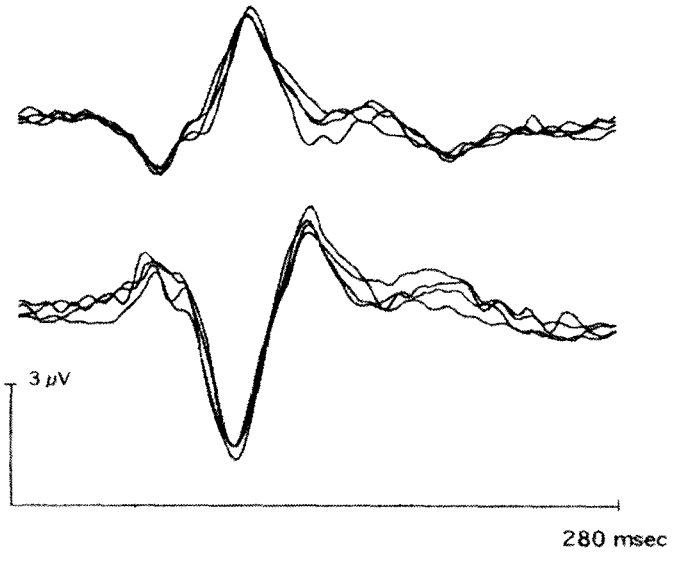


Figure 5 mVEP hemifield responses recorded from one subject on four different occasions – traces are superimposed

There is, however, no polarity reverse or waveform change in bipolar recordings across the vertical meridian of the stimulated visual field. In order to avoid equivocality due to horizontal polarity change, quadrants (instead of the left and right hemi-fields) have been analysed. A typical example, presented in Fig.6 shows that both upper-field quadrants exhibit similar negative-positive waveform while low hemi-field quadrants show a similar positive-negative waveform of opposite polarity.

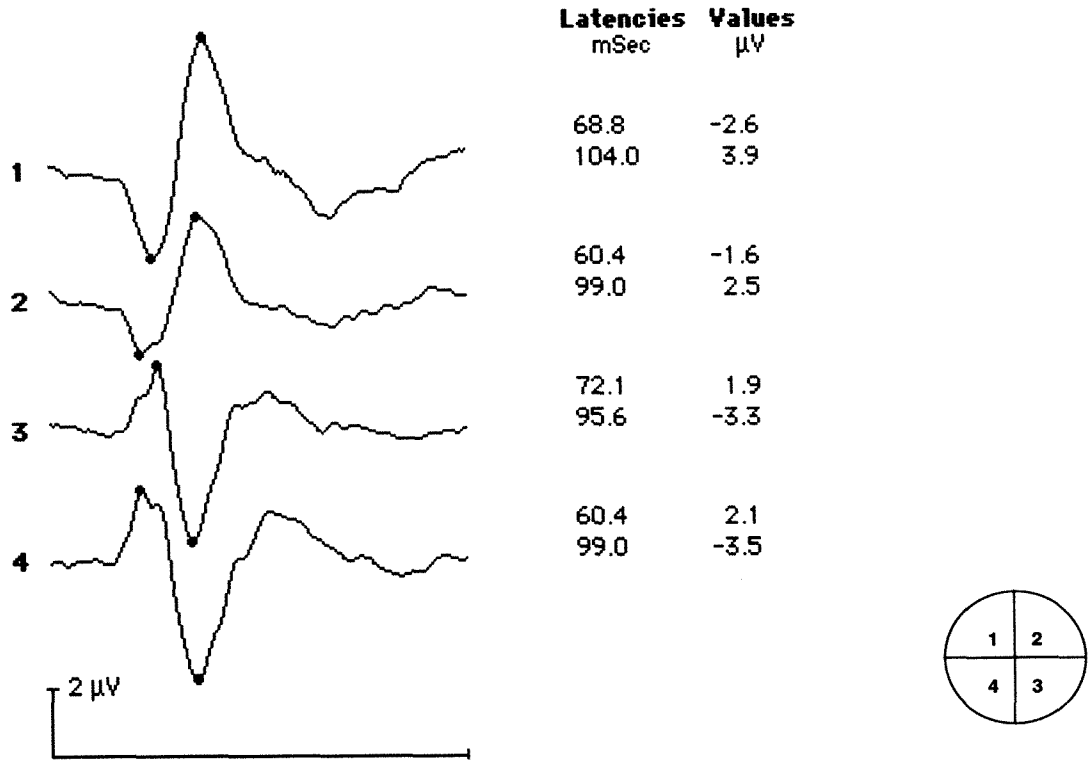


Fig 6 Averaged quadrantic responses from the left eye of a normal subject. mVEPs from both upper-field quadrants exhibit similar negative-positive waveform while low hemi-field quadrants show a similar positive-negative waveform of opposite polarity. Insert shows quadrants.

The polarity reverse across the horizontal meridian has an important effect on the recording of full-field pattern VEP, which has not been properly addressed in the available literature. Based on the additive nature of the Pattern-VEP [30,47] one may assume that the summation of the opposite polarity signals evoked by stimulation of equal areas of upper and lower fields would produce partial or even complete cancellation of the full-field response. We found that the full-field BOS VEP exhibits such behaviour - the larger half-field constituents often produce a much smaller resulting full-field waveform .

Although the degree of cancellation depends to some extent on the relative amplitude of upper and lower hemi-fields VEPs, it is also very dependant on the difference in latency between hemi-field responses: the shorter the difference between opposite peaks of averaged upper and lower hemi-field responses, the smaller the resulting full-field signal.

However, the above mentioned consideration is not applicable to monopolar recordings, where the lower retina contributes little if anything to the full-field response. While the summation of the signals from whole stimulating area recorded using bipolar electrode montage produces an extremely small VEP, the average full-field monopolar VEP demonstrates a substantial amplitude which is practically equal to the sum of lower and upper hemi-field responses (Fig.7). The combined response from the whole stimulated area in this case is mainly determined by the signal from upper hemi-retina, minimising

the phenomenon of cancellation. Thus, although the full field signal is large, the result of the monopolar recording could be misleading in a case of upper visual field defect.

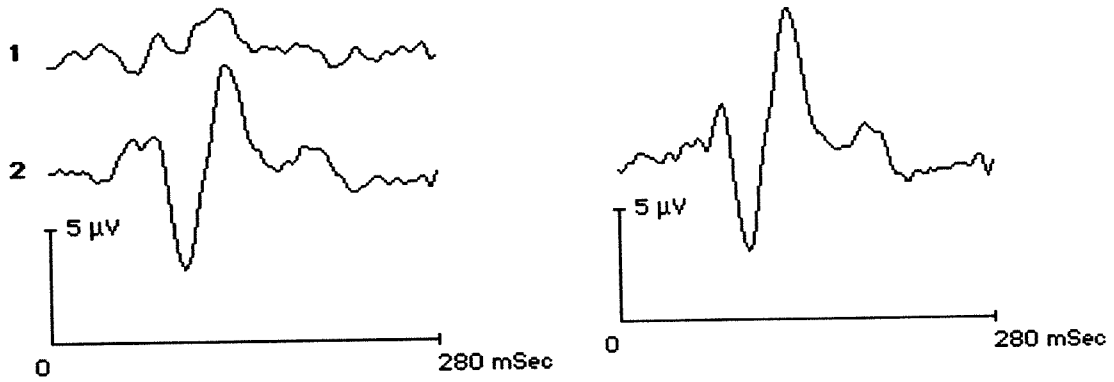


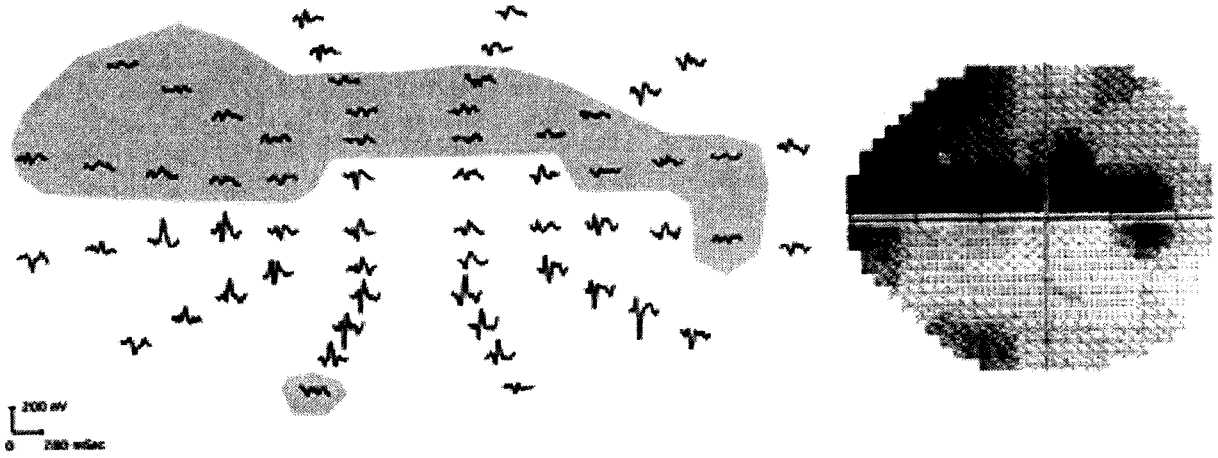
Figure 7 Example of hemifield (left) and full-field (right) mVEP recorded using monopolar electrode placement. The full-field signal predominantly reflects the lower hemifield response.

Another visible consequence of the polarity reverse is an often observed significant reduction in the BOS VEP amplitude at locations along the horizontal meridian (see Fig. 2) which may well be explained by retino-cortical topography (see Discussion).

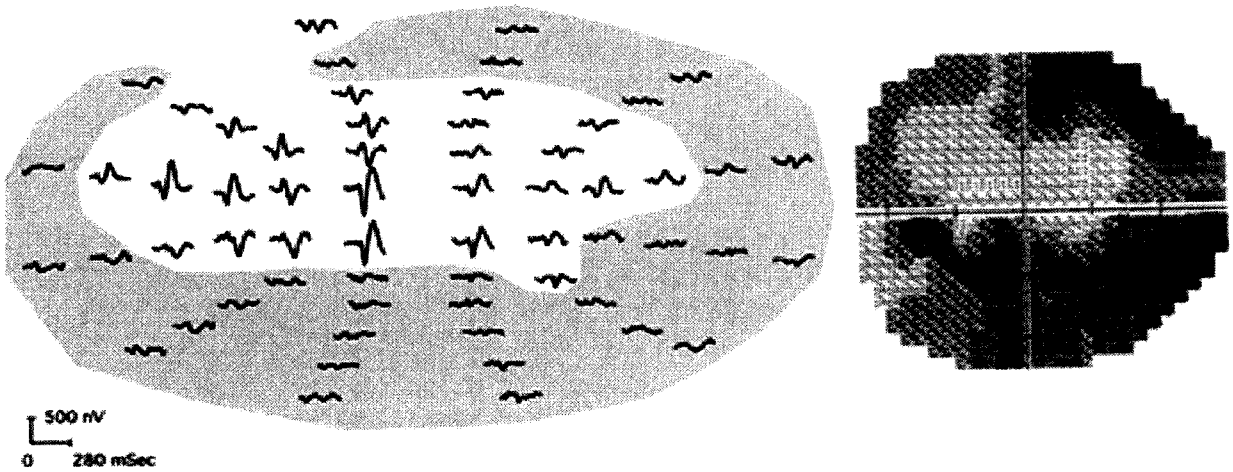
(ii) Multi-focal VEP recorded from subjects with visual field defects.

In order to substantiate the local nature of generated signals, a few subjects with well defined defects of visual field due to a variety of pathological conditions were investigated using the BOS VEP protocol. Figures 8 and 9 illustrate the correspondence between visual field defects and multi-focal VEPs in the above mentioned subjects.

(a)



(b)



(c)

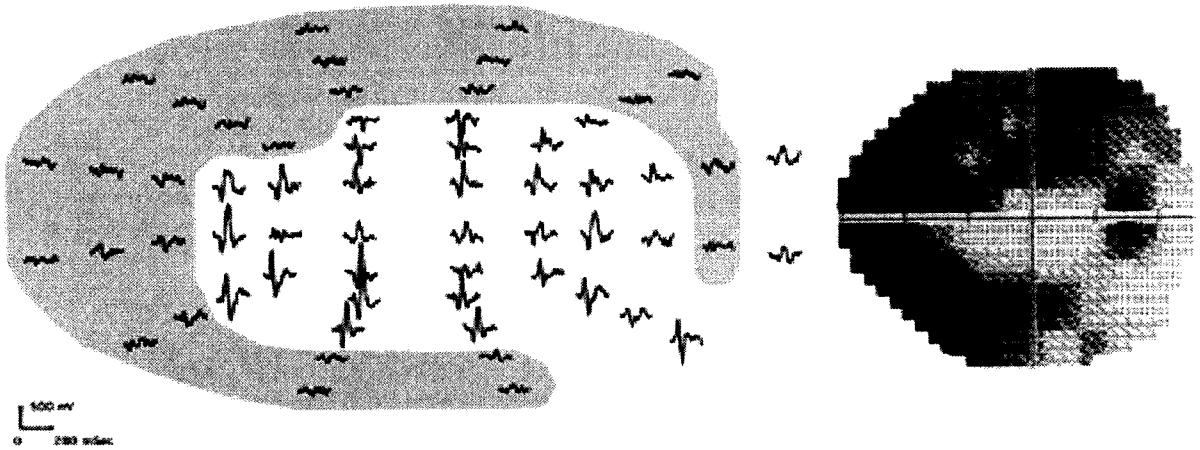


Figure 8a,b,c Examples of multi-focal VEP traces recorded from subjects with glaucomatous visual field defects. The corresponding Humphrey perimeter gray scale is shown. Shaded areas of the VEP array highlight those segments where the responses approach zero. These areas correlate well with the automated perimetry findings. The shading is a subjective addition for the purpose of presentation, but does not represent a statistical analysis. Note that the representative scales for perimetry and VEP plot are not exactly the same - the perimetry plot is linear while the VEP plot is cortically scaled (ie VEP has greater number of test points within central 10° compared to perimetry).

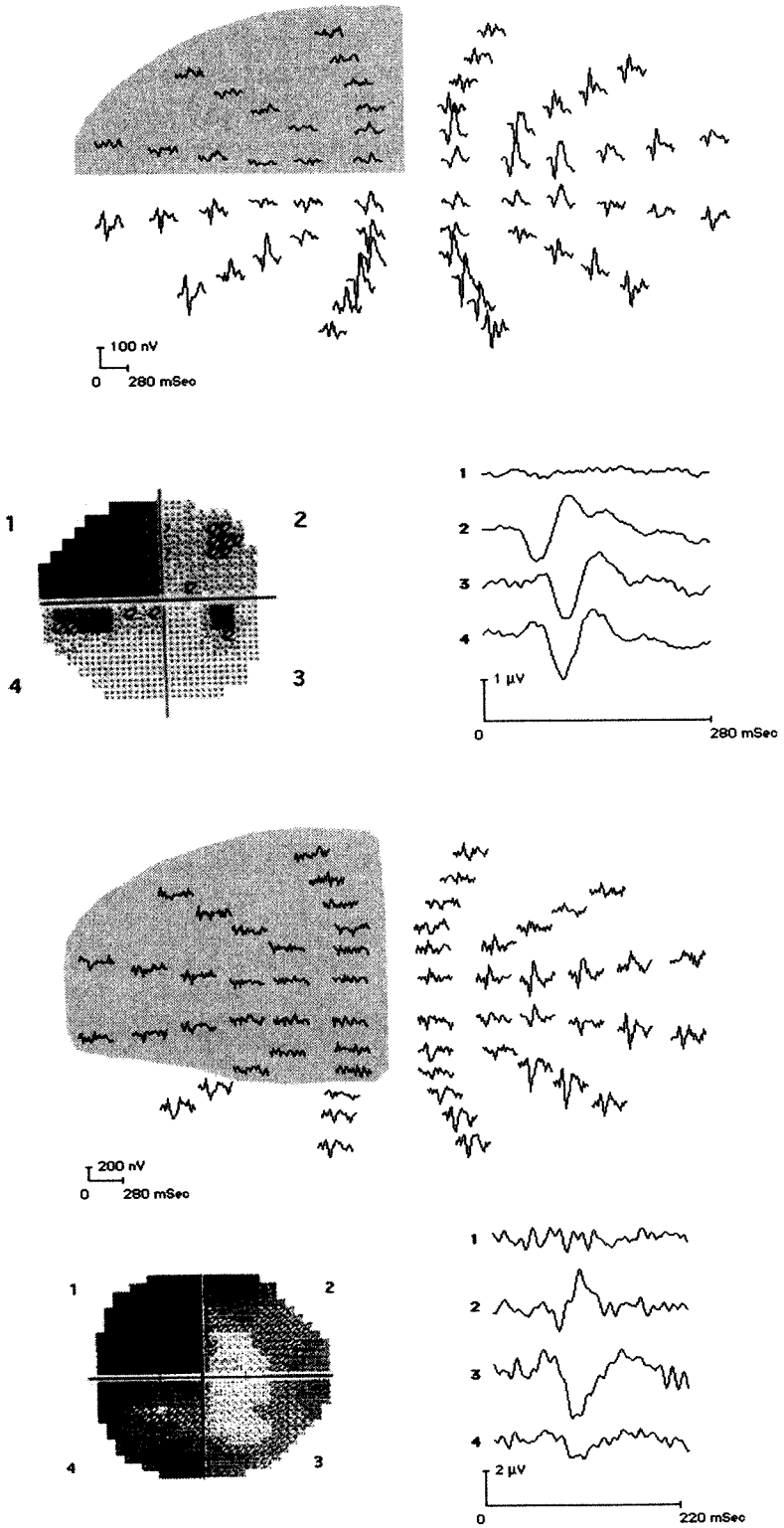


Figure 9 Examples of multi-focal VEP traces recorded from subjects with neurological visual field defects. Fig.9a shows trace array from a subject with upper quadrantinopia due to cerebral infarct. There is no response from affected area of visual field. Fig.10b subject with pituitary adenoma demonstrates clear demarcation of the response across the vertical meridian, despite noisy traces.

Overall there is a good agreement between the topography of the scotoma detected by automated perimetry and the areas of visual field from which no detectable response was recorded. When examining these figures it is important to consider that the representation of multi-focal VEP traces shown is not exactly correlated with visual field test locations, since the VEP stimulus areas are cortically scaled, as seen in the dartboard arrangement and insert of Figure 1. The averaged quadrantic responses, which have been presented for some patients, reflect the area of field loss with smaller amplitudes in the quadrant most affected.

Full field responses, however, do not reliably reflect damage. An example of how misleading interpreting the full field response can be, is shown in Figure 10. This shows the relationship between averaged full field responses and hemifield responses in an eye with, and an eye without, a visual field defect. The traces are from the glaucoma patient represented in Fig.9a. This patient has POAG in the right eye with upper field loss, but no visual field defect in the left eye. While the amplitude of the upper hemi-field response from the right eye is much smaller than the upper hemi-field response of the fellow eye, the full-field VEP averaged over whole tested area of right eye is larger than the response from the whole field of the left eye.

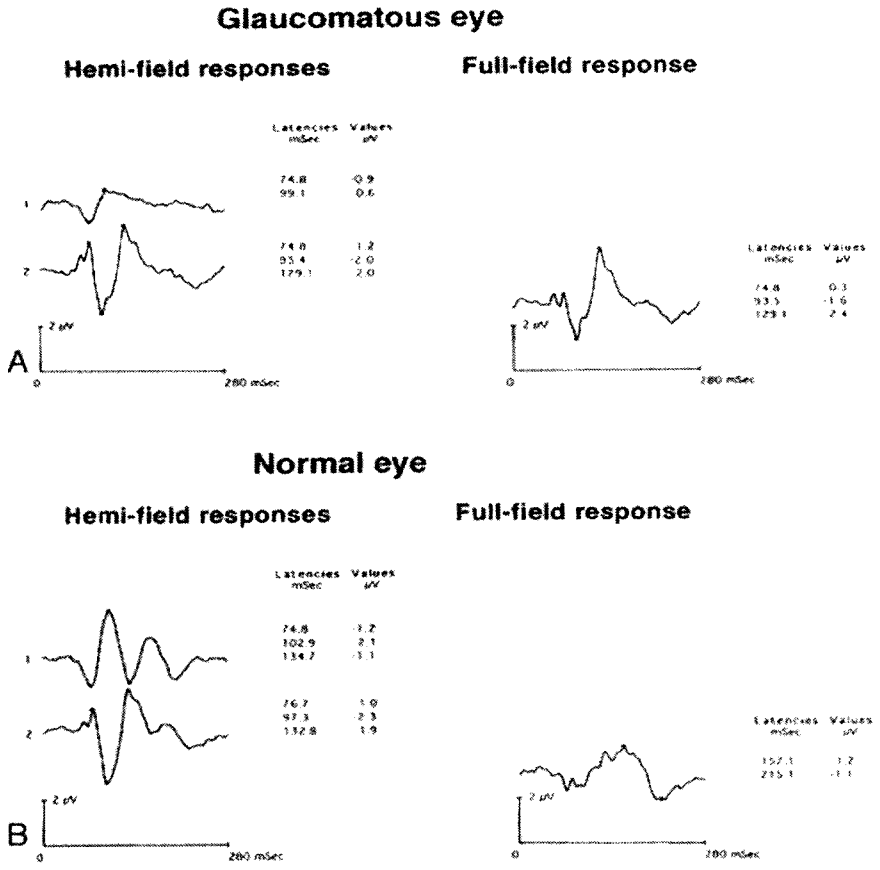


Figure 10 Hemi- and full-field responses from both eyes of the glaucoma patient whose traces are shown in Fig. 8a. The traces from the glaucomatous (right) eye are presented in A (upper row) while B (lower row) shows the responses from the fellow eye which still has a normal visual field. Due to reduced amplitude of the upper hemi-field response in the affected eye, the full-field response is paradoxically larger than in the eye with a normal visual field, in which full-field signal cancellation occurs.

Such a contradictory finding is most likely the result of a reduced cancellation effect due to the smaller amplitude of the upper hemi-field. Thus, in this case, the decrease in amplitude of one of the hemi-fields leads to the averaged full-field amplitude actually being increased.

Discussion.

There are several findings in this study of the multi-focal pattern-VEP which could be of practical interest for the future development of an objective visual field test. Firstly, it is clear that the position of recording electrodes is crucial for extraction of the signal from areas of the cortex corresponding to the peripheral parts of the visual field. Bipolar leads overlying the active occipital cortex appear to be optimal for this purpose [176, 180]. The linking line between recording electrodes vertically straddling the inion (BOS protocol used in this study) is at practically equal (but opposite) angles to dipoles originating in the striate visual cortex subserving upper and lower central to mid-periphery hemi-fields [113]. This produces VEP signals from averaged upper and lower hemi-field of similar amplitude but reversed polarity.

The line between Oz and Fz electrodes (monopolar recording), on the other hand, is almost perpendicular to the upper field dipole, making its contribution minimal. Thus, highly disproportional responses from upper and lower hemi-fields found in some recent studies [118, 182]can be readily explained on the basis of different dipole orientations rather than a reflection of real functional disparity.

As a result of the relationship between amplitude and polarity of the responses of the vertical hemi-fields discussed above, the full-field BOS VEP appears to be partly (or in some cases almost totally) cancelled. This also applies to small central stimulating areas of a few degrees in diameter. The extent of cancellation is largely dependent on the small difference between implicit time of opposite hemi-field peaks. Therefore the larger difference in latencies between hemifields, the greater the full-field response elicited.

It also should be noted that in cases of bipolar recordings where the full-field VEP produces a measurable signal, the waveform of the response often does not resemble responses from single locations, or upper and lower hemi-fields and tends to have a longer latency. This results in a misleading impression of conductivity delay. Thus, it follows that the full-field bipolar VEP has little significance as a true indicator of visually evoked cortical activity. This observation also agrees well with seemingly paradoxical result described in one of the glaucoma patients (see Fig.11) when the upper field scotoma leads to a significant reduction of the cancellation effect, and therefore to an increase in full field amplitude.

However, due to the fact that the monopolar response does not produce a vertical reversal of polarity, and that it is greatly biased towards the lower hemi-field, it also can not be considered as a reliable indicator of full-field testing. Thus, while the monopolar VEP can give an adequate functional assessment of the lower field projections, it is not

sufficiently sensitive to the upper hemi-field losses. The best indicator of true cortical response is therefore achieved with bipolar recording analysing individual points or hemifields.

There is a marked decrease in amplitude along the horizontal meridian seen in some bipolar recordings, for example Fig 2b. This will unfortunately limit the application of the bipolar VEP as a type of objective visual field assessment, especially since glaucomatous field loss often is first recognised along the horizontal. This may be due to one of two reasons: alteration of the dipole orientation or cancellation of upper and lower field components, or a combination of both. It is generally agreed that the horizontal meridian of the peripheral visual field is represented (with slight individual variability [183]) deep within the calcarine banks at the fissure base [101]. This may lead to such an alteration of dipole orientation that it may become much more perpendicular to the linking line between bipolar electrodes, minimising the recorded signal. On the other hand, amplitude decrease may be simply a result of cancellation of the opposite signals from upper and lower hemi-fields which meet each other around the horizontal meridian. Data presented in this study is not sufficient to solve this question - further experiments employing more electrode channels and positions are needed (See Chapter 5).

The latency delay of the responses from the upper field, reported earlier [120, 182], appears to be an intrinsic feature of the human bipolar VEP. It can not be explained on the basis of different dipole orientations, and we would not expect the position of the recording electrode to affect the latency. The VERIS system makes a correction for the raster delay of the screen, compensating for the slight timing differences between top left and bottom right of the computer display. One explanation previously suggested [119, 122, 184-186] is a more important physiological role of the lower hemi-field which may require faster processing of the visual information. While this study has established that the amplitude of the upper and lower hemi-field responses are not markedly different, it is plausible that a temporal characteristic of retino-cortical pathways is that the lower visual hemi-field has some advantage.

The recordings from patients with different visual field defects confirm the focal character (in a sense of stimulating area of the visual field) of generation of the VEP. While it is difficult to reliably assess the degree of amplitude depression quantitatively (due to extreme convolution of the visual cortex), good correspondence is demonstrated between areas where no response was elicited and the location of dense scotoma in the visual field. Amplitude differences between individuals remain a limiting factor in the application of this technique to objective visual field assessment at the present time.

From this study it may be concluded that the multi-focal BOS VEP demonstrates good correspondence with the topography of the visual field. Occipital bipolar (BOS) electrodes placement, instead of the ISCEV standard recommended monopolar electrodes, provides a much more reliable signal from the upper visual field. Cancellation effects due to the different orientation of generating dipoles, relative to electrode positions, need to be considered for correct assessment of retino-cortical pathways.

(ii) Electrode position and the multi-focal visual-evoked potential: Role in objective visual field assessment.

Published in: Klistorner, A.I., Graham, S.L., Grigg, J.R., Billson, F.A. Aust N Z J Ohthalmol, 1998. 26: p. S91-94.

Summary
This additional minor study was undertaken to compare the newly described BOS electrode placement for mVEP recording (above), with the higher position described by Basseler et al and with conventional fronto-occipital placement, in the same individuals. Analysis of the waveform polarity and shape throughout the field was done to look for similar patterns of signal.
Methods of recording were as described above in General Methods and in Study 1 above. Four normals with VA 6/6, normal Humphrey visual field and normal ophthalmic examination were tested with recording electrodes in all 3 positions simultaneously. Three recording channels were used to achieve this. The BOS position used was 2cm above and 2 cm below the inion. The Basseler position was 2cm above and 4 cm above the inion. The conventional position used the Oz (2cm above inion) with the Fz (forehead) as reference. All were grounded to an ear electrode.
The results were as expected from the previous studies, and were verified across all individuals. In the conventional monopolar position the signal from the upper field locations was very small. In the Basseler position the upper field improved, however in the BOS position there was more proportional distribution of signal throughout the field. The summed responses from the upper and lower hemifields show this. Also the opposite polarity of upper and lower fields is more clearly identified on the BOS recordings.
Analysing the waveforms by sectors showed more consistent shape and polarity of waveforms than by quadrants, particularly in the upper vertical meridian compared to the horizontal sectors. This was finding was further analysed in study 3 following.

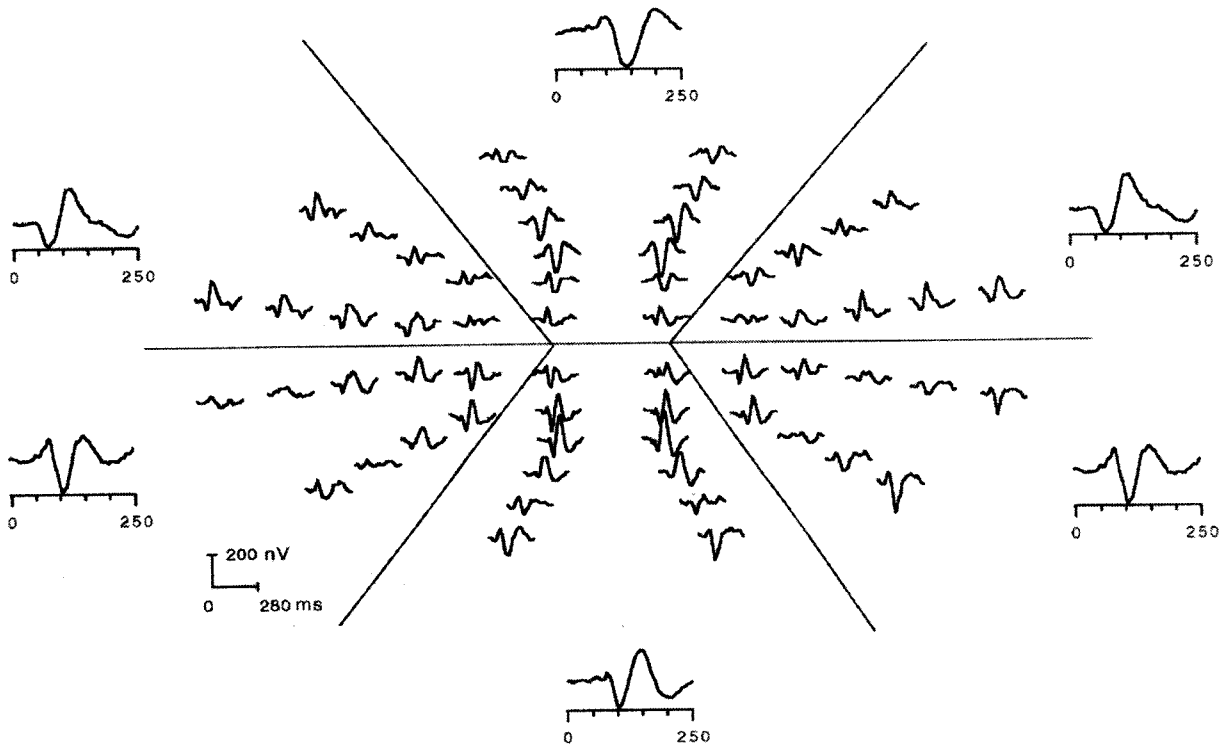


Figure 11 mVEP recorded with BOS electrode placement. Original traces and summed sectoral analysis are shown. Sectoral waveforms are normalised to allow comparison of shape. Note similar polarities within sectors although some points do not have the same shape.

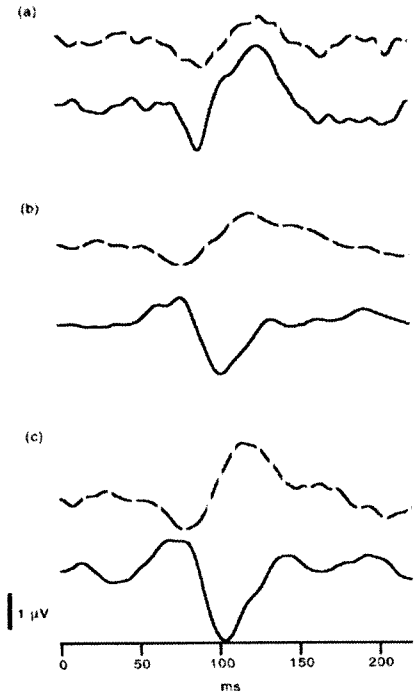


Figure 12 Hemifield signals for 3 different electrode positions (a) frontal-occipital (b) occipital-occipital – Basseler (c) occipital-occipital (BOS) recorded from the same subject simultaneously. Shows that the BOS position generates the best signal from upper and lower fields.

(iii) Multifocal pattern VEP perimetry: analysis of sectoral waveforms

Published in: Klistorner, A.I., Graham, S.L..Doc Ophthalmol 1999, 98,183-96

Summary
The purpose of this paper was to further examine the variations in waveform seen across the mVEP field, to determine the best method for summating responses of similar type within the field. The mVEP recording technique was as described above, with electrodes in the standard BOS position. 12 normal subjects were tested. Inclusion criteria required Vac 6/9 with normal clinical examination, IOP and visual field. Groups of traces from different sectors of the field were analysed with respect to their summed responses. Statistical comparisons between points and between sectors were performed (Statistica 4.1).

As previously observed in the above two studies, upper and lower hemifields produced signals of opposite polarity. In addition, the waveforms showed marked variations within the upper field itself. In a number of subjects the waveform of the segments situated along the horizontal meridian exhibited a negative-positive complex with a major positive deflection around 100-110msec, while in the VEP signals recorded from segments along the upper vertical meridian the positivity reached a peak much earlier (80-90 msec) and then rapidly declined. In the lower field there was more uniform waveform shapes.

To determine the optimal number of segments that could be averaged together to produce maximal amplitudes and minimise cancellation we analysed segments down the vertical meridian adding successively more segments until the whole sector was included. Figure 13 shows that using all segments gave the best amplitude, compared to using just hemifield averages. The improvement in the upper field was significant at 60.3% ($p<0.0001$), but in the lower field was only 11.6% which was not statistically significant.

Therefore when analysing single channel recordings, the variability of the waveform within each hemifield limits the usefulness of summing responses from quadrants or hemifields. If any averaging is done it is best to divide the field into radial sectors, as shown in figure 11 in the previous study. This principle applies to the vertical BOS position, but would not necessarily apply for other electrode configurations. It fits with the anatomical arrangement of dipoles in the striate visual cortex. The lower bank of the calcarine sulcus rolls over more acutely on the medial aspect of the occipital lobe, and this part of the cortex subserves the vertical meridian of the upper field.

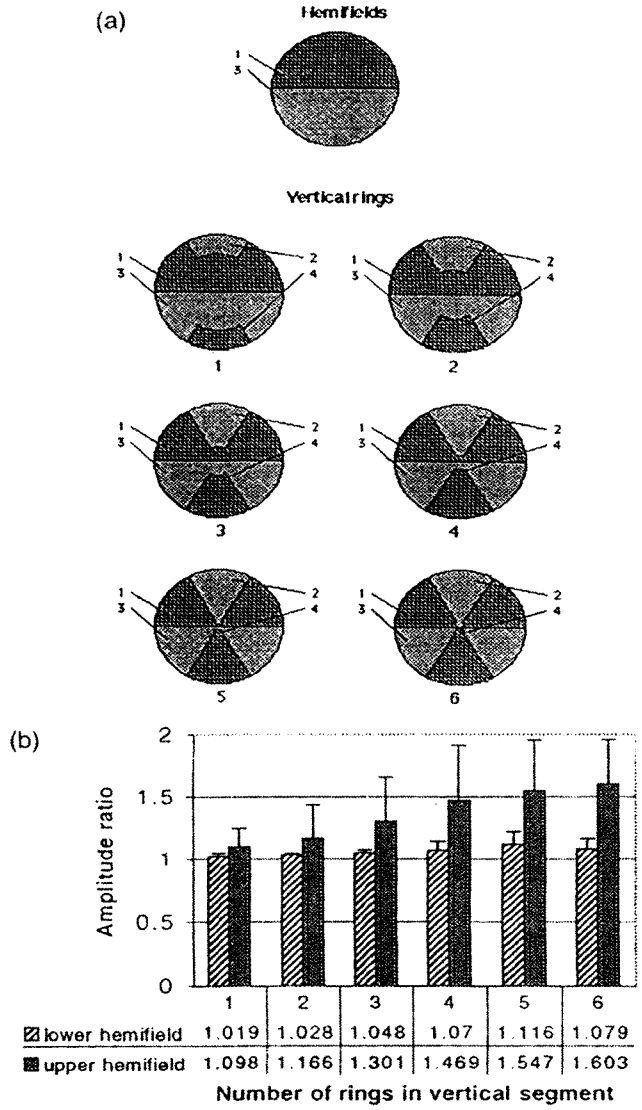


Figure 13 Comparison of hemifield amplitudes using whole hemifield summation versus summed absolute amplitudes of individual sectors within the same hemifield. (a) The size of the vertical sector sampled was gradually increased and the positive (absolute) value of the peak to trough wave for the two sectors was added. The ratio between the sum of the two sectors within each hemifield and the summed signal of the corresponding whole hemifield was calculated. (b) Results for 12 normals. The greatest increase in the total upper field response occurred when all six pairs of segments along the vertical meridian are grouped. This increased the the mean amplitude by 60.3%. a smaller increase for the lower hemifield was seen (11.6%). Grouping waveforms of similar polarity within the hemifield therefore reduces cancellation effects and gives a greater overall amplitude.

Chapter 3

Application of a bipolar single channel mVEP to glaucoma detection

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Summary of paper

In the preliminary studies described in Chapter 2 the multifocal pattern visual evoked potential (mVEP) was adapted to simultaneously record local VEP responses from multiple areas of the visual field. We showed that it potentially could detect visual field loss in glaucoma and other disorders affecting the visual pathway. In this study we applied the technique to a larger group of glaucoma patients. We sought to determine the extent to which the bipolar single channel mVEP amplitudes correlated with subjective perimetric thresholds.

MVEPs were recorded using the VERIS system. Bipolar occipital straddle electrode positions were employed and the visual field up to 26° of eccentricity was investigated. 42 glaucoma patients with reproducible visual field defects were tested. The bipolar mVEP corresponded well with Humphrey visual field defects, showing loss of signal in the scotoma area. For Humphrey quadrant threshold totals and mVEP quadrant amplitudes, the correlation coefficient was strong ($r=0.49$, $p<0.0001$). The mVEP demonstrated good correspondence with the topography of the visual field. This study represents the first practical application of the multifocal pattern VEP to objective detection of visual field defects in glaucoma.

Introduction

The conventional technique for recording the visual evoked potential (VEP) can be modified to record a multifocal topographic pattern VEP (mVEP) using the VERIS - Scientific™ system (Electro-Diagnostic Imaging, Inc., San Francisco). Employing a cortically scaled pattern stimuli, we have been able to record VEP responses from as far as 26° eccentricity using a bipolar electrode, placed 2cm above and 2 cm below the inion. This recording position was termed bipolar occipital straddle (BOS) placement [165, 181]. Several case examples in our preliminary papers have shown the relative amplitudes from each stimulus area of the mVEP to reflect corresponding areas of visual field loss in patients with glaucoma and neurological deficits. These changes have not been assessed in any quantifiable way or compared directly to subjective thresholds. Therefore since the mVEP appeared to have potential for the investigation of glaucoma, we examined a group of glaucoma patients to compare topographic signals with corresponding Humphrey perimetric thresholds.

Methods

MVEP recording was performed according to the General Methods section outlined in Chapter 2. Monocular single channel bipolar mVEPs were recorded using our BOS position for the electrodes. The study included 42 patients. Of these 36 had glaucoma and 6 were glaucoma suspects. Within the glaucoma group, 21 patients had primary open angle glaucoma (POAG) and 15 had normal tension glaucoma (NTG). Data from both eyes was included in the analysis with each eye classified separately according to severity as described below.

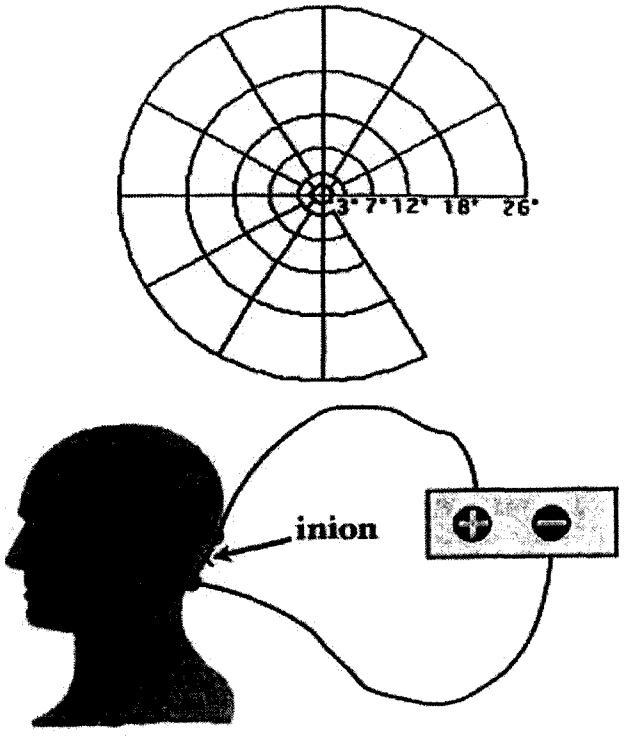

Figure 1a Eccentricity of stimulus segments within the visual field. Fig 1b BOS electrode configuration

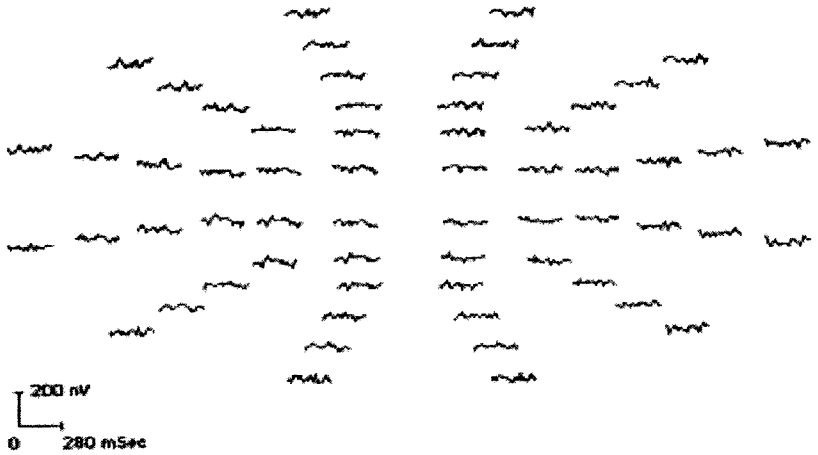

Figure 1b Trace array of first order kernel shows flat responses indicating that pattern reversal stimulus is not producing a luminance change response

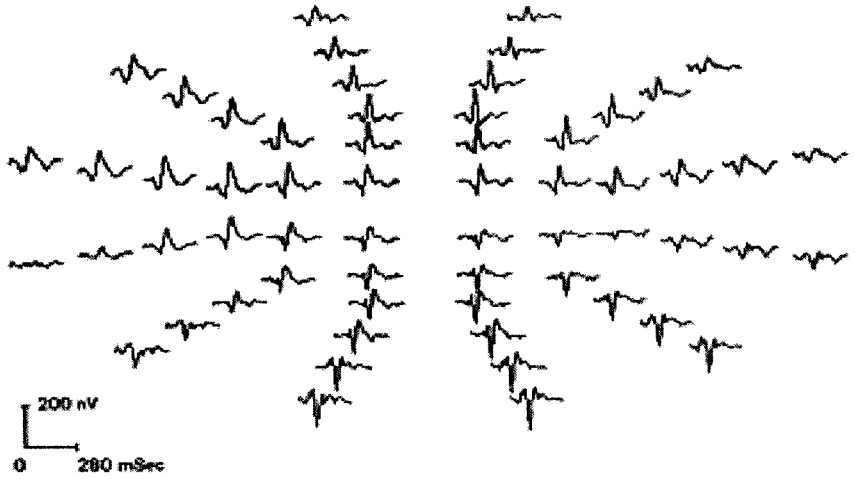

Figure 1c Trace array of second order kernel (first slice)

The inclusion criteria required a corrected visual acuity ≥ 6/12 and pupils ≥ 2mm without dilation. Patients with diabetes, previous cataract surgery and other ocular disorders were excluded. The glaucoma patients had previously performed Humphrey 24-2 fields on multiple (≥3) occasions, and had reproducible field defects. The diagnosis of POAG required the following three parameters: a confirmed visual field defect on Humphrey 24-2, a glaucomatous optic disc as judged by stereo disc photography, an intraocular pressure > 21 mmHg with the applanation tonometer. NTG patients had field and disc changes but had never had an IOP > 20mm Hg. The definition of a field defect used the pattern deviation plot on the Humphrey 24-2 programme. A minimum scotoma required at least 3 adjacent points depressed by at least 5 dB on the pattern deviation, with one of the points depressed at least 10 dB, or two adjacent points depressed by 10 dB. The cluster of abnormal points that included the 10 dB nucleus could not cross the horizontal meridian and points immediately above and below the blind spot could not qualify as part of the scotoma.

Analysis

For the purpose of analysis, the Humphrey threshold quadrant totals were compared with the corresponding summed mVEP amplitude and the mean latency for the same quadrant. The two nasal rim points on the Humphrey 24-2 field were excluded since these were outside the test area of the mVEP. Correlations were examined for each quadrant and hemifield using Statview 4.0 (Abacus Concepts Inc.,1992). Although our preliminary studies had suggested sectoral analysis was better than using quadrants for the mVEP, it was not feasible to divide the Humphrey into corresponding sectors for comparison. Therefore we summed individual amplitudes for each point in the quadrant rather than averaging them together which would have caused cancellation effects.

Results

The first order kernel of the mVEP recordings (example in figure 1C) was flat, which confirmed that there is no luminance change between alternating pattern frames of the screen. The first slice of the second order kernel contained a prominant and reproducible waveform and represents the interaction between the responses to two consecutive frames of the monitor. It is considered to be analogous to the conventional pattern VEP (26) and is the waveform analysed in this paper.

The mVEP trace arrays recorded showed a good correlation with visual field defects seen in glaucoma patients. The case examples shown in figures 2 -6 show the field loss to be reflected in the mVEP trace array. The corresponding area of the mVEP shows flat signals in the distribution of the field defect.

In all the 36 cases with a confirmed scotoma, a subjective assessment of the trace array identified areas where the mVEP signal approached zero, and these corresponded to the scotoma area. In some cases with arcuate defects, we have analysed the signal derived from Bjerrum-like superior and inferior arcuate regions. This mirrors the scotoma more precisely than quadrantic analysis.

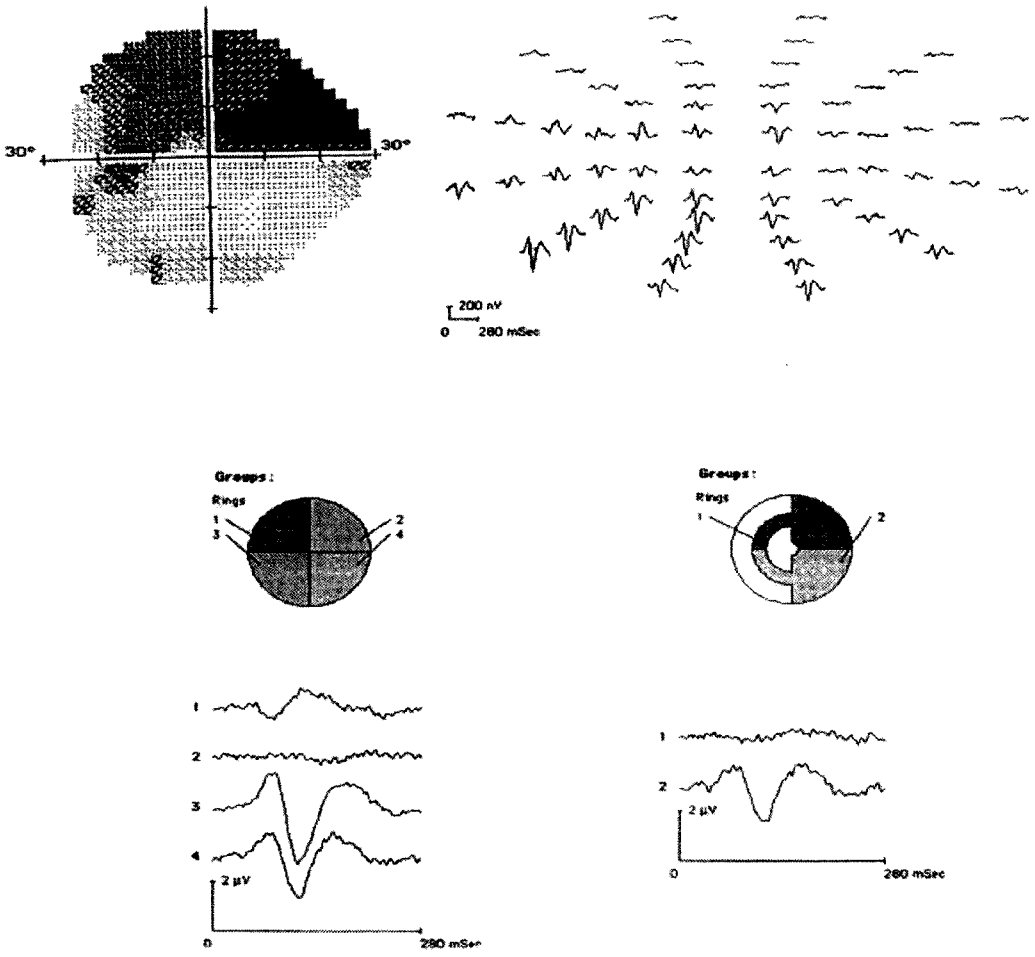

Figure 2 - Example of correlation between multifocal pattern VEP trace array and Humphrey visual field. The superior hemifield defect is demonstrated on the pattern VEP with loss of signal in most of the superior hemifield. Analysis of quadrants shows minimal response from the upper nasal quadrant, and reduced upper temporal. Analysis of arcuate regions shows no response superiorly. Quadrant and Bjerrum zone traces represent sums of all signals from segments within the area.

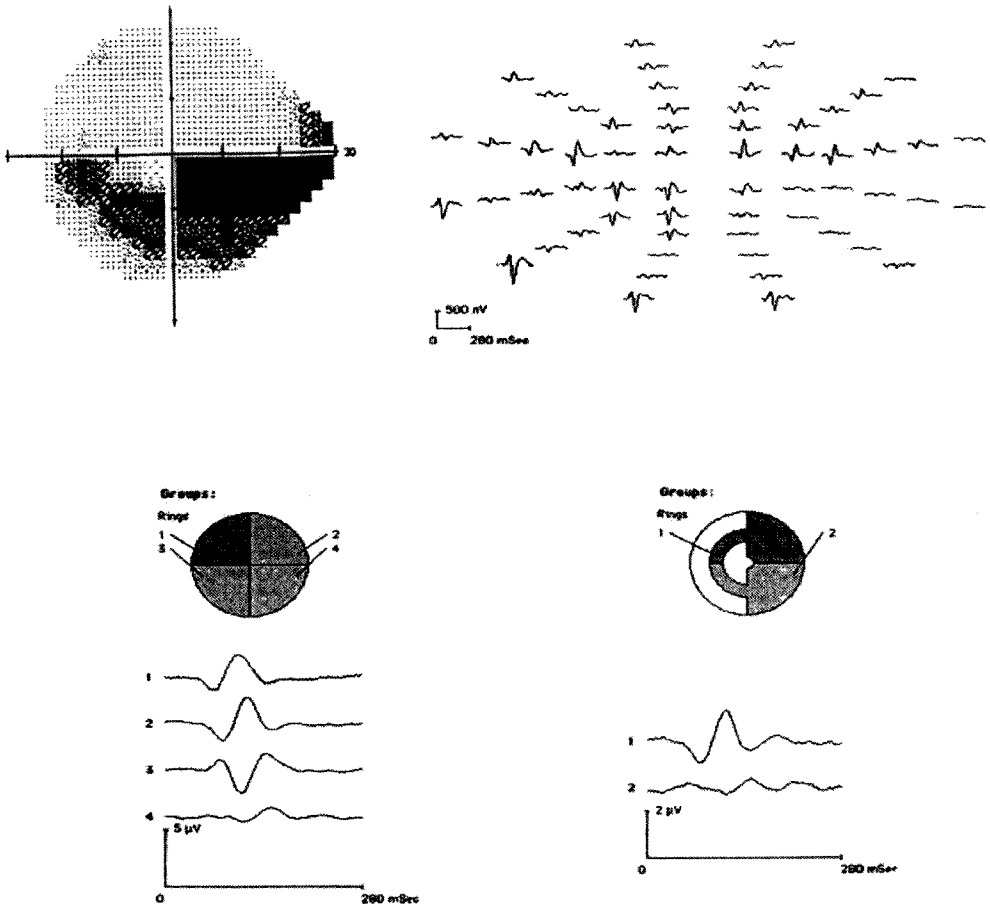

Figure 3 - Inferior arcuate defect demonstrating loss of signal in most of the inferior hemifield, but with preservation of signal below the Bjerrum area. There is also some early superior loss of signal across the arcuate region and the superonasal step is confirmed. Analysis of segments shows no response from inferonasal quadrant and inferior arcuate areas. Quadrant and Bjerrum zone traces represent sums of all signals from segments within the area.

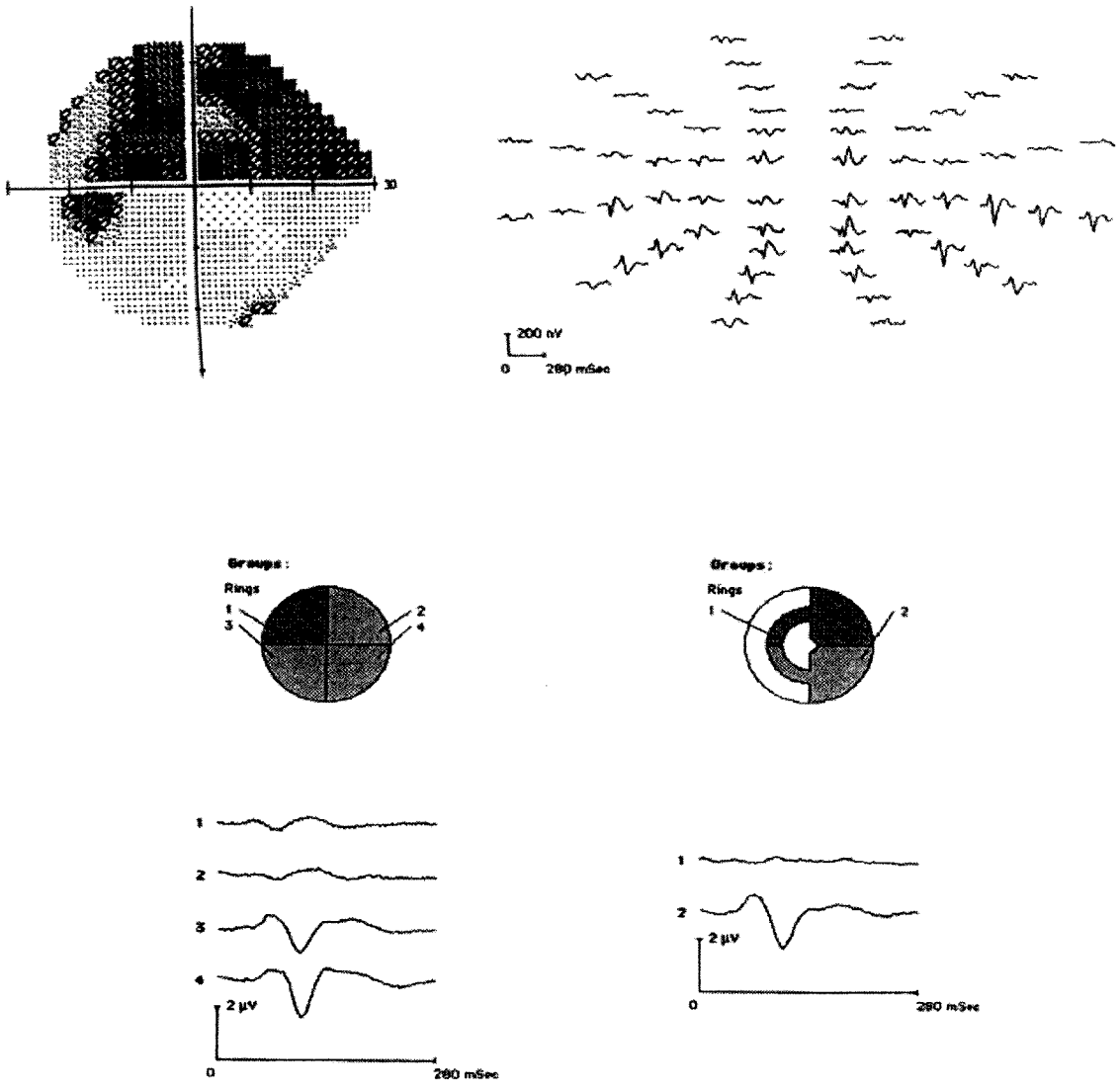

Figure 4 - Superior arcuate defect with pattern VEP suggesting all superior points except the two central points are involved. The corresponding perimetric field showed some areas in the superior field to still have normal thresholds. Quadrant and Bjerrum zone traces represent sums of all signals from segments within the area.

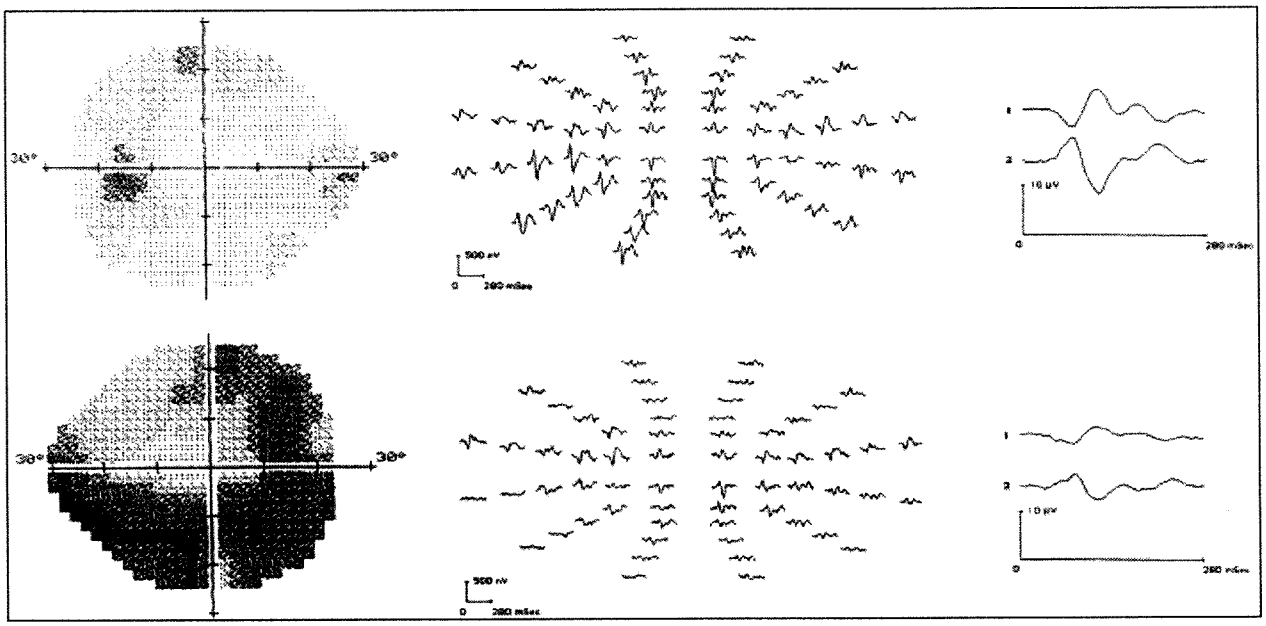

Figure 5 - Pattern VEP recordings from both eyes of a patient with asymmetric disease. The left eye is still relatively normal. The right VEP trace confirms both upper and lower arcuate losses. 1= upper hemifield, 2= lower hemifield response. Hemifield traces represent sums of all signals within the area.

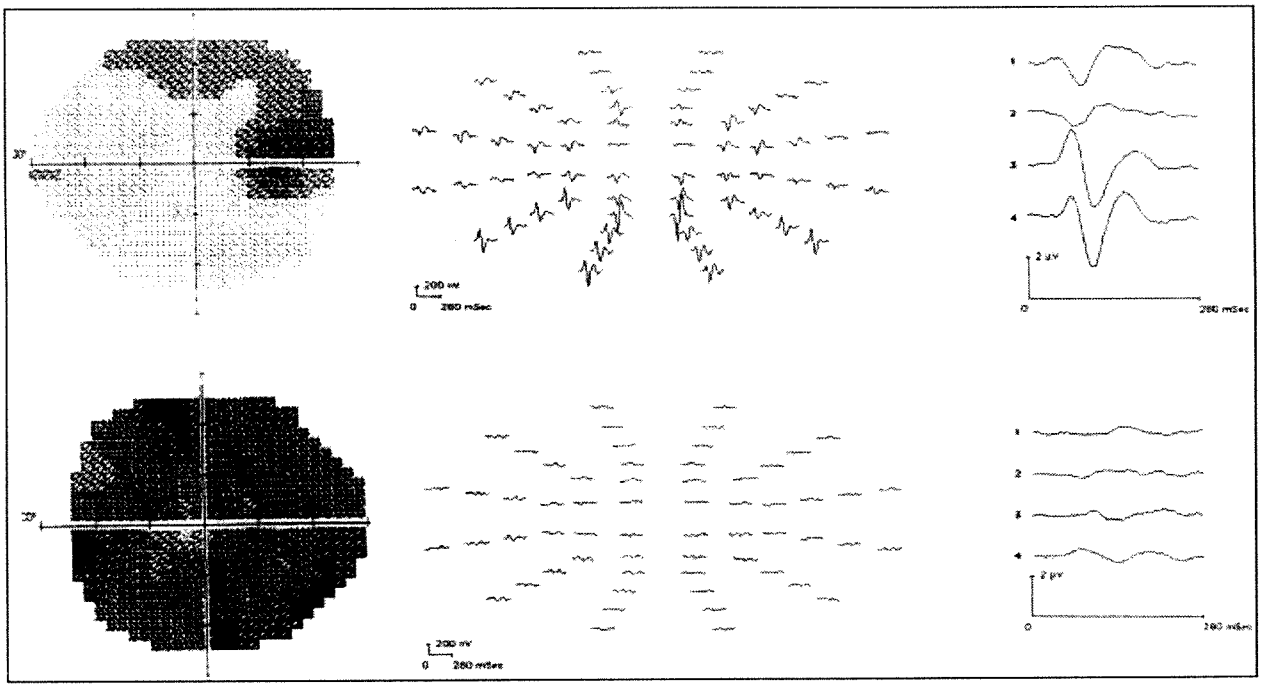

Figure 6 - Pattern VEP recordings from both eyes of a patient with early losses in the right eye, and advanced glaucoma in the left. Visual acuity was still 6/6 in both eyes. The right VEP suggests early losses superiorly, particularly in the superotemporal quadrant. (Quadrant traces numbered as for figures 2-4)

Figure 7 below shows the correlation of all Humphrey quadrant scores for perimetric thresholds with pattern VEP amplitudes and latencies. Amplitude correlation was r=0.49, p<0.0001. Latency of N75 and P100 waves however did not correlate as well with perimetric thresholds for the same quadrant (r= -0.18 and -0.3 respectively). P100 showed the more consistent delays with decreasing perimetric thresholds.

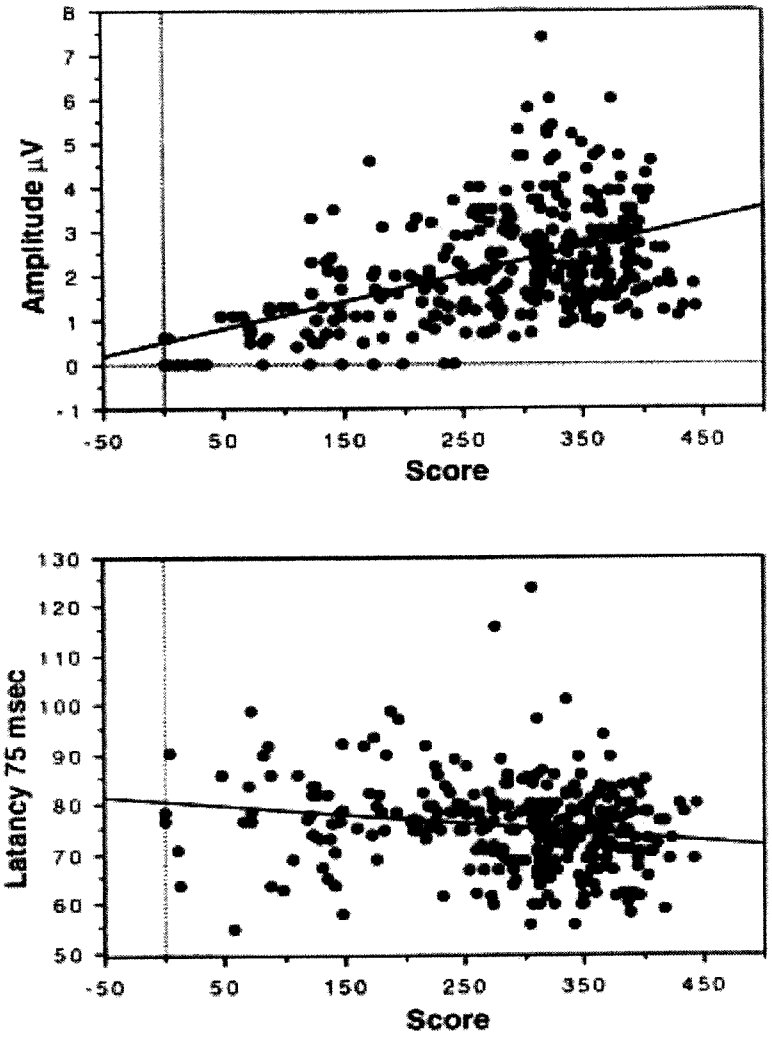

Figure 7a and 7b – see over page for legend

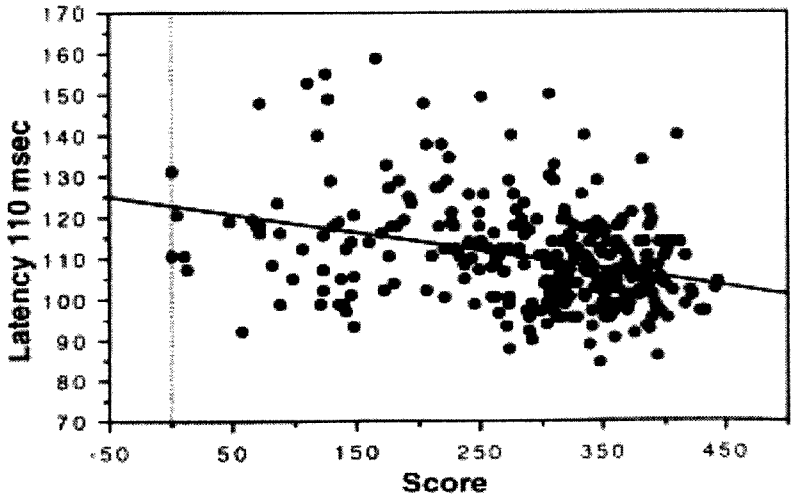

Figure 7a –c Correlations between all Humphrey threshold quadrant total dB scores and pattern VEP amplitude and latency for the same quadrant. Amplitude is the trough to peak measurement, while latency is determined for the waves at around 75msec and 110 msec. Amplitude correlation is strong $r=0.49$, $p<0.0001$. Latencies showed weaker correlation at $r= -0.18$ and $r= -0.3$ respectively

Individual quadrant correlations for amplitudes are shown in Fig 8a-d. The mVEP amplitudes correlated well in all quadrants, with the best correlation in the superonasal quadrant ($r=0.61$). The other quadrants were as follows: superotemporal 0.43, inferotemporal 0.42, and inferonasal 0.44.

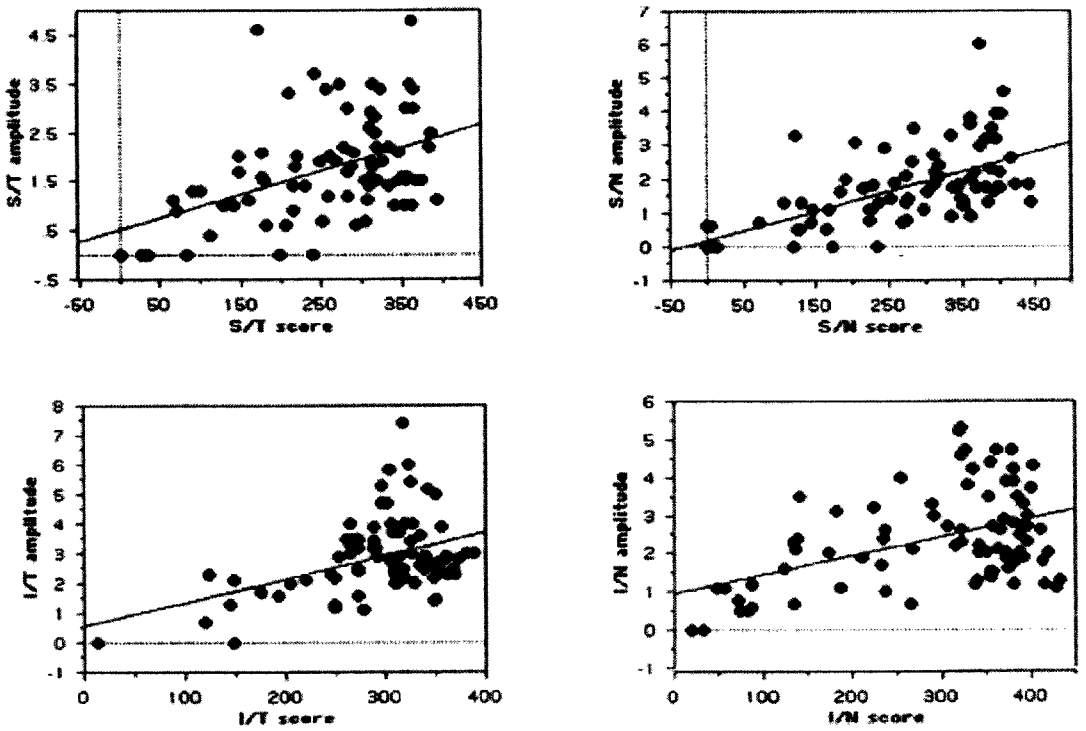

Figure 8 - Correlations between Humphrey threshold quadrant totals and pattern VEP amplitude for individual quadrants. The best correlation was seen in the superonasal (S/N) quadrant r=0.61. The other quadrants were as follows: superotemporal (S/T) r=0.43, inferotemporal (I/T) r=0.42, and inferonasal (I/N) r=0.44.

Discussion

The mVEP amplitudes seem to correlate well with visual field defects seen in glaucoma patients. In this study, assessment of the trace arrays showed good correspondence between areas where no mVEP response was elicited and the location of dense scotomas in the visual field. When amplitudes were compared with the corresponding perimetric quadrant thresholds, there appeared to be a good correlation present. Latency delays however did not show a strong relationship with field loss, being only weakly correlated.

A method to quantitatively assess the degree of amplitude depression for each test location needs to be determined. Since variable amplitudes are achieved in different areas due to the convolution of the underlying visual cortex, and waveforms vary with location, the use of quadrant averages is not ideal. Using Bjerrum-like sectors may improve the performance. Although apparent relative reductions could be seen, it is not yet possible to try and establish probability plots for each location, since we have yet to accurately define the normal variation seen in the general population.

There is a marked decrease in amplitude along the horizontal meridian seen in some bipolar recordings. This may be due to one of two reasons: alteration of the dipole

orientation or cancellation of upper and lower field components, or a combination of both. It is generally agreed that the horizontal meridian of the peripheral visual field is represented with slight individual variability [183]deep within the calcarine banks at the fissure base [101, 187]. This may lead to such an alteration of dipole orientation that it may become much more perpendicular to the linking line between bipolar electrodes, minimising the recorded signal. Data presented in this study is not sufficient to solve this question, and experiments employing more electrode channels and positions are needed.

We observed that in several of the high risk suspects who had abnormal optic discs but still normal visual fields, that there were amplitude reductions in parts of their mVEP. In particular, several cases were seen of asymmetric disease, where there were clear differences between the VEP responses of the two eyes in parts of the visual field that remained with normal thresholds. This may imply that mVEP changes preceed the development of field defects. These patients will need to be followed longitudinally to establish if they subsequently show signs of visual field loss, and if the mVEP changes are consistent. If so, then this form of objective perimetry could prove to be an early marker for glaucomatous damage. The potential for between eye asymmetry analysis was recognised and a specific study was set up to look at this as a means of detecting early change (see Chapter 4).

The mVEP appears to have potential for further investigation in glaucoma. The study demonstrated its ability to detect glaucomatous field loss and there was good correlation with both topography and and depth of field loss. The technique in this simple form provides a major advance in assessing the visual system, and is a significant step towards our goal of objective mapping of the visual field.

Chapter 4

mVEP inter-eye asymmetry analysis to identify early deficits

Published as

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Summary

Purpose The multifocal visual evoked potential (VEP) shows markedly symmetrical responses between the two eyes of normal subjects. Patients with glaucoma and high risk suspects were examined to determine if VEP asymmetry could be identified in these cases and used for diagnosis and detection of early damage.

Methods Multifocal pattern VEP recordings were performed using a single channel bipolar occipital electrode position and the VERIS system. There were 125 subjects including 24 normals, 70 glaucomas and 31 high risk suspects. A between-eye relative asymmetry coefficient (RAC) was determined for each of the sixty test points in the VEP field. Glaucomas and suspects were compared with normal values. Correlation between Humphrey thresholds and RAC scores was performed.

Results Both the suspects and glaucomas showed significantly larger mean quadrant RAC values. When point by point analysis was performed, 69/70 scotomas were identified with a cluster of at least 3 points of $p<0.05$ probability. In the suspects, 10/31 cases had abnormal areas in the VEP field.

There was a strong correlation ($r=0.82$) between quadrantic RAC mean values and Humphrey quadrant threshold scores in an asymmetric glaucoma subgroup. Abnormal VEP responses were identified in parts of the visual field that were still normal on perimetry.

Conclusions Asymmetry analysis correctly identifies patients with glaucomatous field loss and shows abnormalities in many high risk suspects with still normal fields. It is able to objectively identify the extent of glaucomatous damage and may be able to detect changes before subjective field loss occurs.

Introduction.

The application of the multifocal visual evoked potential (VEP) to objectively test the visual field has been confirmed as a potential test for glaucoma [165, 166, 169, 181, 188, 189]. It has been shown that by using appropriately placed electrodes [165, 189], it was possible to obtain an objective representation of the visual field up to 26° of eccentricity.

Analysis of the multi-focal VEP in normal subjects however, revealed that although visual evoked responses are present at every stimulated location of the visual field, the amplitude and waveform of the individual signals varies. Such "within eye" asymmetry of the signal makes the interpretation of a small amplitude reduction more difficult. With early pathological stages of the disease, the amplitude of the evoked cortical signal might only be reduced, not totally extinguished. Several possible factors, such as retinal eccentricity, size of the pattern element, cortical magnification and inter individual variability of infolding of the cortical sulci could account for spatial asymmetry of the locally stimulated VEP [113, 171, 190]. In addition, the large variability in VEP amplitudes seen between subjects also renders detection of mild reductions in amplitude less sensitive, with greater reductions required to reach statistical significance.

Detailed comparison of VEP trace arrays recorded from both eyes of normal subjects, revealed remarkable similarities in the waveform and amplitude of the individual signals between the two eyes of the same individual. This suggested that the major part of the "within eye" asymmetry may be due to underlying cortical convolution. Even though different parts of the retina are being stimulated in the two eyes, the information from similar parts of the visual field of both eyes projects to identical areas of the visual cortex [177, 191]and activates the same cortical cells producing the same electrical dipoles. Therefore it might be possible to compare eyes within individuals to look for localised areas of asymmetry in cases of early unilateral disease.

The aim of this study was to establish the "between eye" asymmetry in normal subjects and examine asymmetry as a potential clinical technique in glaucoma suspects and early glaucoma. A potential benefit of the technique is that it may be able to detect damage in areas of the visual field that are still normal on psychophysical testing.

Method

Stimulation and recording.

Full details of the single channel multi-focal recording technique are described in the General Methods in Chapter 2. The VERIS Scientific™ system (Electro-Diagnostic Imaging, Inc., San Francisco, CA) was employed for all recording, with a pattern dartboard stimulus.

Single channel recording was employed in this study. Two gold disc Grass electrodes (Astro-Med Inc, Rhode Island, USA) were placed on the occipital scalp along the vertical midline 2 cm above and 6 cm below the inion. This position which is a modification of our previously described bipolar occipital straddle (BOS) [165, 189]is termed extended BOS [192]. The lower midline electrode was negative. Electrodes were mounted on a custom-built electrode holder.

Subjects

The study protocol was approved by our regional ethics committee and informed consent was obtained from all patients. The inclusion criteria for the normal, suspect and glaucoma groups required a corrected visual acuity of 6/12 or better and pupils at least 2.5mm without dilation. Subjects with diabetes, previous cataract surgery or any other ocular disorders were excluded. All patients were assessed for asymmetry in their VEP results to determine the diagnostic value of the test on its own.

The study included 24 normal subjects (12 females and 12 males). Mean age was 55.6±14.2 years (range 16 - 79 years). Both eyes were tested sequentially with random choice of right or left being tested first. The normals had normal intraocular pressure and ophthalmoscopy, and no family history of glaucoma or retinal dystrophy. All performed normal Humphrey 24-2 field tests, confirmed by a normal result on the glaucoma hemifield test analysis. Three of these normals were tested on four separate days at least one month apart to demonstrate reproducibility of the RAC values.

One hundred and one patients were recruited from our clinic over a twelve month period and tested with the multifocal VEP. These included 70 glaucomas and 31 high risk glaucoma suspects. Primary open angle glaucoma and normal tension glaucoma were both included, with no attempt made to separate their results. All patients had previously performed Humphrey 24-2 fields on two or more occasions, and all glaucoma patients had reproducible field defects. The diagnosis of glaucoma required a confirmed visual field defect on Humphrey 24-2 and a glaucomatous optic disc as judged by stereo disc photography, with or without an intraocular pressure > 21 mmHg on the applanation tonometer. The definition of a field defect used the pattern deviation plot on the Humphrey 24-2 programme. A minimum scotoma required at least 3 adjacent points depressed by $p<0.5\%$ on the pattern deviation probability plot. The cluster of abnormal points could not cross the horizontal meridian and points immediately above and below the blind spot could not qualify as part of the scotoma. The glaucoma hemifield test was abnormal.

The glaucoma group had a mean age of 60.0±14.6 years (range 15-89) which was not significantly older than the normals ($p<0.1$, t-test). There were 34 males and 36 females. Their Humphrey mean deviation (MD) in both eyes ranged from 0.06 to -25.7, mean -7.14±6.2.

There were 31 high risk glaucoma suspects (18 males and 13 females). They had a mean age of 57.8±10.8 years (range 34-81) which was not significantly different

to the normals ($p>0.1$, t-test). They included 26 patients who had been noted to have either a definite structural change or an asymmetry in the neuroretinal rim, but who had not developed visual field defects, representing "preperimetric glaucoma". The remaining five suspects included had both longstanding ocular hypertension and a family history of glaucoma. The Humphrey glaucoma hemifield test was normal in all suspects. Since these suspects were considered to be at high risk for early glaucomatous damage, they were analysed separately for VEP asymmetry to establish its ability to identify early electrophysiological changes.

Analysis

Raw trace data was analysed using custom designed software. Peak-to-trough amplitudes for each wave within the interval of 50-165 msec were determined. The inter-eye asymmetry was then calculated for every segment of the tested visual field by dividing the difference in amplitude between left and right eyes by their sum:

Response asymmetry coefficient (RAC) = (Amp1-Amp2)/(Amp1+Amp2)

where Amp1 is a maximal peak to trough amplitude of the response at particular segment from the left eye and Amp2 is a maximal peak to trough amplitude of the response of the same segment from the right eye. Using the ratio instead of simple difference helps to avoid dependence on the absolute amplitude of the signal.

The reason for not analysing latency of the VEP was that our previous study of the multifocalVEP in glaucoma had shown only a poor correlation between latency delay and degree of visual field loss in glaucoma. In contrast, amplitude was more strongly correlated with Humphrey visual field threshold scores [192].

For assessment of variability of RAC values, three normal subjects were tested on four separate occasions and RAC standard deviation values were calculated for each subject (a coefficient of variation could not be calculated on the RAC which has both positive and negative numbers).

The high risk suspects' and glaucoma patients' RAC values were compared with the RAC values for the same point in the normal group. Points with statistically significant difference (ie $p<0.05$ and $p<0.01$) from the mean RAC value for that point in the normal database were flagged as abnormal on a probability plot. A cluster of three adjacent points of probability $p<0.05$ was considered as representing a possible scotoma.

A comparison between the normals and high risk suspects was performed in terms of RAC values, to establish if greater asymmetry values occurred in the suspect group. The mean of all 15 points within each quadrant was calculated for normals and suspects, and compared for each quadrant of the field.

From the 70 glaucoma cases, a subgroup of patients with established field defects in one eye only were identified. There were 28 cases of clearly asymmetric glaucoma.

Their mean deviation (MD) in the affected eye on Humphrey fields ranged from -0.79 to -25.7dB, mean -10.4±6.5dB. The Humphrey quadrant thresholds from this group were correlated with the asymmetry values from the same quadrant, to show that the degree of VEP asymmetry reflected the level of perimetric loss.

To demonstrate that VEP abnormalities may precede visual field changes, all congruous quadrants which were still normal in both eyes on the Humphrey were identified in the 70 glaucoma patients. Only quadrants where there were no points on pattern deviation or total deviation with $p<1\%$ in either eye were included. A total of 34 quadrants from 26 patients fulfilled this criterion. VEP asymmetry data from the corresponding visual field quadrants that were still perimetrically normal in both eyes was examined in comparison to the quadrant asymmetry of the normal subjects. This was done to determine if VEP asymmetry could be identified in normal areas of the visual field of glaucomatous eyes.

All statistical analysis was done using Statistica 4.1 (StatSoft, 1994, Tulsa, OK).

Results

The 24 normal subjects tested showed little between-eye asymmetry. To demonstrate the similarity of the response between the two eyes an example of the trace arrays from both eyes is presented in Figure 1 with the traces superimposed. While there is some variation in amplitude of the signal throughout the visual field, the VEP traces from both eyes are almost identical for each field location.

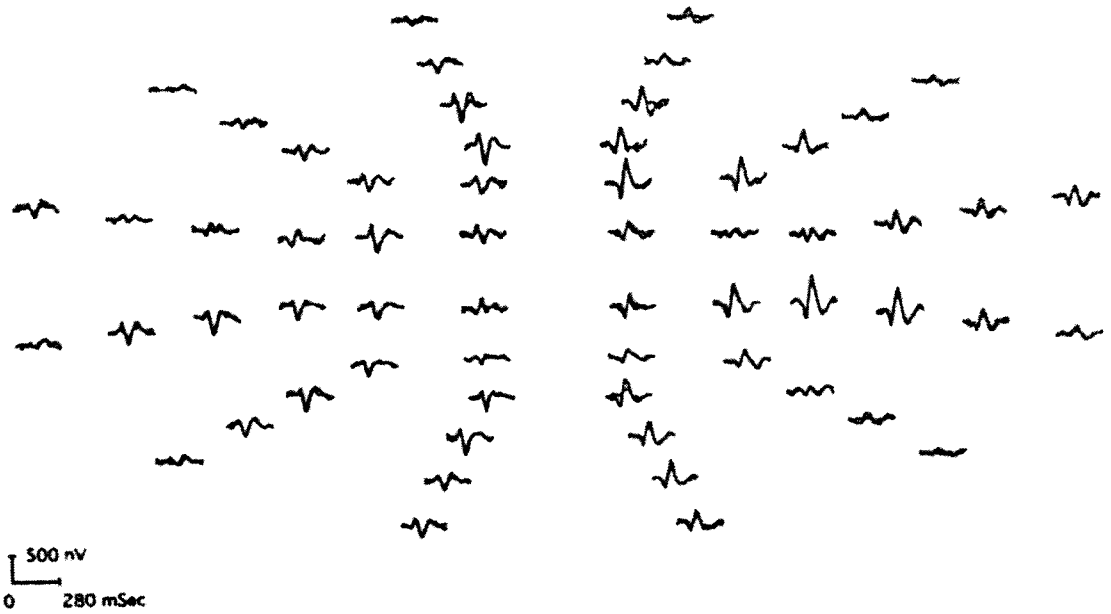

Figure 1 Example of multifocal VEP trace arrays from both eyes of a normal subject with the traces superimposed. While there is some variation in amplitude of the signal throughout the visual field, the VEP traces from both eyes are almost identical for each field location.

Figure 2 represents the mean values and standard deviations of the RAC from all 60 locations of the visual field from the 24 normal subjects. Because the VEP amplitude of the right eye was subtracted from the VEP amplitude of the left eye the positive value of the RAC indicates that the left eye produces bigger amplitude from the stimulation of the particular location of the visual field. A negative RAC value, on the other hand, points out on dominance of the right eye. The RAC tends to be clustered around zero and rarely exceeds value of 0.2 on either side, which indicates strong "between eye" symmetry within each subject. The greatest asymmetry was in segment 42 which includes the projection of the blind spot of the left eye and therefore exhibits a correspondingly negative RAC. Segment 37 includes the right eye blind spot.

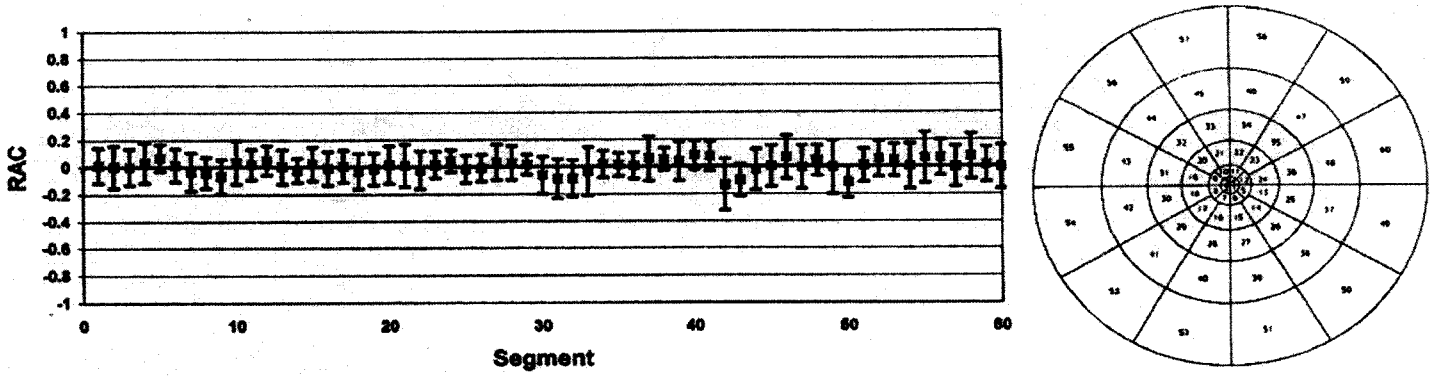

Figure 2 Mean RAC values and standard deviations from all 60 locations of the visual field from the 24 normal subjects. Insert shows location within the field for each of the 60 segments.

There was no dependance of mean RAC (and its variability) on the age of tested subjects, with no statistical correlation identified with age (r=0.1). Asymmetry did not increase in the more peripheral points of the field, with a poor correlation (r= -0.22) showing only a slight trend for greater asymmetry in the more central points.

The RAC values showed little variability between tests in the three normals who were tested four times. Standard deviation values of RAC for three subjects are presented in Fig. 3. For most test points the RAC variation was <0.05. Mean standard deviation of RAC for first subject was 0.034±0.01, for second subject 0.043±0.02 and for third 0.049±0.013.

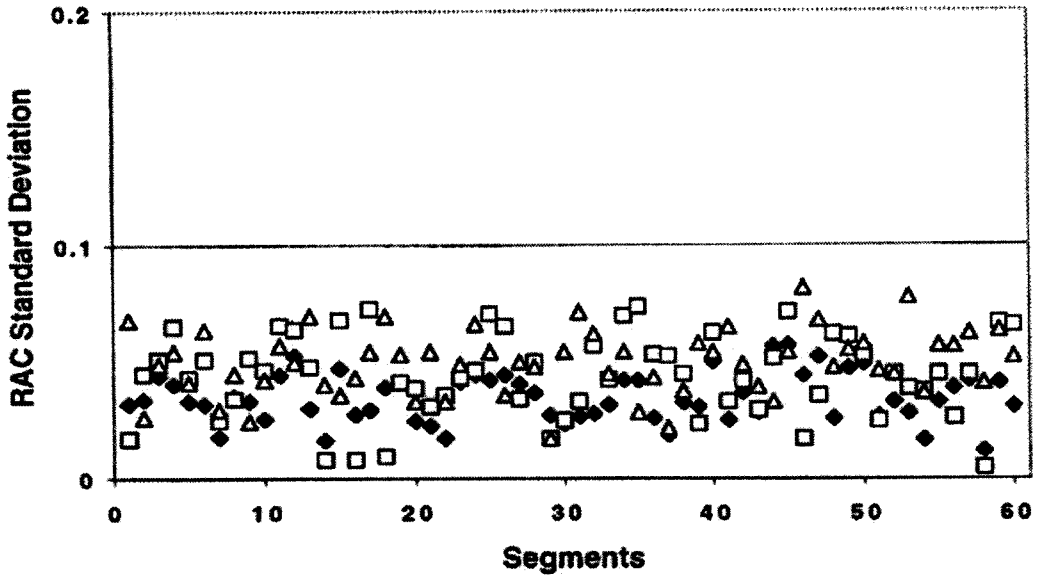

Figure 3 Standard deviation values of RAC for three subjects tested on four different occasions. Each point of the visual field is shown and the three different symbols represent the results for the three normal subjects. (□= subject 1, □□subject 2 and □= subject 3). Variability for individual points is low between tests.

Using the asymmetry values of the normals, the glaucoma and suspects' fields were analysed. Points with a statistically significant difference (ie $p<0.05$ and $p<0.01$) from the mean RAC value for that point in the normal database were flagged as abnormal on a probability plot. A cluster of three adjacent points with $p<0.05$ was considered as representing a possible scotoma. Among the 70 glaucomas, 69 cases had scotomas identified by this criterion. The asymmetry was able to identify abnormalities even though in many cases the scotomas were in similar positions in the two eyes eg bilateral superior arcuate defects. The only case that was not identified had paracentral defects in corresponding locations in the two eyes. In this case a comparison with normal amplitudes identified the localised scotomas.

Figure 4 shows an example of a glaucoma subject. The trace arrays are shaded when the amplitude was less than 120nV (no signal identified from noise levels). The third column shows an asymmetry gray scale for the magnitude of the RAC value for each point (range from 0-1). The fourth column represents a probability of abnormality compared to the RAC values for normals. In this case the superior scotoma is readily identified in the right eye.

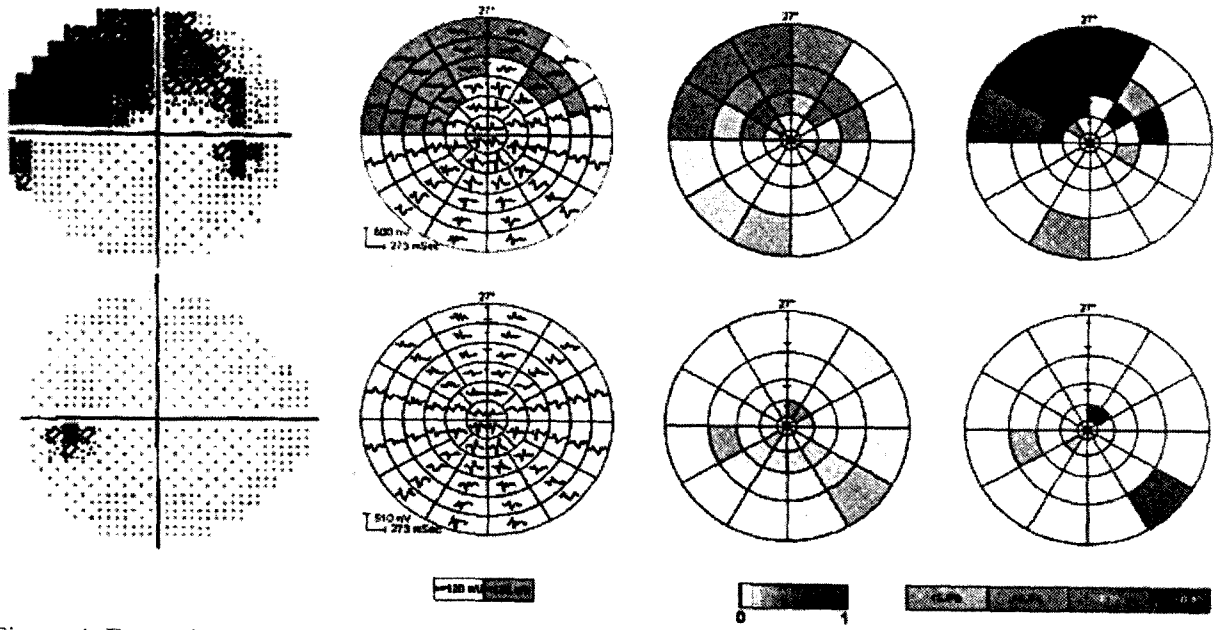

Figure 4 Example of asymmetric glaucoma. First column - Humphrey gray scale. Second column - VEP trace arrays with points shaded if amplitude <120nV ie; no signal identified from noise levels. The central segments are enlarged for graphics purposes to enable their traces to be shown, but in area they correspond directly to the segments shown in fig 2 insert. Third column - intereye asymmetry gray scale for the magnitude of the RAC value for each point. Fourth column - probability of abnormality compared to the RAC values for normals. The superior scotoma is readily identified in the right eye on the various plots.

In the high risk suspects with normal fields 10/31 cases showed a scotoma on the VEP asymmetry analysis. In contrast, when each of the normals' data was assessed using the remaining normals as the data base, only one case showed a false positive result with 3 points being flagged as abnormal ($p<0.05$) on the asymmetry plot. If the strictness of the definition for a scotoma was increased to require 3 points $p<0.01$, there were still 5/31 cases that were positive in a suspect group but no normals. All these suspects will now be followed closely to determine if these VEP changes truly herald subsequent field loss. An example is shown in figure 5, where a superior scotoma is suggested in the left eye on the VEP asymmetry plot. This is recognised even though there are still recordable signals in these locations. Clinically this patient had early left disc changes but normal fields.

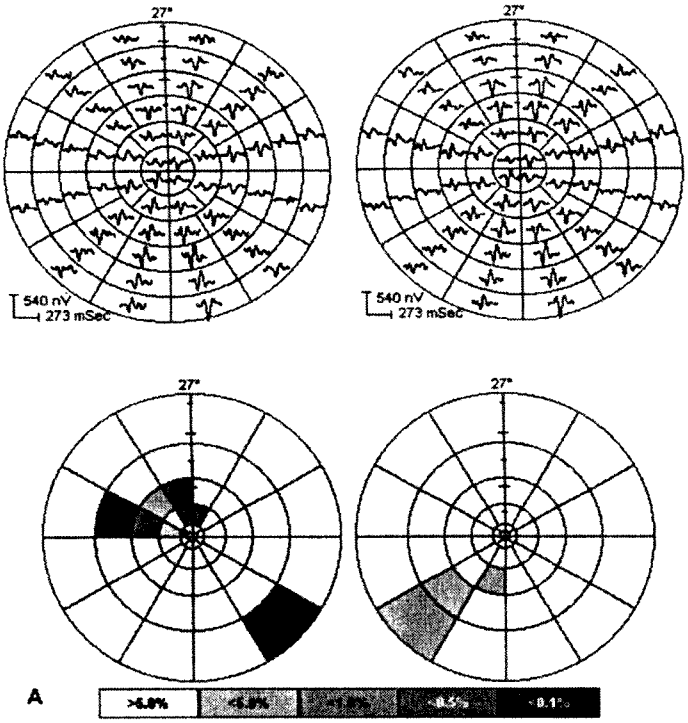

Figure 5 shows example of asymmetry plots from glaucoma suspect. A superior scotoma is suggested in the left eye on the VEP asymmetry plot. This is recognised even though there are still recordable signals in these locations.

The suspects had significantly greater mean quadrant RAC values compared to normals ($p<0.005$ for all four quadrants, t-test). Figure 6 shows the results for each quadrant of the visual field, and suggests that many of these patients are beginning to show VEP changes in at least one eye.

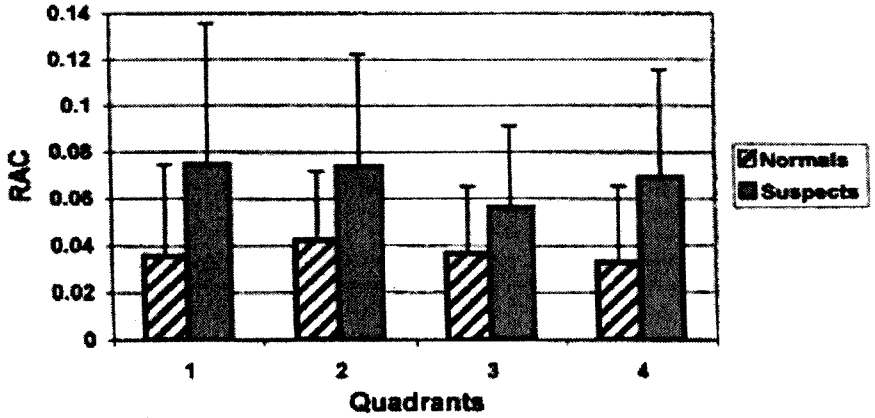

Figure 6 Mean quadrant RAC values from each quadrant of the visual field for normals and suspects. 1= lower right, 2=lower left, 3=upper left, 4=upper right. The suspects had significantly greater mean quadrant RAC values compared to normals ($p<0.005$ for all quadrants) suggesting early glaucomatous damage.

In the subgroup of 28 glaucoma cases with clearly asymmetric disease on perimetry, there was a strong correlation (r=0.82) between quadrantic RAC mean values and Humphrey quadrant threshold scores. Perimetric losses were reflected by corresponding VEP asymmetry. The values and correlation plot are shown in figure 7.

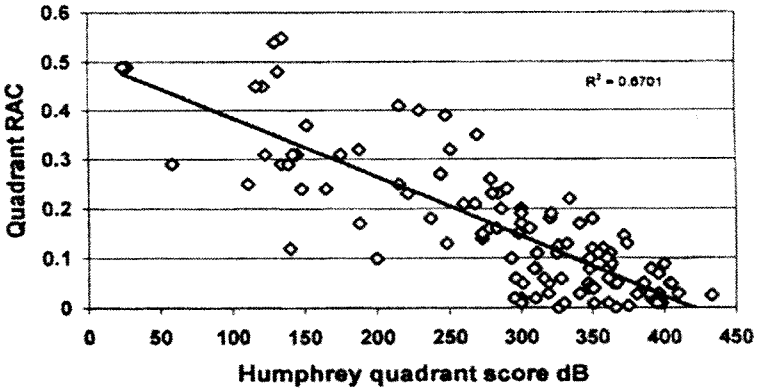

Figure 7 Correlation between quadrantic RAC mean values and Humphrey quadrant total threshold scores in the subgroup of 28 glaucoma cases with clearly asymmetric disease. Perimetric losses were reflected by corresponding VEP asymmetry (r^2=0.67).

There were detectable early VEP asymmetry changes in the perimetrically normal quadrants of the eyes with glaucoma. The RAC values for the normal quadrants of the glaucoma cases was compared with the mean quadrant RAC values for normals. The two groups were statistically different ($p<0.002$). This suggests that in established glaucoma cases, electrophysiological differences are apparent in areas of the visual field not yet manifesting perimetric changes as defined by the Humphrey software.

An example of more extensive changes on VEP asymmetry compared to perimetry is shown in figure 8. This case shows in the right eye that there are greater differences in the inferior quadrant than suggested on the Humphrey. The inferonasal step seen on the gray scale was only supported by a single point of significance $p<0.5\%$ on the pattern deviation, which was just at the edge of the area tested by the VEP stimulus (outer ring extends from 19° to 27°). The asymmetry plot suggested a larger inferior arcuate was developing.

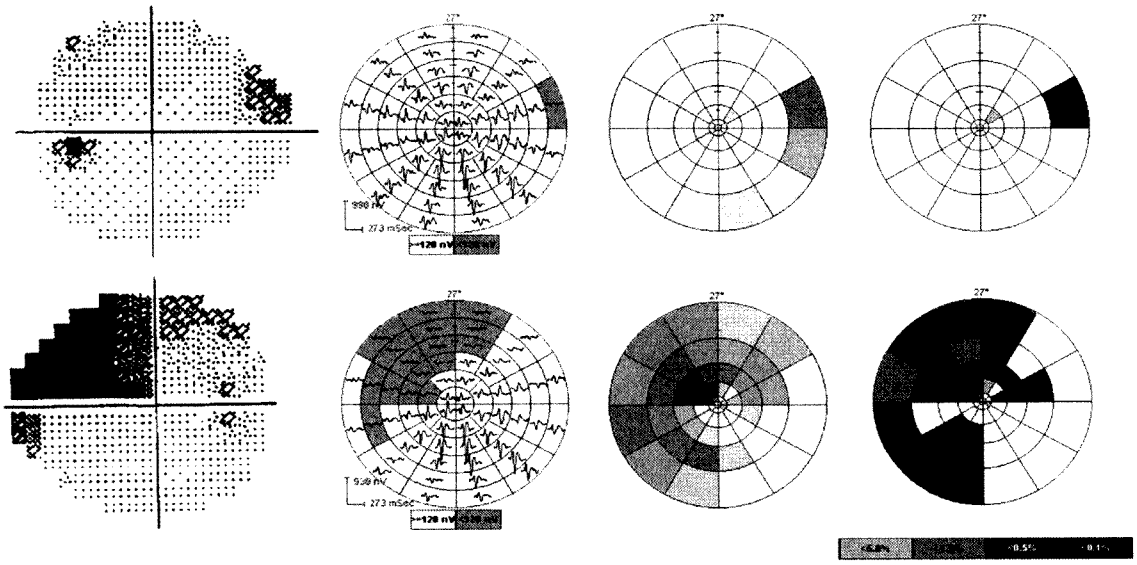

Figure 8. Glaucoma case with greater VEP changes in the inferior quadrant than suggested by the Humphrey field. The inferonasal step seen here on the gray scale was only supported by a single point of significance p<0.5% on the pattern deviation of the Humphrey (not shown) while the asymmetry plot suggested a larger inferior arcuate was developing over many points. The left eye superonasal step is seen on both.

Five glaucoma suspects and seven glaucomas have now been tested 12 months apart to look for changes on asymmetry which might suggest progression. Two of the suspects have shown changes consistent with a new scotoma developing. All glaucomas seem to have remained stable, with one of these shown in figure 9. The suggestion of progression is not based on probability, since we do not yet know the longterm fluctuation of VEP values in normals or glaucoma subjects. At this stage we are commenting only on the appearance of new clusters of asymmetric points, fitting our definition of a VEP scotoma.

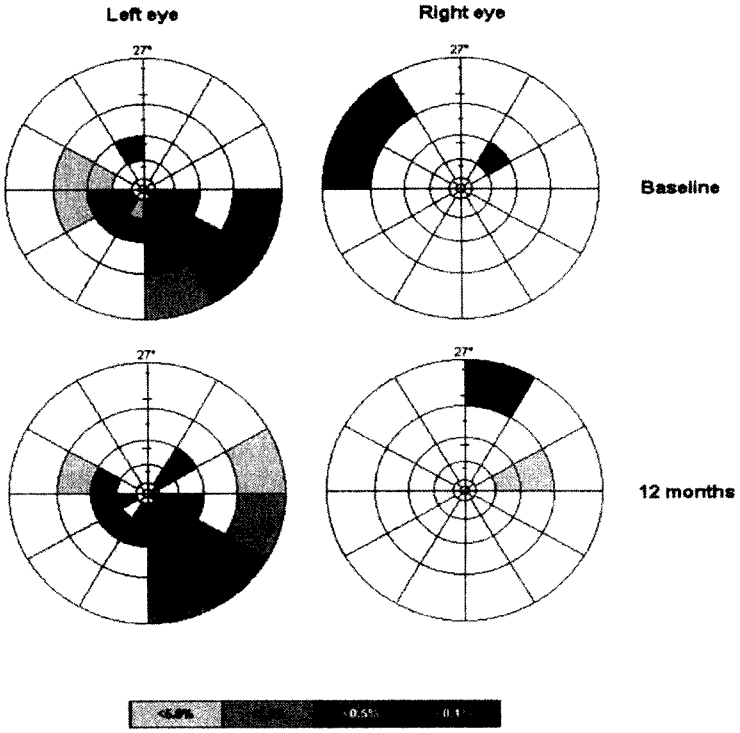

Figure 9 Glaucoma patient tested twice over a 12 month period showing apparent stability of the asymmetry plot.

Discussion.

The multifocal pattern VEP can be used in the detection of glaucomatous field loss. In this study using between-eye asymmetry of VEP responses, 69/70 cases (98.5%) with field defects were detected. More importantly, loss of between-eye symmetry may be useful as a marker for early damage. Abnormalities consistent with early scotomas were seen in almost one third of the high risk suspects, most of whom were already seen to have suspect optic disc contours and to constitute pre-perimetric glaucoma. In addition the normal quadrants of the established glaucomas had significantly higher asymmetry values compared to normal subjects.

Utilising the similarity of VEP waveforms recorded from the same half of the visual field, an inter-eye asymmetry analysis can help to identify subtle changes in the multifocal VEP amplitude. The convolution of the occipital cortex leads to different orientation of the electrical dipoles generating the VEP, which in turn, causes variation of the amplitude and waveform of the responses even if equal cortical areas are stimulated. The observation that in normal subjects "within-eye" asymmetry is almost identical for both eyes suggests that the variation of the individual VEPs recorded from different parts of the visual field is primarily determined by the underlying convolution of the striate cortex. This is based on the fact that identical parts of the visual fields of both eyes project to the same part of the visual cortex. The asymmetry holds even though different parts of the retinas of the two eyes are being stimulated[191]. Similar findings have recently been reported by Hood et al [193].

This study was completed with a relatively small data base of normals (24), which limits the application of probability values. A larger sample size of normals needs to be accumulated, which should tighten our probability limits. Also we cannot judge probability of progression based on our limited reproducibility study, particularly in glaucoma patients where fluctuation may be greater, as it is in perimetry. Further follow-up of normals and glaucoma subjects is required to establish an appropriate data base for this purpose.

Asymmetry analysis has the obvious limitation that it will not detect symmetrical glaucomatous losses, and is limited to those patients who have eyes of similar status in terms of acuity and ability to fixate. In cases where the field loss occurs in the same area (eg superonasally in one eye and superotemporally in the other) the losses will not show up on asymmetry, and would only be detected when they reached significant differences from normal values for the same point in each eye. We are reassured in part by the observation that the only scotoma in this study that was not detected by asymmetry, was identified by comparison with a normals data base for amplitudes, so no established cases of glaucoma were missed. The asymmetry plot in more advanced disease involving both eyes can actually appear only mildly abnormal, but in such cases the amplitude reductions are obvious and are detected by statistical comparison with normal values. The asymmetry plot should therefore never be interpreted on its own, but in conjunction with an amplitude deviation plot.

Examination of the optic disc and fundus should be performed in all cases, since the subject may have congenital differences that would affect the comparison between eyes. We have not yet determined the effect of acuity differences between eyes, or the effect of diseases such as cataract and macular degeneration which commonly confound interpretation of perimetry. Subsequent studies will address this issue.

Further long-term follow-up of suspect cases is needed to determine if asymmetry changes can reliably predict localised areas of early glaucomatous damage. Asymmetry analysis could also be applied in monitoring of progression of the disease or the effects of treatment. The multi-focal PVEP can detect glaucoma damage, not only through amplitude reduction compared to normals, but by using asymmetry analysis between two eyes of the tested subject. It may reflect damage at early stage of the disease process.

Chapter 5

Development of a multi channel mVEP and its application to glaucoma detection

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Summary

This paper introduces the concept of multichannel recording to cover all underlying signal origins more effectively and thus improve signal detection in some areas of the field not adequately sampled with the single channel technique. The multichannel technique is applied to glaucoma detection in suspects and established glaucoma. My role in this study was: discussion and design of study with Dr Klistorner, recruitment of suitable subjects – both normal and disease states, examining and classifying subjects, reviewing visual field data, recording with varying electrode positions in pilot study to determine optimal technique, jointly analysing results and cowriting the manuscript.

Abstract.

Purpose: Objective perimetry in glaucoma is described using the multifocal pattern visual evoked potential (VEP). A multichannel recording technique was utilised to improve signal detection in normals and assess its ability to detect glaucoma and early changes in suspects. **Design:** Comparative observational case series.
Participants: 30 normals, 30 glaucoma suspects and 30 patients with glaucomatous visual field defects and were tested.
Method: The VEP was recorded using cortically scaled multifocal pseudo-randomly alternated pattern stimuli with the VERIS system. An array of four bipolar occipital electrodes provided four differently oriented channels for simultaneous recording. Signals were compared for different locations within the field up to 25° of eccentricity. Normals, glaucoma suspects and glaucoma patients with established visual field defects were tested and results compared to Humphrey visual fields performed on the same day. For reproducibility five normals were each tested on four separate days. Suspects and glaucoma patients were analysed for inter-eye asymmetry of signals and compared to the normals asymmetry values.
Results: Multiple recording channels significantly enhanced the recording of signals from parts of the visual field not reliably sampled with a single channel technique in all normal subjects, particularly along the horizontal meridian ($p<0.001$). Signal amplitude did not decline with age in normals. Recordings showed good reproducibility within individuals. In all 30 cases of glaucoma the Humphrey visual field defects were well demonstrated by the VEP and topographic location was strongly correlated ($r_S=0.79$). Despite large inter-individual variations in amplitude, scotomas were well demonstrated when compared to normal values. In the suspects, smaller changes in signal amplitude

could be identified in parts of the field still normal on perimetry, using inter-eye asymmetry analysis .
Conclusions: The multifocal multichannel VEP can objectively detect glaucomatous visual field defects. The nasal step region can be more reliably tested using multiple channels. Asymmetry analysis has the potential to detect early defects. This technique represents a significant step towards the clinical application of objective perimetry in glaucoma.

Introduction

The objective assessment of the visual field using multifocal stimulation has been described in the earlier studies using a single channel technique [165, 194] . Good correlation has been shown between the multifocal VEP (first slice of the second order kernel) and scotomatous areas of the visual field. Multifocal VEP amplitudes also correlated with Humphrey visual field thresholds [188]. With asymmetry analysis there is the potential to pick up subtle early defects [195].

However, there is a marked decrease in amplitude along the horizontal meridian seen in many single channel multifocal VEP recordings when the BOS electrode position is employed, which has limited the application of the method for objective mapping of the visual field. This is particularly relevant to the assessment of glaucoma since early defects frequently first appear along the horizontal.

The part of the visual field that is situated along the horizontal meridian is represented deep within the calcarine banks at the fissure base [101]. Dipoles generating a response from this region are almost perpendicular to a projected line between vertically situated electrodes and therefore little or no signal is detected. On the basis of cortical topography it was predicted that horizontally oriented bipolar electrodes straddling the inion would be optimal for registration of the horizontally oriented dipoles from the base of calcarine sulcus. This position was tested in a number of normal subjects as well as glaucoma patients with established visual field defects. Two obliquely oriented electrode channels were also included to cover any other dipole orientations in the visual cortex.

The multichannel recordings were analysed in normals to confirm the technique enhanced the recording of signals from parts of the visual field not reliably sampled with the single channel technique. Glaucoma patients were then assessed to ascertain the sensitivity of the technique for detecting field loss. Correlations were performed between the subjective Humphrey visual field results and the objective VEP findings with respect to topography and severity of the field loss.

Analysis of the multichannel multifocal VEP in normal subjects also revealed that although visual evoked responses are present at every stimulated location of the visual field, the amplitude of the individual signals vary. Such "within eye" asymmetry of the signal and the large variability in VEP amplitudes seen between subjects makes the interpretation of a small amplitude reduction more difficult even when comparison with

normal database is made. Since our study of asymmetry analysis using single channel multifocal VEP recording was able to identify defects early in the disease we therefore applied asymmetry analysis to the multichannel method to investigate a number of glaucoma suspects.

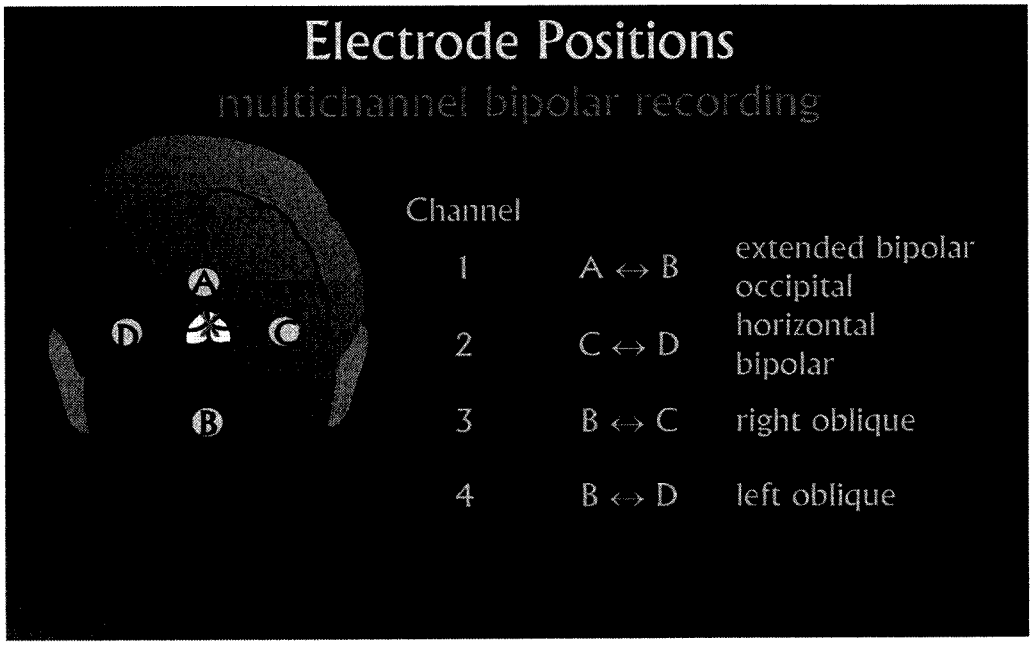
Method

Stimulation and recording

Full details of the single channel recording technique has been reported previously in General Methods Chapter 2. The VERIS Scientific™ system (Electro-Diagnostic Imaging, Inc., San Francisco, CA) was employed for all recording, with a pattern dartboard stimulus. The stimulus parameters used for the multichannel recording were the same as for single channel.

Electrode positions

After preliminary experiments with multiple electrode positions and combinations, 4 bipolar channels of different orientation were determined which consistently produced larger signals, and when combined provided the best coverage of all points in the visual field. These positions were then used for the study. Two electrodes were positioned along the midline 3 cm above and 4.5 cm below the inion. Two additional electrodes were placed 4 cm on either side of the inion. Different combinations of the electrode connections produced four new bipolar channels (see Figure 1): channel 1 (termed vertical or extended BOS) -upper and lower midline electrodes; channel 2 (horizontal) -two electrodes on either side of the inion; channel 3 (right oblique) - electrode on the right side of the inion and lower midline electrode; channel 4 (left oblique) -electrode on the left side of the inion and lower midline electrode. See Figure 1 below:



Gold disc Grass electrodes (Astro-Med Inc, West Warwick,RI) were used. Electrodes were mounted on a custom designed convex occipital-cross electrode holder which standardised positions, improved contact and reduced noise levels. The lower midline electrode was negative for all vertical and oblique channels, while the left horizontal electrode was negative for the horizontal channel.

Subjects

The study protocol was approved by our regional ethics committee and informed consent was obtained from all patients. The study included 30 normal subjects, 30 glaucoma suspects, and 30 patients with established glaucoma. The normals included 16 males and 14 females with an age range 39-75 years, mean 54.1±9.7. For reproducibility we tested five normal subjects on four separate occasions on different days. The normals had normal intraocular pressure and ophthalmoscopy, and no family history of glaucoma or retinal dystrophy. All performed normal Humphrey 24-2 field tests, confirmed by a normal result on the glaucoma hemifield test analysis, and no clusters of points that could constitute a scotoma as defined below for the glaucoma subjects. The inclusion criteria for the normal and glaucoma groups required a corrected visual acuity of 6/12 or better and pupils at least 2.5mm without dilation. Subjects with diabetes, previous cataract surgery or any other ocular disorders were excluded.

Thirty consecutive patients who met the inclusion criteria, with established glaucomatous field defects and varying severity of disease, were recruited from our clinic over a six month period. Both eyes were tested so that asymmetry analysis could be performed but only one eye was evaluated in terms of scotoma detection. The eye selected had a scotoma as defined below. If both eyes had field loss the eye with the lesser defect was chosen. Their mean deviation on Humphrey fields ranged from -0.79 to -25.7dB, mean -10.05±6.4dB. They had a mean age of 58.9±9.5 years (range 42-72) which was not significantly different from the normals ($p>0.05$, t-test). There were 16 males and 14 females. Patients had previously performed Humphrey 24-2 fields on three or more occasions, and had reproducible field defects. Two patients were tested 3 months apart, while one patient who had been tested earlier (during development of the technique) was tested in total 3 times over a 22 month period.

Twenty -five patients with primary open angle glaucoma (POAG) and five with normal tension glaucoma (NTG) were included. The diagnosis of POAG required the following three parameters: a confirmed visual field defect on Humphrey 24-2, a glaucomatous optic disc as judged by stereo disc photography, an intraocular pressure > 21 mmHg with the applanation tonometer. NTG patients had field and disc changes but had never had an IOP > 20mm Hg. The definition of a field defect used the pattern deviation plot on the Humphrey 24-2 program. A minimum scotoma required at least 3 adjacent points depressed by $p<0.5\%$ on the pattern deviation probability plot. The cluster of abnormal points could not cross the horizontal meridian and points immediately above and below the blind spot could not qualify as part of the scotoma. The glaucoma hemifield test was abnormal.

There were also 30 high risk glaucoma suspects (16 males and 14 females). They had a mean age of 53.1±9.6 years (range 25-71) which was not significantly different to the normals ($p>0.1$, t-test). They were patients who had been noted to have either a definite structural change or an asymmetry in the neuroretinal rim, but who had not developed visual field defects, representing "preperimetric glaucoma". Most had ocular hypertension and/or a family history. The Humphrey glaucoma hemifield test was normal in all suspects, and they did not have an identifiable scotoma as defined above. Both eyes were tested and an asymmetry analysis performed as described below.

Analysis

Raw trace data was analysed using custom designed software. Peak-to-trough amplitudes for each wave within the interval of 50-165 msec were determined and compared among channels for every stimulated segment of the visual field. The wave of maximal amplitude from each point in the field was automatically selected and a combined topographic map was created by the software. The interval betweem 50 and 165 msec was chosen to include major deflection of VEP wave at all sites of the visual field in all channels tested.

To demonstrate that the different channels produced amplitudes of greater magnitude in specific parts of the field, comparisons between sectors for different electrode positions were performed with paired t-tests. For analysis of reproducibility of multichannel combined recordings, the coefficient of variation (standard deviation/mean) between repeat tests was calculated for each individual trace point in the combined array for each subject. Data for all normals was examined to look for the effects of age and sex on amplitude of the response. Inter-individual variation in amplitude was determined for all 30 normals for each point in the field. A normal database was compiled, including mean and SD values of VEP amplitude for every stimulated area of the visual field.

To assess the ability of the multifocal VEP to detect glaucoma damage and determine its correlation with Humphrey global indices and local thresholds, the results were analysed in several ways. Firstly, in the combined VEP trace array all individual responses with an amplitude less than 120nV were determined for each patient and the number of these abnormal points was correlated with Humphrey mean deviation (MD) value and corrected pattern standard deviation (CPSD). The arbitrary value of 120nV was chosen since at this level the waveform is difficult to identify from background noise.

Secondly, the glaucoma subjects' VEP amplitudes in the combined trace array were compared with the normals database. Points with a difference in amplitude of more than 1.96 standard deviations (ie $p<0.05$) from the mean value for that point in the normal database were considered as abnormal. The number of abnormal points was correlated with Humphrey MD and CPSD values for the same patient.

Thirdly, the local distribution of VEP points with amplitude <120nV was then compared with the distribution of abnormal points ($p<0.5\%$) on the Humphrey

total deviation plot. Since the distribution and size of test points (ie the test grid) varies with eccentricity for the VEP stimulation but is constant for the Humphrey, direct point by point comparison was not possible. Therefore a correlation between the number of abnormal points within the same quadrant was performed.

Similarly, the number of abnormal points (compared with the normal data base) in each quadrant of the VEP trace array was then correlated with number of abnormal points $p<0.5\%$ in the same quadrant of the Humphrey pattern deviation plot. All statistical comparisons and correlations mentioned above were calculated using Statistica 4.1 (StatSoft, 1994, Tulsa, OK). Since the perimetric and VEP data used ordinal values for the number of points affected in the visual field, all the above correlations were done with the Spearman rank correlation for nonparametric analysis.

For the recognition of scotomas to give a measure of overall test sensitivity, the criterion for abnormality used was at least three adjacent points reduced to <120nV in a similar distribution to the Humphrey scotoma. An alternative criterion required three adjacent points outside 1.96 standard deviations in the same location as the scotoma. Data from each individual normal was also separately reapplied to the data base (with their data excluded) to give an estimate of test specificity.

To detect early VEP changes, in each subject the inter-eye asymmetry was calculated for every segment of the tested visual field by dividing the difference in amplitude between left and right eyes by their sum, within the latency interval of 50-165 msec:

Response asymmetry coefficient (RAC) = (Amp1-Amp2)/(Amp1+Amp2)

where Amp1 is a maximal peak to trough amplitude of the response at particular segment from the left eye and Amp2 is a maximal peak to trough amplitude of the response of the same segment from the right eye. Using the ratio instead of simple difference helps to avoid dependence on the absolute amplitude of the signal.

Mean and standard deviation values of RAC for every point of the tested field were computed for the group of normals. RAC values for each point for each glaucoma suspect were then compared with the RAC values for the same point in the normal group. Points with statistically significant difference (ie $p<0.05$ and $p<0.01$) from the mean RAC value for that point in the normals database were flagged as abnormal on a probability plot. A cluster of three adjacent points of probability $p<0.05$ was considered as representing a possible scotoma.

A comparison between the normals and high risk suspects was also performed in terms of quadrantic RAC values, to establish if greater asymmetry values ocurred in the suspect group. The mean RAC of all 15 individual points within each quadrant was calculated for normals and suspects, and compared for each quadrant of the field.

Results

Normal subjects

A typical four channel recording (figure 2) demonstrates that multichannel recording improved signal detection from certain parts of the field not well sampled by the vertical channel alone. The figure shows a recording from the left eye of a normal subject. The trace array for the vertical channel has been shaded in areas where the signal was poor. The horizontally placed electrodes enhanced the signal detection from areas below the horizontal meridian, in this case mainly on the nasal side. In other cases both temporal and nasal sides showed improved signals in the horizontal channel.

Greater responses from a few isolated locations were seen in the oblique channels, and these varied between individuals. This was presumably due to the underlying cortical dipole being at a different orientation, due to variable cortical anatomy. Since recording the oblique channels requires only two extra channels (in fact they can be derived with only three channels in total), with no additional electrodes or recording time needed, it was considered worthwhile to collect this additional data to help cover all possible orientations. To create a single map of retinotopic cortical activity, all channels were then combined together using the traces of greatest amplitude for each particular location of the visual field tested. An example of a combined trace array is presented in Fig. 2b (derived from the traces in Fig. 2a). If only the vertical channel had been recorded, an impression of a nasal step, superior defect and paracentral defect would have been suggested. The combined array shows good responses throughout the field in this normal subject.

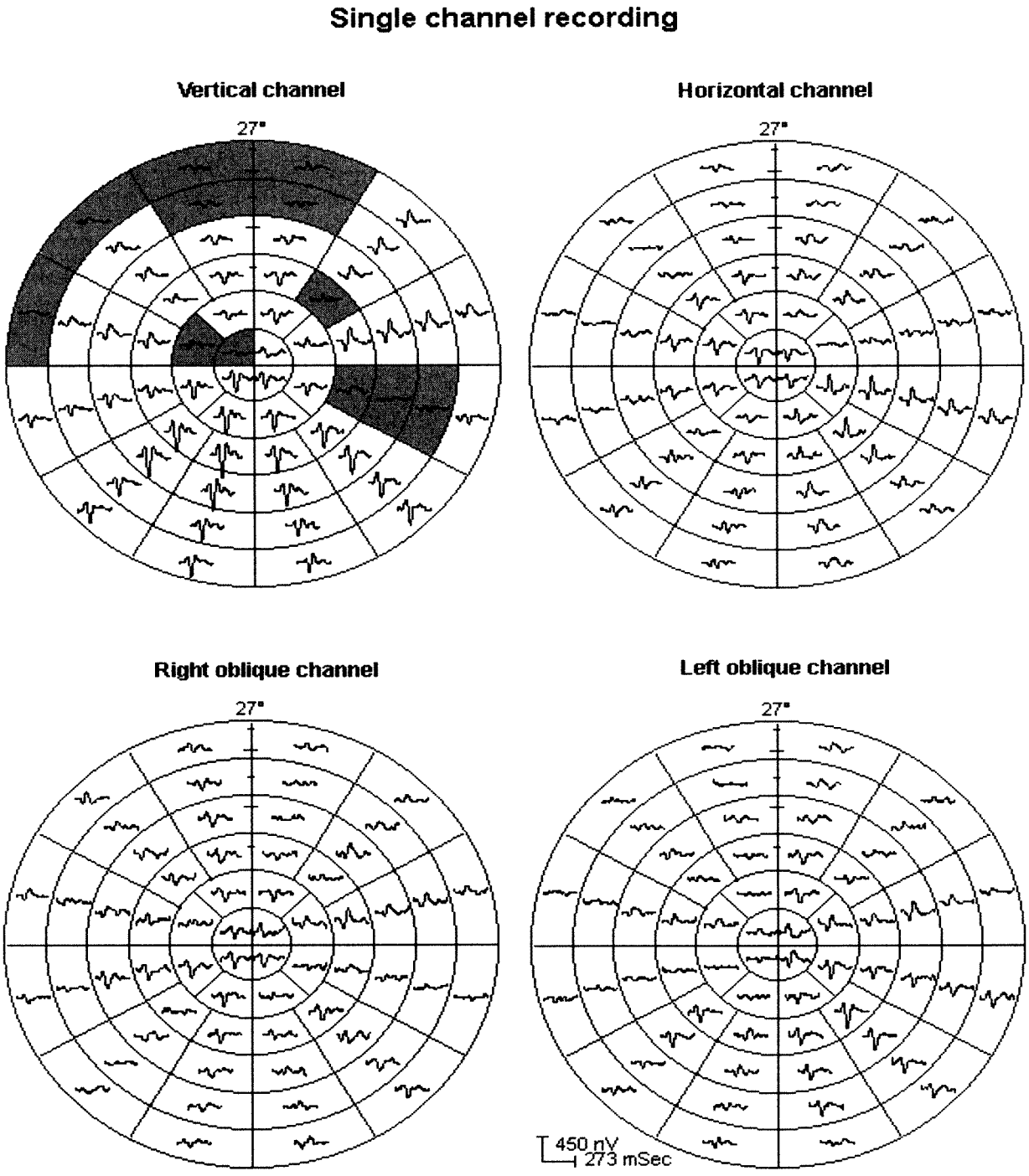


Figure 2a Four channel recording of multifocal VEP. Left eye of a normal subject. Shaded areas in vertical channel demonstrate points with small signals close to noise levels (<120nV). The inferonasal horizontal meridian is better recorded in the horizontal channel. Some peripheral upper field locations have larger signals in the oblique channels.

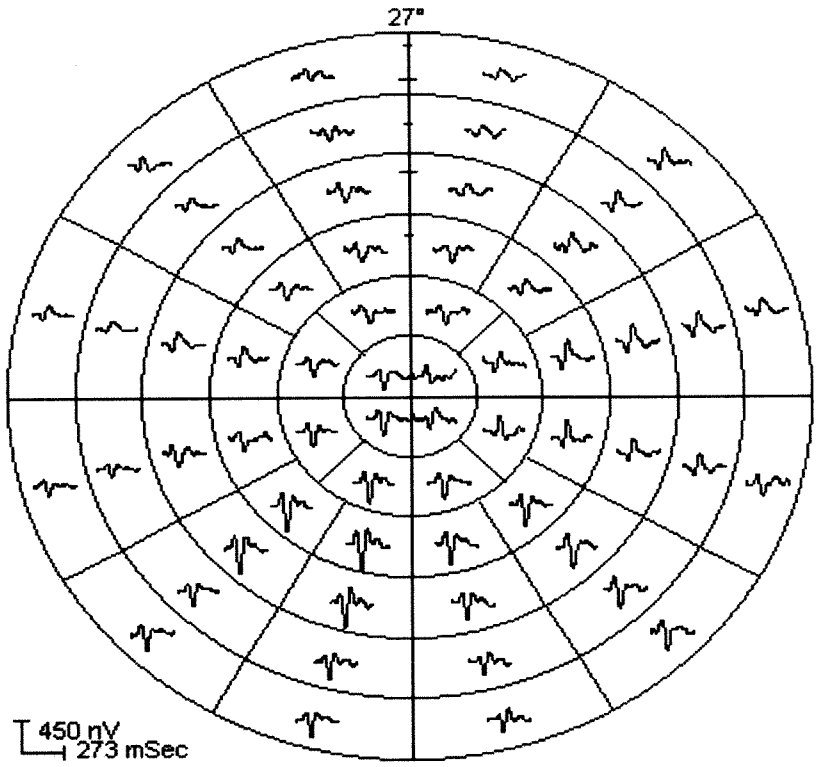


Figure 2b Combined multichannel VEP trace array using all four channels from the same subject as 2a. If only the vertical channel had been recorded, an impression of an inferonasal step, a superior defect and paracentral scotoma would have been suggested. The combined array shows good responses throughout the field. The lower field has consistently larger signals.

Table 1 Comparison of single and combined channel responses along horizontal

	Vertical channel (μV)	Combined channels (μV)	P value
Temporal side	1.59±0.51	2.22±0.68	<0.001
Nasal side	1.71±0.42	2.10±0.62	<0.0001

Quantitative assessment of multichannel recording revealed that horizontal electrode placement improved the summed amplitude of the responses from the segments below the horizontal meridian by 32%. Table 1 shows the combined amplitudes of the response for the six points just below the horizontal meridian calculated for single (vertical) channel and combined trace array for all 30 normal subjects. The temporal and nasal sides of the field are analysed separately. The difference between the responses derived from single channel and combined trace array was highly statistically significant ($P<0.001$ and $P<0.00001$ for temporal and nasal sectors of the visual field respectively, paired t-test). This means that the multichannel recording is more effectively detecting responses along the horizontal, in particular the nasal step region which is extremely important in early glaucoma. The inter-eye asymmetry for normals was seen to be very small, with almost identical traces recorded from the same part of the visual field for the two eyes.

Reproducibility.

To demonstrate reproducibility of multichannel recording, one eye of five normal subjects was recorded on four different occasions. Combined trace arrays for one subject from two consecutive recordings are presented in Fig. 3. This demonstrates high intra-subject reproducibility of the traces practically at every location. The few points where the waveform shape/polarity differs represents those points where a different channel registered a slightly better signal on the repeat test.

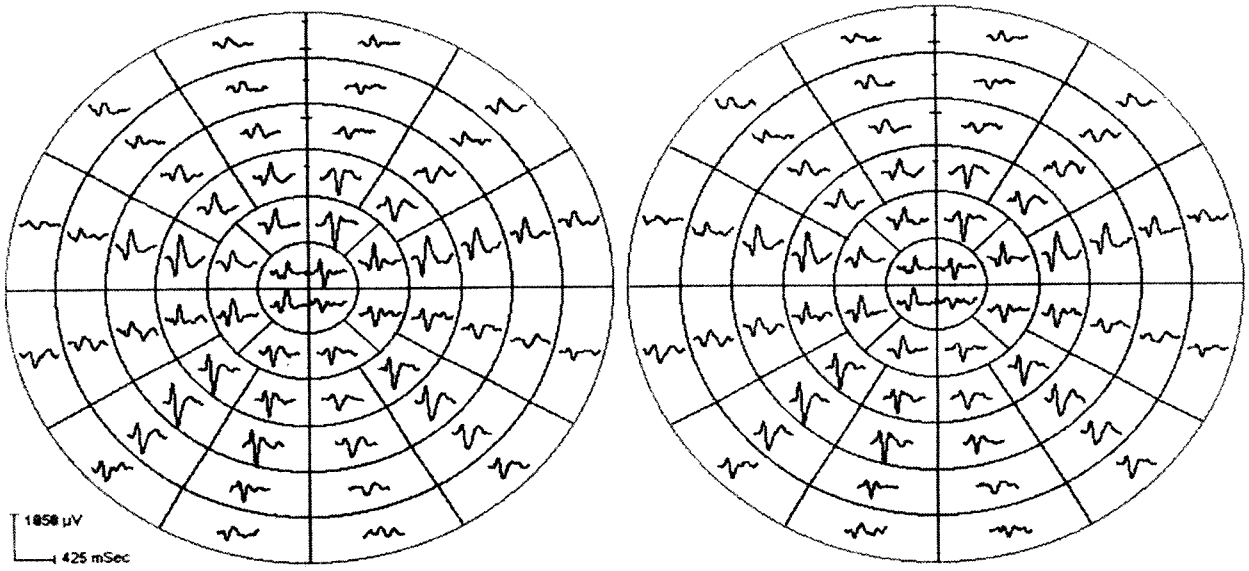


Figure 3. Combined trace arrays for one normal subject from two consecutive recordings Demonstrates high intra-subject reproducibility of the traces practically at every location.

Amplitudes of the individual VEPs (i.e. produced by stimulation of each particular point) recorded at different sessions were compared and the mean, standard deviation and coefficient of variation were calculated. The results for each individual point of the field are shown in figure 4. In most locations of the field the coefficient of variation was under 20% (range 6.8 - 25.9, mean 15.2± 4.5%). It was also evident that the more peripheral parts of the visual field, particularly in the upper hemifield produce greater variability. The multichannel technique had less variability than single channels (eg vertical channel mean coefficient of variation for all points 18.0±5.4%, $p<0.00001$, non-parametric tests) confirming a significant advantage of the multichannel method.

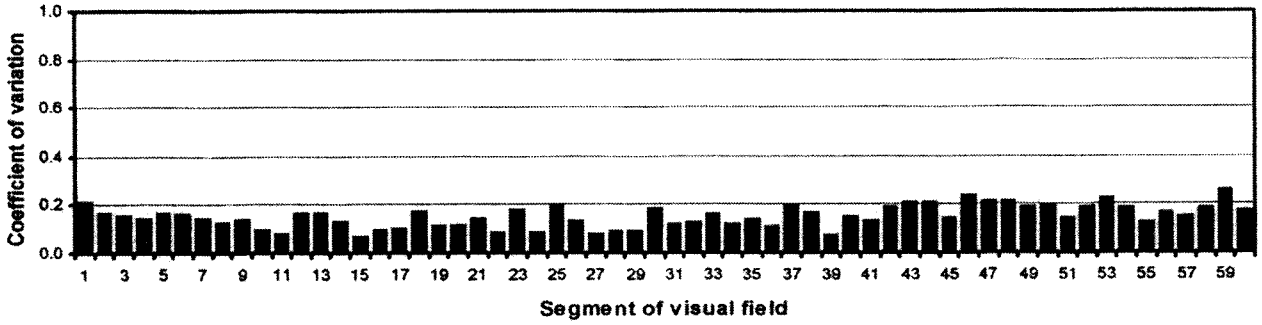


Figure 4. Intra-subject reproducibility for five normals with each tested on four occasions on different days. Graph shows coefficient of variation for each of the 60 test points averaged for the five subjects. Greatest variability (around 20%) was found in the more peripheral areas.

The data from all normals was also assessed to determine the normal population range and inter-individual variation. Age of the subject had no effect on signal amplitude ($r=0.17$). In fact some elderly normals had quite robust signals which were larger than the younger subjects. RAC values also showed no correlation with age ($r_S=0.12$). We found similar results when we analysed the normals data from our single channel study [195]. The lack of an age effect on the multifocal VEP is an advantage over electroretinographic responses which decline with age [33]. There were also no sex differences observed.

The inter-individual variability however, was large with the coefficient of variation for individual points ranging from 23.0 - 52.7%, mean 34.8± 6.9%. The multichannel results showed significantly less variation than the single channels alone (eg vertical channel mean 42.0± 9.2%, $p<0.0001$). This wide variation in amplitudes, which is also observed with conventional VEPs, represents a significant problem for the interpretation of an individual's data when attempting to detect early changes. However, the RAC variability was much less between individuals. Also our recent study on asymmetry analysis in single channel recording showed low intrasubject variability [195]. Therefore to look for more subtle changes, particularly in glaucoma suspects, the asymmetry analysis may be more useful than examining amplitudes alone.

Detection of visual field loss.

In all 30 cases of glaucoma the Humphrey visual field defects were well demonstrated by the VEP. Scotomas were readily identified by loss of signal amplitude to <120nV in at least three adjacent points in the matching area. All subjects' scotomas were also recognised using a criterion of at least three adjacent points outside 1.96 standard deviations from normal's database. This gave a gross sensitivity of 100% by both methods. The latter method was surprisingly effective even though the normal population showed a wide variability. In contrast only one of the normals was identified with three abnormal points when his data was reapplied to the data base, suggesting a specificity of around 97%. The results are shown in Table 2.

Table 2 – Detection rates using different criteria for definition of a mVEP defect

Criteria for abnormality	Normals 30	Suspects 30	Glaucoma 30
Trace amplitude 3pts<120nV	1 (3%)	3 (10%)	30 (100%)
3 pts p<0.05	1 (3%)	2 (6.6%)	30 (100%)
RAC values 3 pts p<0.05	1 (3%)	10 (33%)	30 (100%)
3 pts p<0.01	0	4 (13.3%)	30 (100%)

Four typical examples of correspondence of VEP perimetry with Humphrey perimetry are shown in figures 5-8. They include an inferior arcuate defect, a superior arcuate defect and a paracentral defect. The Humphrey visual field (gray scale and pattern deviation plot) together with multichannel VEP (combined trace array and VEP deviation plot) are presented in each example. Gray areas in the combined trace array plot indicate regions of the visual field where the amplitude of the VEP was less then 120nV (noise level). It should be noted that the trace array as depicted is not identical in terms of the location of VEP and Humphrey test points, since the inner 2 rings of the VEP are actually contained within the central 3° of the field. The trace array as depicted in the figures has the inner points enlarged for graphics purposes only. The VEP deviation plot shown adjacent, unlike the trace array, accurately corresponds to the stimulated areas of the visual field and maps areas with VEP amplitudes statistically different from the normal database. Three levels of significance are shown by the gray scale intensity: $p<0.1$, <0.05 and <0.01.

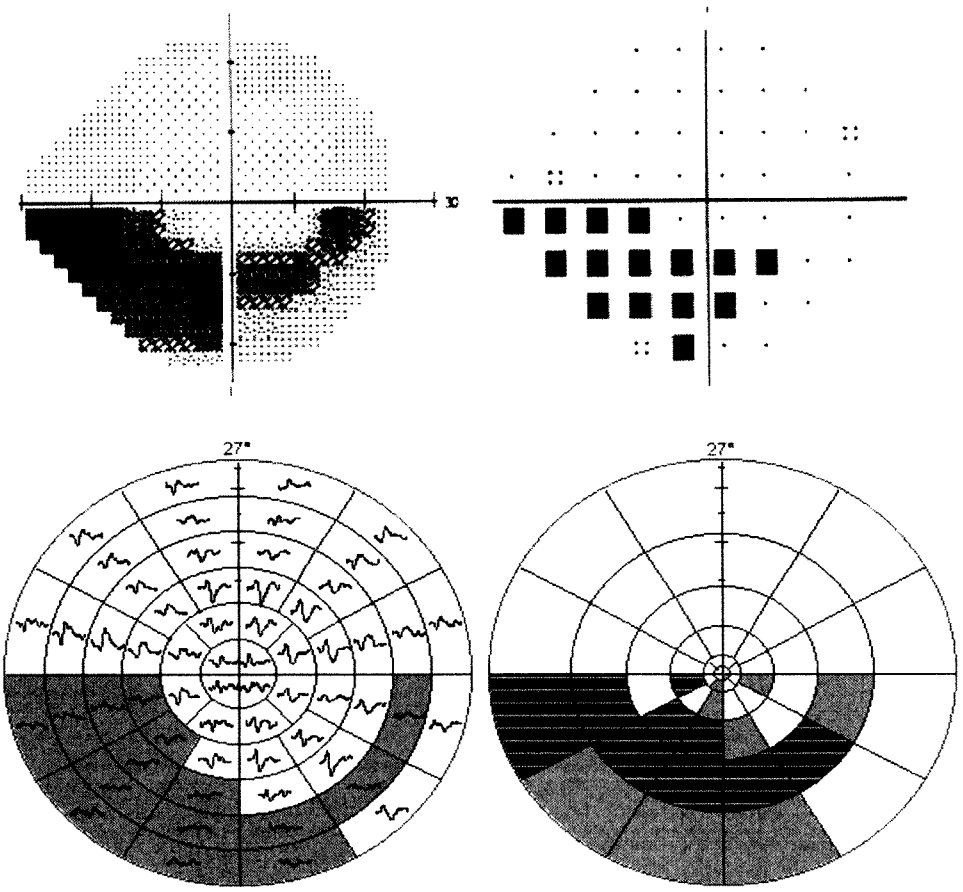


Figure 5. Example of correspondence of VEP perimetry with automated perimetry in a glaucoma patient with inferior arcuate field loss. The Humphrey gray scale and pattern deviation plot are shown, together with multichannel VEP (combined trace array and VEP deviation plot). Gray areas in the combined trace array plot indicate regions of the visual field where the amplitude of the VEP was less then 120nV (noise level). The VEP deviation plot shown adjacent, maps areas with VEP amplitudes statistically different from the normal database. Three levels of significance are shown by the gray scale intensity: $p<0.1$, <0.05 and <0.01.

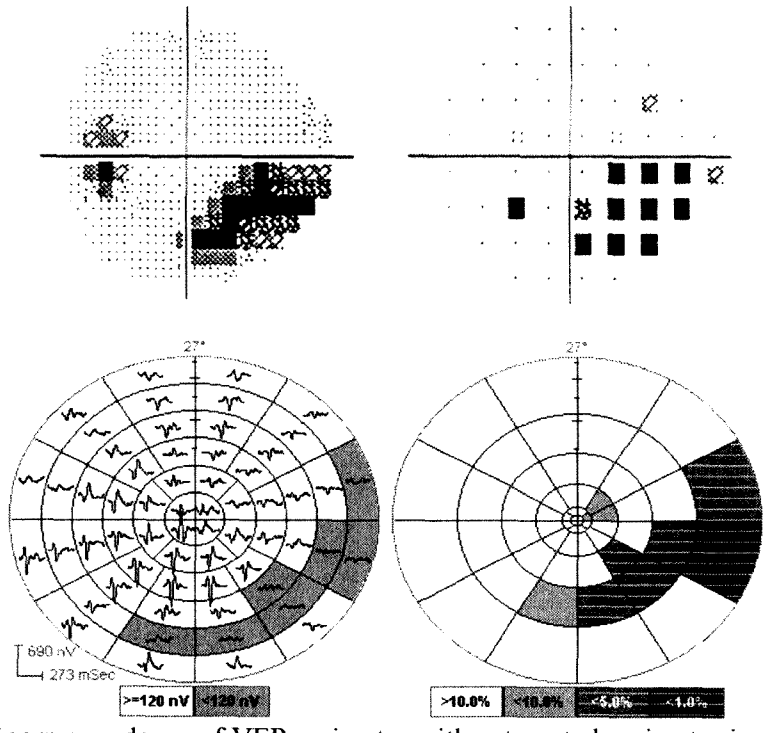


Figure 6. Example of correspondence of VEP perimetry with automated perimetry in a glaucoma patient with an inferionasal step defect. Layout, probability levels and amplitude/time scale as for figure 7.

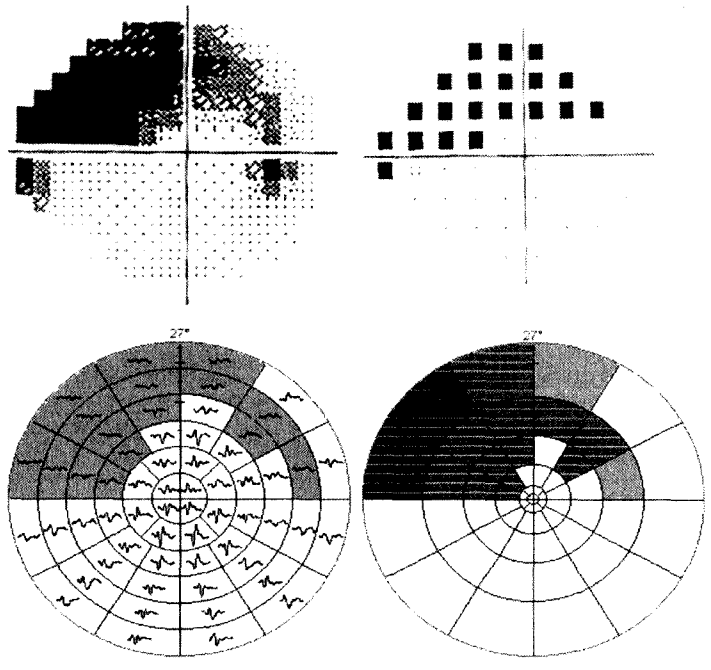


Figure 7. Example of correspondence of VEP perimetry with automated perimetry in a glaucoma patient with superior arcuate defect. Layout, probability levels and amplitude/time scale as for figure 7.

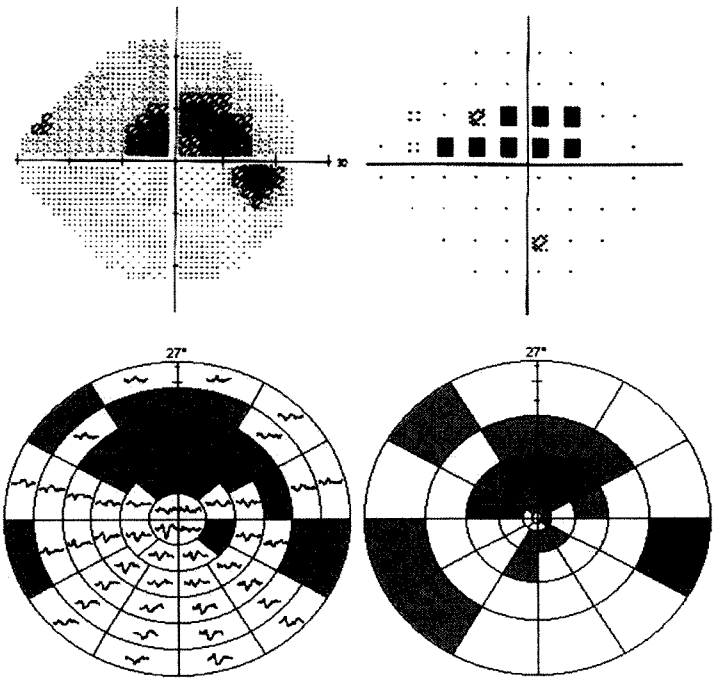


Figure 8. Example of correspondence of VEP perimetry with automated perimetry in a glaucoma patient with superior paracentral defect. Layout, probability levels and amplitude/time scale as for figure 7.

The number of abnormal points on VEP perimetry correlated strongly with the Humphrey MD (r_S=0.89), (Fig. 9a). A high correlation (r_S=0.83) was also found between Humphrey MD and the number of points in VEP perimetry which were statistically different in amplitude ($p<0.05$) from the normal's database (Fig.9 b). The correlation with CPSD also held for both of the above but was weaker (r_S=0.60 and r_S=0.45 respectively).

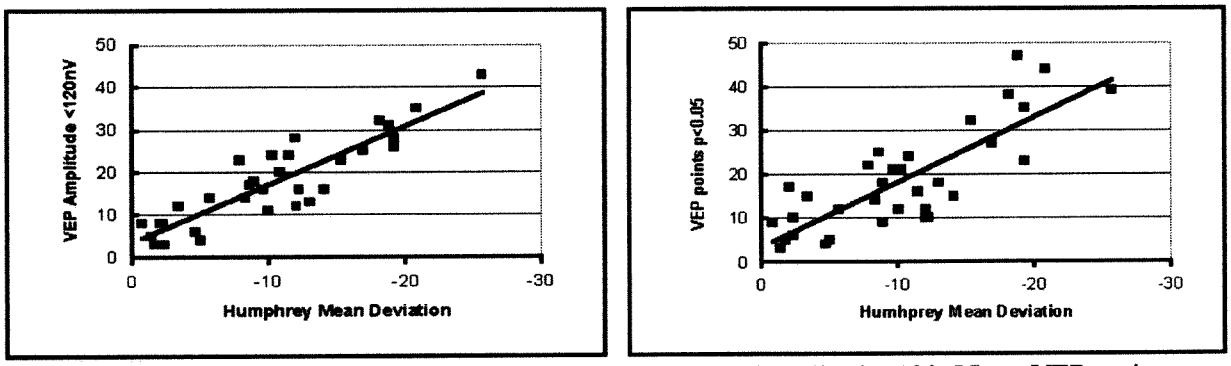


Figure 9a. Correlation between the number of abnormal points (amplitude<120nV) on VEP perimetry with the Humphrey mean deviation (r_S=0.89) for all 30 glaucoma patients. Figure 9b. Correlation between Humphrey mean deviation and the number of points in VEP perimetry which were statistically different in amplitude ($p<0.05$) from the normal's database (r_S=0.83).

Topographic location of scotomas was also highly correlated between Humphrey and VEP perimetry. When analysis was done by quadrants for the number of VEP points with marked amplitude loss (<120nV) compared with number of abnormal Humphrey total deviation test points (p<0.5%) in corresponding quadrants, it produced a correlation coefficient r_S=0.79 (fig 12a). The correlation by location was also high (r_S= 0.71) for number of abnormal (relative to normal database) VEP points (p<5%) compared with the number of abnormal points (p<0.5%) in the same quadrant on the Humphrey pattern deviation plot (fig 12b).

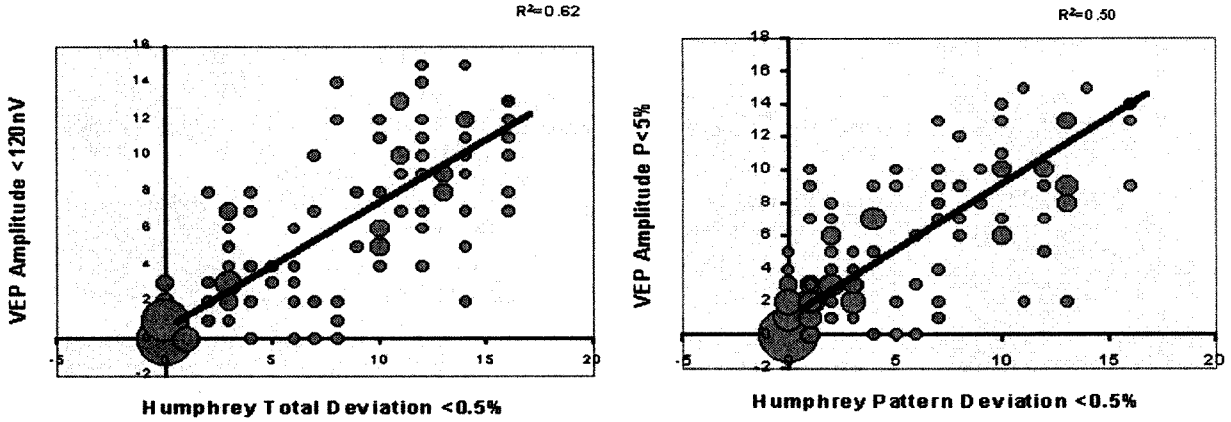


Figure 10a. Topographical correlation by quadrant between the number of abnormal points on VEP perimetry (amplitude<120nV) with the Humphrey deviation plot (p<0.5%) for all 30 glaucoma patients (r_S=0.79). Figure 10b. Correlation by quadrant between the number of abnormal points on VEP perimetry which were statistically different in amplitude (p<0.05) from the normal's database with the Humphrey deviation plot (p<0.5%) for all 30 glaucoma patients (r_S=0.71). Area of the markers is proportional to the number of data points.

We also tested an additional subject with superior field loss three times over a two year period. His results are presented in figure 11. The field loss appeared to be stable, perhaps with a slightly better performance in the Humphrey, while the VEP amplitudes remained stable.

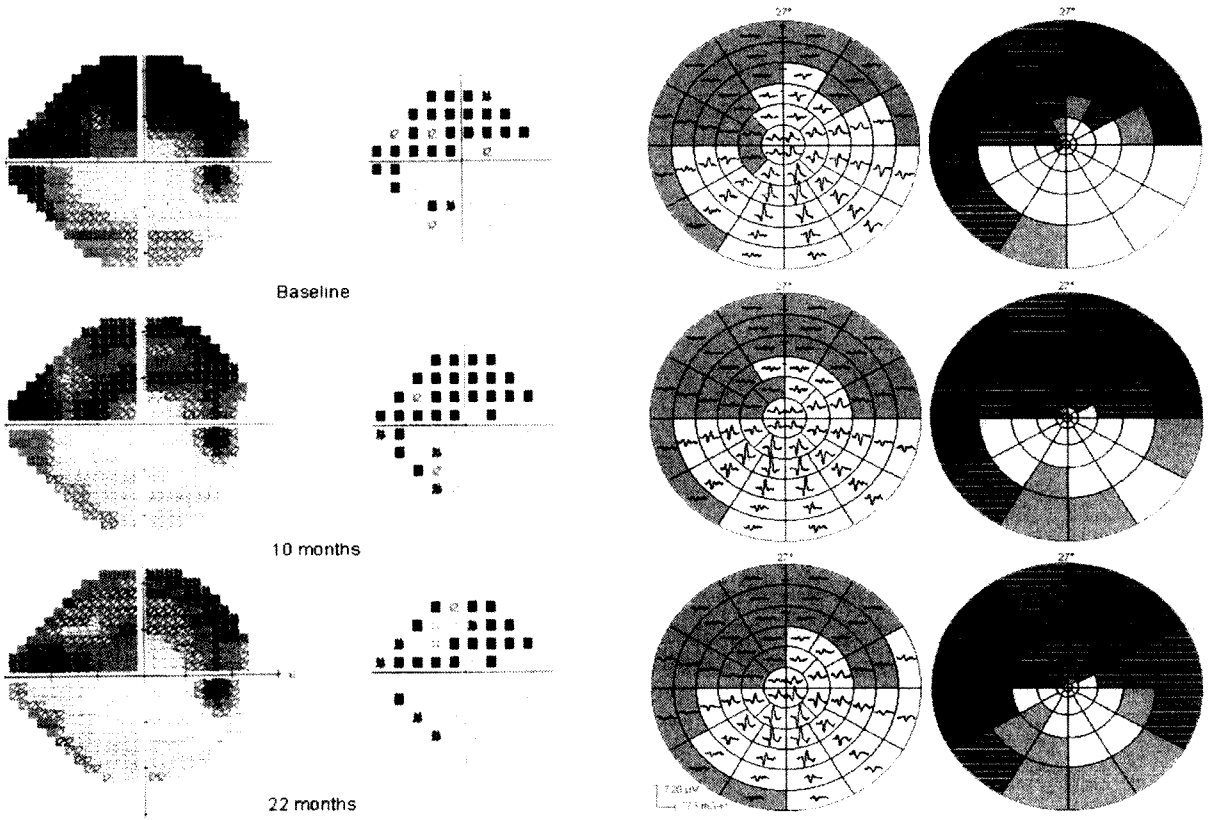


Figure 11a and b. Glaucoma patient (right eye) tested three times over 22 month period. Figure 11a shows Humphrey gray scale and pattern deviation. Figure 11b shows trace arrays and probability plots as for figure 5. While the Humphrey appears to show no progression, the VEP suggests possible worsening of inferior nasal field. Disc progression was documented with cup/disc ratio changing from 0.7 - 0.8 - 0.85 over the three visits despite normalised intraocular pressure.

VEP asymmetry analysis in suspects

Of the 30 suspects with still normal Humphrey fields, 10/30 showed a cluster of three or more points reaching $p<0.05$ probability compared to the normals VEP asymmetry database. If the criteria was tightened to $p<0.01$, there were still 4/30 suspects showing an early defect on the asymmetry plot. Using amplitude values as a criterion for a scotoma, only 3 suspects showed a possible scotoma. When the normals were individually re-assessed by the RAC data base, no false positive scotomas were seen by either criteria. Results are seen in Table 2.

The glaucoma suspects had significantly greater mean quadrant RAC values compared to normals ($p<0.005$ for all four quadrants, t-test). Table 3 shows the results, with the difference for each quadrant being statistically significant. This suggests that there are relative amplitude reductions occurring in at least some of these eyes despite normal perimetric responses.

Table 3 RAC values for quadrants in normals and suspects

Quadrant	Normals	Suspects	P value
Lower Right	0.022± 0.018	0.076± 0.059	<0.001
Lower Left	0.031± 0.032	0.077± 0.051	<0.001
Upper Left	0.027± 0.029	0.059± 0.048	<0.002
Upper Right	0.021± 0.018	0.065± 0.046	<0.001

Mean RAC values for each quadrant of the visual field. Glaucoma suspects have greater values than normals for each quadrant

Discussion

The use of multichannel bipolar occipital recording enhances the ability to detect signals from all parts of the visual field using the multifocal pattern VEP. In all cases of glaucoma with established field defects, the scotoma was detected by this form of objective perimetry. Combining results from at least four channels also reduces the great variability between individuals seen as a result of the underlying convolution of the cerebral cortex, since most dipole orientations are covered by at least one channel. By sampling from different bipolar electrode positions variously oriented around the striate cortex, an optimal response can be determined from each point within the field. Multichannel recording therefore improves the technique for objective VEP perimetry and can be used to identify glaucomatous field loss.

The horizontal bipolar electrodes provide a much greater signal from the test points along the horizontal meridian of the visual field. This fits with the underlying orientation of the striate cortex for this part of the visual field. Improved detection in this area is extremely important for the application of objective perimetry to the detection of glaucoma, since nasal steps often are the first defect to occur. The oblique electrodes can also enhance the signal from various points, in particular along the upper vertical midline of the visual field of the contralateral side.

The lack of a significant decline in the multifocal VEP signal amplitude with age is an interesting finding. Although there is a known dropout of neurones with age, a possible explanation may be that the subcutaneous tissues are thinner in the elderly, providing less resistance to signal transmission. Further expansion of the normals data base to include even older individuals will determine whether this is not simply an effect of a small sample size.

Since the technique can reliably demonstrate visual field loss it may be considered as a useful alternative to psychophysical testing, and may provide objective data for longer term follow-up when looking for disease progression. In the reproducibility study, the individual points showed a variability of around 15% between tests. This is similar to findings for the multifocal ERG previously reported [196]. The reproducible responses allow for follow-up of individuals over time for disease progression. It is known that glaucoma patients often show greater fluctuation on automated perimetry than normals [197] which is factored into the Humphrey glaucoma change probability plots. It will therefore be necessary to examine glaucoma patients over time in a large prospective trial

to determine if they show similarly large fluctuations in VEP amplitudes, before an appropriate change probability algorithm can be devised to follow individuals. In the glaucoma case we have tested 3 times over 22 months (fig 14) there seemed to be little fluctuation in the VEP amplitudes, which is an encouraging finding.

The application of VEP perimetry to glaucoma screening and initial diagnosis is still limited by the significant inter-individual variability in amplitudes seen in the normal population. A large drop in amplitude is currently required to confirm an individual point as outside the normal range (even down to noise levels for some points). Multiple channel recording reduces this range of variation to some extent. However, in order to identify subtle reductions in individual points, methods to overcome this between-individual variation need to be devised. The current technique of using two standard deviations is not ideal. Some normal subjects for example, can have extremely high VEP signals, which creates a large population standard deviation for each point. We are currently examining alternative statistical methods for assessing the data, so that the identification of defects is more robust. A new recording technique is also under investigation for reducing between-individual variation.

For each individual, between-eye analysis looking for asymmetry in signal amplitude at congruous areas of the visual field could be a useful technique. Since the same area of striate cortex receives projections from similar areas of both visual fields, it responds to both eyes with a waveform of identical character. In suspects with pre-perimetric glaucoma we have detected such asymmetry in many cases.

Asymmetry analysis is limited to subjects with eyes of similar status, and does miss cases where a defect appears in the same part of the field in both eyes simultaneously (eg temporal field in one eye and nasal in the other). In such cases the amplitude plots would need to be examined closely. It does seem to provide the possibility of early detection however, and may be a useful strategy for following suspects over time, once long term fluctuation values for RACs are established for glaucomas and normals.

At the present time we only select the greatest amplitude recorded from the four channels as the optimal response, with no relative weighting or scaling. It may be possible to further improve upon this method by looking at relative amplitudes between channels, vector analysis, and/or applying different weighting for different field positions. The reason for not analysing latency of the VEP was that our previous study of the multifocal VEP in glaucoma which was performed using the single channel BOS electrode position, had shown only a poor correlation between latency delay and degree of visual field loss in glaucoma [194].

There are many conflicting reports in the literature about magnocellular and parvocellular losses in glaucoma [138, 146]. The stimulus used in this study has pattern edges and high contrast which would be expected to favour p-cells, but its frame rate is 67 Hz which would favour the faster conducting m-cells. We have recently observed early m-cell losses using a central field flash VEP with low contrast [198] and have also

observed that larger signals can be derived from the more peripheral points with faster frame rates. Therefore, further experiments may be able to devise a multifocal system that is capable of not only varying the pattern, but the speed and luminance throughout the visual field, to optimally target either m-cell or p-cell systems. In its current format the VERIS system cannot do this, and to attempt to target both systems separately would double the test duration.

The study design and the number of patients included are not adequate to make a claim of true test sensitivity and specificity. The high sensitivity of the method is in part related to the fact that many patients had quite advanced scotomas that were easy to detect, but even those subjects with early scotomas were confirmed. Also our jack-knife classification of the normals (using themselves as a data base to reassess their results) does not provide a true measure of test specificity. Further studies will need to look at the test in the clinical setting on larger numbers to gain an appropriate assessment of its performance. A larger range of normals for the data base would also be essential.

Objective VEP perimetry can therefore be used to reliably detect and confirm visual field loss in glaucoma, as defined by standard perimetry. It had good specificity and reproducibility in normals. The test is now in a format that is acceptable for patients and has the potential to be used in clinical practice. Longterm studies of high risk suspects currently underway will determine whether it can detect early deficits prior to subjective field loss, particularly with the use of inter-eye asymmetry analysis, and assess the long term fluctuation amongst established glaucoma patients.

Chapter 6

Development of a new multifocal stimulation system

Summary

Due to some of the limitations encountered when using the VERIS system for multifocal VEP recording, we set out to develop a new system that could be adapted to our specifications. The VERIS system did not provide a means for combining data from multiple channels. It was also limited by the fact that the complete recording sequence had to be completed before any data could be reviewed, either 7 minutes or 15 minutes. There was no input as to the quality of the recording and whether further runs were actually enhancing the data collected. The fixation target was static with no opportunity for a changing stimulus during the runs to maintain patient attention. We needed information on alpha rhythm levels to determine patient concentration and more data on sources of background noise. Furthermore, we wanted to devise a method of scaling the raw mVEP data according to each patient's own unique conduction characteristics, to reduce intersubject variability.

We therefore recruited a mathematician, Dr Iouri Malov, who specialised in data transmission and signal analysis, to devise a means of presenting a multifocal stimulus that could be assessed after each run. We worked closely with him through several concept stimulus modes until we settled on the method described below. His technique is described in a patent "Spread spectrum sequences" #USappl110/148650/PCT AU 00/01483. The patent was licensed to ObjectiVision Pty Ltd, a new company specifically formed with the intention of bringing to market an objective perimetry system using the multifocal VEP.

We also developed a custom electrode cross that could standardise our recording positions for 4 channel recording, and make application of the electrodes easier.

The new ObjectiVision multifocal recording system is described in section A below, and the means of signal scaling using underlying electroencephalogram (EEG) levels is covered in section B. The recording methods described in these sections are standard for subsequent chapters of this thesis unless otherwise specified.

My roles were to work closely with Dr Klistorner and Malov to achieve an algorithm that could be applied to meet our needs for a multifocal stimulus. We had to consider means for minimising cross contamination with the Kasami sequences. We determined through much experimentation that moving the sequence to a different site with each consecutive run was the most effective. I was involved closely with the design company to optimise the design of the electrode cross through several prototypes to make it robust, user friendly and suitable for all patients and head sizes. In the EEG scaling study (Section B) I was involved in assessing all normals, analysing the data and cowriting the paper (and patents) for publication.

A. ObjectiVision multifocal system – spread spectrum sequences

Methods

The ObjectiVision system employs a spread spectrum technique using a family of binary sequences to drive the visual stimulus. The sequences are known as Kasami sequences. The application of these sequences to drive a multifocal stimulus was developed by Dr Iouri Malov, and is described in detail in his Patent #USappl110/148650. Two opposite checkerboard pattern conditions undergo pseudo-random binary exchange at each of 58 sites in the visual field. Each input (stimulation site) is modulated in time according to a different sequence (in contrast to m-sequences where the same sequence is used but shifted in time). The technique permits computation of the resulting signal by cross-correlation of the response evoked by the sequence stimulation, with the sequence itself. Short sequences of 4096 elements are used, which result in 55 seconds of recording time for each run. Further runs then use different sequences for the same stimulation site to reduce the potential for cross-contamination. Results can be viewed on screen after each run, then on-line averaged and the recording terminated when stable signals are achieved.

The visual stimulus is generated on a computer screen (22" Hitachi high resolution display, Hitachi Ltd, Tokyo, Japan) with stimulation rate 75 Hz. 56 close-packed segments in a dartboard configuration are used, with two additional segments located in the nasal step region. The segments are cortically scaled with eccentricity to stimulate approximately equal areas of cortical (striate) surface (see Figure 1). The cortical scaling produces a signal of similar amplitude from each stimulated segment. Figure 1b shows an example of a trace array from a normal subject. Each segment contains a checkerboard pattern (16 checks) with the size of individual checks being proportional to the size of the segment and therefore also dependent on eccentricity.

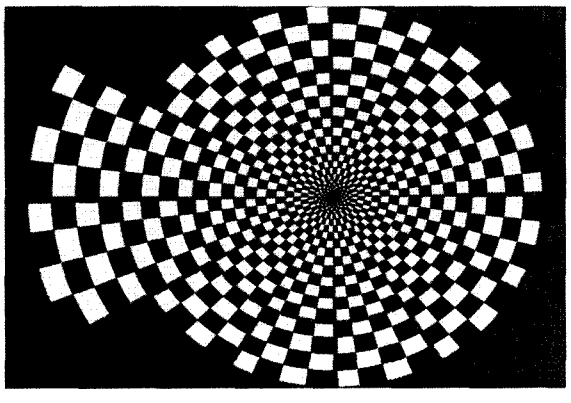


Figure 1a New cortically scaled dartboard stimulus with nasal step region added

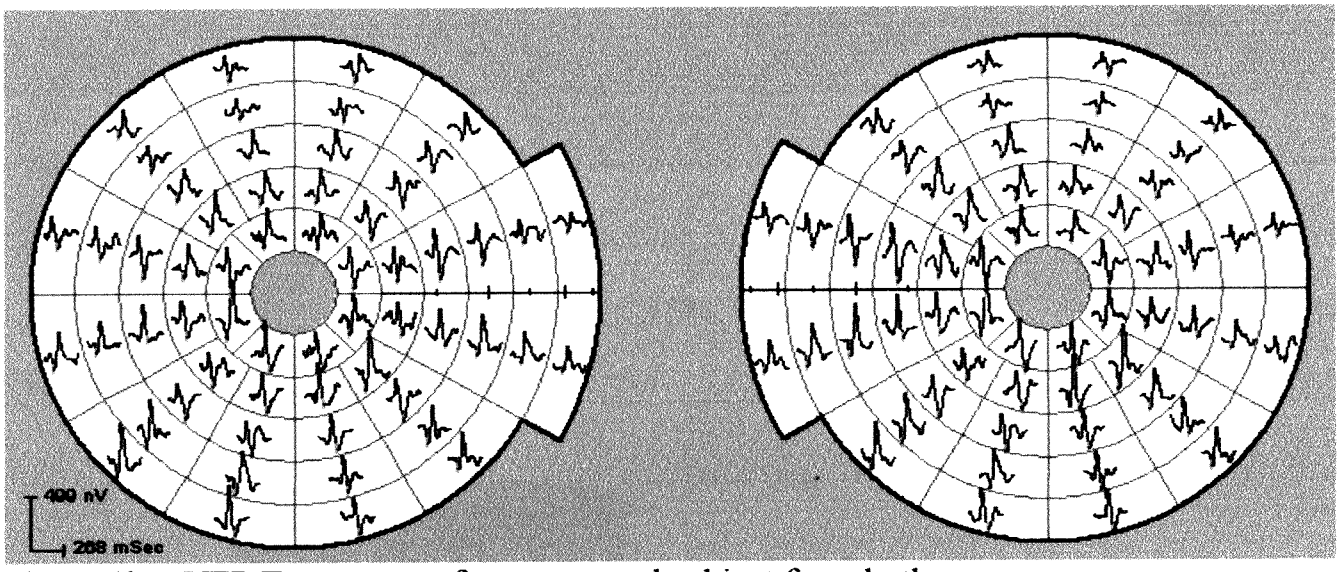


Figure 1b mVEP Trace arrays from a normal subject from both eyes

The central area of 1 degree is not stimulated but is used as a fixation monitor. Numbers of similar shape (3, 6, 8 or 9) are displayed in random sequence and the subject is asked to respond by pressing a button when a particular number appears. This ensures good concentration throughout the recording, and a percentage of missed and incorrect responses is calculated automatically after each run.

Luminance of the white check is 146 cd/m^2 and luminance of the black check 1.1 cd/m^2 producing Michelson contrast of 99%. Background luminance of the screen is maintained at a mean level of 73.5 cd/m^2. A dim room light is always on.

Subjects are seated comfortably in a chair and asked to fixate on the fixation number at the centre of the stimulus pattern. The distance to the screen is 30 cm, corresponding to a radial subtense for the stimulus of 26°, not including the additional nasal step (34°). All subjects are optimally refracted for near and the pupils are not dilated. All recordings are collected using monocular stimulation. Data is recorded using a Grass 4 channel amplifier Model 15 Neurodata (Astro-Med Inc, Rhode Island, USA). The signal is amplified 100,000 times and band-pass filtered between 3 and 30Hz. The data sampling rate is 450 Hz. Raw data is scanned in real time and EEG spikes exceeding 3 SE are excluded from the analysis. Runs contaminated by a high level of noise are also rejected. Usually eight runs are recorded to provide a good signal-noise ratio.

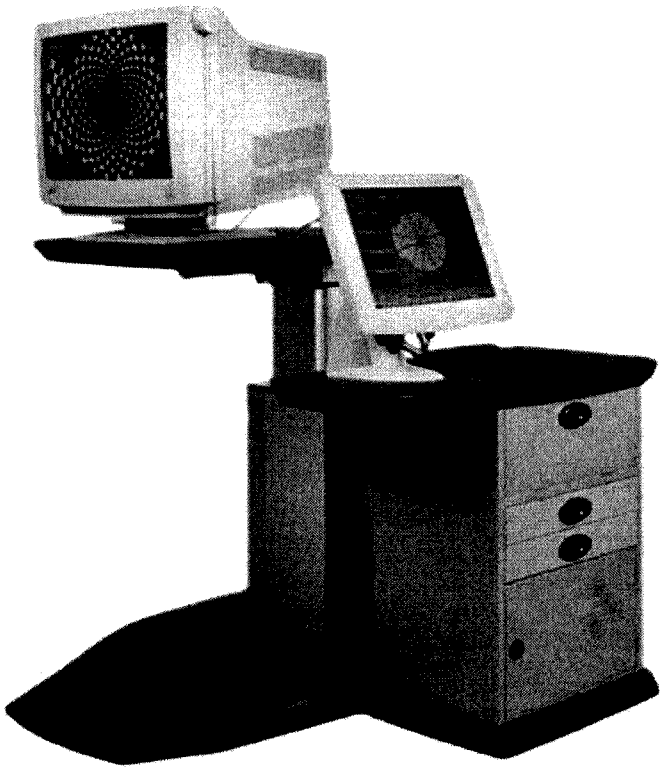
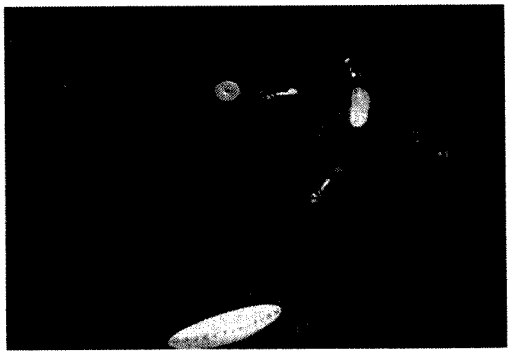


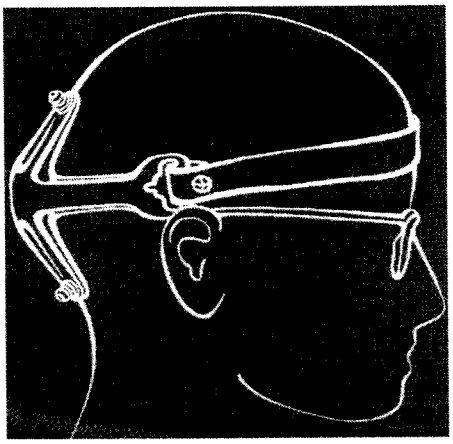
Figure 2 AccuMap system for multifocal VEP objective perimetry

Electrode positions

Gold disc Grass electrodes (Astro-Med Inc, West Warwick, RI, USA) are used. A custom designed occipital cross electrode holder pre-determines the four electrode positions [199] (see figure) It is lightweight and comfortable for the patient, and neck muscles remain relaxed. There are four electrode plugs into which the standard Grass gold cup electrodes are clipped with the wire exiting via the notch. Each plug fits into one of the positioning holes on the cross. The plugs are hollow to permit electrode gel to be injected into the cup once it is positioned. The scalp is cleaned with Nuprep (D.O.Weaver & Co, Aurora, CO, USA) at each site prior to finalising the electrode position. All recordings were performed with the level of resistance between electrodes being under 3 kΩ. Four channels are used as described previously [199] to cover different underlying dipole orientations. The lower midline electrode is negative for the vertical and oblique channels, while the left horizontal electrode is negative for the horizontal channel.

Figure 3a– ObjectiVision headset cross for electrodes
Figure 3b – schematic of cross held by headband





Analysis

VEP traces are analyzed using the custom designed software. Peak-to-trough amplitudes for each wave within the interval of 60-180 msec are determined and compared among channels for every stimulated segment of the visual field. The wave of maximal amplitude from each point in the field is automatically selected and a combined topographic map is created by the software. The combined trace array is then used for further analysis.

The inter-eye asymmetry [195] is also calculated for every segment of the tested visual field by dividing the difference in amplitude between left and right eyes by their sum:

Relative Asymmetry Coefficient (RAC) = (Amp1-Amp2)/(Amp1+Amp2)
Where Amp1 is a maximal peak to trough amplitude of the response at particular segment from the left eye, Amp2 is a maximal peak to trough amplitude of the response of the same segment from the right eye. To analyse asymmetry variations RAC standard deviation values were calculated for each point of the visual field (coefficient of variation could not be calculated for the RAC since it has both positive and negative values).

Once the system was validated in pilot trials a database of normals was collected so that this could be used to prepare probability plots for amplitude deviation, asymmetry deviation and latency compared to normative values.

B. Electroencephalogram-based scaling of mVEP reduces inter-subject amplitude variability

Published as
Klistorner, A, Graham, S.L., *Electroencephalogram-based scaling of multifocal VEP: effect on inter-subject amplitude variability.* Invest Ophthalmol Vis Sci, 2001. 42(9): p.2145-2152.

Abstract

Purpose: The inter-individual variability of the visual evoked potential (VEP) has been recognised as a problem for interpretation of clinical results. This study examines whether VEP variability can be reduced by scaling responses according to underlying electroencephalogram (EEG) activity.
Method: The ObjectiVision™ multifocal objective perimeter provided different random check patterns to each of 58 points extending out to 34° nasally. A multichannel visual evoked potential (VEP) was recorded (bipolar occipital cross electrodes, 7 mins/eye). 100 normal subjects (age 58.9±10.7 years) were tested. The amplitude and inter-eye asymmetry coefficient for each point of the field was calculated. VEP signals were then scaled according to underlying EEG activity recorded using Fourier transform to quantify EEG levels. High alpha-rhythm and electrocardiogram contamination were removed prior to scaling.
Results: High inter-subject variability was present across the visual field with females > males in amplitude. The variability for all left eyes was 42.2± 3.9%, for right eyes 41.7 ±4.4% (co var). There was a strong correlation between EEG activity and the amplitude of the VEP, left eye r= 0.83 (P<0.001) and right eye r=0.82 (P<0.001). When this was used to normalise VEP results the variability coefficients dropped to 24.6±3.1% (p<0.0001) and 24.0±3.2% (p<0.0001) respectively. The gender difference was effectively removed.
Conclusions: Using underlying EEG amplitudes to scale an individual's VEP response substantially reduces inter-subject variability, including differences between males and females. This renders the utilisation of a normal database more reliable when applying the multifocal VEP in the clinical detection of visual field changes.

Introduction.

Recent advances in clinical electrophysiology [137, 165, 166, 169, 181, 188, 193, 194, 199] have extended the possible applications of the visual evoked potential (VEP) to objective visual field mapping. However, a high level of inter-subject variability is one of the major factors limiting clinical use of this technique [169]. This variability has been recognised in standard central field VEPs and has been attributed to many factors such as age, sex, cortical convolution, position of the calcarine fissure relative to external landmarks (inion), eccentricity of the visual field tested, resistance between scalp and electrode, conductivity of underlying tissues (bone and subcutaneous fat thickness and blood circulation), general level of brain activity, and stimulus conditions[169, 193, 200-210]. While some factors (i.e. cortical convolution) are difficult to eliminate, others

(such as age, sex, scalp conductivity and level of brain activity) when compensated for could potentially reduce the amplitude variation.

Basar et al., and Rahl et al., demonstrated an inverse relationship between amplitudes of alpha (8-13 Hz) or theta (4-7 Hz) components of the spontaneous EEG activity measured immediately before stimulation, with subsequent frontal VEP amplitudes [208, 211]. They surmised that a high theta rhythm, (which is associated with drowsiness), was associated with a suppressed VEP. When stimuli were only applied if the root mean square value of the ongoing EEG at the lead F4 was below an individual threshold level (so called 'selective stimulation') the amplitude of the VEP significantly increased.

With relation to sex it was shown that the amplitude of the VEP is larger in females (particularly in older women) compared to males [202, 204, 212]. Interestingly, in was attributed to unusually high responsiveness of the visual system of older females to patterned stimuli [202]or to the level of estrogen [213].

With VEP latency, a significant age effect (increasing latency with age) was demonstrated, but not in relation to gender [214]. The converse held true for VEP amplitude however, with no age effect being observed [212, 215, 216]. Dependence of amplitude variability on visual field eccentricity was demonstrated with the coefficient of variability of amplitudes of the waveforms in mid-peripheral locations being larger than those of the more central areas [207]

There have also been attempts to bypass the problem of between-subject differences in cortical anatomy by inter-eye comparison (asymmetry analysis) in the multifocal VEP interpretation [193, 195]. Underlying cortical convolution and position of the visual cortex relative to external landmarks are major contributors to inter-subject VEP variability, but they influence the signals for the two eyes of a subject equally.

In a pilot study we identified a strong correlation between background EEG levels recorded simultaneously with multifocal VEP stimulation, and the mean amplitude of the VEP. We surmised that the amplitudes of the two responses were correlated because the conduction of electrical signals across the skull, skin, subcutaneous tissue and electrodes was altered proportionately for the two signals. Therefore, it might be possible to use the underlying EEG levels to scale VEP signals for each patient relative to a normal database, and thus reduce intersubject variability. It might also to a lesser extent reduce intra-subject variability if there were variations between tests in tissue/electrode conductivity. Since EEG activity is not totally independent of visual activity, for example alpha rhythm levels vary between subjects and are suppressed by visual attention, other factors in the raw EEG signal must be taken into consideration.

Reducing inter-subject variability is crucial in the identification of normal and pathological results, while a low intra-subject variability is important for detection of progression of the disease. The aim of this study was to investigate variability between

and within subjects for the multifocal VEP, and to determine if an EEG based scaling algorithm could be employed to effectively reduce this variability.

Methods

Subjects

A total of 100 normal subjects (44 males and 56 females) participated in this study. The mean age was 58.9±10.7 years (range 21-80; men 58.2±11.1 yrs, women, 59.0±9.9 yrs). The study protocol was approved by our regional ethics committee and informed consent was obtained from all subjects. All participants were examined by an ophthalmologist. They had normal intraocular pressure and ophthalmoscopy, and no family history of glaucoma or retinal dystrophy. All performed normal Humphrey 24-2 field tests, confirmed by a normal result on the glaucoma hemifield test analysis. The inclusion criteria for the study required a corrected visual acuity of 6/9 or better and pupils at least 2.5mm without dilation. Subjects with diabetes, previous cataract surgery or any other ocular disorders were excluded. To examine intra-subject variability, 15 subjects were also tested on 5 separate occasions on different days.

Stimulation and recording.

A multifocal VEP was recorded using the ObjectiVision™ OV-1 perimeter (ObjectiVision Pty Ltd, Sydney, Australia). In order to quantitatively analyse the relationship between the EEG and VEP a Fourier power spectrum (Fast Fourier Transform-FFT) of the EEG for each recorded channel was calculated (Fig. 4a). It was noted that in some subjects there was a large peak in the FFT at around 8-10 Hz which was attributed to alpha-rhythm (Fig.4b). In some subjects there was also a strong electrocardiogram contribution. The electrocardiogram had previously been noted in several subjects during real time recording, seen as spikes that were synchronous with the subject's pulse. These were identified in the FFT (Fig.4c). In order to exclude the influence of these two components the Fourier power spectrum within the interval 0-30Hz was fitted with a polynomial function of 4^{th} order and the integral of the fit was calculated. Average values of the integral from all 100 subjects were obtained for each channel.

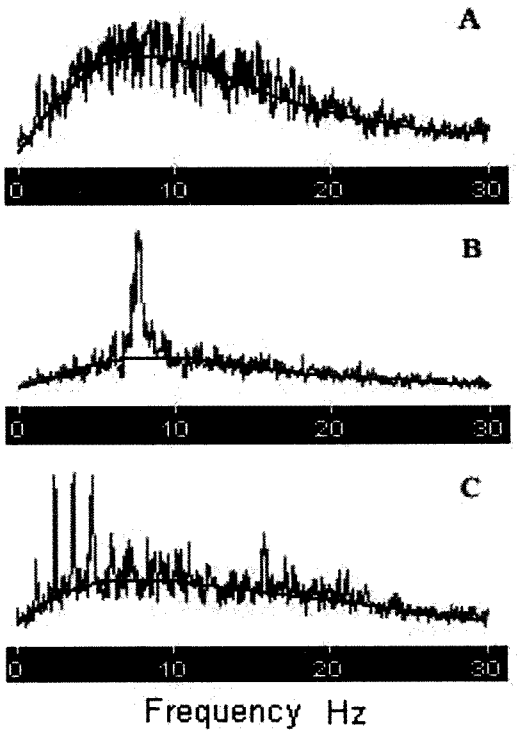


Fig.4 Fourier power spectrum calculated within the interval 0-30Hz and fitted with a 4th order polynomial function. Vertical scale arbitrary.

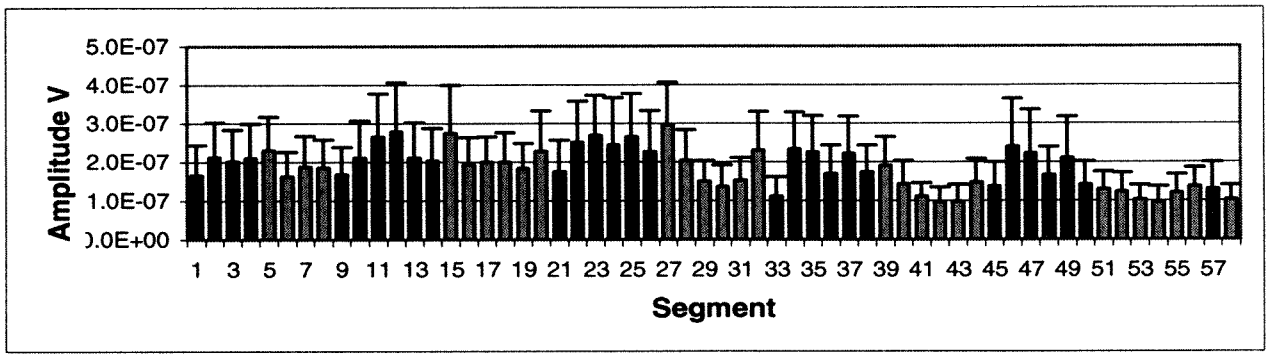
- **a.** typical example in normal subject
- **b.** subject with high alpha-rhythm spike
- **c.** subject with high ECG contamination. The multiple Fourier peaks correspond to the actual frequency of the ECG waveform (not to the pulse rate which is quite different). The distance between peaks corresponds to the pulse rate, for instance in a case presented the interval between peaks is approx. 1.15 Hz, which corresponds to pulse rate of about 70/minute.

Results

Inter-subject variability.

The VEP traces for all 100 normals were examined for both unscaled and scaled data. The mean (± SD) of the VEP amplitude for each segment of the visual field is presented in Fig.5. There is a noticeable reduction of the amplitude in the upper part of the visual field of both eyes, which is in agreement with previously reported results [165, 207]. Generally, the amplitude also tends to decrease toward the periphery.

A



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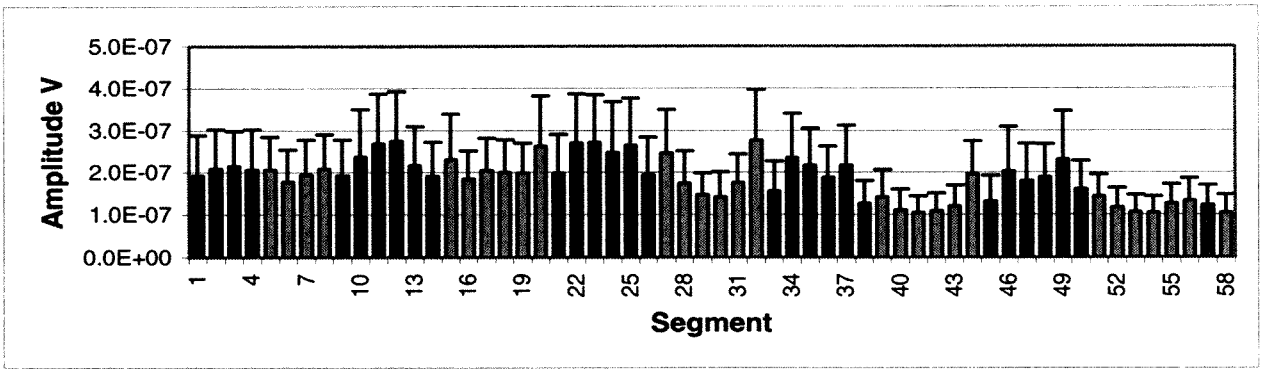

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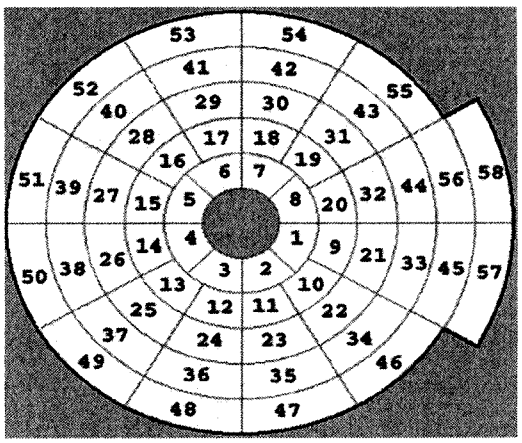


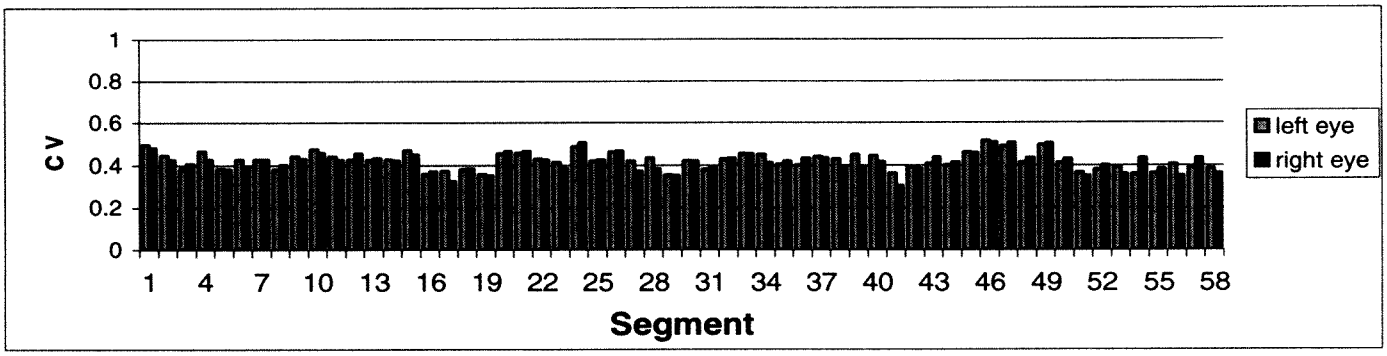
Fig.5 The mean (± SD) of the VEP amplitude for each segment of the visual field for all 100 subjects. The lower hemifield segments are in darker grey, upper field segments light grey.

a. left eyes
b. right eyes
c. topography of the segments

The inter-subject variability in amplitude for each segment of the tested visual field for both eyes is presented in Fig.6a, b. High inter-subject variability was present across the visual field and similar between the two eyes (Fig.6 a). The CV for all left eyes was 42.2± 3.9%, for right eyes 41.7 ±4.4%. There was no change in variability with

eccentricity of the visual field stimulated (Fig. 4 b) despite the fact that amplitudes were smaller with eccentricity.

A



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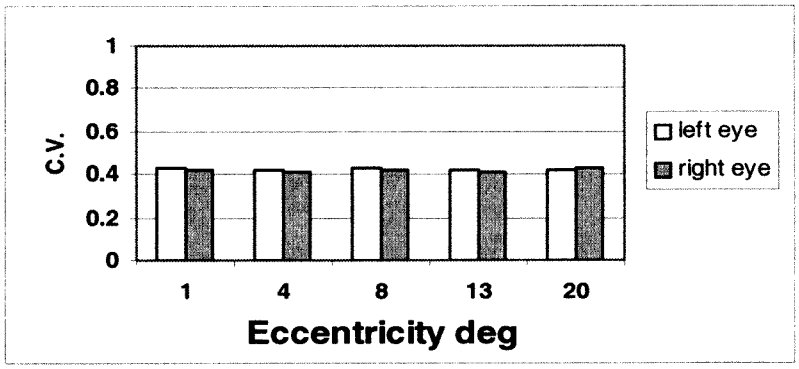

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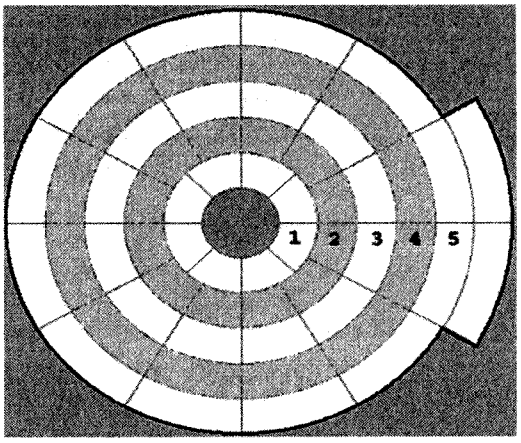

Fig.6 a. Coefficients of inter-subject amplitude variability prior to EEG scaling for each segment of the visual field (100 subjects). Right and left eyes shown. Segment numbers as for Figure 3c.

b. Coefficients of inter-subject variability prior to EEG scaling averaged over rings of different eccentricity. Shows no increase in variability with eccentricity

c. Ring topography

The variability between subjects was also calculated for the mean amplitude averaged across the visual field of each subject. The CVs for the mean amplitude of the left and right eyes were 27.5% and 28.5% respectively.

There was a strong correlation between EEG activity and the amplitude of the VEP. Figure 5 displays the relationship for all subjects tested between VEP amplitude of the vertical channel averaged over the whole visual field and the related Fourier power spectrum of the raw EEG data of the same channel collected during VEP recording. The integral of the FFT fit was used as an EEG measure. The correlation coefficient defined by linear regression analysis was for the left eye r= 0.83 (P<0.001) and for the right eye r=0.82 (P<0.001). The high level of correspondence between the amplitudes of two electrophysiological responses measured simultaneously suggests that VEP amplitude variability is likely be attributed to the same factors which influence EEG amplitude.

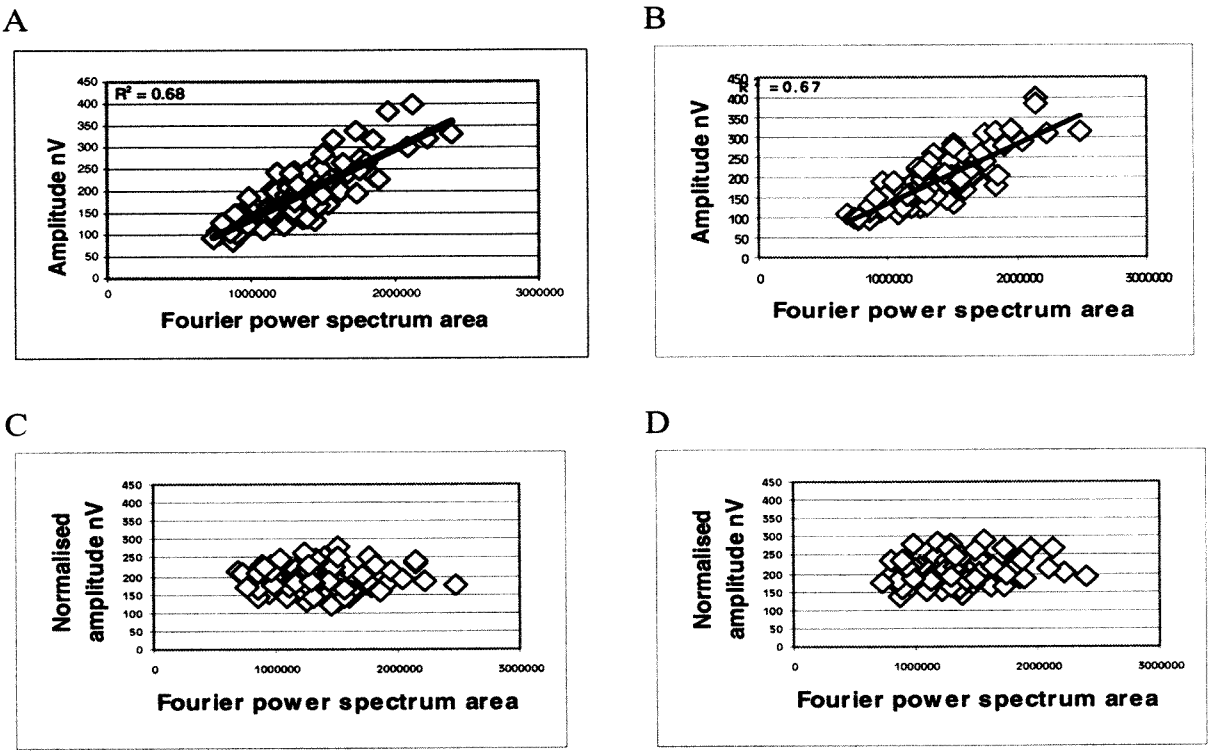

Fig 7a and b Correlation between EEG activity and the mean VEP amplitude for the vertical recording channel. Fourier power spectrum expressed in arbitrary units. (a) left eyes, (b) right eyes

5c and d Amplitude of the mean VEP after EEG-based normalisation. (a) left eyes, (b) right eyes

Normalization of the VEP signal was performed for each run separately. The following formula was applied to raw EEG data:

$$VEPnor = VEPrec \times \{(Faver-Xint)/(Frec-Xint)\}$$

where:
VEPnor - normalised VEP
VEPrec - recorded VEP

Faver – average of Fourier power spectrum from all 100 subjects
Frec – Fourier power spectrum recorded for particular run of particular subject,
Xint – interception of the trend line with horizontal axis.

The result of normalization is presented in Fig.7 and Fig.8. There was a significant reduction in inter-subject variability in both averaged VEP amplitude and VEP amplitude of individual sectors. The variability coefficient of VEP amplitude averaged across the whole visual field (Fig.5) fell by more then 46% for both eyes ($p<0.01$) and reached 15.2% and 14.9% for LE and RE respectively. The amplitudes of the VEP after normalization appear to be much more closely grouped around the mean value.

Variability coefficients of the individual sectors of the visual field were also reduced by normalization procedure by 41.4±5.2% and 42.2±5.8% for LE and RE respectively. Figure 8 shows a comparison between non-scaled (grey background) and scaled (bars) variability, and demonstrates significant improvement in amplitude variability for each stimulated sector of the visual field. Coefficients of variability after amplitude scaling were 24.6±3.1% for LE ($p<0.0001$) and 24.0±3.2% for RE ($p<0.0001$).

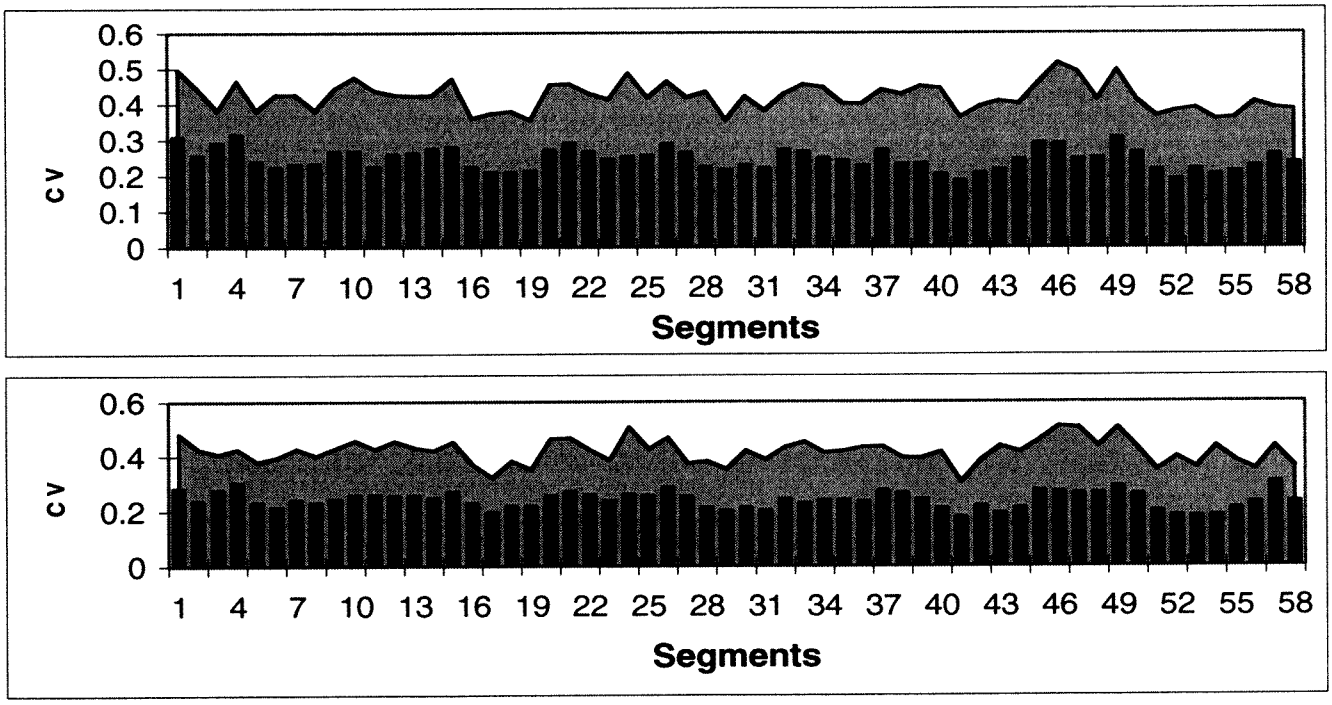

Fig.8 Comparison between non-scaled (grey background) and scaled (vertical black bars) coefficients of variability (CV) for each stimulated segment of the visual field. Segment topography as in Fig.3c. (a) left eyes, (b) right eyes

Examples of EEG scaling are shown in Figure 9. The first subject had a low EEG amplitude and lower than usual VEP signal, which was scaled up by the normalisation

procedure. The second subject had a high VEP signal and very high EEG signal. His VEP traces were scaled down by normalisation. The resultant trace arrays are much more similar in amplitude than they were prior to normalisation.

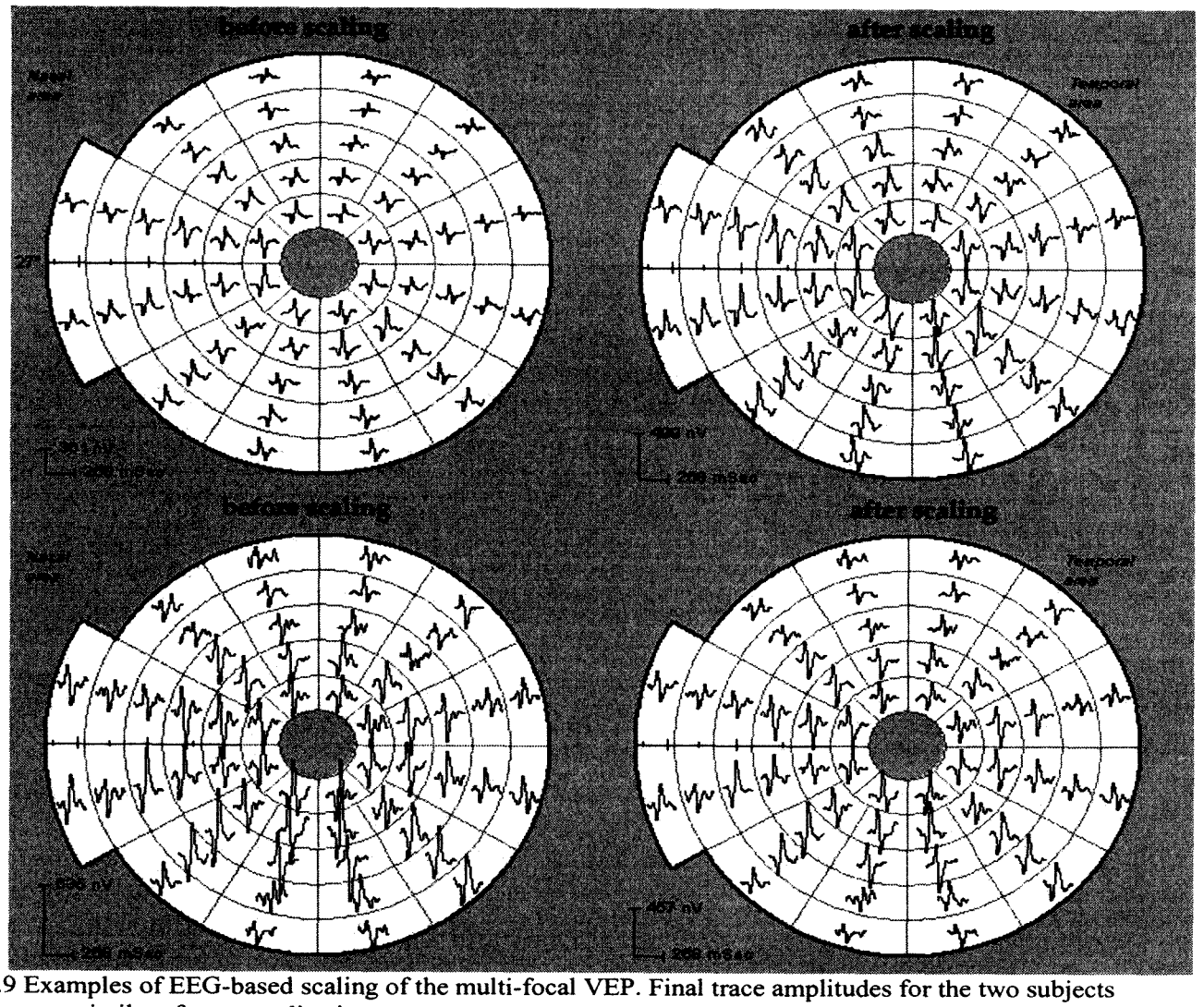

Fig.9 Examples of EEG-based scaling of the multi-focal VEP. Final trace amplitudes for the two subjects shown are similar after normalization.

a. Small VEP amplitude scaled up based on smaller than average EEG signal in that subject
b. Large VEP amplitude was scaled down based on high EEG signal in that subject

There was practically no relationship between the amplitude of the VEP recorded in this study and the age of the subjects neither before nor after scaling (see Fig. 10 a, b,c,d). Age vs amplitude correlation coefficients before scaling were r= 0.049 (P>0.5) and r=0.0094 (P>0.5). and after scaling r=0.1 (P>0.5) and r=0.05 (P>0.5) for the left and right eye respectively.

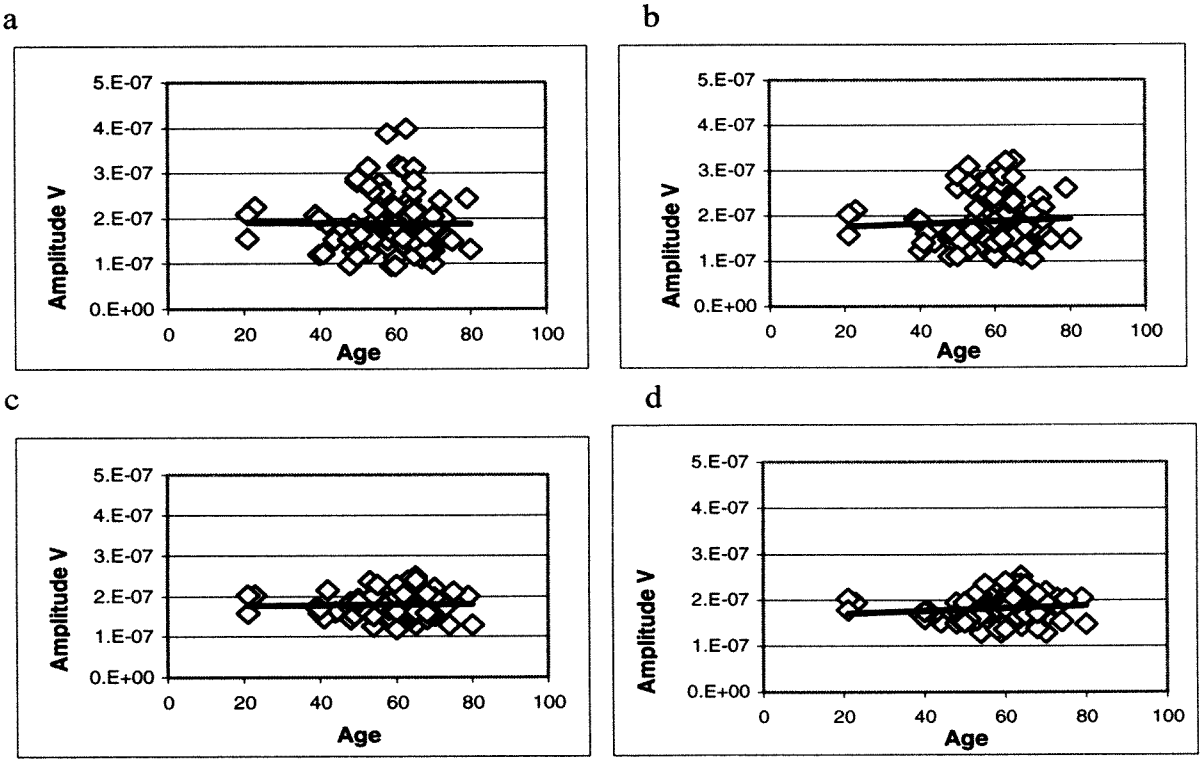

Fig.10 Mean VEP amplitude vs age of the subjects before (a-left eye, b-right eye) and after (c-left eye, d-right eye) EEG scaling.

There was however a significant difference in amplitude between male and female groups before scaling (Fig.8). The averaged amplitude in females was 209□V and 210□V compared to 158□V and 156□V in males for left and right eye respectively ($p<0.00001$ for both). The variability was less after subjects were separated into gender groups (Table 1). The gender-based amplitude difference was removed by EEG scaling. Mean amplitudes in females group were reduced to 180□V for both eyes, while mean amplitudes in males group increased to 182□V and 178□V for left and right eye respectively ($p=0.69$; $p=0.16$).

Table 1 VEP variability for groups before and after scaling

Groups	Individual segments pre-scaling	Individual segments after scaling	Averaged response pre-scaling	Averaged response after scaling
Whole group				
LE	41.4±5.2	24.6±3.1	27.5	15.2
RE	42.2±5.8	24.0±3.2	28.5	14.9
Female				
LE	39.8±5.4	24.2±2.8	25.8	15.0
RE	40.1±5.2	24.1±3.0	25.9	14.9
Male				
LE	39.3±4.8	24.4±2.9	24.9	14.8
RE	39.8±4.4	23.8±3.2	25.3	15.0

Data are percentages. Columns 2 and 3 show mean±SD for CV. Columns 4 and 5 show mean±SD CV of mean amplitude. Note CVs for all groups are less after scaling.

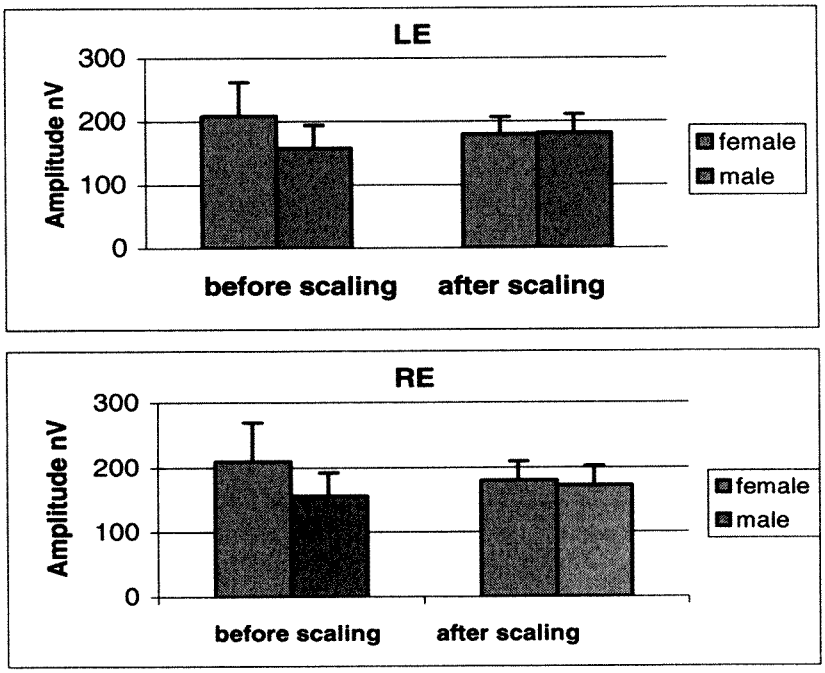

Fig.11 The mean VEP amplitude in females and males before and after EEG-based scaling. Shows amplitudes of the two groups are similar after scaling, and standard deviation within groups is reduced.

Discussion.

Multi-focal VEP recording provides a unique opportunity for objective detection of the visual field defects. However, high inter-subject variability due to variation in cortical anatomy made some authors to conclude that it would not be useful for clinical testing [169]. It was argued that in some locations even extreme damage of the visual pathway may not be reliably distinguished from normal values [193]. This study demonstrates that using underlying EEG amplitudes to scale an individual's VEP response substantially reduces this variability, including differences between males and females. This makes utilisation of a normal database viable in the clinical detection of disease.

The inter-subject variability assessed after derivation of the VEP by cross-correlation of the raw EEG data with stimulating sequences was evenly distributed across the visual field and very similar between the two eyes. VEP amplitude did not correlate with age, which is in accordance with previous reports [212, 215, 216]. However, there was a well-defined difference in VEP amplitude between genders with the female group demonstrating significantly higher amplitudes than the males. Slight decreases in variability of gender groups compared with the tested population as a whole indicated

that some of the inter-subject VEP variability was gender-based. EEG scaling of the VEP was able to effectively remove the differences between the sexes.

The VEP amplitude was highly correlated with raw EEG data collected during the recording. The high correlation (r^2>0.65) suggests that similar influences account for the amplitude variability of these two electrophysiological measures [217]. Since the VEP signal is tiny in comparison to the EEG (approx 1000x) it does not make a significant contribution to the overall EEG amplitude and is only identified by cross-correlation techniques. We propose that normalisation of the VEP by the EEG removes the common source of variability. We suspect that the differences between individuals are to a large extent conductivity differences affecting transmission of the signal from the cortex to the scalp.

The scaling applied in this study to the VEP amplitude reduced inter-subject variability greatly. An inter-subject variability coefficient of the averaged VEP amplitude dropped by more then 46% while individual segments of the stimulated visual field improved variability by around 40%. The scaling procedure did not affect the independence of the VEP amplitude with age. However, the clear difference in amplitude of the VEP between genders before scaling was eliminated by EEG-based normalization.

It is critical to determine the components of the raw EEG signal before applying any scaling. Some individuals have high alpha-rhythm activity even when they are visually attentive. If this is included in the scaling the VEP response will be artificially scaled down. High alpha-rhythm may also indicate that the patient is not concentrating, and can provide some real time feed-back to the recording technician, especially if it appears half-way through the recording. It could also be present in malingerers who are deliberately defocusing. Our system cannot differentiate the cause of a high alpha-rhythm, but it needs to be excluded from any scaling algorithm employed.

There are other measures which can be taken to attempt to reduce variability. The randomly changing fixation number in the central screen helps to keep the subject concentrating and mentally alert. It provides a partial index of reliability, and reduces the mesmerizing effects of the multifocal display which can cause some patients to fatigue. Breaks between runs and the presentation of an alternative image between runs (eg scenic photographs) also help in relaxation and preventing fatigue during the test.

The upper band pass filter is set to 30 Hz. This is relatively low and outside ISCEV standards for conventional VEP recording. We have deliberately chosen this since it removes high frequency noise that can ruin some recordings. We have performed comparisons on the same subjects, both normals and subjects with glaucoma, and find minimal differences between 100Hz and 30Hz cut-off other than a slight increase in latency. Fourier analysis shows no high frequency components in the VEP that have been removed by filtering (unpublished data).

The remaining variability is probably due to differences in the cortical convolutions of the striate cortex. Our multichannel recording technique proved superior

to single channel methods [199] and helped to some extent to overcome this by sampling dipoles of different orientation. The fixed electrode cross used in recording standardises electrode positions, which may also help to overcome differences. Standardising distance to the screen may increase reproducibility, and a tracking device has been developed for this purpose. However, we still need to derive a source localisation technique which will accurately pick up responses from all underlying anatomical variations. Several different approaches to this problem are currently under review.

In conclusion, by the application of EEG scaling to VEP responses, a considerable problem in objective visual field mapping can now be largely overcome. Inter-individual variability is halved, which allows meaningful comparisons with normal databases and increases the sensitivity of the test to the detection of disease.

Chapter 7

Application of the new mVEP technique to glaucoma detection

Published as Goldberg, I., Graham, S. and Klistorner A. *Multifocal objective perimetry in the detection of glaucomatous field loss.* **Am J Ophthalmol.,** 2001, 133, p:29-39

Abstract

Purpose: To test the ability of a new type of multi-focal objective perimetry (MOP) to identify glaucomatous visual field defects.
Design: Cross-sectional study
Methods: The ObjectiVision™ System provides different random patterns to each of 58 zones extending out to 32° nasally. A multichannel visual evoked potential (VEP) was recorded (bipolar occipital cross electrodes, 7 mins/eye). 100 patients with confirmed glaucomatous visual field defects (age 62.2±9.8, mean MD –6.5±4.17dB) were tested and compared with the normal database of 100 normals (age 58.9±10.7). Both eyes were tested, but for determining sensitivity the eye with the lesser field defect was chosen if both had a scotoma that qualified. The amplitude and inter-eye asymmetry coefficient for each zone of the field was calculated. A MOP glaucoma severity index was calculated for each subject.
Results: In 95(95%) glaucomas Humphrey field defects were demonstrated by VEP amplitude reductions identifying a cluster of ≥3 abnormal zones. In 2/5 remaining cases the defect was detected on the inter-eye asymmetry analysis. Topographic location was strongly correlated with Humphrey fields. Mean amplitude was significantly reduced in 86(86%) of the glaucoma cases. There were 37 glaucoma cases with a perimetrically normal fellow eye. In 22 (59.4%) of these the MOP showed an abnormality in the second eye. The glaucoma severity index was abnormal in 93 glaucoma cases and showed a strong correlation with Humphrey MD (r=0.67 right, 0.69 left eyes).
Conclusions: MOP can assess the visual field and identify glaucomatous visual field defects. It may have the potential for identifying defects earlier than conventional perimetry.

My role in this study included : discussion and design of the study together with Dr Klistorner, recruitment of suitable glaucoma subjects together with Dr Goldberg. I personally examined all 100 normals in the database, and verified the classification of all 100 glaucoma subjects, jointly analyzing all results and cowriting the manuscript with both Drs Goldberg and Klistorner. Our technician did the actual recordings.

Introduction

Objective assessment of the visual field using multifocal visual evoked potentials (MVEP) has now been described as a potential clinical technique [165, 188, 195, 199, 218]. The objective nature of this type of test has definite clinical advantages over subjective methods. This study presents early results using the ObjectiVision™ multifocal objective perimeter, a system designed specifically for the investigation of glaucomatous visual fields. The technique is termed multifocal objective perimetry (MOP).

Sutter's group first recorded a MVEP [169]. They used the method of pseudo-randomly presented multifocal stimulation together with cortical scaling of the size of stimulated patches. They concluded that it could not be applied clinically due to the high variability of responses. We modified the technique and recorded successfully MVEPs from all areas of the field up to 26 degrees of eccentricity in normal subjects using a bipolar electrode position straddling the inion, termed bipolar occipital straddle (BOS)[165]. The electrode configuration was then further enhanced to a four-channel system employing an occipital cross electrode holder. This provided better signals from many zones, particularly along the horizontal meridian. It also reduced variability between subjects and standardised the electrode positions to improve reproducibility [199].

Comparison of VEP trace arrays recorded from both eyes of normal subjects had revealed remarkable similarities in the waveform and amplitude of the signals between the two eyes of the same individual [193, 195]. Even though different parts of the retina are being stimulated in the two eyes, the information from similar parts of the visual field of both eyes projects to identical areas of the visual cortex [173, 178, 183]. This activates the same cortical cells producing the same electrical dipoles. Therefore it is possible to compare eyes within individuals to look for localised areas of asymmetry in cases of early unilateral disease. This technique was able to identify VEP defects in eyes where the visual field remained normal[195, 199].

The ObjectiVision system was developed to provide further improvements on the above MVEP recording technique, which had utilised the VERIS™ system. The ObjectiVision system uses a spread spectrum technique to drive the stimulus, with different pseudorandom sequences for each stimulated area of the field, and different sequences for each zone with each consecutive run. The sequences are derived from specific families of pseudorandom binary sequences with low cross-correlation between family members and good auto-correlation properties. All sequences can therefore be presented simultaneously with no time delay. This allows on-line monitoring of the signal and review of results after short sequences of recording, which can then be included in the averaging if acceptable. This is in contrast to the VERIS system, which requires the full m-sequence to be completed before any results can be calculated and reviewed.

The ObjectiVision system also employs a scaling algorithm during the recording, which reduces inter-individual variability of signal amplitude. This is based on

underlying electroencephalogram amplitudes reflecting characteristic signal conduction for each individual [219].

This study presents the results of a trial of 100 glaucoma subjects tested with the ObjectiVision system. The results are compared with the ObjectiVision normals' database which has been collected by testing 100 normal subjects. A reproducibility study was also performed in a subgroup of normal subjects.

Methods

Subjects

The glaucoma subjects were recruited from a private tertiary referral glaucoma ophthalmic practice and normals from both the practice and the general community. Informed consent was obtained from all subjects and the study followed the principles of the Declaration of Helsinki. The study included 100 patients with glaucoma established on clinical diagnosis and field-testing, and 100 normal subjects.

The same ophthalmologist examined all normals. They had normal intraocular pressure and ophthalmoscopy, and no family history of glaucoma or retinal dystrophy. All performed normal Humphrey 24-2 SITA threshold field tests, confirmed by a normal result on the glaucoma hemifield test analysis [220], and showed no clusters of points that could constitute a scotoma as defined below for the glaucoma subjects. The inclusion criteria for the normal and glaucoma groups required a corrected visual acuity of 6/9 or better and pupils at least 2.5mm without dilatation. Subjects with diabetes, previous cataract surgery or any other ocular disorders were excluded.

Glaucoma patients who met the inclusion criteria, with established glaucomatous field defects and varying severity of disease, were consecutively recruited from the clinic over a six-month period. Patients had previously performed Humphrey 24-2 fields on two or more occasions, and had demonstrated reproducible field defects. Both eyes were tested so that asymmetry analysis could be performed but only one eye was evaluated for assessment of scotoma detection and overall sensitivity. The eye selected had a scotoma as defined below. If both eyes had field loss the eye with the lesser defect was chosen. For the study eyes the mean deviation on Humphrey fields ranged from -0.79 to –18.58 dB, mean –6.5±4.2dB. They had a mean age of 62.2±9.8 years (range 42-72), which was not significantly different from the normals 58.9±10.7($p=0.32$, t-test). There were 55 males and 45 females. To examine reproducibility, 15 of the normal subjects were each tested on 5 separate days.

The diagnosis of glaucoma required the following parameters: a confirmed visual field defect on Humphrey 24-2 and a glaucomatous optic disc as judged by stereo disc photography, with or without an intraocular pressure > 20 mmHg with the Goldmann applanation tonometer. The definition of a field defect used the pattern deviation plot on the Humphrey 24-2 program. A minimum scotoma required a cluster of 3 or more abnormal points including at least 2 points depressed by $p<0.5\%$ on the pattern deviation probability plot. The cluster of abnormal points could not cross the

horizontal meridian and points immediately above and below the blind spot could not qualify as part of the scotoma. Peripheral rim points could qualify as part of the overall scotoma, but at least 2 of the points qualifying as the nucleus had to be non-rim. The glaucoma hemifield test needed to be abnormal.

MOP recording.

A multifocal multichannel VEP was recorded using the ObjectiVision™ perimeter (ObjectiVision Pty Ltd, Sydney, Australia). The ObjectiVision system and recording methods are described in chapter 6. Briefly, the visual stimulus was generated on a high-resolution display with 56 close-packed segments in a dartboard configuration, with two additional segments located in the nasal step region (58 in total). The central area of 1 degree was not stimulated but was used as a fixation monitor. Numbers of similar shape (3, 6, 8 or 9) were displayed in random sequence and the subject was asked to respond by pressing a button when a particular number appeared. This ensured good concentration throughout the recording, and the percentage of missed and incorrect responses was calculated automatically after each run.

The distance to the screen was 30 cm, corresponding to a radius of the stimulus of 26° with an additional nasal step out to 32°. All subjects were optimally refracted for near, the pupils were not dilated and monocular stimulation was used. MVEPs were recorded using a four channel system as described previously [199] to cover different underlying dipole orientations. The custom designed occipital cross electrode holder pre-determined the four electrode positions [199]. Usually eight runs of 55 seconds each were recorded to provide a good signal-noise ratio.

Analysis

Raw trace data were analyzed using custom designed software. A Fast Fourier analysis is performed on the data. Alpha-rhythm spikes and electrocardiogram effects are removed, before calculating a scaling factor for each run, to scale results up or down depending on the characteristic signal conduction for each individual[219]. See full description in Chapt 6 Section B.

Maximal peak-to-trough amplitudes for each wave within the interval of 60-180 msec were determined and compared among channels for every stimulated segment of the visual field. The wave of maximal amplitude from each zone in the field was selected and a combined topographic map created by the software. Figure 1 shows an example of an MOP trace array from a normal subject. From the 100 normal subjects a normal database was compiled, including mean and SD values of VEP amplitude for every stimulated area of the visual field.

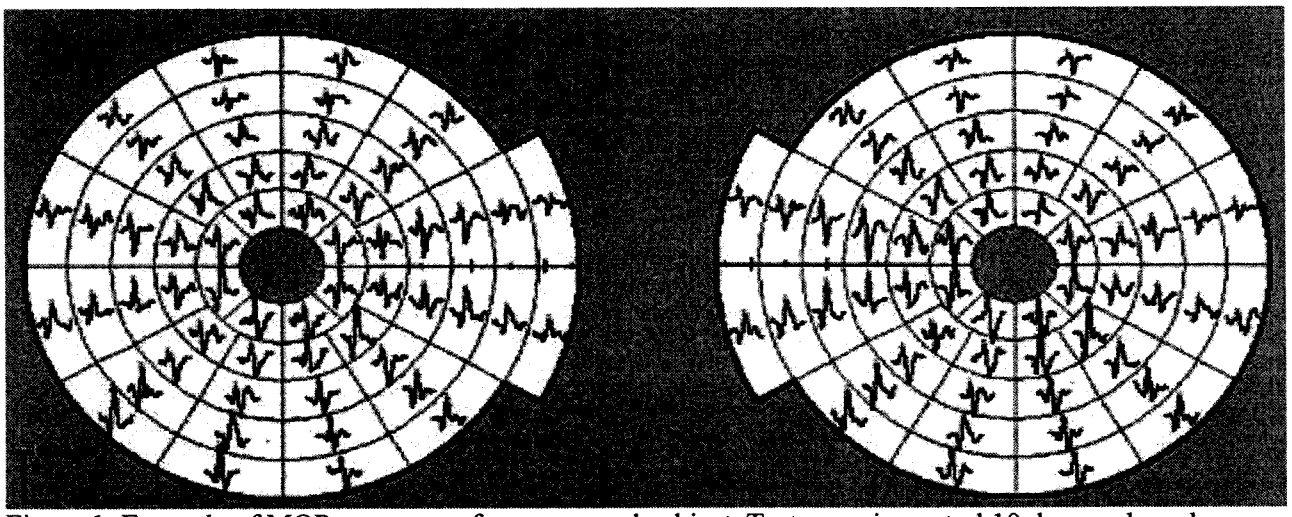

Figure 1 Example of MOP trace array from a normal subject. Test areas in central 10 degrees have been relatively enlarged for graphical representation, but are derived from the smaller central check areas.

Mean amplitude for all zones across the field was calculated and compared for the two groups. VEP amplitudes for each individual zone in the combined trace array were compared with the normals database, and probability of abnormality plots were constructed. Zones with a difference in amplitude $p<0.05$ for that zone in the normal database were considered abnormal. A cluster of 3 abnormal zones on the threshold plot with at least one zone $p<0.02$ was classed as a potential scotoma. Peripheral rim zones could qualify as part of the overall scotoma, but at least 2 of the zones qualifying as the nucleus had to be non-rim.

The inter-eye asymmetry [195] was also calculated for every segment of the tested visual field as previously described in Chapt 4. A probability plot for asymmetry was also constructed based on the normal group's distribution of asymmetry between the two eyes. The definition of a scotoma on the asymmetry plot was a cluster of 3 abnormal zones on the threshold plot $p<0.05$, with at least 2 of the zones qualifying as the nucleus, non-rim zones.

An MOP severity index, which takes into account the total number of abnormal zones, their relative severity, and the asymmetry values, was calculated for each subject. This provides an overall index of whether the MOP result is within normal limits ($p>5\%$), borderline ($5\%>p>3\%$) or outside normal ($p<3\%$).

Results

The glaucomas showed substantial changes in their MOP results in almost all cases. Table 1 shows the mean values for the two groups for age, Humphrey global indices, MOP mean amplitude and the calculated severity index. Table 2 shows a summary of the sensitivity and specificity of various criteria used in the identification of the glaucoma cases.

Table1. Comparison of global indices of Humphrey visual field and MOP.

	Normals n=100	Glaucomas n=100	*p* value
Age	58.9±10.7	62.2±9.8	0.32
Humphrey MD	0.19±0 .97 dB	-6.5±4.17 dB	<0.000001
Humphrey CPSD	1.46±0.25	7.06±3.7	<0.000001
MOP mean amplitude	255 ±40 nV	157±42 nV	<0.000001
MOP abnormality index	5.6±8.0	112.9±74.6	<0.000001

Table 2. Sensitivity and specificity of MOP in glaucoma detection.

	Normals n=100	Glaucomas n=100
MOP averaged amplitude	5	86
MOP amplitude scotoma	3	95
MOP asymmetry scotoma	2	74
MOP amplitude and/or asymmetry scotomas	4	97
MOP index: abnormal	4	93

The mean amplitude of the MOP, which is a relatively global measure of function similar to Humphrey MD, showed a highly significant reduction ($p<0.00001$) compared with normals. Figure 2 shows the mean amplitudes for all eyes for both normals (one eye random choice) and glaucoma study eyes separately. For the normals mean amplitude was 255±42nV, while for glaucoma subjects mean amplitudes was 157±42nV ($p<0.000001$). The mean VEP amplitude was therefore substantially lower in the glaucoma eyes. However, this could not identify all individual cases. Figure 3 shows the individual values for mean VEP amplitude for all study eyes, and for all normals. The horizontal line represents two standard deviations from the mean of the normals. There are 5 normals below the line, and 86 glaucomas. The sensitivity of calculating the mean amplitude as a marker for glaucoma was thus 86%, if the specificity is set at 95%.

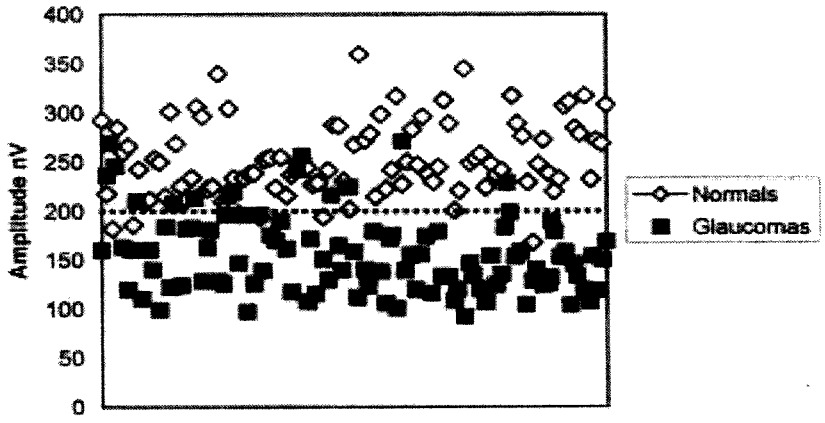

Figure 2 Mean MOP amplitudes across all zones for 100 normal eyes and 100 study eyes with glaucoma. Error bars show standard deviations.

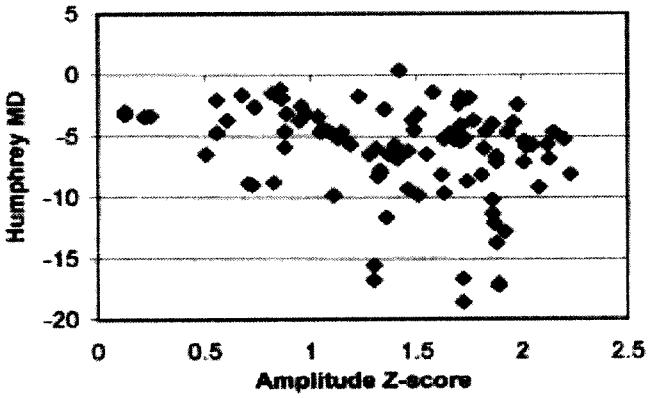

Figure 3 Individual subject mean MOP amplitude across all zones for each normal and glaucoma study eye. The line represents two standard deviations from the mean of the normals. The sensitivity was 86% if the specificity is set at 95%.

To identify localised field defects the criteria of a cluster of 3 zones $p<0.05$ with at least one zone $p<0.02$ (when compared to the normals) was used. This identified 95/100 study eyes. When asymmetry analysis was used the sensitivity was lower at 74/100, because the eye with the lesser defect was chosen for the analysis and in many cases this defect occurred in a part of the field already damaged in the other eye. When the criteria for abnormality included **either** the amplitude plot scotoma **or** asymmetry plot as abnormal then 97/100 cases were identified. The three cases that were not detected had early but definite defects that did not reach statistical significance on the MOP plots.

Figure 4 shows some examples of the range of Humphrey scotomas present in the glaucoma cases, and how they were identified by MOP. There was very close topographical correlation between the two tests in terms of the areas of the visual field that were affected.

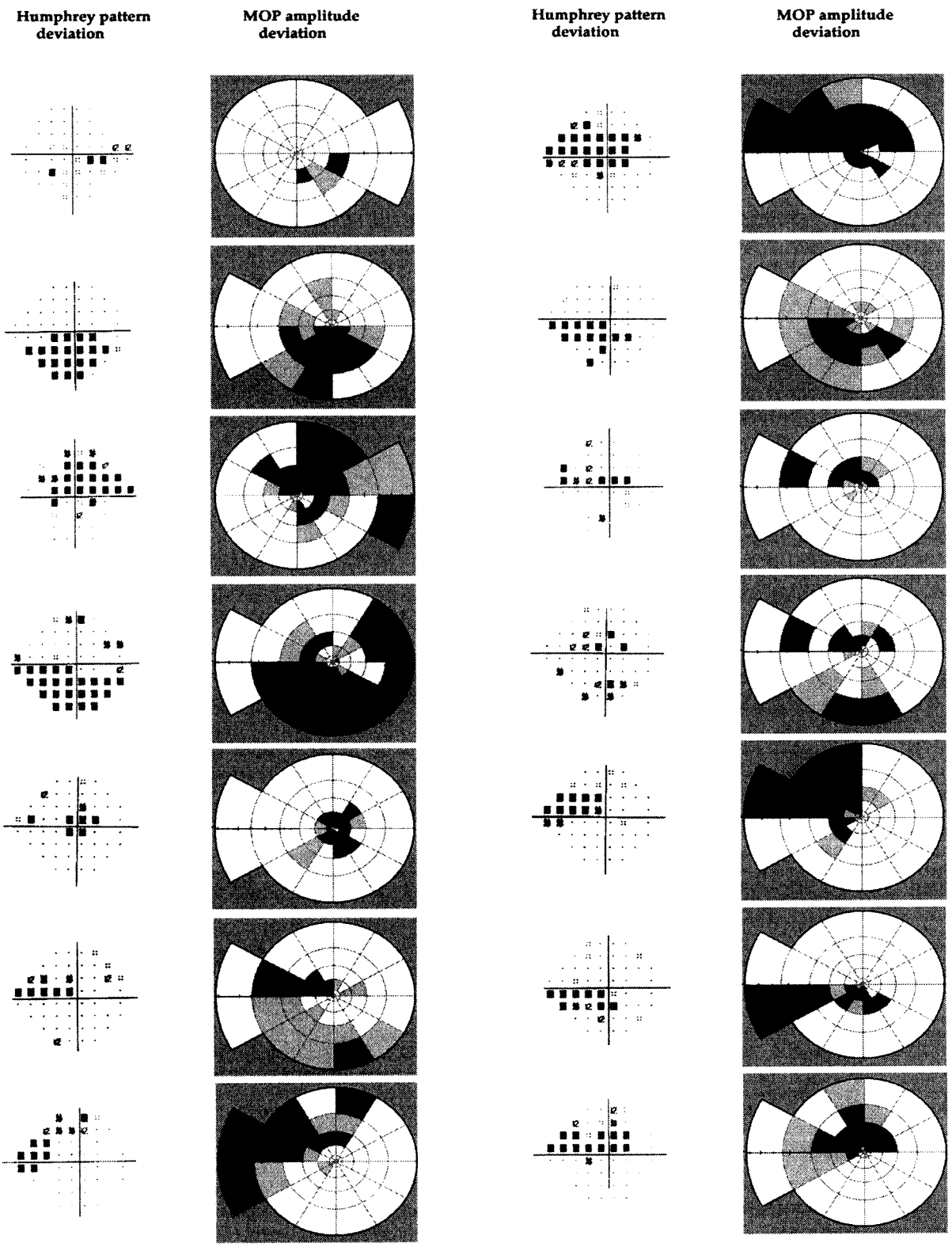

Figure 4 Pattern deviation plots of Humphrey scotomas with corresponding MOP amplitude deviation plot. There is close topographical correlation between the two tests in terms of the areas of the visual field affected.

When all 100 cases were inspected for comparison between tests for topographical location and size of scotomas, there was generally good concordance. In many cases the MOP defect was more prominent, but in a few others the Humphrey defect was more apparent. Figure 5 shows an example where the MOP result was worse than the Humphrey for both the study eye (with a very early scotoma that only just qualifies) and the "normal" fellow eye.

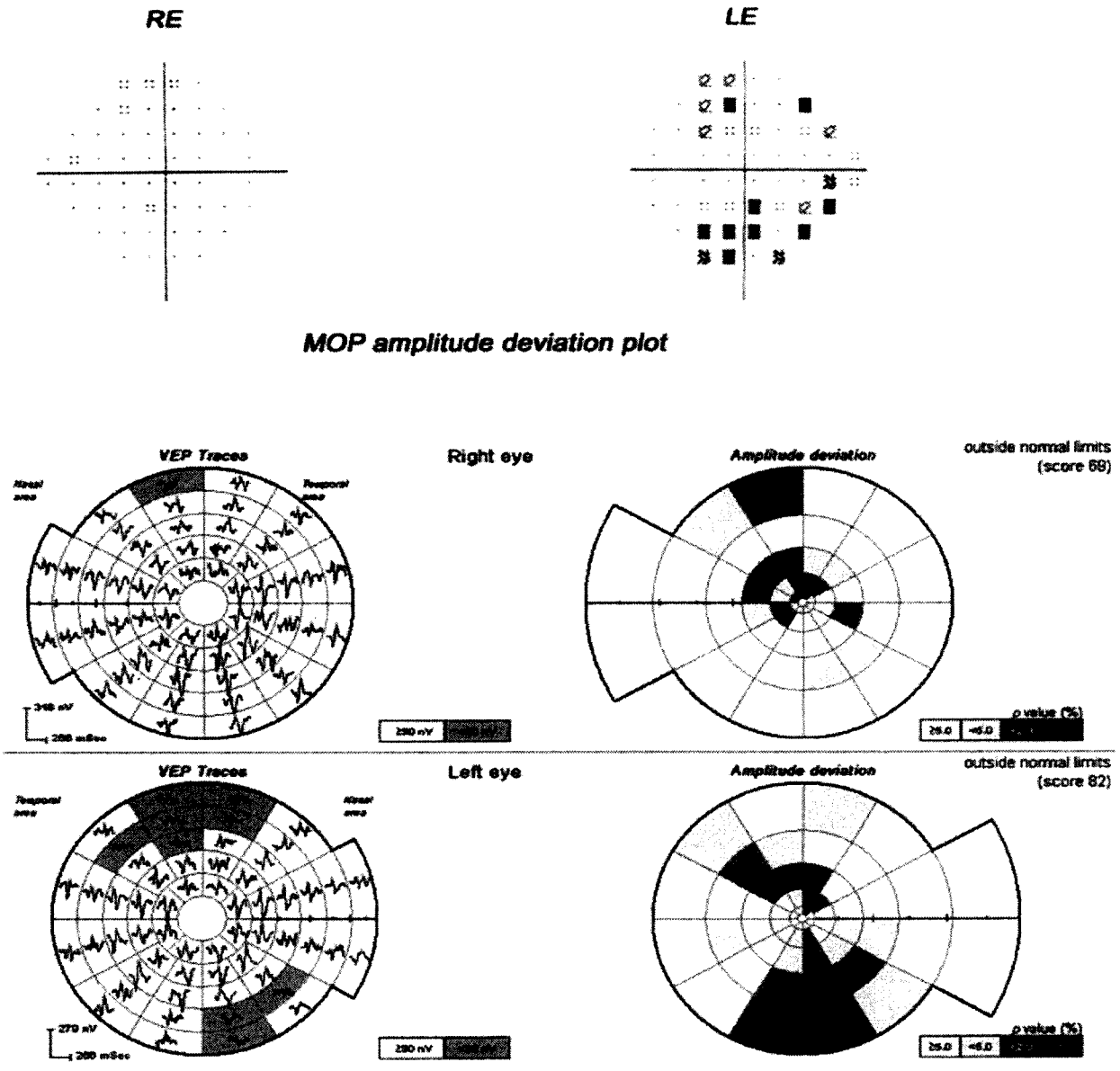

Figure 5 Example of early glaucoma where the MOP result for both eyes was more significant than the Humphrey. The left eye has a very early scotoma that only just qualifies and fellow eye is perimetrically normal. Both show defects on the MOP.

The severity index was designed to give an overall score based on the number of abnormal zones on both the amplitude plot and the asymmetry plot, with relative weighting for severity, and to score zones for clusters in certain locations within the field. The glaucomas showed a score >40 in 93% of cases, while in the normals there were 4 false positives. The severity index correlated well with the Humphrey MD (r=0.67 right, 0.69 left eyes) and showed a smaller correlation (r=0.42) in the study eyes with lesser defects. Figure 6 shows the severity index for all the study eyes, with good separation from normals. The cut-off lines are an index of 30, and 2 SDs above the mean Humphrey MD.

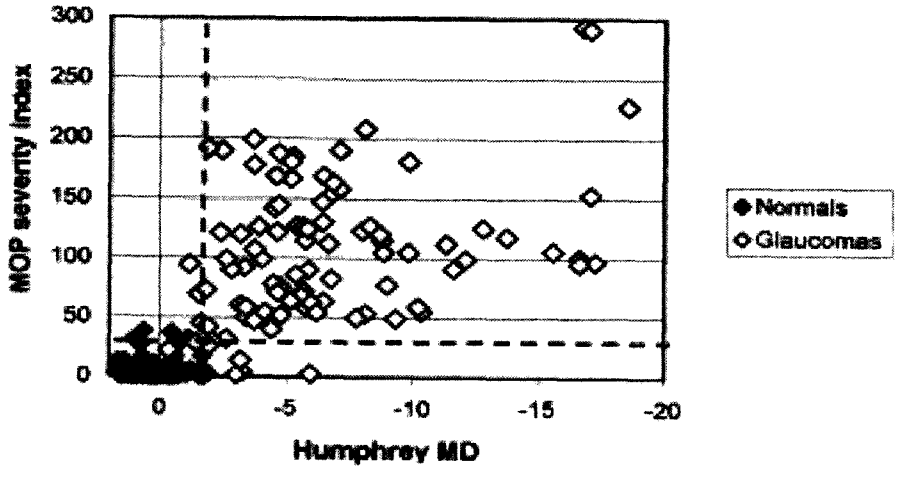

Figure 6 MOP severity index for all study eyes, showing good separation from normals. The horizontal cut-off line is a severity index of 30 ($p<0.05$), and the vertical line shows 2 standard deviations above the mean Humphrey MD for the normals.

In 37 glaucoma cases the fellow eye had a normal Humphrey visual field with no scotoma identified according to the definition. In 22 (59.4%) of these cases the MOP showed an abnormality in the second eye. Of these 19/22 cases had been assessed clinically as having a suspicious optic disc appearance, and many were already on treatment in the second eye as "pre-perimetric" glaucoma. Table 3 identifies where the abnormality was seen in these 22 subjects. In most cases it was on the amplitude deviation plot. Two cases had a potential scotoma that could be recognised on the Humphrey total deviation plot, but not on the pattern deviation (using the same definition for a scotoma. Four cases had a GHT result that was borderline, and 7 cases had an MD that was flagged as abnormal, so some of these cases are showing possible early subjective changes as well. Two cases (23 and 24) had normal MOP values but an abnormal Humphrey MD. Unfortunately there were still some subjects with clinically abnormal discs who maintained a normal visual field and normal MOP (in subjects 25-37), suggesting further room for improvement in detection. Some of the remaining cases may of course be normal. A smaller number were identified on asymmetry analysis, because these were eyes with milder defects and were being compared with the eye with greater damage. In contrast, all 37 cases showed an abnormal asymmetry analysis in the worse eye, as expected.

Table 3. Abnormalites in fellow eyes of the 37 patients without a qualifying scotoma.

	MOP	MOP	MOP	Humphrey Field			
Pt	Amplitude scotoma	Asymmetry scotoma	Abnormal severity index	MD	GHT result	Total Deviation Scotoma	Cup / disc ratio
1	+	+	+	-0.49	N	-	0.8
2	+	+	+	-0.26	N	-	0.6
3	+	+	+	-0.65	N	-	0.6
4	+	+	+	-1.4	N	-	0.7
5	+	+	+	-2.3 p<5%	B	-	0.8
6	+	+	+	-0.99	N	-	0.7
7	+	+	+	-3.72 p<1%	N	-	0.7
8	+	-	+	-2.16 p<5%	N	-	0.8
9	+	-	+	1.43	N	-	0.6
10	+	-	+	-2.56 p<5%	N	+	0.7
11	+	-	+	2.48	N	-	0.8
12	+	-	+	-2.04	N	-	0.7
13	+	-	+	-2.01	N	-	0.7
14	+	-	+	-0.03	N	-	0.8
15	+	-	-	0.0	N	-	0.8
16	+	-	-	-1.42	B	-	0.5
17	+	-	-	1.02	N	-	0.5
18	+	-	-	0.92	N	-	0.6
19	+	-	-	-3.12 p<2%	B	-	0.8

20	+	-	-	-1.94	N	-	0.7	
21	-	+	+	-0.42	N	-	0.7	
22	-	-	+	-0.04	N	-	0.6	
23	-	-	-	-2.43 p<5%	B	+	0.9	
24	-	-	-	-2.74 p<2%	N	-	0.4	
25-37	-	-	0-26 mean 7.0±7.6	+0.74 to -2.78 mean -0.69			0.4 to 0.9 mean 0.67	

MOP findings in the fellow eye (non study eye) of the 37 glaucoma cases without a qualifying scotoma. B = borderline, N = normal limits, MOP severity index 30-39 = borderline, greater than 39 = outside normal limits.

The results of the 15 normal subjects tested on 5 occasions are presented in Figure 7. The coefficient of variation for the amplitude of the VEP signal for all 58 zones is shown for the right and left eyes. The mean for the right eyes is 0.161 +/- 0.02 and for the left eyes is 0.163 +/- 0.02. The variability therefore ranges from around 10%-20% with the more peripheral superior test zones showing the greatest variability.

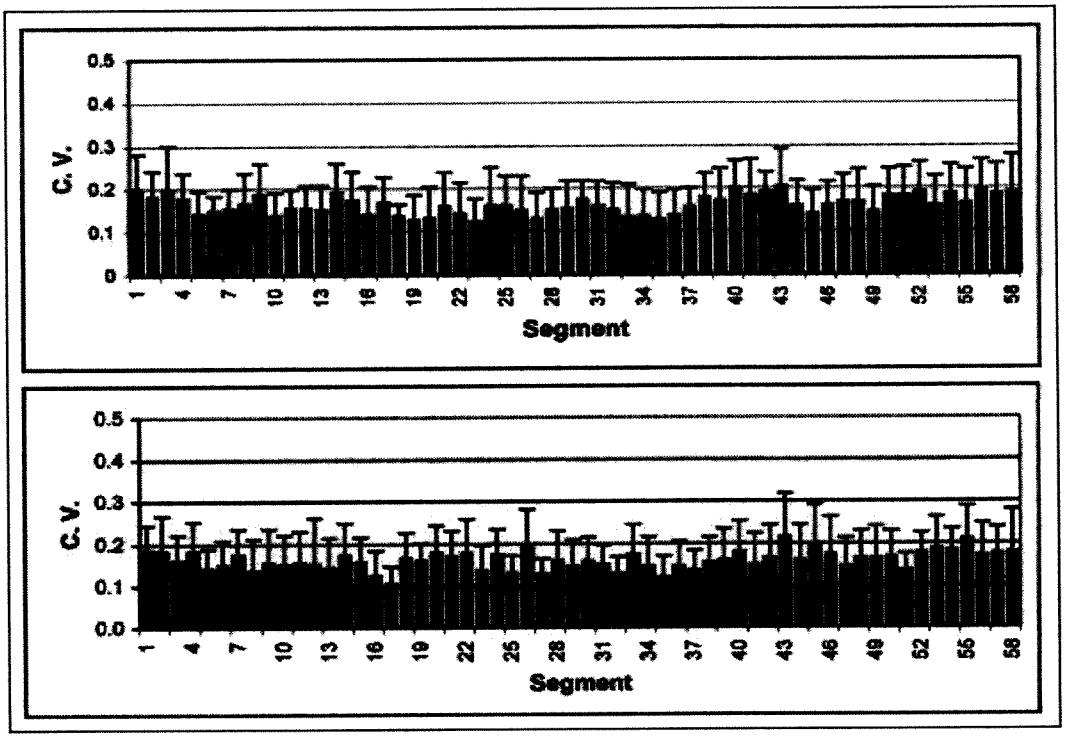

Figure 7 – Reproducibility of MOP amplitudes in 15 normal subjects tested on 5 separate occasions. The coefficient of variation (CV) for the amplitude of the MOP signal for all 58 zones is shown for right and left eyes. Mean R 0.0161+/-0.02 , mean L 0.0163+/-0.02

Discussion

The technique of multifocal objective perimetry (MOP) used in this study provides an objective measure of visual field loss. The ObjectiVision system utilises a new method of MVEP recording to sample local VEP responses from 58 segments of the visual field. The study indicates that this form of objective testing detected the scotoma(s) in nearly all cases of glaucoma with established field defects (97%), and is the largest clinical study in glaucoma using the mVEP to date.

Current methods of detecting field loss depend on the subjective confirmation of visual field defects. Many patients perform poorly on subjective tests, particularly the elderly. There is also a learning curve associated with subjective perimetry that complicates interpretation in new patients, such that 2-3 field tests need to be done before a reliable result is achieved. In contrast there is no learning curve for MOP. To be eligible for this study, patients had to be able to perform reliable subjective tests with reproducible field defects. In our clinical practice many patients are unable to do this, but can perform MOP. Patients also find this form of perimetry less stressful, since it does not involve decision-making.

Up to 25 to 50% of nerve fibres can be lost before a field defect is detected on subjective perimetry [221]. In this study a large number (22/37) of the glaucoma subjects who had a fellow eye with a normal visual field demonstrated abnormal VEP responses

in that eye. Perhaps MOP is detecting functional changes before they are identified on conventional perimetry. The defect was most commonly seen in the amplitude plot rather than the asymmetry analysis since in these subjects the asymmetry comparison is being made against the more affected eye, and is therefore less likely to identify a relative reduction. Asymmetry analysis is more useful in glaucoma suspects where the eyes are still only mildly affected, and subtle differences are more easily seen. Once significant defects develop, asymmetry will not be useful in the eye with the lesser defect unless changes occur in a part of the field not affected in the other eye.

One of the problems previously recognised with multifocal VEP recording was the high inter-individual variability in VEP amplitudes. This led to some glaucoma cases being missed because of high overall signal amplitudes. In these cases localised areas of depression did not reach the threshold for detection. In other cases the signal was very small due to poor conductivity. The ObjectiVision system uses a scaling algorithm based on underlying EEG signal conductivity recorded during the VEP, to overcome the inter-individual variability. It also removes the influence of alpha-rhythm and electrocardiogram spikes before calculating a scaling factor. The reduced variability has meant a major improvement in the application of the normal database to the analysis.

The MOP technique provides an objective measure of visual function in glaucoma. In its current format it shows a high level of sensitivity for detecting glaucoma diagnosed by current gold standards. It has a high patient acceptance with no learning curve. Future longitudinal studies will determine its ability to diagnose glaucoma early in the disease process.

Chapter 8

Clinical Application of mVEP in Glaucoma Practice

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Graham S.L, Klistorner A.I. and Goldberg I., *Clinical Application of Multifocal VEP Objective Perimetry in Glaucoma Practice*, **Archives Ophthalmol** 2005, 123,729-793

Abstract.

Purpose: To evaluate the role of objective perimetry using multifocal visual evoked potentials (mVEP) in glaucoma practice, and to assess its utility in patients with inconclusive standard automated perimetry (SAP) performance.
Methods: A retrospective case series of 436 consecutive subjects referred for glaucoma investigation who were tested with the AccuMap™V1.3 system within a defined 12 month period. Sensitivity was determined compared to SAP and in a subgroup using masked stereo optic disc photos as an alternative reference standard. Overall clinical diagnostic outcomes were assessed.
Results: The mVEP changes were correlated with both stage of disease and with Humphrey MD (r =0.78). The overall sensitivity for detecting glaucoma with established subjective field loss was 97.5%, (early glaucoma 95%) while 92.2% of low risk suspects had normal mVEPs. When masked disc assessment alone was used for diagnosis of abnormality, sensitivity for both mVEP (80.6%) and HVF (81.9%) were similar, but mVEP specificity was better (89.2% vs79.5%). The mVEP was particularly useful in assessing excessive subjective field loss (45 eyes) showing a much closer correlation with the clinical picture.
Conclusions: MVEP is an effective method for detecting visual field loss in glaucoma. It provides a valuable aid to the clinician in categorising patients with unreliable, variable, unconfirmed or excessive subjective field loss.

Introduction.

In an attempt to provide an objective and clinically feasible test for glaucoma visual field assessment, the multifocal visual evoked potential (mVEP) has recently been developed [165, 169]. With the use of multiple recording channels, signals from all areas of the visual field can be detected to provide an objective map of visual function. Signal amplitudes are compared to normal population values and probability plots can be created. Using this technique, sensitivity has been reported to be >90%, depending on the criteria used to define abnormality[195, 199, 222-226]. Hood et al have demonstrated that mVEP amplitude correlates with subjective visual field threshold[227]. However, in contrast to the amplitude, mVEP latency did not demonstrate good correlation with visual field loss despite the promise it held in earlier works based on full-field stimulation [228-230].
The multifocal method can also be useful in other optic neuropathies such as optic neuritis[193, 231] or ischemic optic neuropathy[227]. Studies have described mVEP in

subjects with cortical lesions [232, 233] and in assessment of the visual field in children[234].
Several reports have shown the ability of the mVEP to confirm subjective scotomas in established glaucoma but there have not been any reports of its utility in the clinical setting where a wide variety of subjects are encountered. Additionally, all mVEP studies to date have applied selection criteria (visual acuity limits, reproducible field defect, unilateral damage, other ocular pathology) for their selection of subjects [199, 222, 223, 226, 227, 235, 236]. In particular they usually required reliable confirmed subjective visual fields, which masks some of the limitations of standard perimetry encountered in normal clinical practice.
This report examines the results of mVEP testing in a busy glaucoma referral practice over a 12-month period to further investigate its sensitivity in glaucoma detection and to evaluate its role in the outcomes of patient assessment. All patients tested during the study period have been included regardless of their subjective field performance or ocular status. The overall role in investigation of the glaucoma patient, particularly in cases of poor, unreliable or suspicious subjective testing, can then be reviewed.

Methods

A retrospective review was performed of all subjects referred for glaucoma assessment within a defined 12-month period who were tested with the Accumap™ (ObjectiVision Pty Ltd, Sydney, Australia).

Subjects

There were 436 subjects referred for glaucoma investigation who also had mVEP testing. None of these subjects' results were used in previous publications. Of the 436 patients there were 83 "low risk" glaucoma suspects, 107 "high risk" glaucoma suspects in at least one eye and 220 patients with glaucoma in at least one eye. There were also 25 patients who were referred for investigation with excessive field loss or uninterpretable fields in at least one eye. All subjects had performed Humphrey 24-2 (SITA or full threshold) field tests at least once. Visual field criteria for inclusion in the different subgroups is described in the analysis section below.

The initial classification was based on the diagnosis made by the clinician. Low risk suspects were those with ocular hypertension (IOP>21mmHg) and/or family history of glaucoma, but normal optic discs and visual fields. Corneal thickness measurements were only available in a limited number of subjects, therefore this was not used as a discriminator.

High risk suspects had a suspicious or abnormal optic disc appearance in at least one eye (eg large cup >0.6, nerve fibre layer thinning, notch or hemorrhage) and/or asymmetrical disks with Cup/Disc (C/D) ratio difference of more than 0.2, +/- raised IOP, +/-family history, but still normal visual field. This group therefore included some "pre-perimetric" glaucoma. The glaucoma subjects had (at least in one eye) an abnormal optic disc with characteristic glaucomatous cupping with corresponding visual field

defect, +/- raised IOP and +/-family history. The fellow eyes of glaucoma patients that still had a normal visual field were identified and analysed as a separate group.

Informed consent was obtained from all subjects prior to mVEP testing and the study followed the principles of the Declaration of Helsinki.

Table 1. Glaucoma subgroups

Group	Number (eyes)	Humphrey MD (dB)	Cup/Disc ratio
Low Risk suspect	180	-0.44+/-1.33	0.46+/-0.13
High Risk suspect	205	-0.64+/-1.46	0.7+/-0.08
Fellow glaucoma eye (normal subjective field)	81	-0.94+/-1.37	0.62+/-0.15
Early glaucoma	211	-5.11+/-1.9	0.76+/-0.12
Moderate glaucoma	79	-10.0+/-2.2	0.80+/-0.14
Advanced glaucoma	69	-20.5+/-4.7	0.87+/-0.11

Analysis

The analysis was approached in several ways. Firstly the clinician's diagnosis was used to categorise eyes as low risk, high risk or glaucoma as per the definitions above. The clinician's diagnosis was based on the full clinical picture at the time of referral including optic disc appearance (by slit lamp stereoscopic disc assessment in all cases) and visual fields, but prior to recording mVEP. Due to the heterogeneity of the glaucoma group (for instance, a patient can have glaucoma in one eye and be suspect in the other) the data was analysed with each eye classified separately, and data for all eyes is included. Table 1 shows the breakdown of all eyes in the study according to the level of severity. Humphrey mean deviation (MD) values were recorded and used to subgroup glaucoma into early (MD<7 dB), moderate (MD 7-13 dB) and advanced (MD≥14 dB) stages. While it is acknowledged that some early glaucomas with focal defects may have low MD and high PSD values, the opposite can be true of advanced cases. Currently mVEP amplitude is evaluated in a total deviation plot (analogous to total deviation plot of Humphrey) and a corresponding PSD based score is yet to be developed.

For Humphrey visual fields the Glaucoma hemifield test (GHT) was used to classify fields as normal or abnormal. A confirmed field defect was a field "Outside normal limits" on 2 consecutive occasions with a field defect in the same location. These subjects were used for sensitivity assessment (see below). Reliability of the field test, and in the case of repeat tests, any variability in location of scotomas or fluctuation in GHT rating, was noted. MVEP records were analysed separately with the investigator masked to both clinical data and subjective visual field results.

To investigate the relationship between stage of disease and mVEP results, mean values for the new modified AccuMap Severity Index (ASI), (proprietary technique, see below) for each subgroup were determined. A correlation between the Humphrey Mean Deviation (MD) and ASI values was performed for all glaucoma patients.

Secondly, for the purpose of verifying the sensitivity of mVEP in glaucoma, a subgroup of the glaucoma patients who had more than one reliable Humphrey field available and had reproducible visual field loss (same location in field) were identified. There were 286 glaucomatous eyes that met these criteria and their ASI results were reviewed to determine sensitivity of the AccuMap™. Further analysis was done based on the detection of a scotoma on the mVEP amplitude deviation plot or the asymmetry plot. A scotoma for the amplitude deviation plot was defined as a cluster of 3 points in one hemifield with $p<2\%$ and at least one point $p<1\%$. For asymmetry the cluster was 3 points with $p<1\%$ or 2 points $<0.5\%$. The 4 central superior rim points were excluded from the clusters.

Since there were no normal subjects tested in this study, a true specificity could not be determined. Therefore, as an indirect measure of specificity, the rate of abnormality in low risk suspects (180 eyes) was determined for both ASI and for the presence of a scotoma (as defined above for sensitivity, normal on GHT on all occasions tested to qualify as normal). This provides an estimate of the lower limit of mVEP specificity since it is anticipated that very few of these subjects had true glaucoma at the time of testing.
In high risk suspects and fellow eyes (still with normal visual field) of glaucoma patients, the rate of mVEP abnormality was determined to assess its potential as an early marker of disease.

There was a subgroup of eyes, which had variable Humphrey fields. Variable fields were defined as fields classified differently by GHT on at least three consecutive occasions (eg normal/abnormal/normal or abnormal/normal/abnormal), or alternatively fields which had different quadrant location of abnormal points (upper vs lower, or nasal versus temporal) on at least the last two tests. The situation where the visual field gradually improves (for example Ab/Bor/Nor), which could be due to a learning effect was not considered variable but classed as a normal field and the eye classified according to disc appearance. There was also a group of patients with unconfirmed visual field loss, with a new scotoma not yet confirmed on repeat testing. This group was not included in sensitivity estimates described above, but since this is a common problem encountered in the clinical environment, outcomes based on mVEP findings were determined. Confirmation would ultimately require repeat subjective and/or mVEP testing. Furthermore subjects with excessive visual field loss, as determined by the clinician, compared to their optic disc appearance were identified. These subjects had either undetectable or mild structural disc change with visual field loss that was greater than expected. The role of mVEP in these three groups, (variable fields, unconfirmed field changes or excessive loss) was assessed separately.

Finally, the morphology of the optic disc was used as an independent reference of disease to obtain an estimate of sensitivity and specificity. All subjects who had stereo disc photographs performed within the last 12 months were identified. Photographs were graded in randomized masked fashion by two glaucoma specialists (SG and IG) as either normal, suspect or glaucoma. Photos were graded independently (masked from each other) and when classification differed, photos were then reviewed by both clinicians and

consensus reached. Visual field and mVEP results were then evaluated based on this classification. Although older photos could have been used in glaucoma cases, since the changes once present are permanent, the same did not apply to suspect eyes with normal or borderline discs, which could have progressed over any longer period of time. Therefore, the photos were restricted to the prior 12 months. The sample size available was also limited by the fact that many patients now have alternative forms of disc imaging performed (mainly scanning laser with Heidelberg Retinal Tomograph (HRT). Statistical analysis was performed using Statistica 4.1 (StatSoft, Tulsa, OK,1994)

mVEP Recording.

The method of mVEP recording is the same as previously reported in the paper by Goldberg et al.[223] and as described in Chapter 6. We used the AccuMap™perimeter V1.3 (ObjectiVision Pty Ltd, Sydney, Australia). The only differences were that the AccuMap™ had a new four-channel amplifier to replace the Grass amplifier and the filtering settings in the new amplifier were tightened (see below).

Subjects were seated comfortably in a chair with the chin slightly elevated to relax neck muscles. They were asked to fixate on the small randomly changing number at the centre of the stimulus pattern. The distance to the screen was 30 cm, corresponding to a radius of the stimulus of 25° with an additional nasal step out to 33°. All subjects were optimally refracted for near and the pupils were not dilated. All recordings were collected using monocular stimulation. Data was recorded using a new ObjectiVision four channel amplifier, with band-pass filter between 1 and 30Hz. The signal was amplified 100,000 times and then digitally filtered between 1 and 20Hz. The new amplifier uses vertical rather than sloping cut-off filters. The data-sampling rate was 450 Hz.. Usually 7-9 runs of 55 sec each were recorded to provide a stable signal as indicated by the trace improvement software in the OPERA program.

An ObjectiVision occipital cross electrode holder pre-determined the four electrode positions[199]. Data was analyzed using OPERA™ V1.3 software (ObjectVision Pty Ltd, Sydney, Australia). Raw data for each run was cross-correlated with Kasami sequences to extract the mVEP signal for each segment of the visual field stimulated and then scaled according to underlying EEG activity to reduce inter-subject variability [219]. The procedure was repeated for each run and results averaged for each channel independently. Maximal peak-to-trough amplitudes for each wave within the interval of 60-180 msec were determined and compared among channels for every stimulated segment of the visual field. The wave of maximal amplitude from each segment in the field was selected and a combined topographic map created by the software.

MVEP amplitudes for each individual zone in the combined trace array were compared with the normals database and probability of abnormality plots were constructed. The inter-eye asymmetry[195] was also calculated for every segment of the tested visual field. A probability plot for asymmetry was constructed based on the normal database distribution of asymmetry between the two eyes.

Finally, a new modified AccuMap Severity Index (ASI version 2) was calculated by the OPERA™ software for each subject. The ASI assigns scores to individual abnormal points and clusters of points with a weighting for location and whether they are present on the asymmetry plot. The ASI provides an overall index of whether the mVEP amplitude results are within normal limits (score 0-11), borderline (score 11-19) or outside normal (score 20 and higher)

Results

Correlation with glaucoma severity

The glaucoma subjects showed substantial changes in their mVEP results depending on severity of the disease. Figure 1 shows the mean ASI values for all groups. There was a statistically significant difference between all groups except for the group of fellow eyes of glaucoma patients, which was not statistically different from both low risk and high risk groups (Table 2, one-way ANOVA, Tukey HSD test). The fellow eye group might have been expected to show a higher rate, but it included a broad mix of disc appearances – see below for discussion.

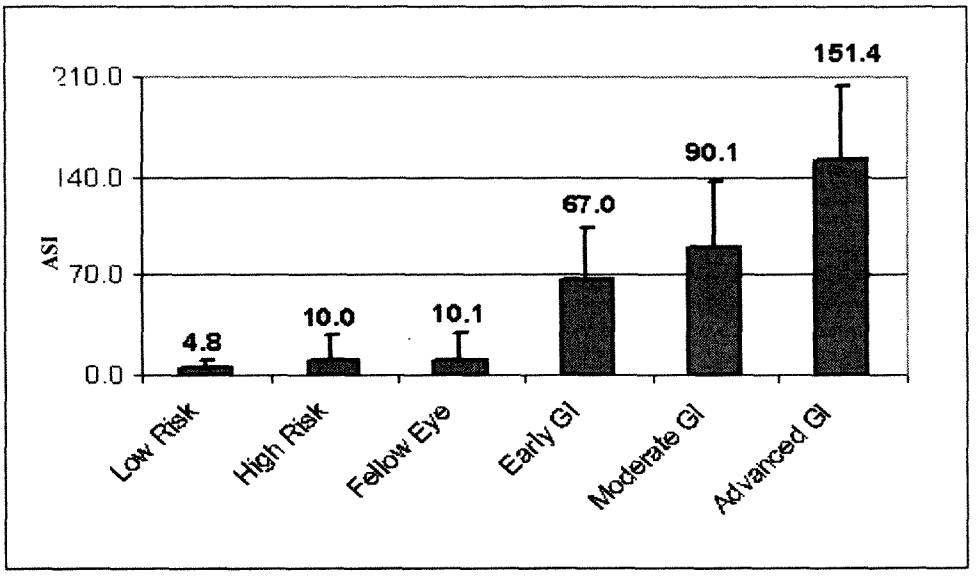


Figure 1. Mean ASI values (+/-SD) for different glaucoma groups. Bars indicate Standard Deviations.

A strong correlation (linear regression coefficient r=0.78) was demonstrated between ASI and Mean Deviation (MD) of Humphrey fields for all glaucoma patients, as shown in Figure 2. This is similar to previously reported studies[199, 223].
Since the ASI increased through all stages of the disease process, this suggests it reflects the severity of disease. It also correlated to the severity of visual field loss as detected by the Humphrey MD global index.

Table 2. Probability values (p) for statistical comparison of ASI between different glaucoma groups.

Groups	High Risk	Fellow Eye	Early Glaucoma	Moderate Glaucoma	Advanced Glaucoma
Low Rrisk	0.021	0.42*	0.000	0.000	0.000
High Risk		0.32*	0.000	0.000	0.000
Fellow Eye			0.000	0.000	0.000
Early Glaucoma				0.003	0.000
Moderate Glaucoma					0.000

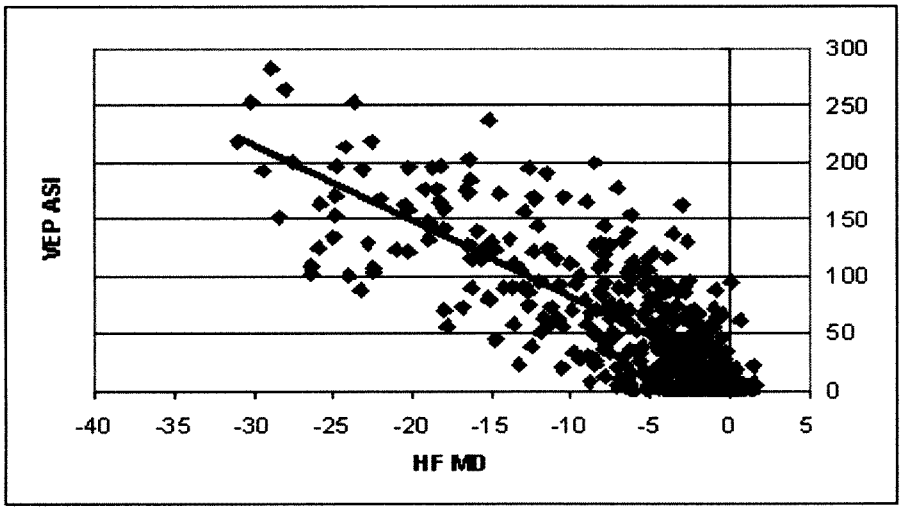


Figure 2. Correlation between Humphrey field MD and mVEP ASI. Line indicates best linear regression fit.

Sensitivity and specificity

There were 286 glaucomatous eyes that had more than one reliable Humphrey field available and had reproducible visual field loss (same location in field). For these subjects the sensitivity of the mVEP ASI in detection of visual field defects was calculated as 97.5% (279/286 eyes). When analysed by disease severity, all eyes with advanced (69 eyes) and moderate (79 eyes) stages of glaucoma were identified (100% sensitivity) while in the early glaucoma group the sensitivity was 95% (131/138 eyes). Figure 3 shows examples of early, moderate and advanced glaucoma, with Humphrey printouts and AccuMap™ trace arrays and amplitude and asymmetry (for early glaucoma only) deviation plots.

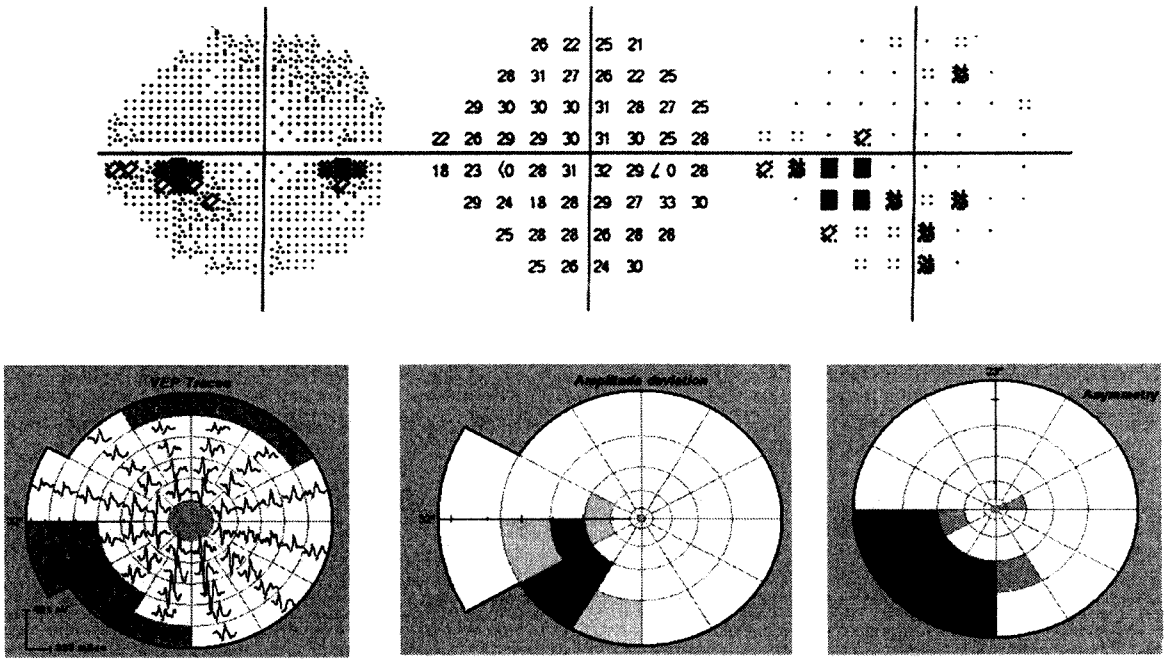
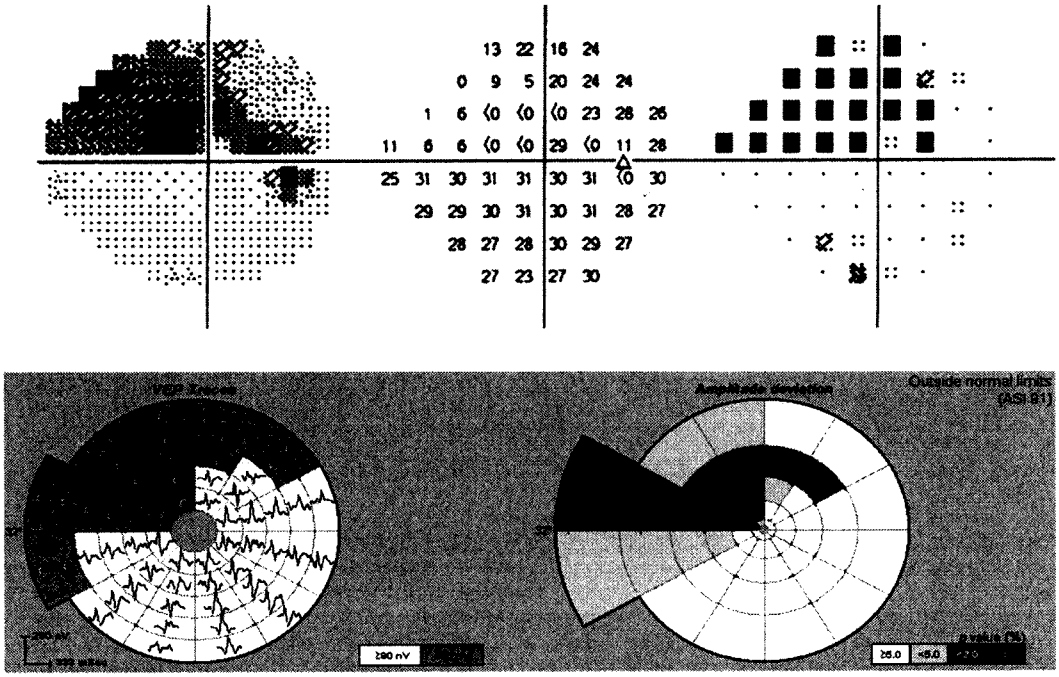
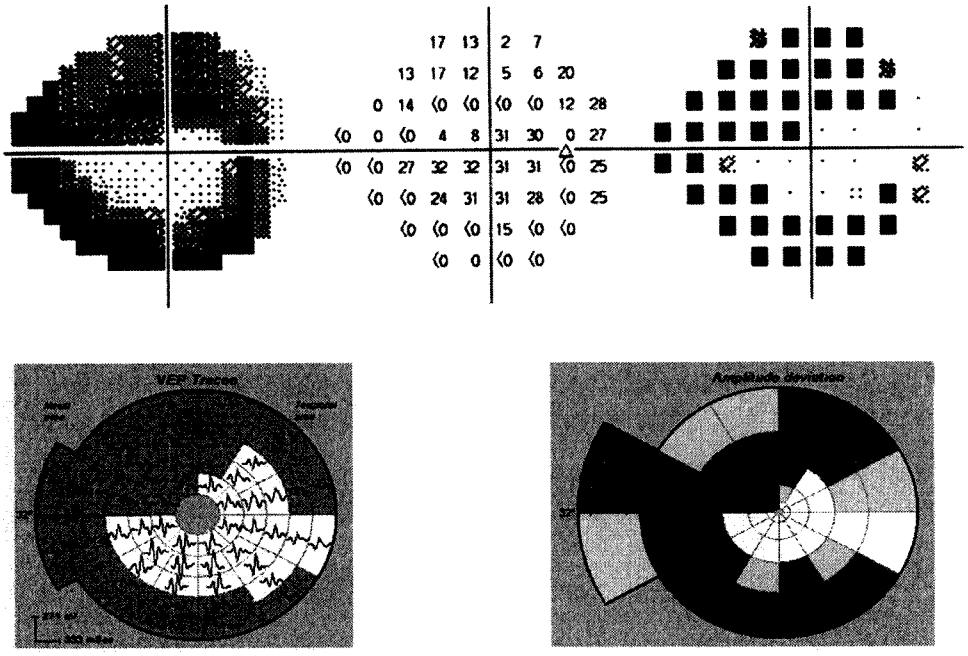


Figure. 3 Examples of correspondence between Humphrey (upper row) and AccuMap (lower row) visual field results.
a) example of early glaucoma (Humphrey MD=-4.18, AccuMap ASI=41)



b) example of moderate glaucoma (Humphrey MD=-8.49, AccuMap ASI=67)



c) example of advanced glaucoma (Humphrey MD=-18.1, AccuMap ASI=107)

Of the 7 cases of early glaucoma where the mVEP did not detect abnormality three had shallow defects (MD ranging from -1.26 to -1.86) and the other four had more significant defects (MD range: -1.9 to -5.1), but advanced disease in the second eye, therefore reducing the relative contribution of asymmetry to the ASI score. Examples are presented in Figures 4a and 4b.

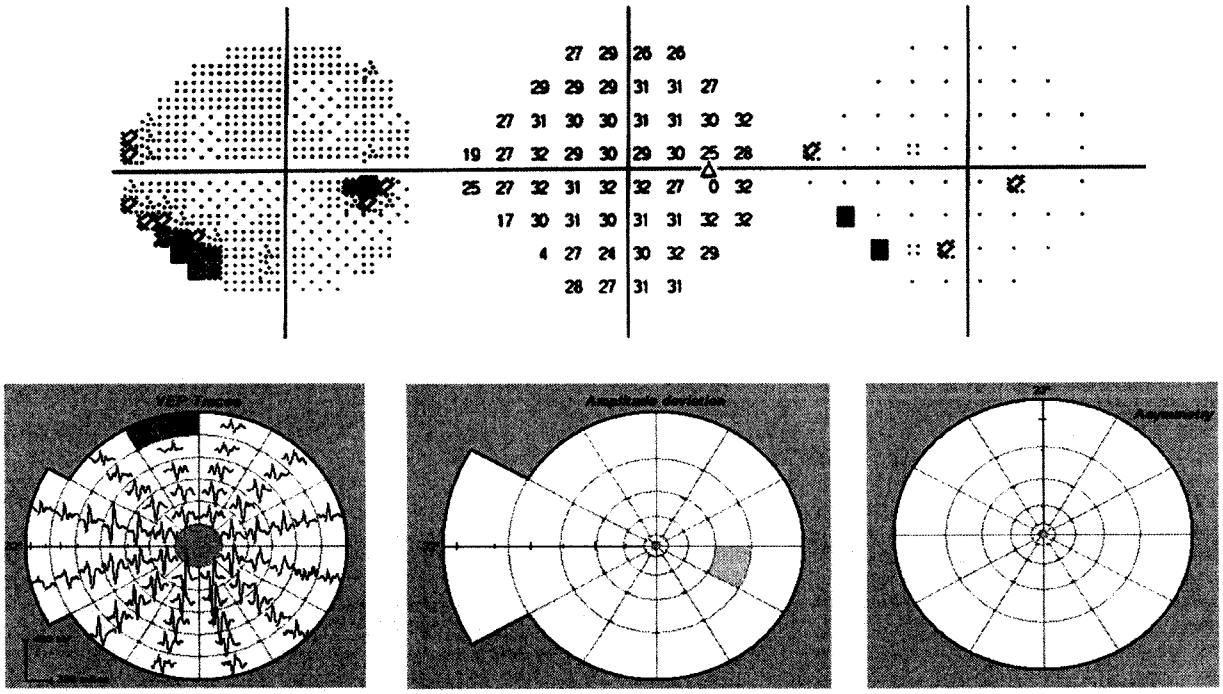


Figure 4 Examples of mVEP misses.
Fig 4a mVEP miss of a very early defect (Humphrey MD=-1.48).

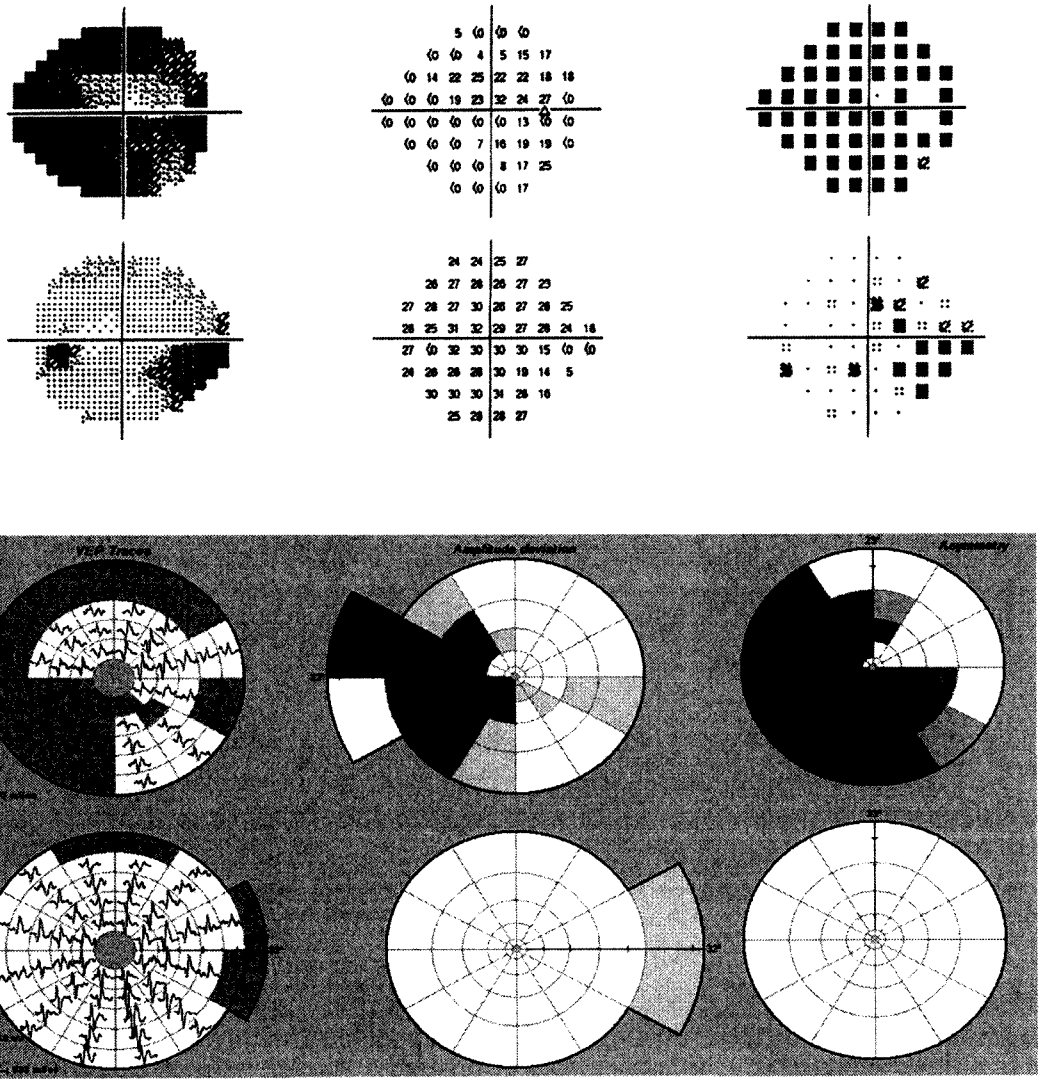


Fig 4b mVEP miss (left eye-low row in both Humphrey and AccuMap) with the fellow right eye having advanced glaucoma (Humphrey MD=-5.1).

An estimate of the lower limit of mVEP specificity was determined based on the group of low risk suspects. Of the 180 low risk eyes, 13 had variable Humphrey field (SAP) results and another 13 had an unconfirmed defect and were analysed separately as for the glaucoma group (see sections below). Of the remaining 154 eyes, 92.2% (142 eyes) had a normal mVEP. In 12 eyes the mVEP was borderline or abnormal. However, in eight of these eyes there was a moderate to high refractive error, which is a known cause of reduction of mVEP central amplitude and may explain the abnormal result in at least some cases [237].
When scotoma criteria described above were used as the basis for abnormality on the monocular amplitude deviation plot or the asymmetry plot, the sensitivity was slightly lower than for ASI. In the glaucoma eyes, there were an additional 6 cases where the asymmetry plot did not register an appropriate cluster, while only one additional case was detected that was not

flagged by the ASI (sensitivity 95.5%). For the monocular plot a cluster was missed in a further 18 cases, revealing that it is not as sensitive as asymmetry in early disease (sensitivity 88.8%). Specificity (using low risk suspects), however, remained the same as when ASI was used. There were 7 eyes abnormal on both monocular and asymmetry plots, with an additional 3 abnormal on asymmetry only and 2 abnormal on monocular only. If the criteria of either being abnormal is used this gives a specificity of 92.2% which is the same as that determined using ASI.

Disc analysis as reference gold standard

The morphology of the optic disc was used as an independent reference of disease, instead of the usual Gold Standard of subjective visual fields. There were 111 subjects (218 eyes) who had stereo disc photographs performed within the last 12 months that were of sufficient quality to grade.

There were 83 eyes whose disc photos were graded as normal, 63 suspect and 72 glaucoma eyes. In 87% of eyes the observers were in agreement as to the disc grade with their first view. In the remaining 13% a consensus was reached after conferring and second viewing. Subjective visual field and mVEP results were then evaluated based on this classification to determine sensitivity and specificity.

For those eyes graded as having normal discs, 74 eyes (89.2%) had normal mVEPs and 66 eyes (79.5%) had normal Humphrey fields. Therefore the specificity was better for mVEP than for subjective visual fields (SAP) when an independent reference standard was used.. It should be noted that within this group there were no true normals since they consisted mainly of low risk suspects and it is possible that some of them may have had early glaucoma despite normal discs.

Sensitivity however was very similar for both mVEP and SAP tests. In those eyes with discs graded as glaucoma, 80.6% had abnormal mVEPs and 81.9% had abnormal Humphrey fields. For those eyes graded as suspect (63 eyes), 7 eyes (11%) had abnormal mVEPs and 13 eyes (20.6%) had abnormal Humphrey fields. All eyes with normal mVEP had normal SAP. However, out of 5 eyes with abnormal SAP but still normal mVEP 4 had unreliable subjective fields, suggesting they were probably false positives.

In summary, the performance (sensitivity) of the mVEP was equivalent to subjective Humphrey fields for detection of glaucoma based on masked classification of optic discs, with a better specificity. The structural changes in the disc which can be detected by a trained observer are known to occur in advance of functional losses. Therefore it is not surprising that the sensitivity of the two functional tests was less than 100% when relying on disc appearance only.

High risk suspects

High risk suspects are of particular interest since these eyes are more likely to develop glaucoma. In fact, some "pre-perimetric" eyes already have early stages of the disease, despite the subjective field remaining normal.

The high risk suspect group comprised 205 eyes. Of those: 163 eyes (79.5%) had normal SAP, 16 eyes (7.8%) had unconfirmed SAP defect on their most recent field and 26 eyes (12.7%) had variable SAP. High risk eyes with a variable or unconfirmed SAP defect were analysed separately (see below).

From the 163 eyes with a normal SAP, 133 eyes (81.6%) had a normal mVEP, therefore reassuring normal function. However, 30 eyes (18.4%) demonstrated various degrees of abnormality in mVEP with 16 eyes classified as borderline and 14 eyes as abnormal based on ASI. Further analysis revealed that 22/30 (74%) of these eyes had other indications of early glaucoma (were classified as pre-perimetric glaucoma or had asymmetric discs). Therefore, it is possible that in at least 22 cases the mVEP has detected changes of early glaucoma prior to change on the subjective field test. If the results from the low risk suspects is accepted as an estimate of the lower limit of test specificity (92.2%), then these cases are more likely to be definite glaucoma and not false positives (as we would expect no more than 3 cases (around 10%) to be false positives).

Analysis of fellow eyes in glaucoma patients

There were 81 fellow eyes in glaucoma patients that still had normal visual fields on SAP. Fourteen of them (17.3%) were classified as abnormal or borderline by mVEP. Of these, 7 out of 14 eyes were considered "pre-perimetric" due to the appearance of the optic disc. It was expected that the fellow eyes might have a higher rate of abnormality than in high risk suspects, but this was not the case. One possible explanation is that in HR suspects the mVEP abnormality is mainly revealed by inter-eye asymmetry, while in the fellow (normal) eye of glaucoma patients asymmetry often does not produce detectable changes when the second eye is significantly damaged or the scotoma arises in the same part of the visual field. In these cases we have to rely on the monocular amplitude deviation plot. When using the definition of a scotoma rather than the ASI, the rate of abnormality was slightly higher at 21%.

Analysis of eyes with variable fields

Assessment of variable fields (in terms of severity or/and localization of the defect) commonly presents a clinical challenge. The rate of variable fields in the study was similar for all groups. For low risk there were 16 eyes with variable fields (8.9%), high risk 26 eyes (12.4%) and early glaucoma 28 eyes (13%). Fig. 5 shows distribution of mVEP results in these three subsets. Moderate to advanced glaucoma with variable fields were not analysed separately as mVEP results did not change clinical outcomes.

a. Low risk suspects.

As can be seen from the figure 5, all eyes with variable SAP clinically classified as low risk suspects had a normal mVEP, therefore confirming normal status of those eyes. This serves to reinforce the clinical impression that the eye is still normal.

b. High risk suspects.

Only a small proportion of variable high risk suspect eyes (4/26 eyes) were borderline or abnormal on mVEP ASI. Of the 22 remaining eyes none were considered clinically as pre-perimetric and in 7 cases the abnormal SAP thought to be caused by rim artefacts. Therefore the normal mVEP in these subjects reveals that the variability in the subjective field is less likely to be progression of glaucoma.

c. Early glaucoma.

In the 28 eyes classified as early glaucoma with variable fields, 13 eyes had a normal mVEP. All 13 of these eyes had been classified as early glaucoma based on the fellow eye having definite glaucomatous field defect (they could not, however, be classified into the "fellow eye of glaucoma patient" group because this group by our definition had to have a normal field and they had performed an abnormal SAP at some stage). These eyes mostly had only mild disc changes, but our definition placed them into the early glaucoma group. The mVEP results did not detect any definite functional change in these 13 eyes.

In comparison, the other 15 eyes with variable fields and classified as early glaucoma had borderline or abnormal mVEPs. There was a statistically significant difference between the C/D ratio and Humphrey MD ($p=0.02$ and $p=0.01$ respectively) of the group of patients who had an abnormal mVEP (mean C/D 0.77, MD=-4.3+/-1.4) and those who still had a normal mVEP (mean C/D 0.67, MD =-2.6+/-1.1). In 7/10 eyes with variable field and definitely abnormal mVEP (ASI>20) there were additional indications of abnormality such as notched or asymmetrical disk or nerve fibre layer (NFL) defect. Therefore, in these patients the mVEP is confirming what is suspected by the disc appearance and the fluctuating fields – that there is evidence of early glaucoma.

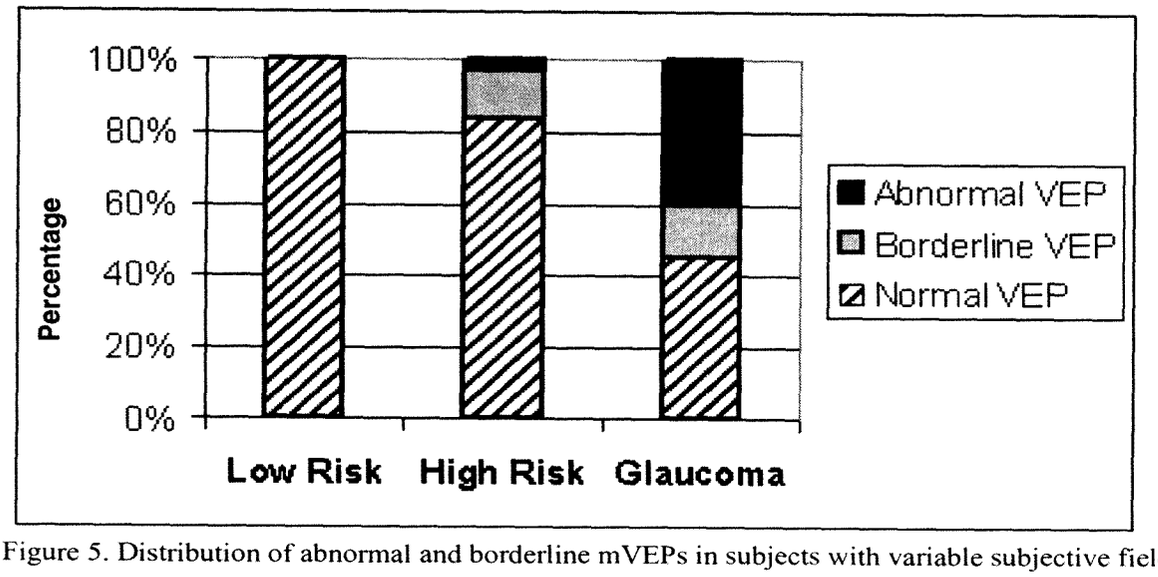


Figure 5. Distribution of abnormal and borderline mVEPs in subjects with variable subjective fields.

Unconfirmed field defects

Evaluation of unconfirmed or newly diagnosed visual field defects is another area of uncertainty in diagnosis, since the specificity of first time subjective perimetry is low, and even experienced observers can show large fluctuations between tests. Figure 6 shows the distribution of mVEP results in low risk suspects, high risk suspects and glaucoma patients whose last (or only) subjective field was abnormal.

a. Low risk suspects

In the low risk suspect group, of the 13 eyes with an unconfirmed SAP defect (10 eyes-borderline on GHT and two outside normal limits, mean MD=-0.4+/-1.1) all had a normal mVEP. Since the optic disc was normal in these eyes the mVEP result shows better correlation with disc appearance.

b. High risk suspects

There were 16 high risk eyes with unconfirmed SAP defect. None of these 16 eyes was considered truly glaucomatous clinically by the reviewing clinician. Of these, 13/16 eyes (81%) had a normal mVEP, suggesting still normal function. In three cases the mVEP was classified as abnormal, which may represent early glaucoma. Follow up with both mVEP and subjective fields would be required to confirm this.

c. . Early glaucoma

There were 45 eyes with an unconfirmed scotoma in the last (or the only) SAP and in the majority of cases this was a first attempted visual field . In 58% (26/45) of these eyes (SAP mean MD=-4.2+/-4.1) an abnormal mVEP was found that corresponded to the defect, thereby supporting the abnormality found on Humphrey.

However the remaining 19 eyes (SAP mean MD=-2.8+/-2.2) demonstrated a normal mVEP .. In 10 of these the SAP was unreliable and a likely false positive. In two cases the fellow eye had advanced glaucoma, which reduced the relative contribution of asymmetry analysis (and therefore ability to detect early glaucoma). These 2 cases are probable mVEP false negatives. In the remaining 7 cases, the mVEP result tends to suggest that the eye is still functionally intact, again repeat testing is needed to confirm.

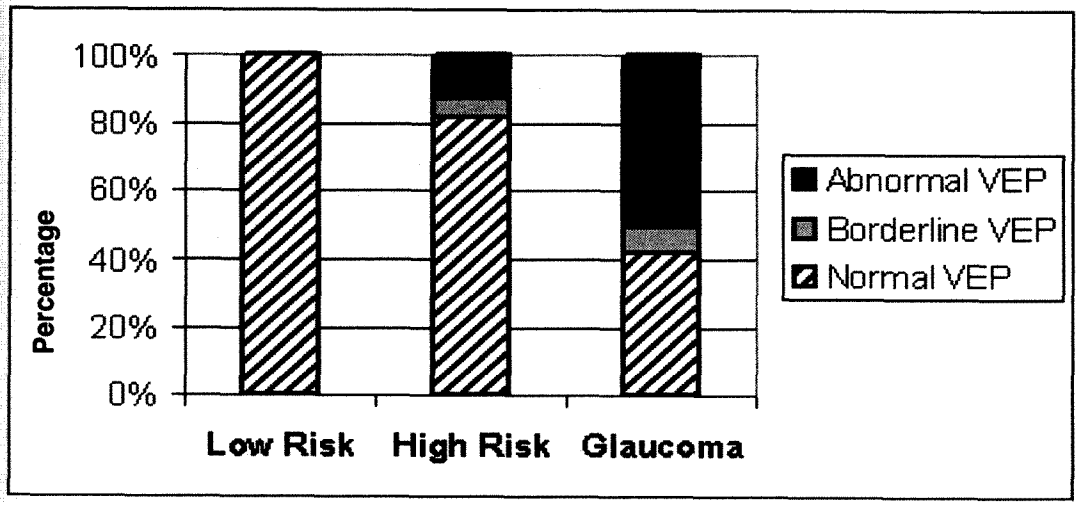


Figure 6. Distribution of abnormal and borderline mVEP in subjects with unconfirmed subjective field defect.

Eyes with excessive visual field loss compared to disc

There was a group of patients (24 patients, 45 eyes) in which the loss of the subjective visual field was much more excessive than expected from the optic disc appearance. The referring clinician highlighted this fact in their assessment.
Of these patients, based on disc appearance, eight eyes were classified as early glaucoma (C/D=0.73+/-0.08). All had subjective visual field testing on two or more occasions and if SAP criteria was used all would have been inappropriately classified as moderate or advanced glaucoma (mean MD= –13.1+/-6.7dB, range -7.15dBto-23.88dB). While the mVEP was abnormal in 7/8 cases, it demonstrated very mild abnormality with ASI ranging from 23 to 46. (see Figure 7 for an example). This was in keeping with the clinical picture, and the expected severity of field loss based on the structural changes in the disc and NFL.

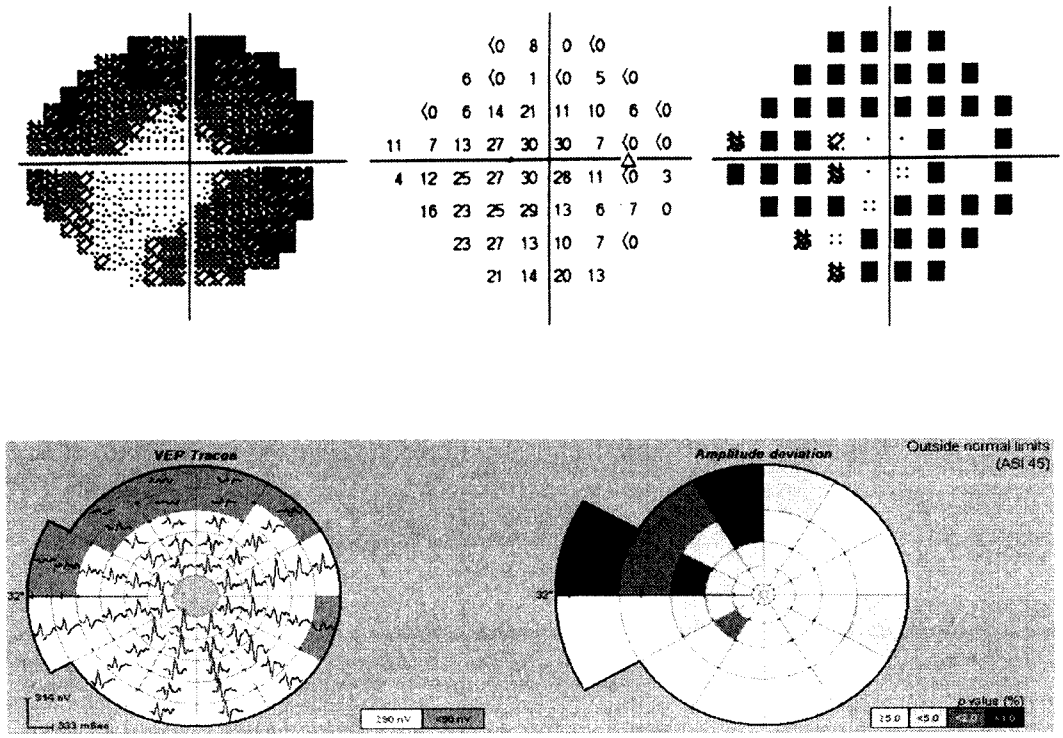


Figure 7. – Example of early glaucoma with mild disc cupping (inferior notch) but advanced subjective field loss. The mVEP confirms a superior arcuate defect that is consistent with the structural findings.

The remaining 37 eyes were classified as glaucoma suspects (C/D=0.59+/-0.1) with SAP MD range –1.57to-27.8 (mean MD= –10.6+/-7.9). Based on optic disc and nerve fiber layer appearance these eyes should not have had a significant visual field defect. However it is possible that some individuals may not show a structural change prior to field loss or that structural change was present but not detectable by routine clinical examination. The mVEP ASI identified 34/37 eyes (92%) as being within normal range, with 3 remaining-only as borderline cases. Two of those borderline eyes had mild central depression of mVEP amplitude due to cataract, while in contrast the SAP demonstrated very advanced reduction of sensitivity all across the field (MD=-29.1 and – 23.8) that was not consistent with the clinical status.

Of the same 37 eyes, the fields of 18 eyes were rated as unreliable by Humphrey (Figure 8) and many were variable in their severity (Fig.9). However the remaining 19 eyes demonstrated substantial, consistent and "reliable" subjective visual fields. (Fig.10) Some of these were "rim" or "upper lid" type SAP artifacts, but some had marked constrictions. An experienced clinician may be able to exclude some of these as artifacts at a glance, or by repeating subjective testing with closer supervision of the patient. However in most cases the test had already been repeated with the same result, so the objective results help to interpret the changes as not significant.

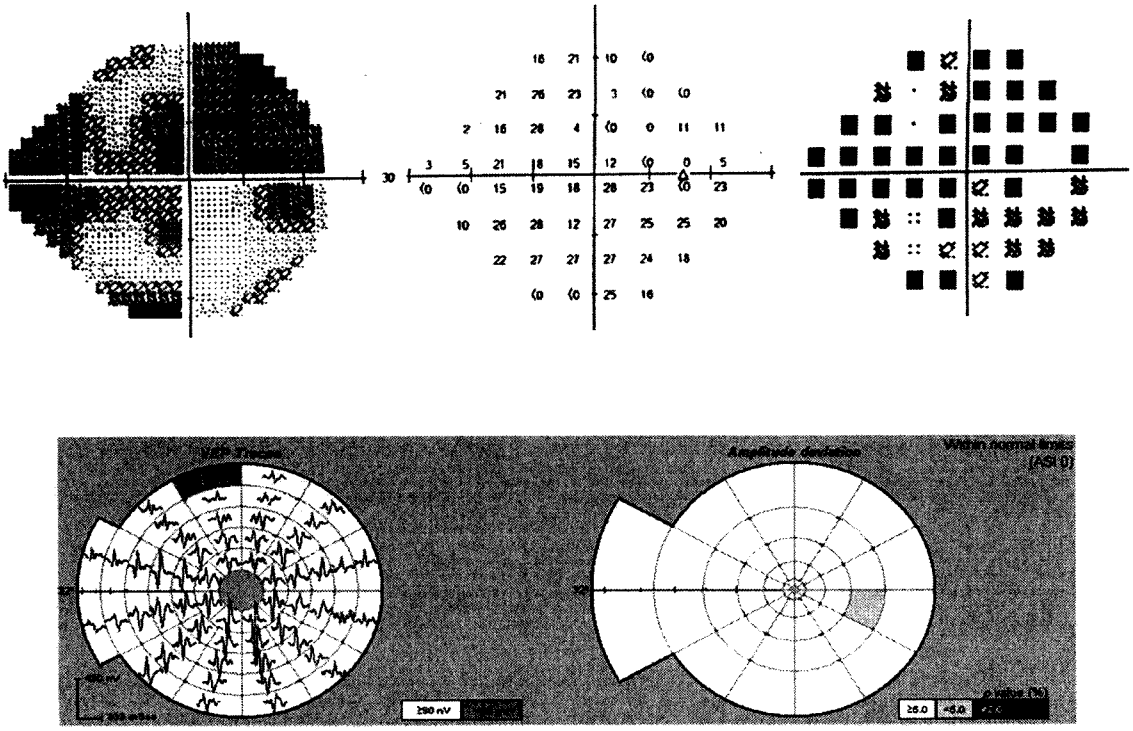
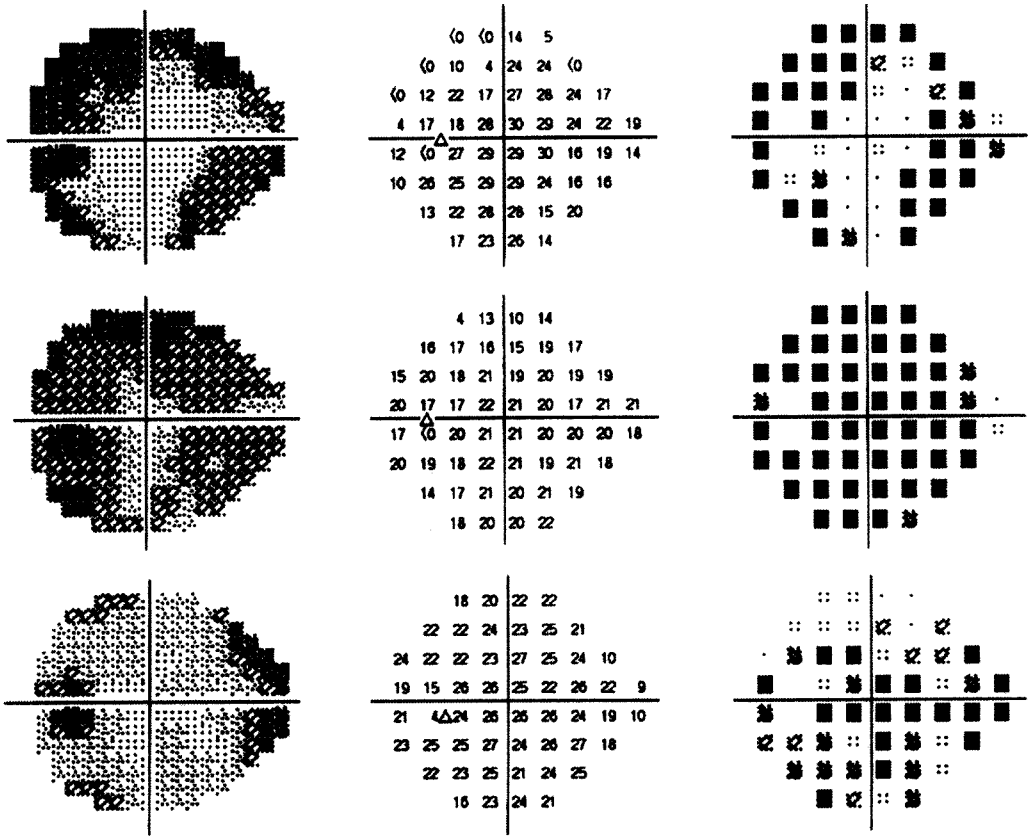


Figure 8. Example of unreliable subjective field (66% false positive) with advanced "losses". Subject had normal disk witch was confirmed functionally by mVEP.



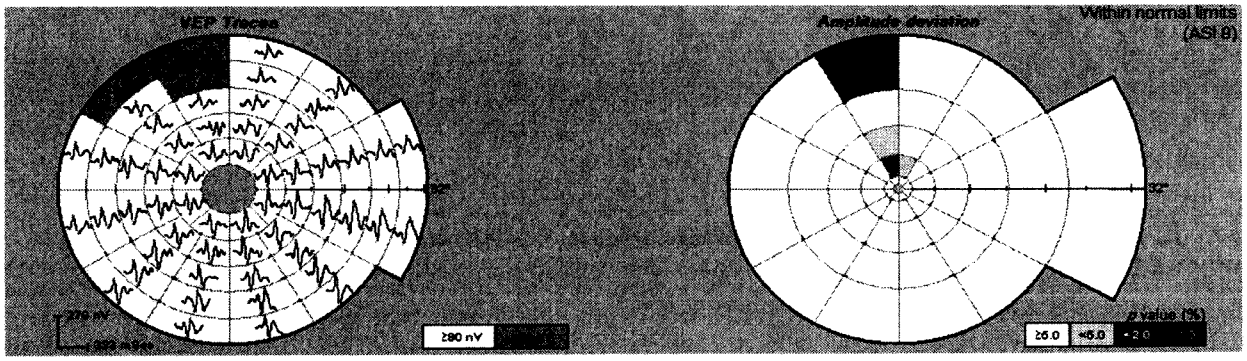


Figure 9. Example of a variable but constantly abnormal Humphrey field with excess loss compared to the disc – Objective result on mVEP is normal

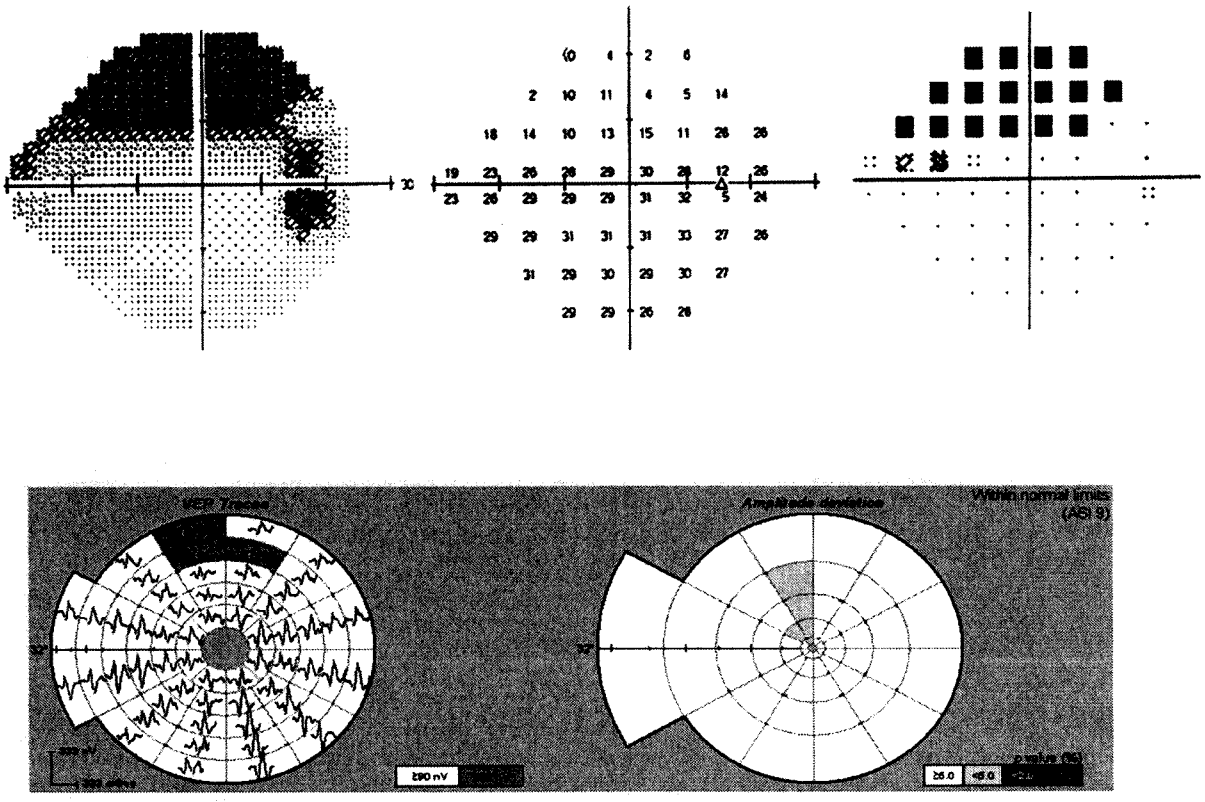


Figure 10. Example of a consistent upper field defect on subjective testing. Disc normal. MVEP is within normal limits.

Clinical outcomes.

The clinical outcomes of mVEP objective perimetry in assessing the glaucoma patient are summarized in the Glaucoma Investigation Model patient flow chart (figure 11). In a large proportion of subjects the mVEP was able to support suspected defects, or exclude defects where the SAP was unreliable, variable or excessive. Total numbers of eyes falling into each category are included. The breakdown does not report numbers of unreliable fields within each category, but many of these fell within the variable and excessive loss groups. In these subjects the mVEP appears to have a particularly important role.

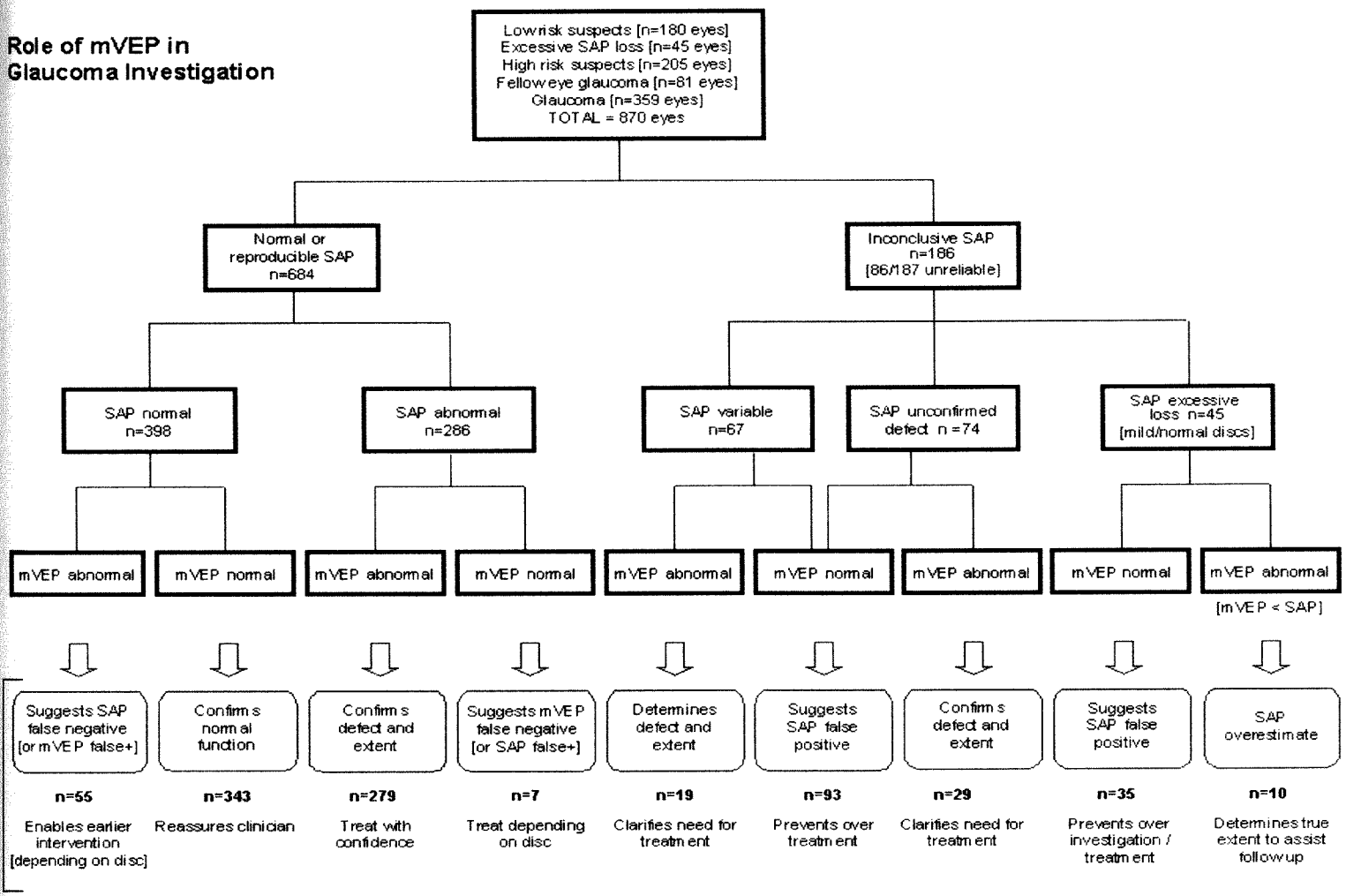


Figure 11. Glaucoma Investigation Model patient flow chart.

Discussion

In this retrospective study the mVEP recorded with the AccuMap™objective perimeter was evaluated across a wide range of clinical presentations in the investigation of glaucoma. It was closely correlated with the severity of the disease both in diagnostic category and directly with Humphrey MD. It achieved a high level of sensitivity in detecting abnormal visual field when compared to the Humphrey (SAP) gold standard. The sensitivity was 97.3% with the only cases missed being in the early glaucoma category. In addition, a significant proportion of high risk suspects (18.4%) and fellow eyes of glaucoma patients with normal visual fields (16.0%) were abnormal on mVEP. This is supportive of earlier detection of defects, especially when it is considered that in the low risk suspect group only 7.8% were abnormal, providing an estimate of the lower limit of specificity. Some of these could actually be early glaucomas who are not

showing a structural change as yet, with follow up being required to determine true status.

The new ASI performs better than setting criteria for the identification of clusters on the monocular amplitude deviation or asymmetry deviation plots. The asymmetry plot is limited when the fellow eye has significant glaucoma, however since the ASI also relies to a significant extent on asymmetry it will also be less sensitive in these cases. The rate of abnormality in fellow eyes (17 to 21%) is lower than in the previously published study. We believe this is due to a higher proportion of fellow eyes that were actually of relatively lower risk, but also to the fact that we have tightened our diagnostic criteria for a scotoma on the deviation plots and in the ASI, as recommended by Hood and Fortune (personal communication), to improve specificity.

When masked assessment of disc photos was used as the comparator both mVEP and SAP performed similarly, however mVEP demonstrated a significantly higher specificity (89% vs 79%). The lower sensitivity (81.6% mVEP, 82.9% SAP) for both types of functional test is expected, since structural changes are known to precede functional changes. In addition, suspect discs can also be misinterpreted as glaucoma even by trained observers. Therefore it is not surprising that the sensitivity of the two functional tests was less than 100% when relying on disc appearance only. Based on the changes seen in some high risk suspects we had expected to see a higher sensitivity for mVEP than for SAP but may have been limited by the small sample size of those with photos available.

The study has limitations by the fact that it is retrospective, and that the data available for patients (eg number of SAP fields) varied widely between subjects. However, since all consecutive cases were included for analysis , with no entry criteria exclusions , the results are representative of a large clinical practice where all levels of disease and experience are encountered and typical patients who perform poorly or variably on subjective testing need to be evaluated. Clinical decisions hinge on visual field data, which is often flawed. In the cases described with variable field loss, excessive field loss compared to the disc appearance, and in those with new but as yet unconfirmed defects, the mVEP was very helpful in determining the functional status.

The GHT was used since it is a simple method for evaluating fields and is used by clinicians regularly in clinical practice. We acknowledge many other factors should be considered when interpreting fields (such as PSD) and many definitions exist in the literature, but there is no agreement as to which is the ideal. In addition, since we were comparing the GHT to a 3 way classification of Normal, borderline and abnormal on ASI scores, the results were more easily compared.

Both eyes were included for the analysis despite the known statistical problems associated, since in the clinical setting there is frequently different performance on SAP between eyes, and a selection criteria which chose only the better eye or the eye with the more reliable field would not give us an overall view of how patients are performing.

Random selection of an eye would exclude a substantial amount of data from the different subgroups. Many subjects had their two eyes classified into different subgroups.

Almost all SAP visual fields were performed in the same glaucoma clinic by trained technicians, who are instructed to alert subjects when they are performing badly or not fixating, and to recommence the test. Therefore there is no known deficiency in the way the SAP fields were conducted.

In the glaucoma cases where the SAP showed excessive loss compared to disc appearance, most had more appropriate levels of change on the mVEP which matched the degree of structural change. In the eyes with normal or only suspect discs but advanced field changes, the mVEP was nearly always normal. It is possible that some individuals may not show a structural change prior to field loss, or that some structural change was present but not detectable on clinical examination. We believe it is unlikely that those cases with advanced losses, however, would still have a normal appearing disc. Across all diagnostic groups of the study between 12-16% of subjects showed variable SAP results over their last 3 tests. In these subjects the mVEP was able to clarify their functional status. In particular, all subjects clinically classified as low risk suspects had normal mVEP, while their subjective field fluctuated considerably from normal to abnormal.

In many cases it is not simply enough to repeat an abnormal or unreliable subjective field test, as clinical trials have shown large fluctuations in defects from test to test and patients may be repeatedly poor performers. There is also a learning curve associated with subjective perimetry that complicates interpretation in new patients, such that 2-3 field tests need to be done before a reliable result is achieved. The fact that in a recent study of the performance of SITA vs full threshold visual field testing [1], the specificity of SITA was only 73.7% after two tests, confirms that in many cases the clinician will be faced with results that either cannot be interpreted or will be misleading as to the extent of disease. However, SAP is now the accepted gold standard with which we investigate glaucoma, and is used in most major clinical trials despite its known limitations.

Alternative subjective tests have been shown to detect glaucomatous change early in the disease, in particular methods such as short wavelength automated perimetry (SWAP)[238] and frequency doubled perimetry (FDT) [239]. Being subjective in nature, they are still subject to the same limitations relating to patient co-operation and understanding of the test technique.

Finally in terms of patient outcomes, the roles of objective perimetry in assessing the glaucoma patient are summarized in the Glaucoma Investigation Model patient flow chart (Fig.11). The mVEP supports or helps rules out subjective field loss, it reassures the clinician and patient when results are inconsistent, variable or excessive changes are seen, and may help prevent over-treatment. In some early or high risk glaucoma cases it may detect functional damage earlier enabling commencement of treatment at an earlier stage. Many cases with excessive field loss might otherwise be sent for diagnostic imaging with

CT scan or MRI, since the clinician is uncomfortable accepting the disparity between structural and functional loss. More rarely, should both subjective and objective tests be out of proportion to disc changes (not seen in this study sample) then further pathology should be suspected (eg intracranial tumor) and CT/MRI should be considered.
The mVEP provides some significant advantages over subjective testing of the visual field. It presents objective results, removing the effects of patient indecision. It does not seem to have a learning curve[234] and has a high level of patient acceptance[240]. Unlike other electrophysiological tests such as the pattern ERG, the set-up is non-invasive. Its main limitation remains cases of noisy recording which can lead to false positives, and increased variability.

Further developments in mVEP technology should be directed towards improving signal to noise ratios and thereby both increasing sensitivity and reducing variability. Reproducibility studies with AccuMap V2.0 are currently underway to determine if reproducibility is good enough to enable application to glaucoma progression analysis. The ultimate goal is to provide an objective measure of functional change over time. At present the mVEP provides a valuable diagnostic aid in many different clinical settings involving the assessment of the glaucoma patient.

Chapter 9

Application of mVEP to cortical lesions

Published as:
Klistorner, AI, Graham,SL, Grigg, J., Balachandran, C Objective perimetry using the multifocal VEP in central visual pathway lesions **Br J Ophthalmol 2005,** 89,739-744

Abstract.

Background/Aims: To examine the ability of the multifocal pattern visual evoked potential (mVEP) to detect field loss in neurological lesions affecting the visual pathway from the chiasm to cortex.
Method: The mVEPs recorded in the clinic were retrospectively reviewed for any cases involving central neurological lesions. Recordings had been performed with the AccuMap V1.3 objective perimeter, which used an array of four bipolar occipital electrodes to provide four differently oriented channels for simultaneous recording. Nineteen patients with hemianopias were identified. Of these there were 10 homonymous hemianopias with hemifield type loss, two bitemporal hemianopias and 7 homonymous hemianopias with quadrantinopic distribution. A comparison with subjective field results and CT/MRI findings was done to determine the relationship between the two methods of visual field mapping and any relationship with the anatomic location of the lesion and the mVEP results.
Results: In all hemianopic type cases (12) the defect was demonstrated on the mVEP and showed good correspondence in location of the scotoma (nine homonymous and two bitemporal). The topographic distribution was similar but not identical to subjective testing. Of the 7 quadrantinopic type hemianopias, only 4 were found to have corresponding mVEP losses in the same areas. In the 3 cases where the mVEP was normal, the type of quadrantinopia had features consistent with an extra-striate lesion being very congruous, complete and respecting the horizontal meridian.
Conclusions: The mVEP can detect field loss from cortical lesions, but not in some cases of homonymous quadrantinopia where the lesion may have been in the extra-striate cortex. This supports the concept that the mVEP is generated in V1 striate cortex and that it may be able to distinguish striate from extra-striate lesions. It implies caution should be used when interpreting "functional" loss using the mVEP if the visual field pattern is quadrantic.

My role in this study included : discussion and design of the study together with Dr Klistorner, identifying suitable neurological subjects and verifying their diagnosis. I jointly analyzed all results and cowrote the manuscript with Dr Klistorner. Our technician did the actual recordings.

Introduction

The visual evoked potential (VEP) has traditionally been an important method for assessing the state of visual pathways. It has been most useful in the diagnosis of conditions such as optic neuritis and other optic neuropathies. There have been several reports of VEP abnormalities in cortical lesions, which demonstrated reductions in amplitudes when the affected part of the visual field was stimulated. Sequential quadrantic or hemifield stimulation was used in these studies[108, 111, 241-244].

The clinical findings from these studies were limited by the fact that the conventional pattern VEP is predominantly generated by cortical elements receiving projections from the central retina and it has been estimated that the central 2° of visual field contributes 65% of the response[99-101]. Therefore the conventional VEP had limited ability to reflect field loss in non-central areas. Recent developments in multifocal stimulation techniques have provided a new method for assessing visual function, and expanded the scope of the VEP to enable assessment of multiple sites in the field out to 26 degrees eccentricity[165, 169]. Results published using multifocal techniques have demonstrated a good correspondence between visual field thresholds and the amplitude of the local multifocal VEP (mVEP) response. Scotomas can be identified and field defects in glaucomatous optic neuropathy and optic neuritis have been well defined[195, 199, 222, 223, 226, 231].

There are a few reports of mVEPs in cortical lesions, which have suggested that hemianopic and quadrantic defects can be identified and topographically represented with the mVEP responses[233] [232]. This has led to the notion that if a normal mVEP response is obtained from stimulation of the "blind" area of the field that the visual loss is "functional" rather than pathological[233]. If true then this would have implications for the investigation of suspected malingerers and cases of medicolegal compensation.

We have investigated a sample of confirmed visual pathway or cortical lesions with varying types of field defects to determine if the mVEP does represent the subjective losses seen on perimetric testing. Based on previous conventional VEP studies, which were thought to be a good differentiating tool for functional versus organic field loss, and limited mVEP research, it had been anticipated that all types of lesions would be detected. However, since the mVEP is thought to be derived from the striate V1 cortex [245-247] (although exact origins are yet to be confirmed), the possibility that extra-striate lesions might remain undetected still remains.

Methods

Subjects

A retrospective review was performed of all subjects tested in the clinic with mVEP. Nineteen subjects were identified who had various forms of homonymous or

bitemporal field loss from lesions of the posterior visual pathway (pituitary to visual cortex). All subjects had subjective visual fields (Humphrey visual field SITA standard 24-2) - and 17/19 had some form of neuroimaging (CT or MRI) available. Three of the subjects had been tested twice on the mVEP.

Informed consent was obtained from all subjects prior to mVEP testing. The study had ethics approval and followed the principles of the Declaration of Helsinki.

Stimulation and recording.

The AccuMap^{TM} system (ObjectiVision, Sydney Aust) has been described in detail in previous publications[223] and in Chapter 6.

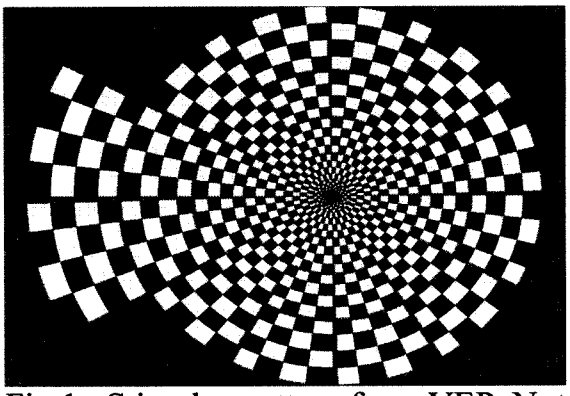


Fig.1 . Stimulus pattern for mVEP. Note central check sizes and stimulus areas are much smaller than peripheral (cortical scaling of stimulus).

Subjects are seated comfortably in a chair and asked to fixate on the fixation number at the centre of the stimulus pattern. The distance to the screen is 30 cm, corresponding to a total subtense of the stimulus of 52° with an additional nasal step. All subjects are optimally refracted for near and the pupils are not dilated. All recordings are collected using monocular stimulation. Usually seven or eight runs of 55 seconds are recorded to provide a good signal-noise ratio.

Gold disc electrodes are positioned in an occipital cross electrode holder positioned over the inion, (with electrodes 3cm above, 6cm below and 4 cm either side)[199]. Four channels are derived from different pairs of electrodes (vertical, horizontal and 2 oblique). Raw trace data are analyzed using the ObjectiVision software. Peak-to-trough amplitudes for each wave within the interval of 60-220 msec are determined and compared among channels for every stimulated segment of the visual field. The wave of maximal amplitude from the four channels, for each point in the field is selected and a combined 4-channel topographic map is created by the software. Amplitude and latency of the mVEP for each point in the field is then determined (for details see Klistorner and Graham[199]).

The mVEP amplitudes in the combined trace array are compared with the normals database percentiles, and probability of abnormality plots are constructed. Student's t-test

are used to calculate statistical difference between averaged mVEP amplitudes derived from affected and non-affected quadrants of the visual field.

Results

There were 19 cases identified where the diagnosis and clinical findings were consistent with a posterior pathway (chiasm to cortex) lesion. These resulted in 12 cases with hemianopic and 7 quadrantinopic type defects. Of these, there were 14 definite cerebrovascular events, one presumed CVA having no lesion identified on CT scan, one cerebral aneurysm, one traumatic head injury, a pituitary tumor, and a craniopharyngioma. A CT scan or MRI had been able to confirm a lesion in 15/19 cases. In 2 cases no lesion was seen on CT scan, but the patient had been presumed to have had an ischaemic event (which is not always identifiable by CT scanning). A further two cases were longstanding deficits (cerebral aneurysm and trauma) and did not have old CT scans available. Table 1 summarizes the subjects and the clinical findings.

In 16 out of 19 cases (12 hemifield type hemianopias and 4 quadrantic type hemianopias) the mVEP result was abnormal according to the Accumap severity index[223] (ASI) and showed a scotoma of >10 points on the probability plot of $p<0.1$. In all those cases there was clear topographic correspondence between subjective visual field and mVEP. However, the distribution of the field defect did not always show as clear a vertical demarcation as the subjective test, and in some cases did not fully extend to the periphery. Despite this, it was always identifiable as a hemifield defect, and was not obscured by surrounding noise. When average quadrantic amplitude of mVEP from subjectively normal quadrants was compared with amplitude derived from quadrants which were affected on Humphrey visual field (HVF) (at least 75% of test points p <0.5%) the difference was highly statistically significant ($p<0.0001$, Student's t-test) (Fig.2.).

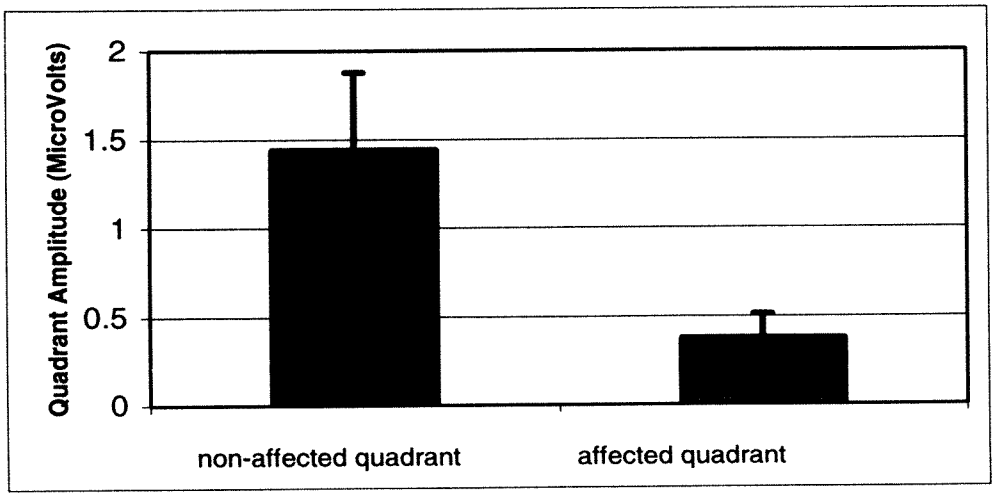


Fig.2 Average quadrantic amplitude of mVEP from normal quadrants on Humphrey visual field (HVF) SITA 24-2 compared with mVEP amplitude derived from quadrants which were affected on HVF (at least 75% of test points p <0.5%). Data from the 10 cases where mVEP was abnormal. ($p<0.0001$)

Examples showing subjective fields and corresponding mVEP are presented in Figs 3-5, with one heminanopic type and 2 quadrantic type hemianopias. The heminanopic defect is well defined on both subjective and objective tests (Fig.3). The quadrantic field defect in figure 4 just crossed the horizontal midline in the left eye making it incongruous, although in the mVEP it respects the horizontal. Figure 5 shows an incomplete left homonymous quadrantinopic defect, with an additional small defect in the superonasal field of the left eye which was due to an old retinal lesion.

However, in three cases (all congruous quadrantinopic types) the amplitude of mVEP was normal across the whole field despite clear abnormality seen on HVF (see example in Fig 6). On examining quadrantic mVEP amplitudes there was no statistical difference ($p=0.46$, Student's t-test) between the mVEP amplitudes of the affected and unaffected quadrants.

Therefore in all of the hemianopic type cases (12/12) but only 4/7 quadrantinopic type lesions, corresponding mVEP losses were found in the same areas of the visual field. The topography of the field loss in the corresponding 15 cases showed very similar distributions, as seen in the figures 3-5. However, the vertical midline demarcation was not as clear in the mVEP as it was on subjective testing, with some abnormal points seen in several cases adjacent to the midline in the normal hemifield.

To determine if artifactual signals could be generated in a quadrant with no signal input we occluded a quadrant of the screen and recorded the mVEP in a normal subject (Figure 7). This clearly showed loss of signal in that quadrant, so we do not believe the normal signals in the 3 cases with quadrantinopic type defects with normal mVEPs are generated by cross-contamination or artifact. It is important to realize that occluding a segment of the screen does not simulate a real scotoma, it merely confirms that the software is not generating artificial signals by cross contamination.

Table 1. Summary of clinical, mVEP and CT/MRI findings of all patients.

Pt #	Age	Sex	Diagnosis	CT or MRI Confirmed	Field defect	MVEP defect	Repeat MVEP defect
1	56	M	L Occipital infarct	Yes	R hemianopia incomplete	Yes	-
2	80	F	L Occipital infarct	Yes	R complete hemianopia	Yes	-
3	53	M	L Occipital infarct	Yes	R hemianopia congruous, macular sparing	Yes	-
4	65	M	Pituitary tumor	Yes	R hemianopia, incomplete, plus L optic atrophy	Yes	-
5	43	M	Embolic CVA - partial recovery	No lesion on CT	L incomplete congruous hemianopia	Yes	-
6	53	M	Blunt head injury 20 years ago	Not available	L hemianopia, complete, congruous	Yes	Yes
7	80	F	R medial occipital lobe infarct	Yes	L incomplete hemianopia, congruous	Yes	-
8	45	F	Craniopharyngioma with chiasmal compression	Yes	Bitemporal hemianopia	Yes	-
9	74	M	L occipital infarct	Yes	R Hemianopia incomplete	Yes	-
10	57	M	Massive R middle cerebral CVA/ craniectomy decompression	Yes	L Hemianopia, R complete field loss and optic atrophy	Yes	-
11	66	F	R occipital infarct	Yes	L Hemianopia incomplete	Yes	-
12	69	F	Left parietal and right occipital infarcts	Yes	L Heminanopia, R incomplete incongruous quadrantinopia	Yes	-
13	62	M	Cerebral aneurysm	Not available	RS Quadrantinopia incomplete non-congruous	Yes	-
14	51	F	Occipital infarct	Yes	RI Quadrantinopia incomplete, noncongruous	Yes	-
15	61	F	R Occipital infarct	Yes	LS Quadrantinopia non-congruous	Yes	Yes
16	73	F	L inferior pole occipital lobe infarct	Yes	RS Quadrantinopia incomplete, macular sparing	Yes	-
17	51	F	L inferior pole occipital lobe infarct	Yes	RS Quadrantinopia complete, congruous	No	-

18	66	F	Hypotensive episode ?infarct	No lesion on CT scan	LS Quadrantinopia Complete, congruous	No	No
19	80	M	L Occipital infarct	Yes	RI Quadrantinopia incomplete, congruous	No	-

R= Right, L=Left, S=Superior, I=Inferior, CVA=cerebrovascular accident, CT=Computerised axial tomography Scan, MRI= magnetic resonance imaging, Repeat mVEP defect shows result if 2nd test was done (3 cases only)

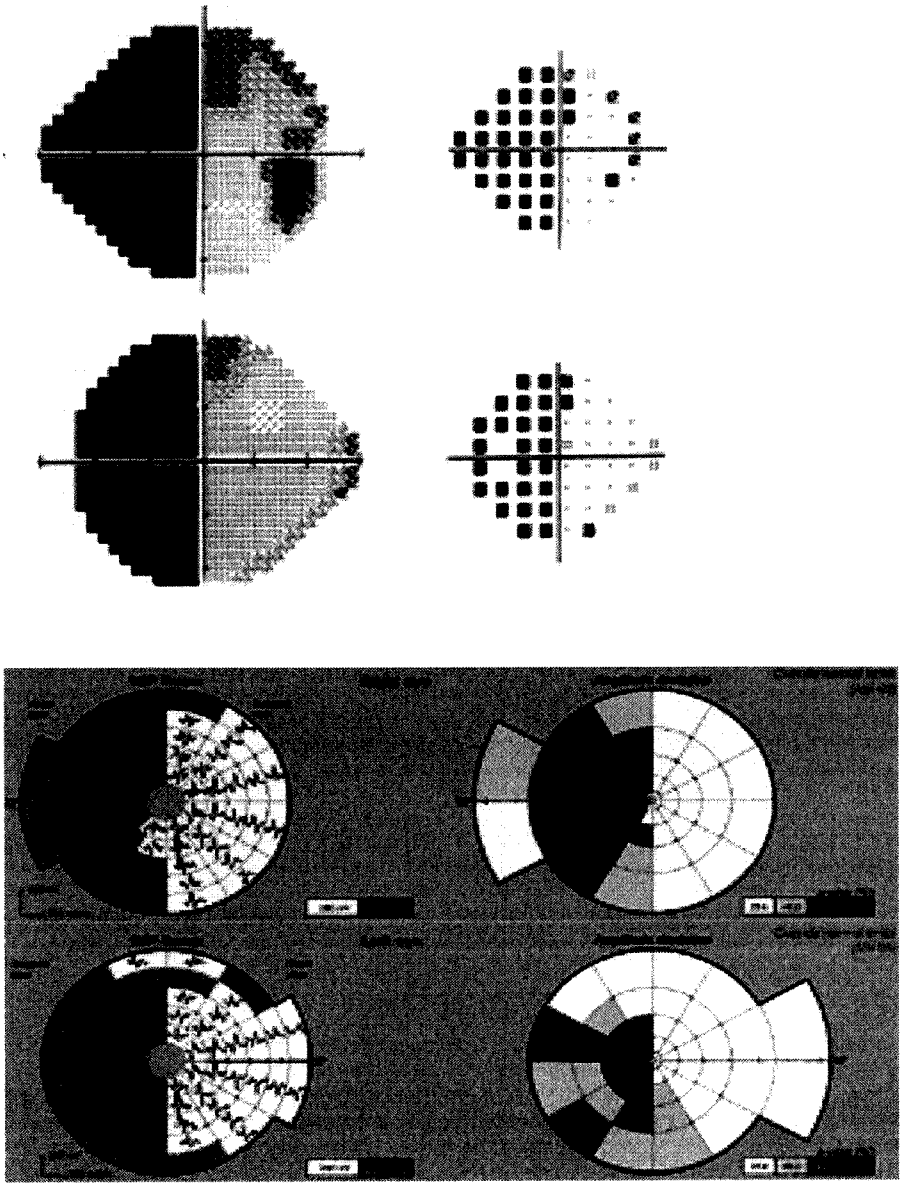
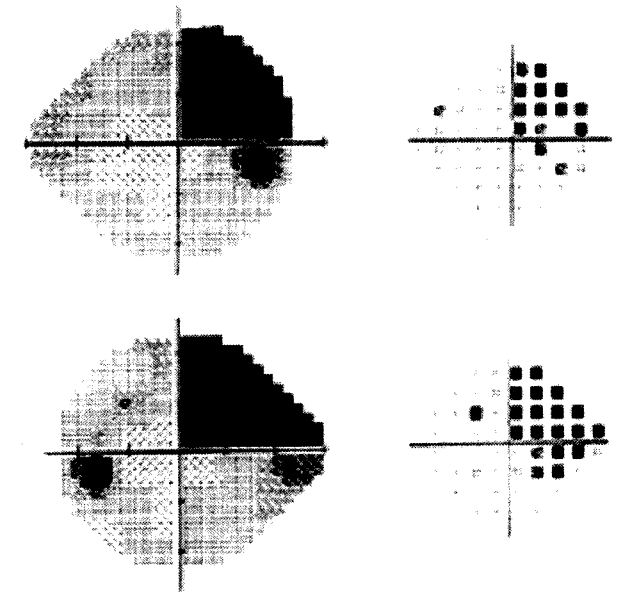


Figure 3. Subject with hemianopia from head trauma. Shows HVF grayscale and pattern deviation plots (SITA 24-2) for right and left eyes (upper2 rows) and corresponding mVEP trace arrays and probability plots (lower 2 rows) from AccuMap. There is good correspondence between HVF and mVEP defects.



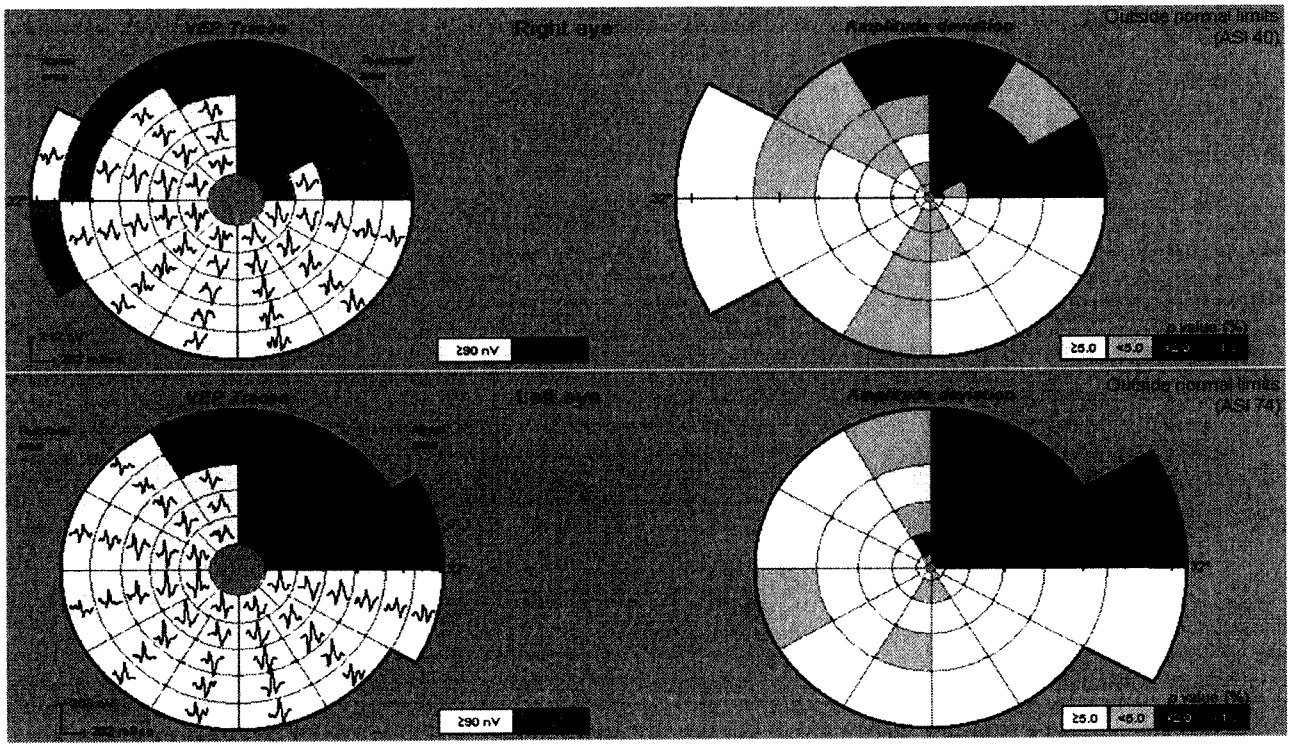


Figure 4. Subject with right superior quadrantic field defect. The defect just crosses the horizontal meridian in the left eye making it not completely congruous, (although in the mVEP it respects the horizontal).

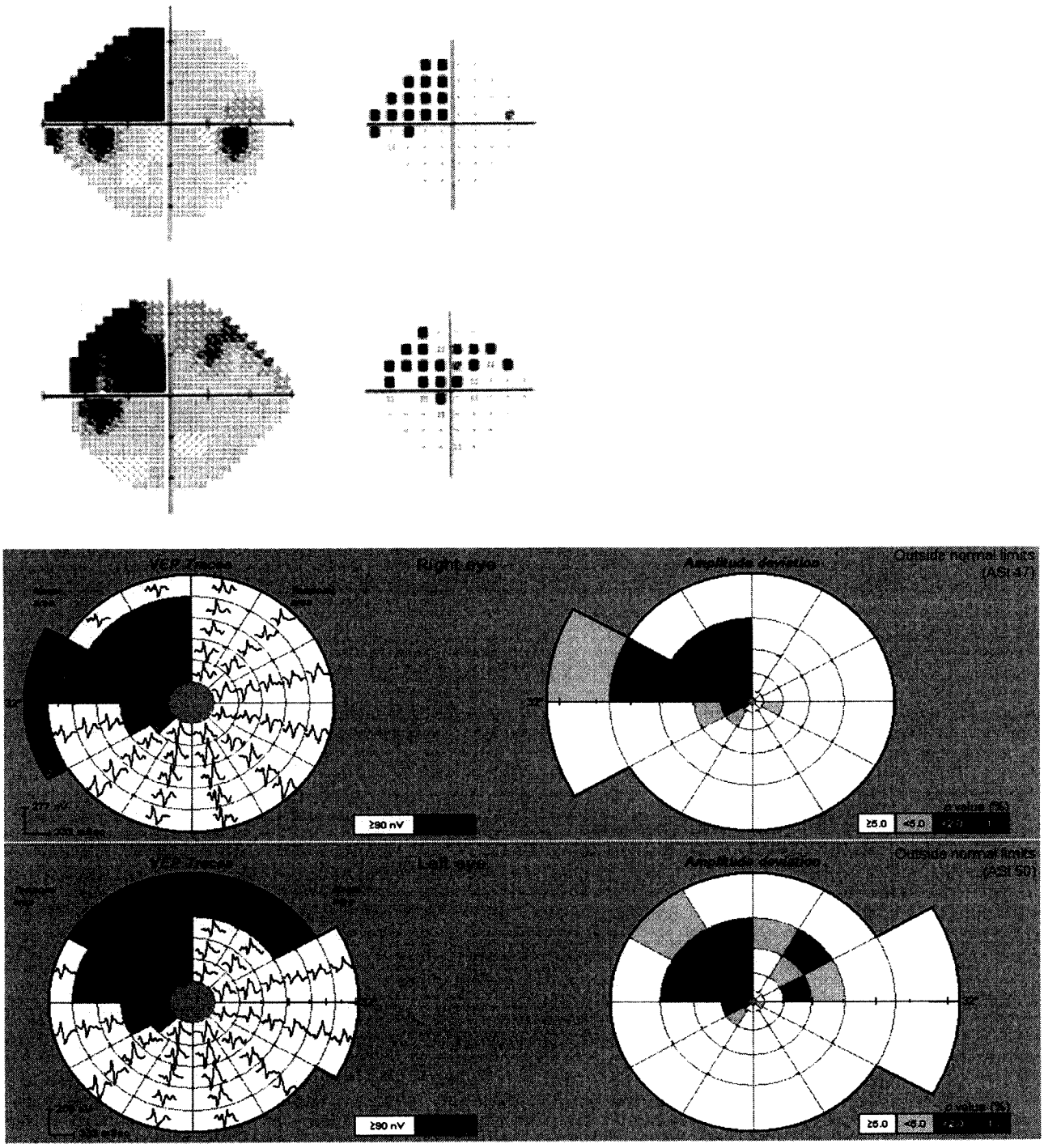


Figure 5. Subject with an incomplete incongruous left quadrantinopia, with an additional small defect in the superonasal field of the left eye which was due to an old retinal lesion.

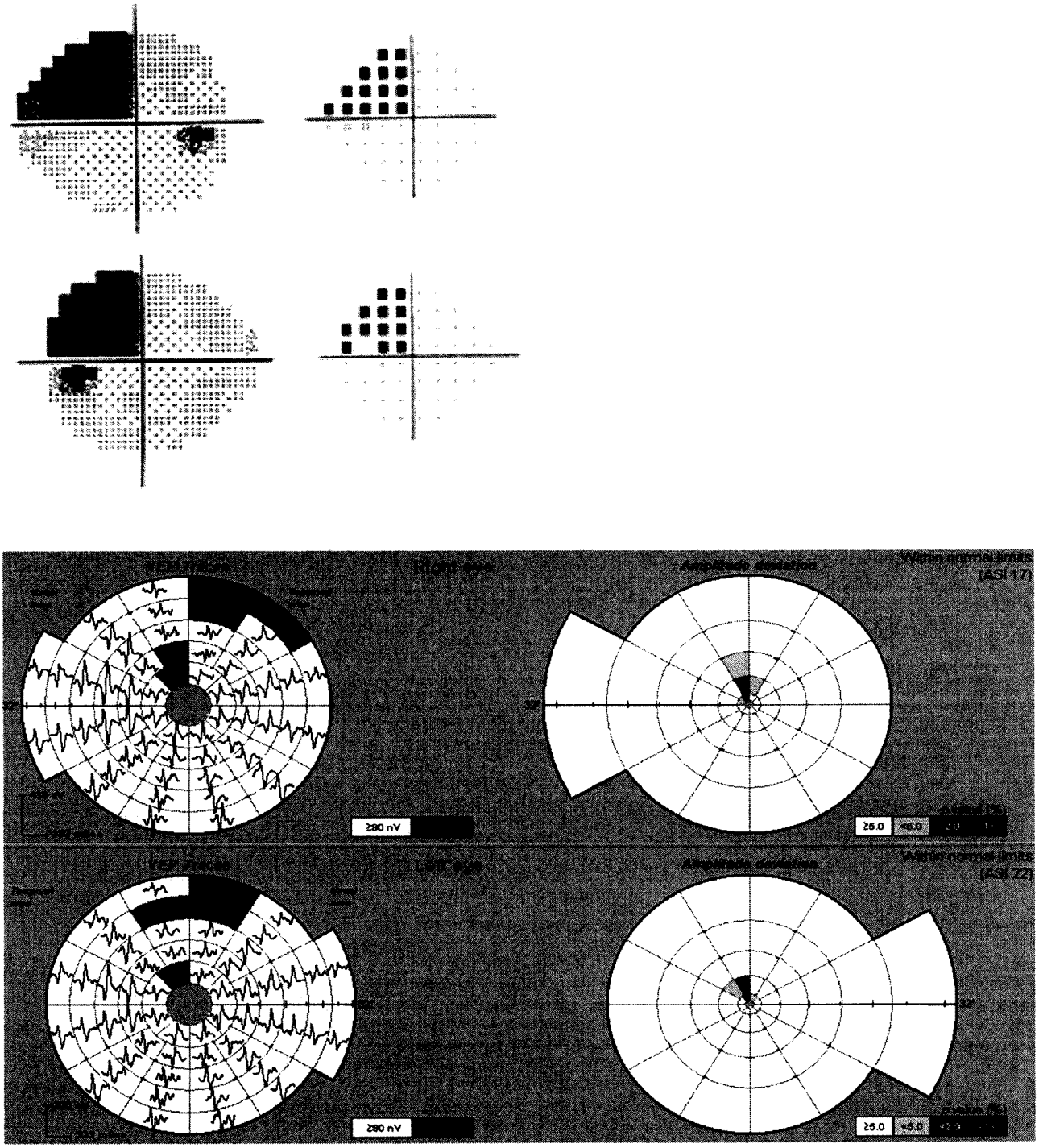


Figure 6. Subject with complete, congruous left superior quadrantinopia from a presumed vascular event with normal CT scan. Repeat visual fields on greater than 5 occasions confirmed distribution. A normal mVEP was recorded twice, with only a few paracentral points reaching borderline significance. Finding is consistent with an extra-striate lesion.

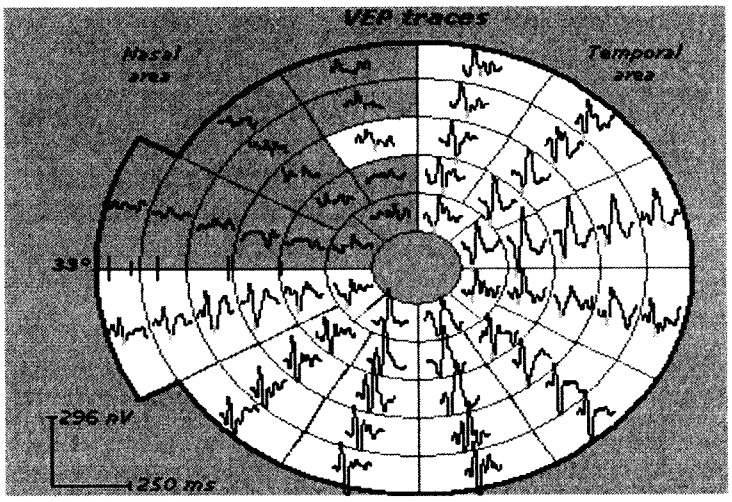


Figure 7. Artificial quadrant defect in a normal subject produced by occluding the upper left quadrant of the stimulus screen. Confirms loss of mVEP signal and excludes cross contamination that could mask field loss. Grey shading indicates areas where signal is indistinguishable from noise or <60nV. There is slight signal spillover across the vertical (in one segment) possibly due to fixation shifts. It is important to realize that occluding a segment of the screen does not simulate a real scotoma, it only confirms that the software is not generating artificial signals by cross contamination or processing errors.

Discussion.

The use of multichannel multifocal VEP recording provides a new means of assessing the integrity of the visual pathway through to the visual cortex. There have been many previous studies on the ability of the conventional VEP to detect field loss and hemianopias [108, 111, 241-244]. While it can be achieved, the topographical relationship is difficult to define without the new dimension that multifocal techniques provide. Thus, two recent reports by Ringger (ARVO abstract #248, 2004) and Wall[248] indicated that hemianopic lesions can be detected by mVEP, but that subcortical lesions are more readily identified than cortical.

In this study all of the 12 hemianopic cases with hemifield type defects were found to have corresponding mVEP losses in the same areas. These hemianopic cases showed definite occipital lesions in 8 cases, 2 more were from chiasmal compression, one from clipping of a cerebral aneurysm more than 15 years earlier and one from old head trauma from a motor vehicle accident. In all these cases the heminanopia was demonstrated on the mVEP, even if the topographic correspondence was not exact.

However, only 4 out of 7 hemianopias with quadrantinopic type defects showed corresponding defects on mVEP. In 3 quadrantinopia cases the mVEP was normal across the whole field. A more detailed examination of the Humphrey visual field in these cases was undertaken, and revealed a noticeable difference between the two groups. While in the four cases where the mVEP was affected the subjective scotoma did not quite reach horizontal meridian (3 cases) or was slightly over it (1 case), in the remaining 3 cases with a normal mVEP, the HVF defect respected horizontal meridian.

The mVEP is thought to be mostly derived from the striate cortex (area V1) [222, 226, 245, 247]. Thus, recent studies based on principal component analysis have confirmed that the electrical dipole that generates the mVEP response follows the shape of V1 folding[247]. We therefore would expect that any lesions prior to or including V1 would lead to reductions in signal. Alternatively, the presence of a mVEP should indicate that there is no abnormality present at least up to the level of neurons of the striate cortex. However, the possibility remains that focal cortical lesions involving extra-striate areas (which are also retinotopically organized) could produce subjective visual field loss but retain a normal amplitude mVEP signal.

While there is no information available about the topography of extra-striate V2/V3 areas in humans, animal experiments indicate that V2 surrounds V1 with projections of upper and lower hemifields being separated by the striate cortex, while V3 is wrapped around V2, being therefore with its upper and lower hemifields separated even further{Van Essen, 1978 #1181;Gattass, 1981 #1182;Weller, 1983 #1198}. Based on anatomical topography it has been suggested, and clinically confirmed in a few cases, that lesions on the border between V2/V3 or V1/V2 will manifest quadrantanopic type scotomas, which would exactly respect the horizontal meridian[103, 245, 249, 250]. Furthermore, it was proposed that for localized damage in V1 to produce a scotoma strictly respecting the horizontal meridian, the lesion must transect striate cortex exactly along the base of the calcarine fissure all the way through to incorporate central and peripheral parts of the field[250]. This is very unlikely for both vascular and neoplastic type lesions. The idea that lesions in extra-striate areas can produce dense perimetric scotomas has been, however, questioned by Merigan and co-workers[251]. Based on animal studies using chemical deactivation of neurons in area V2, they suggested that a quadrantic type scotoma is more likely to be caused by damage to fiber tracts leading from V1 to extra-striate areas, rather than neuronal damage in extra-striate areas themselves. While this matter still remains controversial, it is accepted that a quadrantanopic type scotoma, which strictly respects the horizontal meridian can be caused by damage beyond the neurons of V1.

As was indicated, in the 3 cases of quadrantanopic type scotoma, which were not manifested on mVEP the defects strictly respected the horizontal as well as the vertical midline. This was consistent with a lesion beyond the striate cortex [250]. The CT or MRI showed small localized occipital pole infarcts in 2 cases, and no detectable lesion in the third. Standard neuroimaging is not accurate enough to confirm an exclusively extra-striate lesion, but the small occipital infarcts were not inconsistent. A recent study using functional MRI has demonstrated in a human with a congruous quadrantic defect the loss of MRI signal in the corresponding extra-striate area, but preservation of the MRI signal in V1[245].

In the 4 other cases of quadrantanopic type hemianopia where the mVEP was reduced, the field defects were more characteristic of lesions at or prior to the striate cortex. They had less congruous defects that did not respect the horizontal meridian. In these cases the mVEP was abnormal in the quadrants involved.

The observation that in many cases the mVEP was not as clearly respecting the vertical midline is important in terms of applying the mVEP technique to investigating neurological lesions. This may be due to the fact that the stimulus extends right up to the midline in the mVEP, whereas in the Humphrey visual field the test points are 3 degrees either side of the vertical. Shifts of fixation therefore can have a greater impact. A similar problem was reported in frequency doubled threshold (FDT) perimetry[252] and has been adjusted for in the latest version of the FDT system.

A further reason for lack of exact correspondence between mVEP and subjective fields is that in some areas the background noise (including alpha rhythm) is being incorrectly recognized as true signal, so the probability plot is not flagging the area as abnormal. Improvements in signal recognition will help reduce this problem.
In conclusion, in the investigation of optic pathway or cortical lesions the mVEP can be used to confirm hemianopic defects, in lesions up to and involving the V1 striate cortex neurons. We propose that the 3 cases with quadrantinopic type defects with preserved mVEP (which showed scotomas respecting horizontal meridian) may have been lesions in extra-striate areas or the tracts leading from V1, as they had field defects typical for this. The findings are consistent with the notion that the majority of the mVEP signal appears to derive from V1. It also means that preservation of amplitude implies an intact pathway to V1, which helps localize the lesion. However, a quadrantic subjective field loss respecting horizontal meridian should not be judged as "functional" or malingering just because the mVEP is preserved, since it could be of extra-striate origin.

Chapter 10

Effect of slow stimulation rates on mVEP

Klistorner, AI, Graham,SL Effect of eccentricity on pattern-pulse multifocal VEP.

In Press Doc Ophthalmol

Abstract

Purpose: The sparse pattern-pulse stimulation has been suggested to produce better cortical evoked responses compared to pattern reversal stimulation. This study examines varying pattern-pulse states and the effect of eccentricity of the stimulated visual field on the response.

Method: The multifocal visual evoked potential (mVEP) was recorded using Accumap™ (ObjectiVision Pty Ltd, Sydney, Australia). 58 close-packed cortically scaled checkerboard segments in a dartboard configuration were used. The best configuration for pattern-pulse stimulation was determined by altering relative duration of the "pattern on" and "pattern off" states. This optimal stimulus condition was then compared to pattern-reversal stimulation at different eccentricities of visual field.in terms of signal/noise ratio (SNR) of mVEP amplitude

Results: The maximal response was seen when each element "1" of the binary sequence was represented by two "pattern on" frames (when the pattern was displayed) followed by two "pattern off" frames (when the stimulated area was diffusely illuminated) while each element "0" of the binary sequence is represented by four "pattern off" frames. There was a significant overall increase (27.5%+/-16.5%, $p<0.0001$,) of SNR using this pattern-pulse stimulating mode (SNR=15.5+/-3.8) compared with pattern-reversal stimulation (SNR=12.4+/-2.6). However, this was strongly dependant on eccentricity. In rings 1, 2 and 3 SNR improved by 48%, 43% and 26% respectively (p=0.0003, p=0.0002, p=0.004,) with ring 4 the effect was marginal (p=0.048) and ring 5 was not significantly different (p=0.65). In 4 out of 10 cases S/N ratio in the outermost ring was higher when pattern-reversal stimulation was used.

Conclusions:

The pattern-pulse method offers some advantages for achieving larger mVEP signals from the central visual field. However, the more peripheral field where it is the most difficult to obtain signals, does not show any benefit.

My role in this study included : discussion and design of the study together with Dr Klistorner, examining all normals, jointly analyzing all results and cowriting the manuscript. Dr Klistorner and our technician did the actual recordings.

Introduction.

In recording the mVEP there are several factors which limit the power of the technique. Small signal amplitudes and significant inter- subject variability results in considerable standard deviation of normal values, which limits the ability to detect some early cases. Significant intra-subject variability leads to difficulties in monitoring progression of the disease. While a certain portion of variability can be attributed to intrinsic factors such as cortical convolution and individual variations between external cranial marks and the brain[253], it is partially caused by noise, both biological and electronic. Reduction of noise or alternatively, increase in signal amplitude will result in improvement of both intra-and inter-subject variability. It will also increase the dynamic range of the mVEP. Hood and co-workers recently demonstrated that mVEP recording with large SNR could be more sensitive than subjective testing in detection of early visual field deficit [227]. An increase in the SNR would be particularly beneficial in the peripheral area of the upper field where often the signal is so small that it is practically indistinguishable from noise.

There have been reports recently indicating that slowing down the stimulation rate [246], particularly when it is associated with pattern-pulse rather than pattern-reversal stimulation [254], considerably increases amplitude of the mVEP and significantly improves SNR. James has demonstrated that using pattern-pulse multi-focal stimulation of the central 32 degrees (16 degrees of eccentricity) improves SNR on average by 1.9 times. The author's suggested explanation for the observed improvement was that with traditional pattern-reversal stimulation spatial contrast is present at all times, which, together with high rate of stimulation, increases adaptation of the visual system and leads to reduction in amplitude of the response. Pattern-pulse stimulation, on the other hand, allows an interval of zero contrast between stimuli, which reduces the adaptation and leads to a full recovery of the contrast sensitivity of the response, therefore increasing the amplitude.

However, the question of whether the benefit of reducing the effect of "adaptation" is equally manifested at different part of the visual field and particularly in the periphery (where the amplitude of mVEP is smallest) still has not been addressed. Thus, the aim of the current study is to investigate the effect of slow pattern-pulse stimulation on amplitude of mVEP at different retinal eccentricities (up to 24 degrees).

Method.

Stimulation.

The multifocal multichannel VEP was recorded using Accumap™ (ObjectiVision Pty Ltd, Sydney, Australia). The ObjectiVision system employs a spread spectrum technique (as described in Chapter 6) using families of binary sequences to drive the visual stimulus. Each sequence consists of two elements (element "0" and element "1"- see below) distributed pseudo-randomly. The visual stimulus was generated on a

computer screen (22" Mitsubishi high resolution display, stimulation rate 75 Hz). The standard 58 close-packed segments in a dartboard configuration were used. The segments were cortically scaled with eccentricity to stimulate approximately equal areas of cortical (striate) surface (see Figure 1). Luminance of the white check was 146 cd/m^2 and luminance of the black check was 1.1 cd/m^2 producing Michelson contrast of 99%. Background luminance of the screen was maintained at a mean level of 73.5 cd/m^2.

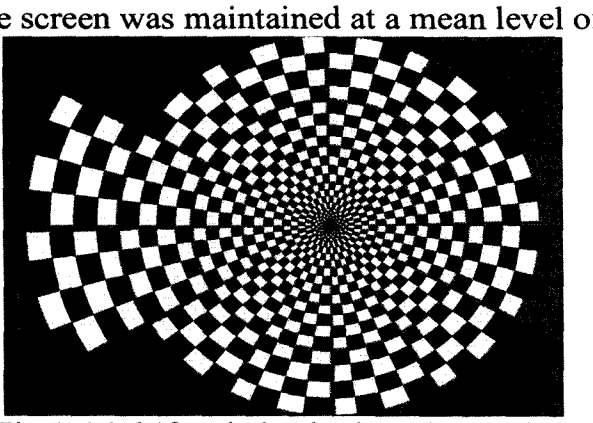


Fig.1 Multifocal checkerboard stimulus.

For pattern-reversal stimulation two opposite checkerboard pattern conditions undergo pseudo-random binary exchange at each of 58 sites in the visual field with a probability of reversal being 50% at each frame of the monitor (Fig.2). For each segment of the visual field the pattern reverses each time the binary pseudo-random sequence assigned to the segment has element "1" and stays unchanged when the sequence has element "0".

For pattern-pulse stimulation each element "1" of the binary sequence is represented by two consecutive states: state "pattern on"-checkerboard pattern and state "pattern off"-diffuse illumination of the whole segment with an intensity of a mean luminance between the black and white check (73.5 cd/m^2). The relative duration of the two states may be altered. For the element "0" of stimulating sequence the state "pattern off" is always active.

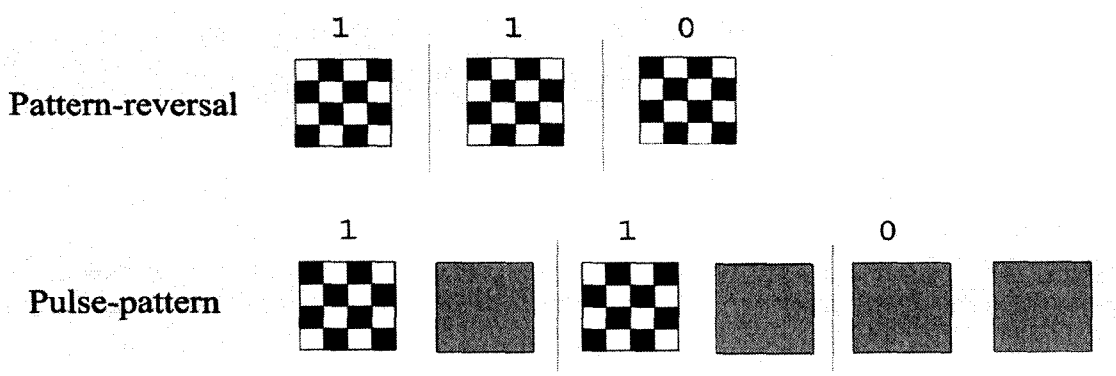


Fig.2 Examples of pattern-reversal (upper row) and pattern-pulse (lower row) stimulation mode. Number indicates element of the stimulating sequence.

For both types of stimulation each input (stimulation site) is modulated in time according to a different sequence. The technique permits computation of the resulting signal by cross-correlation of the response evoked by the sequence stimulation, with the sequence itself. Short sequences of 512 elements are used. Several runs are usually performed and the results are averaged to increase SNR. While duration of a single run varies depending on the combination of states "pattern on" and "pattern off" in the pattern-pulse type of stimulation, the total recording time for each experiment is approximately equal.

In order to establish best stimulation parameters for the pattern-pulse stimulation a pilot experiment (Experiment 1) was performed (5 subjects). It had two steps. Firstly, the optimal number of frames during which the pattern was continuously displayed ("pattern on" frame) was determined. It was noted that if one frame was used to present the pattern, the perceived contrast of the stimulus was low (which is possibly due to the very short period of presentation-decay rate of CRT monitor, which is about 2 msec). Three different conditions were tested (Fig.3). Condition one was represented by 1 "pattern on" (pattern) frame 1 "pattern off" (diffuse grey) frame for each element of stimulating sequence. Condition two had 2 "pattern on" frames and 2 "pattern off" frames and condition three –3 "pattern on" and 3 "pattern off" frames.

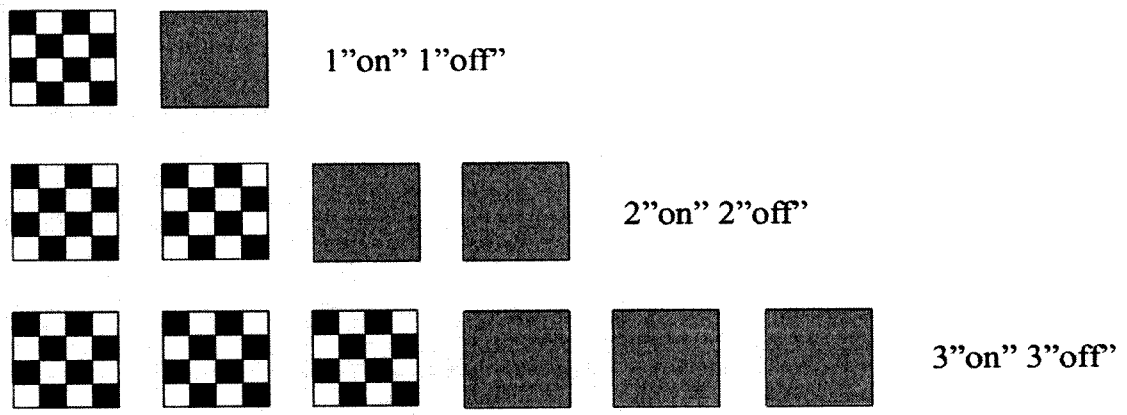


Fig.3 Types of single "pattern-on" elements of stimulating sequence used in first part of Experiment 1

Secondly, the optimal duration of an interval between pattern appearances ("pattern off" frames), which are represented by the diffuse grey of the mean luminance was defined (Fig.4).

The optimal number of "pattern on" frames were kept constant while the number of "pattern off" frames changed between 1, 2, 6, 9 and 14 which were selected to keep total recording time for each test approximately equal (10 runs were recorded in the first stimulus condition, 8 runs in second, 4 runs in third, 3 runs in forth and 2 runs in fifth).

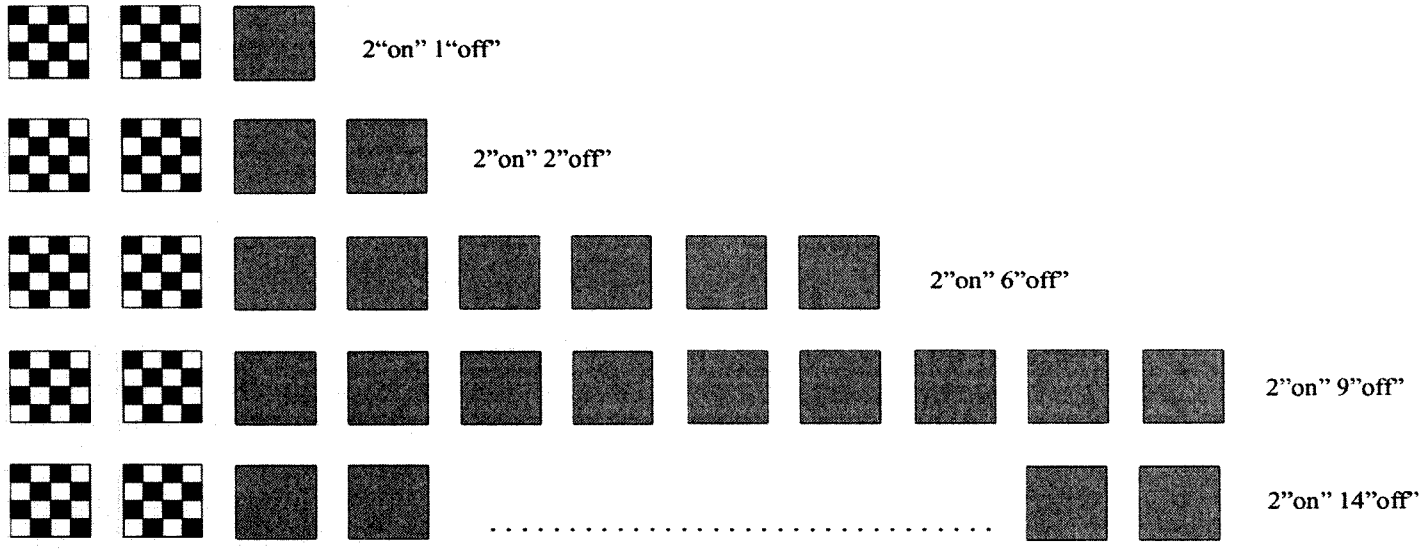


Fig.4 Types of single "pattern-on" elements of stimulating sequence used in second part of Experiment 1.

In Experiment 2 the best pattern-pulse stimulation condition, as determined above, was used for comparison with standard pattern-reversal stimulation. 10 subjects participated in this experiment. Recording of both pattern-reversal and pattern-pulse mVEP were done one after the other in random order on the same Accumap machine under the same conditions. The electrode cross was not removed between the tests.

Subjects and recording.

10 healthy volunteers (5 male and 5 females, mean age 46.2+/-8.6) participated in this study. The study protocol was approved by our regional ethics committee and informed consent was obtained from all subjects. Subjects were seated comfortably in a chair with the chin slightly elevated to relax neck muscles. They were asked to fixate on the small randomly changing number at the centre of the stimulus pattern. The distance to the screen was 30 cm, corresponding to a radius of the stimulus of 25°. All subjects were optimally refracted for near and the pupils were not dilated. All recordings were collected using binocular stimulation. Data was recorded using an ObjectiVision four channel amplifier, with band-pass filter between 1 and 30Hz. The signal was amplified 100,000 times and then digitally filtered between 1 and 20Hz. The data-sampling rate was 450 Hz.

An ObjectiVision occipital cross electrode holder pre-determined the four standard electrode positions[199].

Analysis.

Data was analyzed using OPERA™ V1.3 software (ObjectVision Pty Ltd, Sydney, Australia). Raw data for each run was cross-correlated with pseudorandom Kasami sequences to extract the mVEP signal for each segment of the visual field stimulated. The procedure was repeated for each run and results averaged for each

channel independently. Maximal peak-to-trough amplitudes for each wave within the interval of 60-250 msec were determined and compared among channels for every stimulated segment of the visual field. The wave of maximal amplitude from each segment in the field was selected and a combined topographic map created by the software.

SNR for each trace of the combined array was determined by dividing maximal peak-to-trough amplitude within the interval of 60-250 msec by the noise level. Noise was defined as standard deviation of the response within the interval of 660-1100 msec.

Analysis of amplitudes averaged across the whole stimulated visual field was performed. To investigate the effect of pattern-pulse stimulation at different eccentricities the segments then were grouped into rings for separate analysis. The inner ring covers the area from 0.5° to 2° and contains 8 segments, the second ring-from 2° to 5° (12 segments), third ring-from 5° to 9.5° (12 segments), fourth ring-from 9.5° to 15° (12 segments) and fifth ring-from 15° to 23° (12 segments).

Results.

Experiment 1– determining optimal parameters.

While only the first and second conditions in the first part of Experiment 1 were statistically different (p=0.03, one-way ANOVA, Tukey HSD test),**(1 and 3(p=0.15), 2 and 3 (p=0.65)),** there was a clear maximum of SNR ratio at 2 "pattern on"/2 "pattern off" stimulation (Fig.5a). This remained unchanged at all eccentricities (Fig.5b). Therefore the 2 "pattern on" frames condition was considered to be optimal and was used in the second part of the experiment, where the number of "pattern off" frames were changed in several steps.

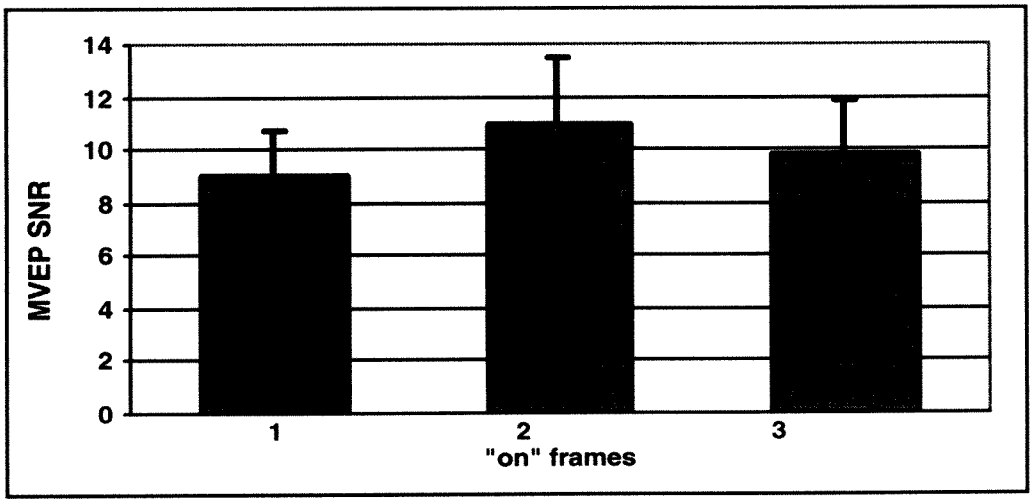


Fig.5a MVEP SNR recorded using 1 "pattern on"/1 "pattern off", 2 "pattern on"/2 "pattern off" and 3 "pattern on"/3 "pattern off" stimulation conditions

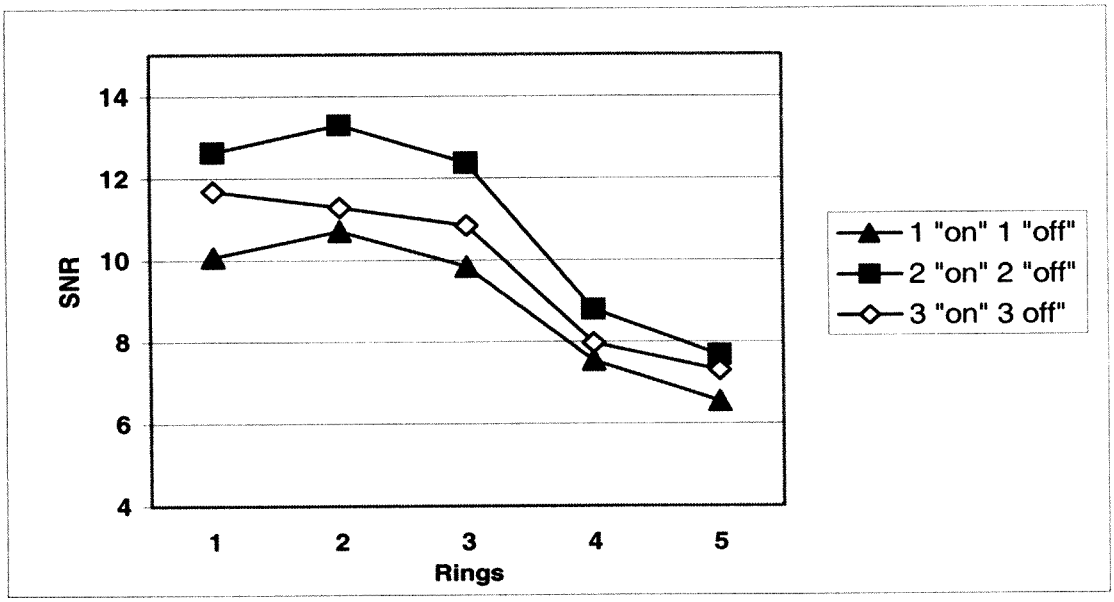


Fig.5b MVEP SNR by ring eccentricity recorded using 1 "pattern on"/1 "pattern off", 2 "pattern on"/2 "pattern off" and 3 "pattern on"/3 "pattern off" stimulation conditions. Rings 1-3 are central rings

Again, while only the first condition was statistically different from the others ($p<0.001$, one-way ANOVA, Tukey HSD test), there appears to be no apparent benefit in extending "pattern off" part of the stimulus beyond 2 frames (Fig.6a). Analysis performed at different eccentricities confirms this trend (Fig.6b).

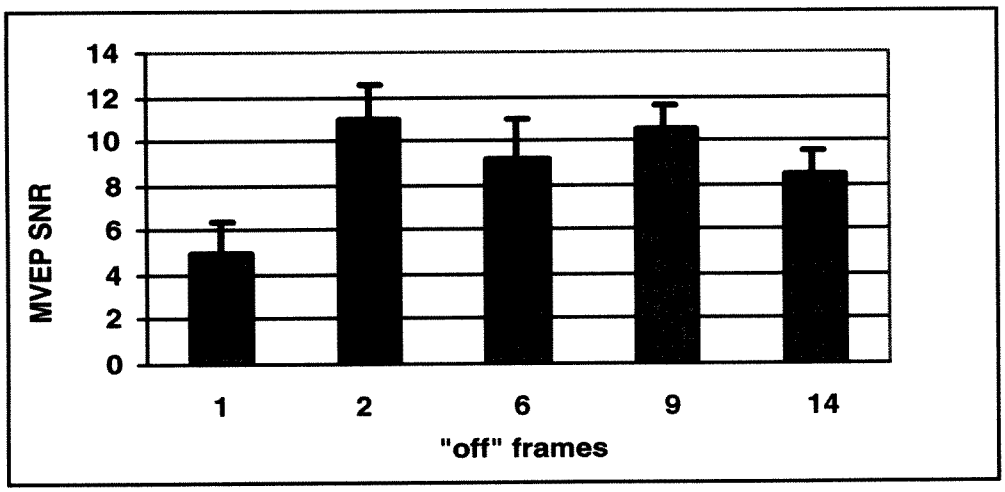


Fig.6a mVEP SNR for varying the number of "pattern off" frames in several steps

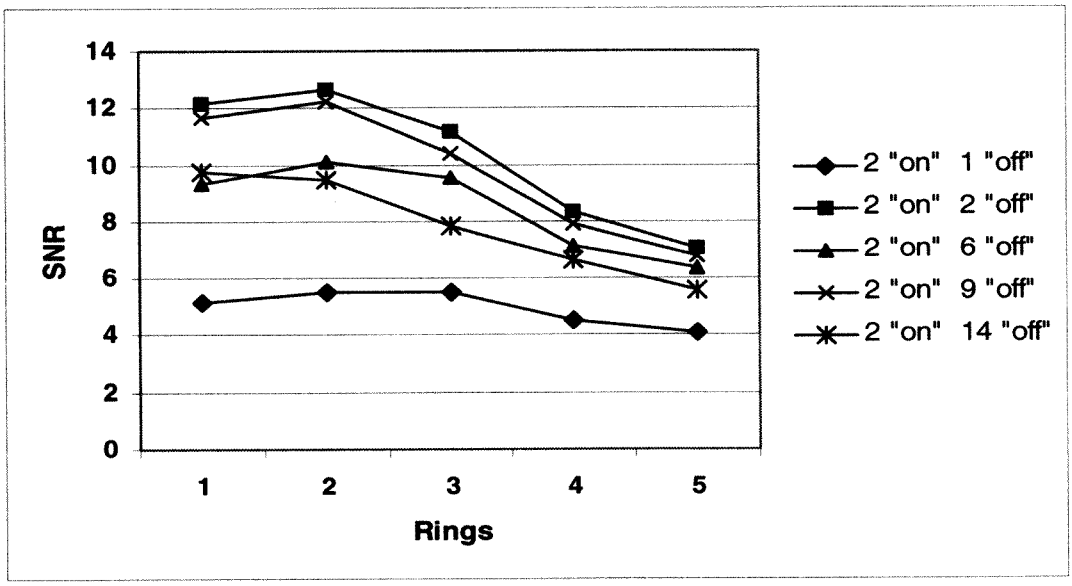


Fig.6b mVEP SNR by eccentricity for varying the number of "pattern off" frames in several

Based on the results of this experiment, the following optimal stimulation conditions were chosen for experiment 2. Each element "1" of the binary sequence was represented by two "pattern on" frames (when the pattern was displayed) followed by two "pattern off" frames (when the stimulated area was diffusely illuminated) while each element "0" of the binary sequence is represented by four "pattern off" frames.

Experiment 2 – Pattern pulse vs standard reversal

The above conditions were then compared with conventional pattern-reversal stimulation where the checkerboard pattern changed polarity at each element "1" of the stimulating sequence.

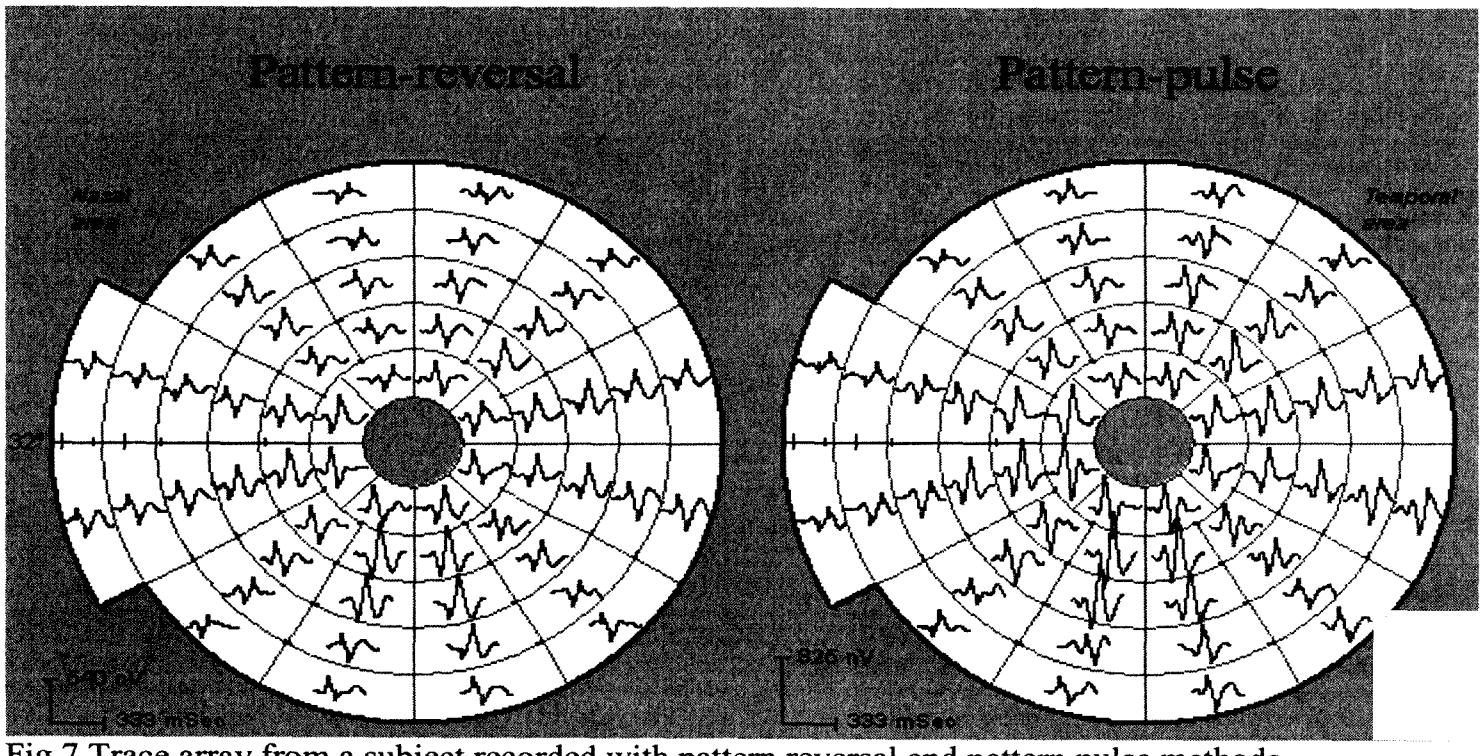


Fig.7 Trace array from a subject recorded with pattern reversal and pattern pulse methods

There was a significant overall increase (27.5%+/-16.5%, $p<0.0001$, Student's t-test) of SNR using the pattern-pulse stimulating mode (SNR=15.5+/-3.8) compared with pattern-reversal stimulation (SNR=12.4+/-2.6). An example of mVEP traces recorded using the two stimulation modes is presented in Fig.7.

This effect, however, was strongly dependant on eccentricity (Fig.8). Thus, the increase was very prominent in the central areas of the visual field tested (rings 1, 2 and 3), where SNR improved by 48%, 43% and 26% respectively ($p=0.0003$, $p=0.0002$, $p=0.004$, Student's t-test). However, improvement just reached significance at ring 4 ($p=0.048$). While there was slight overall improvement in the most peripheral ring (4%), it was not statistically significant ($p=0.65$). In fact, in 4 out of 10 cases SNR in outermost ring was higher when pattern-reversal stimulation was used.

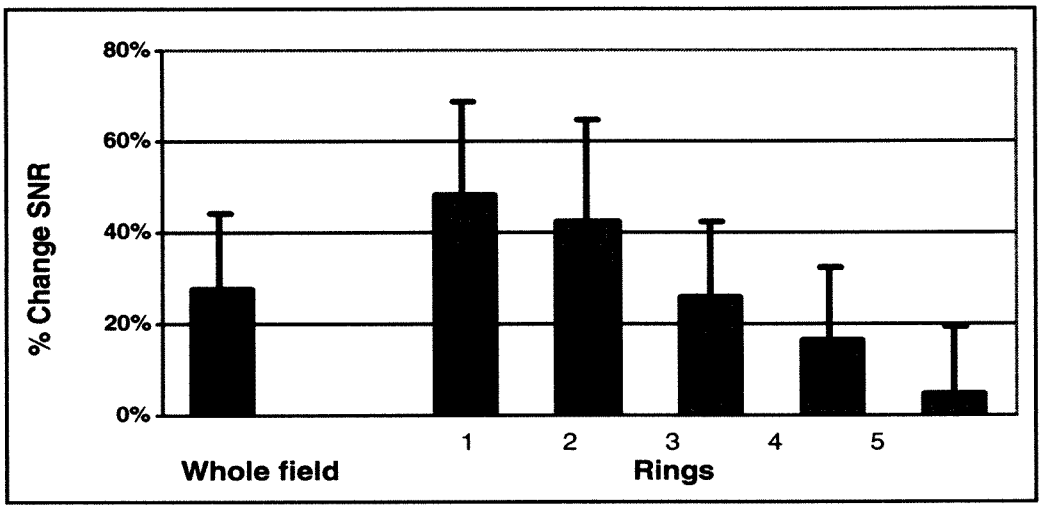


Fig.8 Effect of eccentricity on change in SNR. Main effect is in inner rings with less improvement seen in outer zones.

Discussion.

This study confirmed a significant overall improvement in the SNR of the mVEP when pattern-pulse stimulation is used in comparison with conventional pattern-reversal stimulation. This benefit, however, is limited to the central area of the visual field (up to 9.5°), being only marginal at midperiphery (9.5°-15°) and not significant further out (15°-23°). As was suggested by Jeffreys and Axford [255] the advantage of employing a relatively long inter-stimulus interval could be attributed to reduction of the "adaptation" effect of pattern pre-exposure on succeeding responses. It allows the visual system to fully recover its contrast sensitivity before the next stimulus is displayed [254]. Based on our findings, however, this "adaptation" mechanism is eccentricity-dependant. It implies that temporal resolution of the underlying physiological process is gradually increasing from the center of the visual field to the periphery.

The results of the current investigation are consistent with psychophysical studies of temporal processing using Critical Flicker Fusion (CFF), which also demonstrated increase in temporal resolution with retinal eccentricity[256-259]Thus, Rovamo and Raninen, for instance, showed that, once the cortical magnification factor is taken into consideration, CFF increases from about 36Hz in central area to about 59Hz at eccentricity of 34°[257].

It is well known that the information in the visual system is processed along several parallel pathways. There are two major retino-geniculo-cortical pathways, namely parvocellular (P-cell) and magnocellular (M-cell) with the former responsible for processing of high contrast, high spatial frequency and colour information and the latter-for low contrast, low spatial frequency achromatic images[140, 260, 261]. M-cells also have significantly higher temporal resolution than P-cells[262].

While the number of parvocellular cells is considerably greater across the whole retina, the relative distribution of M ganglion cells increases with retinal eccentricity by the factor of ten[263, 264]. Thus, Dacey and Peterson reported that the ratio of human

parvocellular to magnocellular ganglion cells changes from about 30:1 in the central area to about 3:1 in the periphery[265]. Therefore, one could hypothesize that while reducing the temporal frequency of stimulation by both slowing down the rate of stimulation and reducing the effect of adaptation from the previously displayed pattern (insertion of gray interstimulus intervals) can potentially increase contribution of slow cells (P-cells) to predominantly central mVEP, the effect could be far less evident for M-cells and consequently for peripheral mVEP.

This study did not explore the effect of very slow stimulation rates, due to the fact that even the parvocellular response should be adequate at 5Hz, and a slight increase in the central response would be the only benefit likely, with minimal if any effect in the periphery.

Therefore, in practical terms, the use of pattern-pulse stimulation is likely to be beneficial in detection and monitoring of pathological conditions which predominantly affect the central visual field (for example, optic neuritis). However, the small amplitude of peripheral mVEP still remains a limiting factor in objective detection of visual field abnormality in diseases predominantly affecting peripheral field, such as glaucoma, regardless of the stimulating method used.

Furthermore, in glaucoma, there could be a benefit of using fast rather than slow stimulation. There are a number of morphological[142, 266], physiological[123, 267, 268]and psychophysical[269] studies in recent years indicating possible selective loss of magnocellular cells in early glaucoma. While this matter still remains highly controversial[138, 270], if this is true at least to some extent, then fast stimulation may have an advantage in detection of early glaucoma. In addition, if a faster stimulus is more dependant on magnocellular responses, the fact that even in the periphery these ganglion cells are less numerous should mean that detection of defects is more likely (reduced redundancy).

In conclusion the pattern-pulse method offers some advantages for achieving larger mVEP signals from the central visual field. However, the more peripheral field where it is the most difficult to obtain signals, does not show any benefit.

Chapter 11

Other mVEP studies

Summary

Several other projects were undertaken by our research team to explore different aspects of mVEP recording that were relevant to the design and performance of the technique. In most cases these will be described in full by the first authors in their respective theses or publications, so only the relevant abstracts and/or brief summary/figure are included. Some of these studies were not published as full papers (abstracts only) while others have been submitted for publication.

Research was undertaken into each of the following;

1. Stimulus check size
2. Pupil size
3. Fixation tasks
4. Electrode position
5. Variability of mVEP
6. Blue/yellow mVEP
7. Relationship to Structure – HRT disc imaging
8. mVEP in Optic Neuritis

These projects looked at the effects of variables on the recording technique, or examined alternative stimuli such as blue/yellow check patterns. The relationship to structural change as seen on the scanning laser ophthalmoscope (HRT) was examined. Pilot studies into the application of mVEP to the diagnosis and monitoring of Optic Neuritis were conducted.

1. Check size

Published as: Balachandran, C, Klistorner, AI, Graham, SL. *Effect of stimulus check size on multifocal visual evoked potentials*. **Doc Ophthalmol** 2003, 106: 183-188

In this study we examined the effects of varying stimulus check size on the mVEP. We also evaluated the currently used cortical scaling of stimulus segments. The ObjectiVision multifocal objective perimeter stimulates the eye with random check patterns at 56 cortically scaled segments within the visual field extending to a radius of 26 degrees, with an additional nasal step. All cortically scaled segments have an equal number of checks, which gradually increase in size from the centre to the periphery, proportional to the size of the segment. Stimuli with 9,16,25,36 and 49 checks/segment were tested on 10 eyes of 10 normal subjects. The check size varied inversely with the number of checks per segment. VEP was recorded using bipolar occipital cross electrodes (7 mins/eye). The amplitude and latency of responses obtained were compared with the check size at different eccentricities. Our findings suggest that the existing setting of 16 checks/segment subtending 26' to 140' from centre to periphery is the most effective amongst all check sizes producing the best VEP responses. Decreasing the check size prolongs the latency in the central field only. Cortical scaling of segments generates responses of the same order of magnitude throughout the field, but could possibly be improved slightly to enhance the signal from the outer 2 rings by using a larger stimulus area.

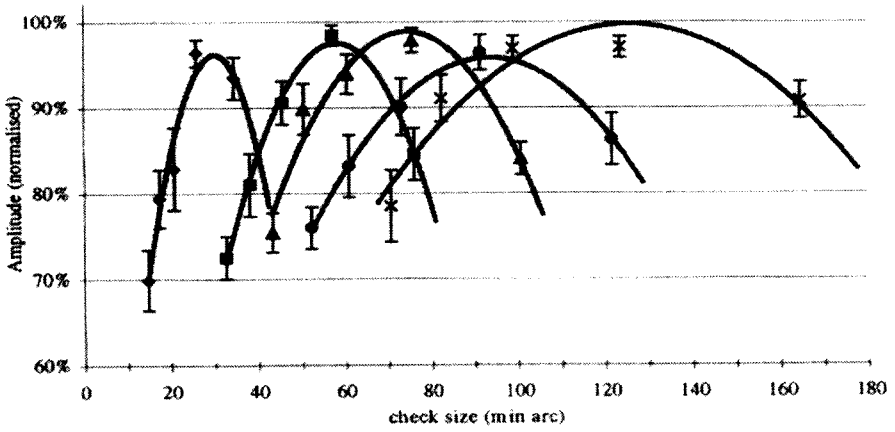


Figure Check size vs normalized amplitude at different eccentricities. Curves represent different rings; diamonds inner ring (eccentricity 1° to 3°) squares second ring (eccentricity 3° to 7°) triangles third ring (eccentricity 7° to 12°), circles fourth ring (eccentricity 12° to 18°) and crosses outermost fifth ring (eccentricity 18° to 26°). Five stimulus sizes were tested for each ring corresponding to 49, 36, 25, 16 and 9 checks/segment of the ring. The size of the corresponding checks varied with size of the ring and is detailed in Table 1. Error bars represent SEM. Curve fitting was done using 2nd order polynomial function.

2. Pupil size

Published as: Martins A, Balachandran C, Klistorner AI & Graham SL *Effect of pupil size on multifocal pattern visual evoked potentials* **Clinical Exp Ophthalmol,** 2003. 31(4): p. 354-356

ABSTRACT

EFFECT OF PUPIL SIZE ON MULTIFOCAL PATTERN VISUAL EVOKED POTENTIALS

Purpose: The purpose of this study was to investigate the influence of pupil diameter on the amplitude and latency of multifocal visual evoked potentials (mfVEP).
Methods: The multifocal objective perimeter (Accumap™; Objectivision) was used to stimulate the visual field at 56 sites extending to 32° using a pseudorandom pattern stimulus. mfVEPs were recorded using bipolar occipital electrodes, 7 min/eye. Ten normal subjects were recruited from the community and one eye was randomly selected for testing. mfVEPs were recorded at four different pupil diameters (2mm, 4mm, 6mm, 8mm), obtained by applying tropicamide (0.5%) or pilocarpine (2%) in different dilutions. Appropriate refractive correction was provided to overcome cycloplegia and achieve a visual acuity of 6/7.5 or better.
Results: Analysis revealed that at most pupil diameters the normalized full field amplitude did not show significant variation, except at the most miotic pupil diameter (2mm), where the amplitude became reduced, based on 2-way ANOVA and Tukey's T Method. There was however, significant correlation between latency and pupil area (corr coeff, upper field –0.63, and lower field –0.76).
Conclusions: The above results suggest that even in the presence of mydriatics or miotics, the m-VEP test can be used to assess diseases that affect amplitude, provided near correction is used. The interpretation of latency, however, must be made with caution, as a borderline conduction defect with a dilated pupil may appear normal.

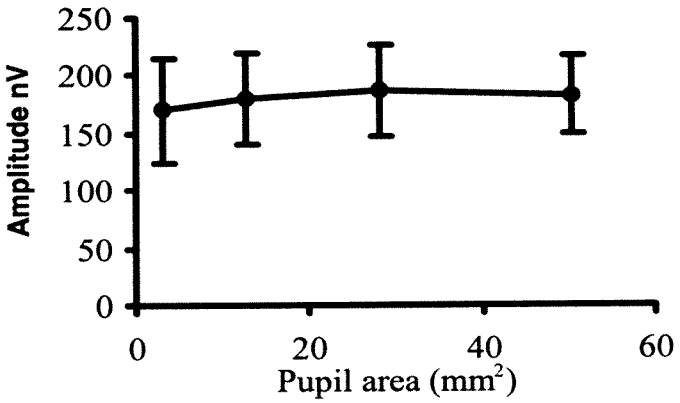


Figure 2 - Amplitude vs pupil area. Amplitude shows no significant difference at 4, 6,and 8mm. There is only a significant variation in amplitude at extreme miosis, 2mm (P=0.007, 2-way ANOVA and Tukey's T Method). Error bar represents the standard deviation of amplitude over the ten subjects at each pupil size.

3. Fixation tasks

ABSTRACT Presented at ARVO 2004 / Submitted to Br J Ophthalmol

EFFECT OF FIXATION TASKS ON MULTIFOCAL VISUAL EVOKED POTENTIALS
Martins, A., Klistorner, AI, Graham, SL, Billson, FC

Purpose: In this study we investigated the effects of cognitive influence on the multifocal visual evoked potential (mVEP) at different levels of eccentricity. Three different foveal fixation conditions were utilized involving varying levels of task complexity. A more complex visual fixation task has been known to suppress peripheral signals in subjective testing.
Methods: Twenty normal subjects had monocular multifocal VEPs recorded using the AccuMap^{TM} objective perimeter. This allowed simultaneous stimulation of 58 segments of the visual field to an eccentricity of 24 degrees. The mVEP was recorded using three different fixation conditions in random order. During Task 1 the subject passively viewed the central fixation area. For Task 2 alternating numbers were displayed within the fixation area; the subject on viewing the number '3' in the central fixation area indicated recognition by pressing a button. Throughout Task 3, numbers were displayed as in Task 2. The subject had the cognitive task of summating the numbers.
Results: Analysis revealed that the increased attention and concentration demanded by Task 2 and 3 in comparison to Task 1 resulted in significantly enhanced central amplitudes of 9.41% (Mann-Whitney p=0.0002) and 13.45% (p=0.0002) respectively. These amplitudes became reduced in the periphery and approached those of Task 1, resulting in no significant difference between the three tasks. Latencies demonstrated no significant difference between each task nor at any eccentricity (p>0.05). As the complexity of each task increased the amount of alpha rhythm was significantly reduced.
Conclusions: Our findings indicate that Task 1 required a minimal demand of cognition and was associated with the greatest amount of alpha rhythm. It was also the most difficult to perform due to loss of interest. The other two tasks required a greater demand of higher order cognitive skills resulting in significantly enhanced amplitudes centrally and the attenuation of alpha rhythm. Therefore, amplitudes are increased around the area of attention.

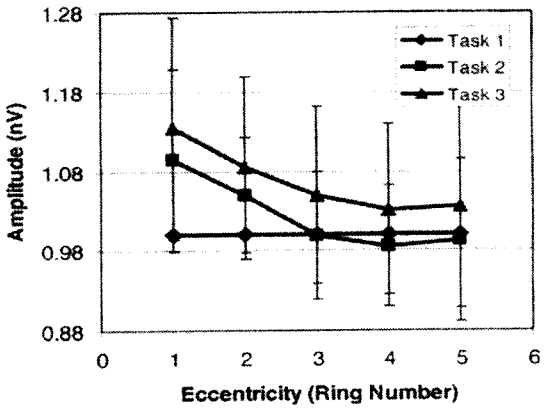


Figure 3 – Illustrates the relative amplitude of each task to Task 1. Bars represent standard deviations

4. Electrode position

ABSTRACT Presented at ARVO 2003

RELATIONSHIP BETWEEN PERIPHERAL MULTIFOCAL VEP SIGNALS AND LOCATION OF MAXIMAL MACULAR RESPONSE

Valencia-Estrada,A, Balachandran, C, Klistorner, AI, Graham, SL

Purpose: To determine the recording location for the largest macular VEP response in normal individuals. To determine whether utilizing the best position for macular signals (BMP) results in improved amplitude of the multifocal VEP in the peripheral field.
Methods: m-VEP was recorded from 20 normal subjects using 4 occipital electrodes (positioned at the inion,10% of the nasio-inion distance below, 10% and 20% of the nasio-inion distance above the inion) and one frontal electrode (Veris-Science® Software). After determining the position associated with the largest macular amplitude, a m-VEP was re-recorded with a 4 channel bi-polar occipital straddle electrode cross configuration (Acumap® system) centered at this BMP and compared with the responses obtained with the cross centered over the inion (standard position). Differences in amplitudes and signal to noise between "On Inion" and BMP were analyzed.
Results: 19 of 20 subjects had the best macular signal recorded with the occipital electrode at 10% of the nasio-inion distance above the Inion. With the electrode cross centered over this BMP the average amplitude of all field locations was 7.1% better than in the standard position ($p=0.002$). Eleven subjects had 13.9% significant improvement in amplitude, 7 subjects had no significant change and in two cases there was significant 8.3% reduction of the amplitude. The group mean amplitude showed a significant improvement of the inferior hemifield of 10.9% ($p=0.002$), both centrally [8.6% ± 2.9% ($p=0.02$); eccentricity 1° to 12°] and peripherally [17.5% ± 2.7% ($p<0.001$); eccentricity 12° to 26°]. Considering all points averaged across the superior hemifield there was no significant improvement in amplitude (2%, $p=0.07$) but, there was a significant improvement in amplitude at the peripheral superior field [17.5% +- 3.6% ($p<0.001$); eccentricity 12° to 26°). The changes were more significant in men.
Conclusions: Changing the position of the electrodes to the BMP improves the amplitude of peripheral m-VEP signals in many but not all cases. If a bipolar occipital electrode cross is used for mVEP recording, the optimal position for centration would appear to be slightly above the inion, although this will still not be ideal in all patients.

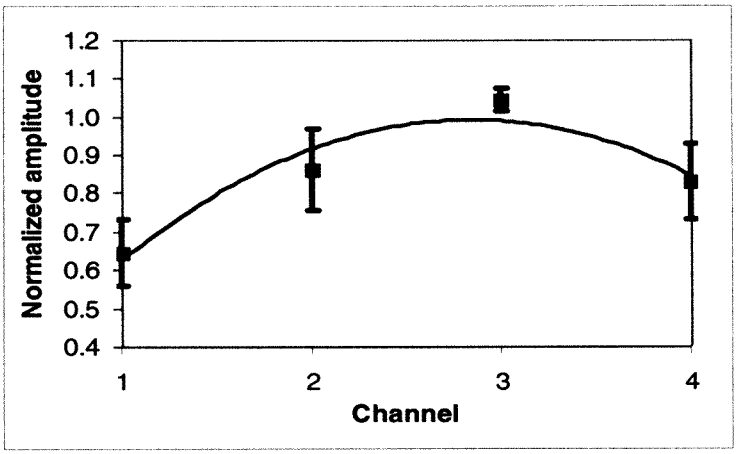


Figure 3 The Macular mVEP amplitude in 20 normal subjects. Values normalized for the highest amplitude. Nineteen of 20 subjects had the position associated with the largest macular amplitude (BMP for "best macular position") in channel 3 (above inion). Channel 2 is the on-inion position (standard). ANOVA, $P < 0.001$

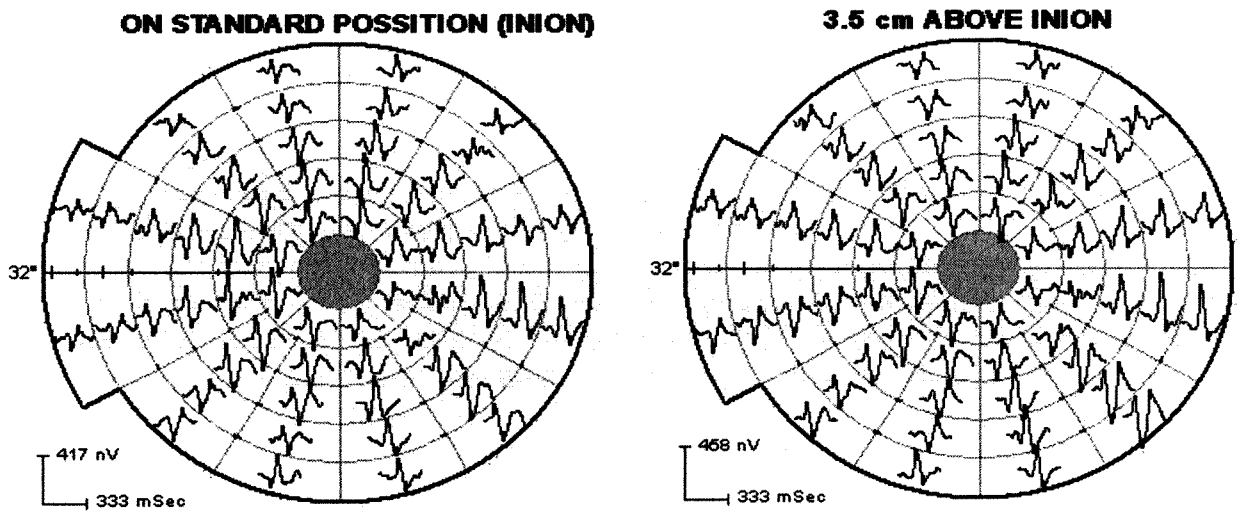


Figure 4 Example of tests done with the electrodes centered at inion and 3.5 cm above inion (BMP)

5. Variability of mVEP

ABSTRACT Presented at ARVO 2003

INTRASUBJECT VARIABILITY OF MULTIFOCAL VEP IN NORMALS AND GLAUCOMA

S.L. Graham, A.Klistorner, C.Balachandran, I.Goldberg.

Purpose: To examine the intrasubject variability of objective perimetry using the multifocal visual evoked potential (mVEP).
Methods: In 22 normals and 22 glaucomas the mVEP was recorded on 2 separate occasions 4 weeks apart using the AccuMap system (ObjectiVision,Sydney Australia). The test recorded multichannel mVEP responses from 58 zones out to 32 degrees eccentricity. Four channels were recorded using a fixed electrode cross position applied over the inion. The cross was reapplied at the same site for the second test and verified by measuring the vertical distance from the cross to the nasal bridge. Humphrey visual fields (SITA 24-2) were also performed. Comparison of amplitudes between tests was then conducted, and a coefficient of variability calculated by using log Test 2/Test1 for each of the 58 points of the trace array.
Results: Average coefficient of variability for the normals was 0.185 +/- 0.03, variability for the glaucoma group was 0.187+/- 0.03. The difference was not significant p=0.7. The variability was slightly increased with eccentricity in normals (0.175-0.195 inner to outer rings), but there was no change in the glaucoma group
Conclusions: The variability is similar to that previously reported for normals with an earlier version of the AccuMap system (Goldberg et al, AJO 2002). In glaucomas we found similar test-retest variability. Further analysis of variability within scotomas needs to be performed to determine the utility of the test in detecting change.

6. Blue/yellow mVEP

In press, Clin Exp Ophthalmol

EFFECT OF CHECK SIZE AND FRAME RATE ON BLUE-YELLOW MULTIFOCAL VISUAL EVOKED POTENTIALS

Martins' A, Klistorner, AI, and Graham,SL

Purpose: To determine the effect of different stimulus frame rates and check sizes on the Accumap blue-yellow multifocal visual evoked potential (mVEP). **Methods:** Experiment 1: 5 adult subjects underwent binocular stimulation by the multifocal objective perimeter. The eyes were stimulated with a cortically scaled dartboard pattern consisting of equiluminant blue and yellow checks. These were arranged in three concentric rings extending to an eccentricity of 26 degrees in the visual field. The stimulus pattern was driven by binary sequences resulting in pseudorandom binary exchange of two opposite checkerboard patterns at each of the 32 sites in the visual field. The mVEPs were recorded at two different rates of display of the pattern stimulus. Experiment 2: mVEPs were tested on 10 normal subjects. Each of the 36 stimulation sites contained a checkerboard pattern of 20, 30, 42 or 56 checks/site, the stimulation pattern was displayed at the optimum rate found in experiment 1. The size of the checks was inversely proportional to the number of checks per site. **Results:** Experiment 1: The slow frame rate significantly increased the average amplitude throughout the field tested by 50% +/ -10.1% (p=0.001). Latency was significantly shortened by 6.3% (p<0.01). Experiment 2: The average amplitude peaked at 30 checks per segment, however this was only calculated to be significantly different from the smallest check size ($F_{crit\ range 4,27}$=0.09 p<0.05, ANOVA, Tukey's T method). A similar difference was found in ring 1 ($F_{crit\ range 4,27}$=0.09, p<0.05, ANOVA, Tukey's T method). In ring 2 however, there was also a significant difference between 56 checks and 20, 35 and 42 ($F_{crit\ range\ 4,27}$= 0.09, ANOVA, p<0.05). Altering the check sizes did not significantly alter the amplitudes in ring 3 nor were the latencies significantly affected. **Conclusion:** Our findings suggest that slowing the stimulation rate and displaying 30 checks per stimulation segment optimizes the blue-yellow mVEP stimulus.

ABSTRACT Presented at ARVO 2003

COMPARISON OF ISOLUMINANT BLUE/YELLOW MULTIFOCAL VISUAL EVOKED POTENTIAL (M-VEP) WITH BLACK/WHITE M-VEP

C.Balachandran, A.Martin, A.Klistorner, S.L. Graham

Purpose: To compare the responses generated using black/white multifocal visual evoked potential (m-VEP) with isoluminant blue/yellow m-VEP.
Methods: 10 normal subjects were recruited from the community. A 25Hz cortically scaled pattern reversal stimulus consisting of 3 rings (eccentricity 1°to3°, 3°to12°, 12°to26°, identical to the m-VEP stimulus) with fixed maximum blue luminance and variable yellow luminance was presented on a computer monitor. For each ring the subject determined the luminance at which there was a minimally distinct border between

the blue and yellow checks. The isoluminant point thus obtained was measured 6 times for each ring. Using the averaged isoluminant valuess for the 3 rings, blue/yellow m-VEP was recorded. The amplitude and latency of signals were compared with those obtained with a black/white m-VEP.

Results: In all individuals there was a gradual increase from the centre to periphery in the amount of yellow luminance needed to achieve isoluminance. There was no significant difference in the amplitude obtained using blue/yellow and black/white m-VEP for the full field ($p=0.49$) and at different eccentricities ($p>0.1$). There was a significant delay in the blue/yellow latency of 19 ± 3 ms ($p<0.001$) when compared with black/white m-VEP. Similar delay was present in all 3 rings in the superior and inferior fields.

Conclusion: Isoluminant blue/yellow pattern reversal m-VEP generates signals of similar amplitude to black/white m-VEP. This may be useful in the early diagnosis of glaucoma.

7. Comparison of objective methods in glaucoma – Multifocal Objective Perimetry and Heidelberg Retinal Tomography

Presented at ARVO 2002 / Submitted for publication J Glaucoma

Balachandran, C, Graham, SL, Klistorner, AI, Goldberg, I *Comparison of objective methods in glaucoma – Multifocal Objective Perimetry and Heidelberg Retinal Tomography*

ABSTRACT

Purpose: To compare sensitivity and specificity of functional and structural changes in glaucoma using two objective tests : the multifocal visual evoked potential (m-VEP) and Heidelberg Retinal Tomograph II (HRT).
Method: 41 glaucoma patients, recruited from tertiary referral practice and 25 normal subjects from the community participated in the study Individuals were evaluated with Humphrey Visual Field (HVF) perimeter 24-2 program (Humphrey System, Dublin), m-VEP (ObjectiVision Accumap™ perimeter, ObjectiVision, Sydney, Australia) and HRT (Heidelberg Instruments, Heidelberg, Germany). "Moorfields Regression Analysis" findings of HRT were compared with presence of scotoma on m-VEP. Linear regression analysis of quantitative variables, such as HVF mean deviation (MD), m-VEP discriminant score (Accumap Severity Index; ASI) and, global HRT parameters was also performed.
Results: m-VEP sensitivity and specificity were 95% and 94% respectively. HRT sensitivity and specificity based on "Moorfields Regression Analysis" were 79% and 92% respectively. The area under the receiver operating characteristic curve (ROC) for m-VEP was 0.97 and for HRT varied from 0.81 to 0.84 depending on the parameters used. If results from the two objective tests were considered, no glaucoma cases were missed. Linear correlation between MD and ASI score was -81% (95% CI -87% to -74%), while that between HRT global parameters, ASI and MD were at best around 50%. Topographic comparison of the presence of scotoma on HVF and m-VEP in different areas of the visual field showed moderate to very good agreement (Kappa statistic 0.56 to 0.81). Comparison of optic nerve head structural abnormality with corresponding areas of field defects on HVF (0.14 to 0.60) and m-VEP (0.14 to 0.54) showed poor to moderate agreement.
Conclusion: The objective test of optic nerve function (m-VEP) and structure (HRT) can detect glaucomatous damage, with limited correlation. The 2 functional tests, HVF and m-VEP correlate better with each other than with HRT. It remains important to look for both functional and structural changes in order to detect all glaucoma cases.

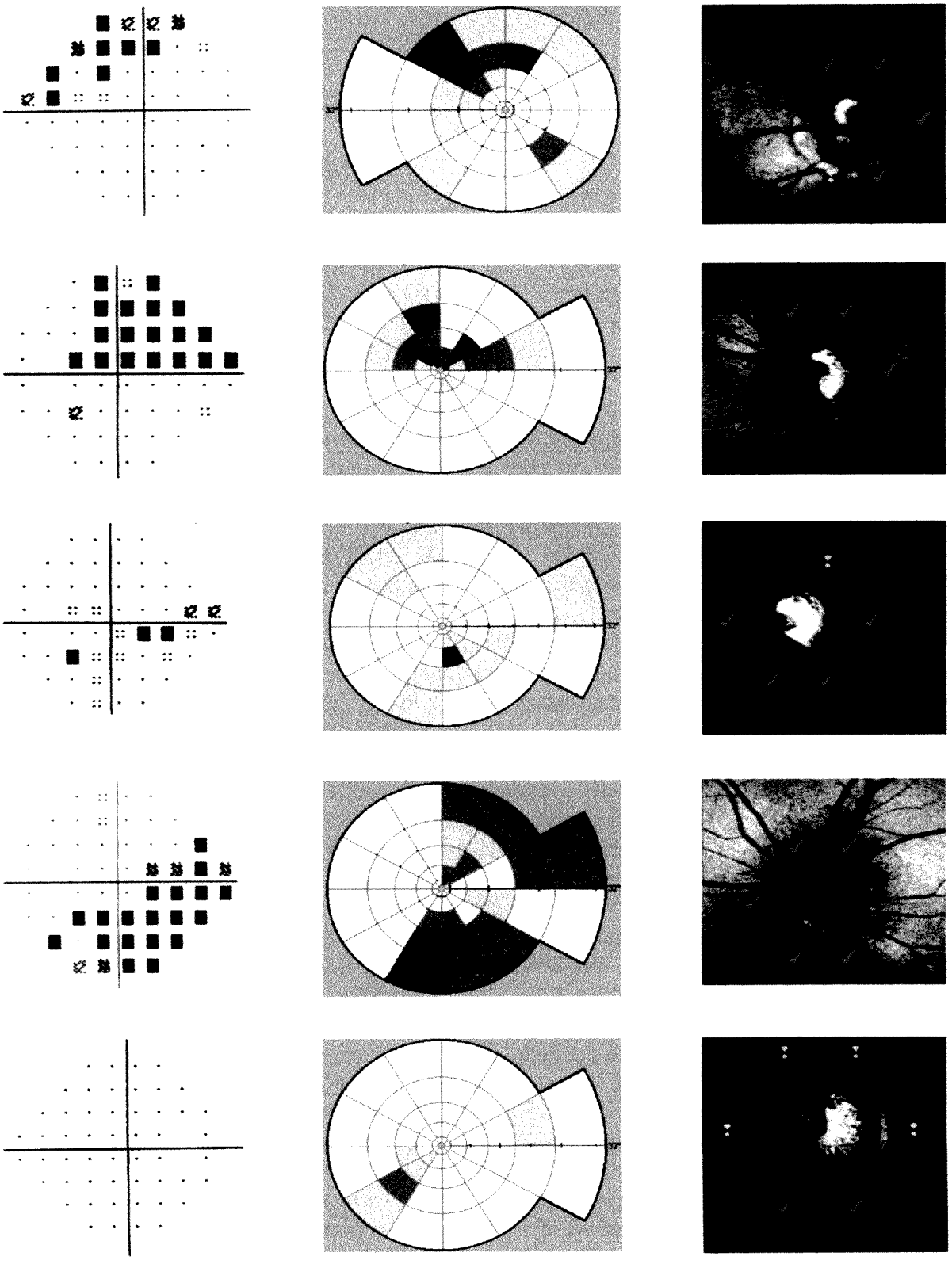


Figure 5 – Examples of 5 glaucoma cases Humphrey total deviation (left column) mVEP amplitude deviation (middle) and HRT Moorfields regression (right). In most cases there is correlation between the tests, but in case 4 the 2 functional tests show a field defect but the HRT is still within normal limits. For the HRT √= normal, ! = borderline, and x = abnormal

8. mVEP in Optic Neuritis

Presented at MS Society 2004

MULTIFOCAL VISUAL EVOKED POTENTIALS IN PATIENTS WITH PREVIOUS OPTIC NEURITIS

Fraser, C [1]Garrick, R [2], Klistorner, A [1], Graham, S [1], Grigg, J [1].
1. Dept Electrophysiology, Save Sight Institute, Sydney University, Sydney.
2. Dept Neurology, St Vincent's Hospital, Sydney.

Aim: to document the changes seen in patients with a history of optic neuritis using the new multifocal visual evoked potential (mVEP) technology.
Methods: 10 patients with optic neuritis over 1 year previously underwent mVEP testing in conjunction with routine visual testing. Patients were recruited through the St Vincent's neurology clinic or the Sydney Eye Hospital. The monocular mVEPs were recorded using the Accumap system. An array of four bipolar occipital electrodes provided four differently oriented channels for simultaneous recording. Visual stimulus consists of 56 closely packed segments in a black and white dartboard configuration, with 16 checks per segment. The segments are cortically scaled to stimulate approximately equal areas of cortical (striate) surface, and extend to 26 degrees of eccentricity in the visual field. Both amplitude and latency, with inter-eye asymmetry for each eye was included. The inbuilt Accumap normal patient database was used for comparison of amplitudes and latencies throughout the visual field, and a probability plot used to identify possible scotomas. Patients also underwent Humphrey subjective visual field testing. Those referred from St Vincent's neurology clinic had conventional VEP performed at some point in their disease course. All had cranial MRI scans performed.
Results: 10 patients (5 male and 5 female, mean age of 35 +/- 10.7) with a mean time since onset of optic neuritis of 45 months (range 12-131) were tested. Of these 2/10 have a diagnosis if multiple sclerosis, 5/10 are at high risk of developing multiple sclerosis with MRI changes over time, and 3/10 patients have a low risk of MS with white matter lesions on MRI not diagnostic of demyelination. Of the 10 patients, 2 had bilateral optic neuritis, and two have had a recurrence in the same eye. All patients had abnormal amplitude of multifocal visual evoked potentials, with an elevated Accumap Severity Index (ASI-based on amplitude deviations) and increased latency, despite 5 patients having visual acuity 6/9 or better in the affected eye. Only 3 patients had an abnormal Humphrey perimetry examination (2 MS patients and the patient with documented extensive optic atrophy). There were significant sectoral latency delays in the affected eye when compared to the fellow eye ($p<0.02$) and the normal population ($p<0.02$). There were, however, no significant latency delays between the fellow eyes and the normal population ($p<0.4$). The average ASI scores indicate that the amplitude of mVEPs worsen as the likelihood of MS increases.
Conclusions: The multifocal VEP can reliably detect evidence of past optic neuritis, in many cases for patients that have normal Humphrey perimetry and visual tests. Further studies are required to determine if mVEP severity index can be used as an indicator of likelihood for progression to MS. The mVEP provides more information on the state of retino-geniculo-cortical pathways than the conventional VEP. The mVEP could also be

used to document visual improvement and remyelination in response to current and new therapeutic options.

ARVO Abstract 2005

ACUTE OPTIC NEURITIS FROM PRESENTATION TO ONE YEAR POST DIAGNOSIS: A MULTIFOCAL VISUAL EVOKED POTENTIAL STUDY

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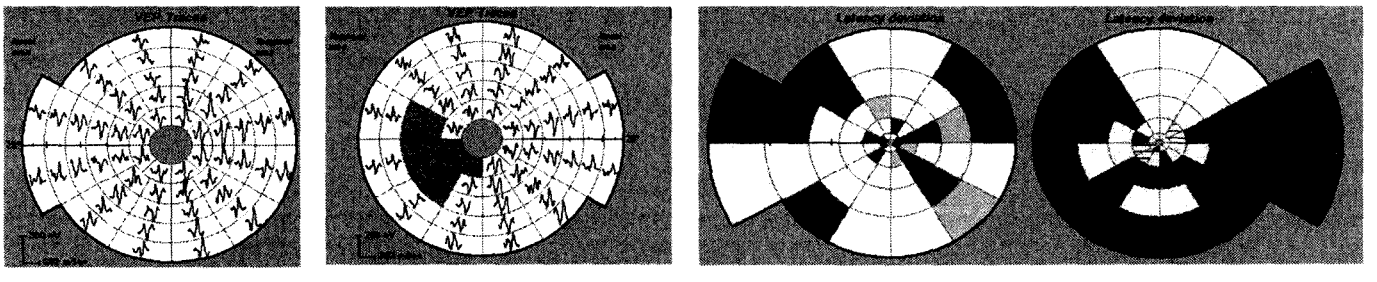
Purpose: We follow 5 patients with a diagnosis of acute optic neuritis with serial multifocal visual evoked potentials (mVEP) for a year to track the changes seen in amplitude, latency and topography of their disease.
Methods: Diagnosis of acute optic neuritis was made by a consultant neuro-ophthalmologist, with all other ocular and neurological pathology excluded. At each visit patients had visual acuity, colour vision, brightness perception, afferent pupillary defect test, Humphrey visual field 24-2 SITA fast and Accumap mVEP performed. All patients had at least two cranial MRI scans over the year. One patient developed optic atrophy, had a normal cranial MRI and was classified as very low risk of MS. The other 4 patients all had evidence of demyelination on cranial MRI, with 2 being given a diagnosis of multiple sclerosis (MS) based on clinical history. Patients were reviewed within the first three months since diagnosis, and again at 3, 6 and 12 months post diagnosis.
Results: The patient with normal cranial MRI and a pale optic disc consistent with optic atrophy did not have significant latency delays. However, central amplitudes in the affected eye were decreased and the area involved enlarged during the year. Visual acuity has remained at 6/60. All of the 4 patients with demyelination on cranial MRI had initial amplitude loss, which recovered to near normal over the one year period. Improvement in visual acuity paralleled increasing amplitudes. Each of these patients had significant latency delays which persisted over the year, with z-scores of >2.5 at each test in the affected eye. In the fellow eyes, the latency z-scores remained <0.5 in 3 patients. The other patient had an episode of optic neuritis in his fellow eye, latency delays were seen to worsen in this eye to z-score of 3.0. Humphrey visual field testing was only abnormal in 3/5 patients and remained abnormal in the same 3 patients.
Conclusion: The mVEP can detect changes of acute optic neuritis with greater sensitivity than Humphrey visual field testing. By analyzing the mVEP latency data we can distinguish between patients with demyelination and those with optic atrophy. We were also able to detect the onset of optic neuritis in a fellow eye.

Fig 6 – Case of acute left optic neuritis shows latency delays in fellow right eye as well



Summary of additional mVEP studies

From the above studies several important findings were reached. Firstly the stimulus check sizes and stimulus areas for the segments were confirmed as close to optimal for achieving good VEP responses throughout the field. It was not considered necessary to change the stimulus design at this stage. Secondly there was very little effect of pupil size on the signal amplitude. This was an important variable to examine as it could potentially have been a problem in clinical practice. There was a slight latency change (decrease) with pupil dilation which would need to be considered if investigating conditions where latency is important eg optic neuritis.

The study on fixation tasks showed the importance of designing a task which keeps the patient concentrating to reduce alpha rhythm. The latter can degrade signals and produce a false positive result. The more difficult tasks enhanced the signal amplitude from the paracentral rings and reduced alpha rhythm. The study on electrode position suggests that although the electrode cross configuration with 4 channels helps cover most underlying signal origins in the cortex, The optimal position may be to centre the cross 3 cm above the inion, still with the lower midline electrode below.

The intrasubject variability of objective perimetry using the mVEP was examined and found to be around 18% with no significant difference between normal subjects and glaucomas. This level of test/retest variation will need to be reduced if the technique is to be used for attempting to detect change over time. Improving the signal to noise ratio is the best way to reduce variability, and we have commenced further studies to attempt to achieve this goal. The sparse stimulus design in chapter 10 may help, but also moving the preamplifier closer to the subject (eg on the headset itself). Also it will be possible with a newer version of the software to analyse individual runs post hoc and delete any noisy runs that may have contaminated the data.

The blue-yellow stimulus is an exciting new development and now being further studied as a possible means for earlier detection of glaucoma. It has been shown to be feasible to record a blue/yellow mVEP but with a reduced number of stimulus segments since the overall signal is smaller.

The comparison with the HRT showed that there was some limited correlation between structural measures assessed by the HRT and changes on the mVEP. There was also a similar correlation found for Humphrey and HRT. There were some cases of glaucoma missed by either HRT or mVEP, however all cases were abnormal on one of the tests suggesting that it may now be possible to detect most glaucoma cases using these 2 objective tests – mVEP for functional deficits and HRT for structural change.

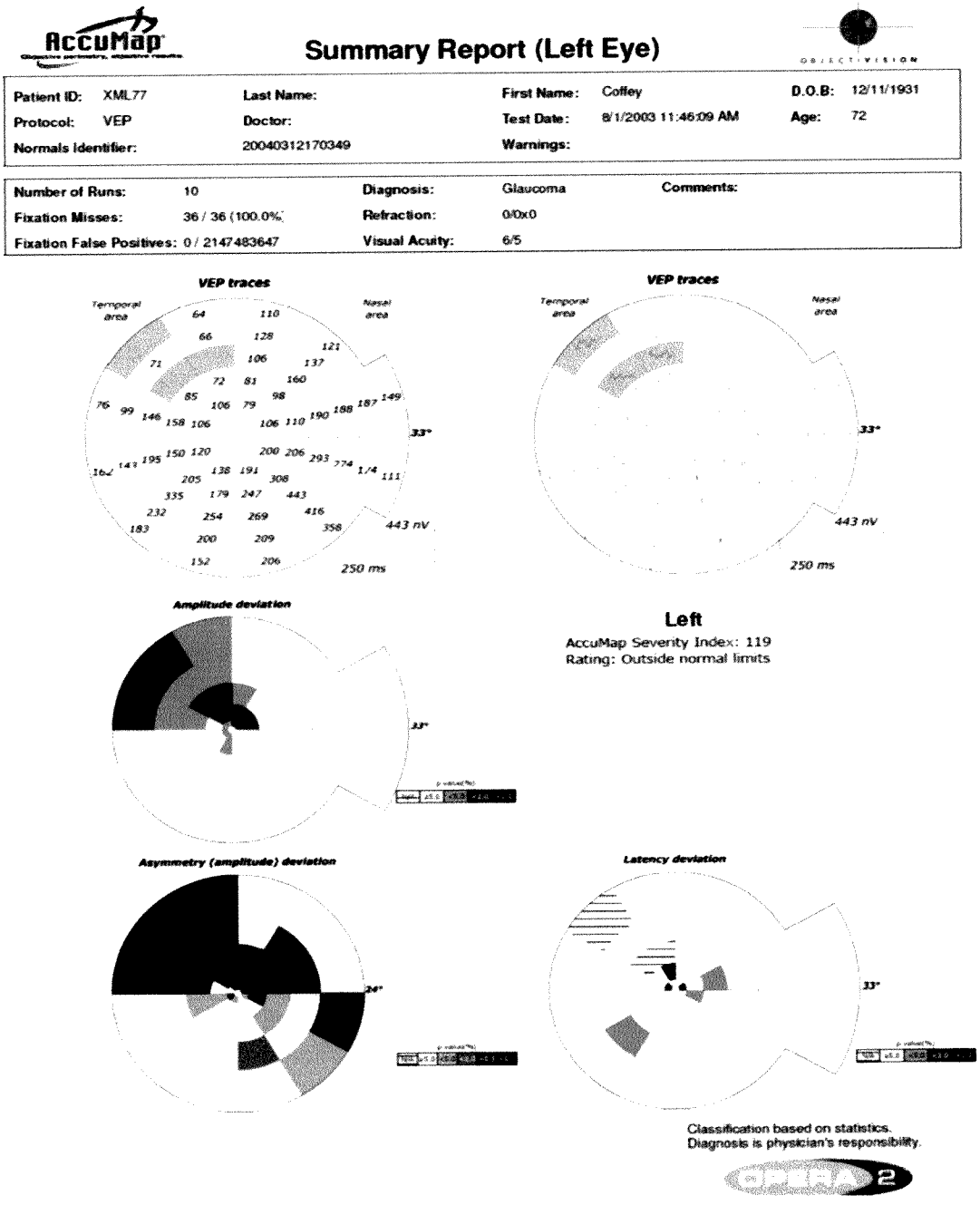
Finally the multifocal VEP can reliably detect evidence of past optic neuritis, in many cases for patients that have normal Humphrey perimetry and visual acuity. Latency delays are present and often in the fellow eye that may not have manifested a symptomatic episode. The mVEP provides more information on the state of retino-

geniculo-cortical pathways than the conventional VEP, with localised latency data. The mVEP could potentially be used to document visual improvement and remyelination in response to current and new therapeutic options. The role of mVEP in optic neuritis is discussed further in the next section in "Future Directions".

Chapter 12

Future Directions and Conclusions

The concept of objective perimetry is still in its infancy, but based on the success we have had so far in applying it to the clinical setting, I am confident that it will continue to evolve as a useful tool in ophthalmology and neurology. The software and hardware configurations of the AccuMap system have undergone continued improvements, and we have plans for further modifications to aid in both recording and interpretation. The figure below shows an example of the most recent results printout from AccuMap V2.0, which includes signal amplitudes, trace array, amplitude deviation plot, asymmetry deviation plot, latency deviation plot, a severity index (ASI) and a noise index with warnings if certain noise levels are exceeded.



Multifocal VEP in Children

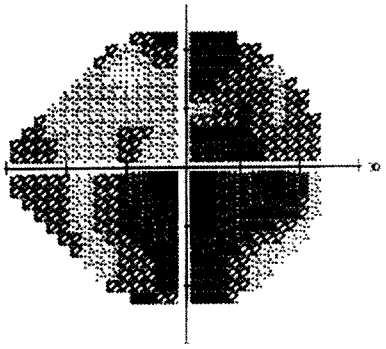
In a pilot study conducted in our lab by Dr Balachandran involving 50 children aged between 5 and 18 years [271] he was successful in recording the multifocal VEP to 32 degrees of the visual field. The average amplitude for this population was found to vary with age, rapidly rising between 10 and 13 years of age. These findings differ from those reported in full field pattern VEP, where it is reported to show a small increase before the age of 10years. These findings need further investigation and may reveal new information about visual maturation at different eccentricities

A further study of 150 normal children is planned. This would provide an age-sex matched control group, for comparison with children with ocular pathology, especially those with optic nerve glioma (ONG), optic sheath meningioma, glaucoma and optic neuritis. Since ONG has its onset and progression under 10 years of age it is particularly important to develop a reliable method of visual field assessment in this age group. A proportion of these children have been shown to have tumour regression over time, therefore demonstration of reliable form of monitoring may save these children from unnecessary chemotherapy or major and sometimes life threatening surgery. In optic sheath meningioma our preliminary study indicated that functional changes can be detected prior to anatomic changes detected on MRI facilitating early intervention. Four patients with NF2 have been followed over at least a year using mVEP perimetry to monitor their visual fields. Two of these patients developed a sudden decline in visual acuity, no change in tumor size was seen on cranial MRI scans. However, the mVEP perimetry was able to objectively map out an area of increasing scotoma in the affected eye. Thus we were able to show functional changes before MRI could resolve anatomical changes.

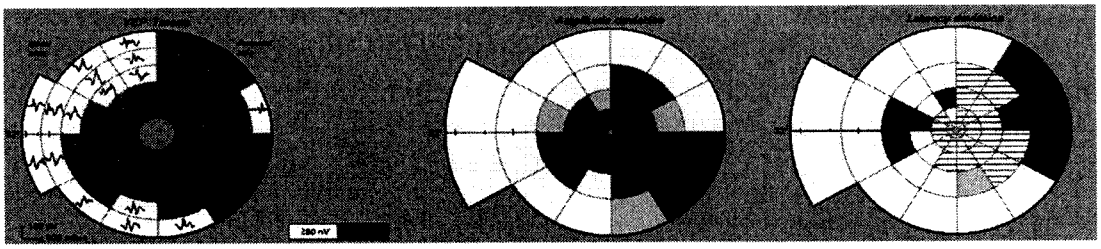
Application to Optic neuritis

Optic neuritis is a demyelinating condition, which impairs vision by disrupting nerve conduction from the eye to the brain. It is often the first presentation of MS, but in the majority of cases a cause is not identified. At 5 years, following further episodes of demyelination, 30% of patients are diagnosed as having MS[272, 273]. The diagnostic confirmation of optic neuritis relies on delays in the conventional VEP. VEPs have been shown to be predictive of the subsequent development of MS [273] and to show high sensitivity in detecting already established MS. The conduction delays are long lasting and thus useful in diagnosis, even following recovery of vision but they are less useful in differentiating between non-specific exacerbations and true recurrences of optic neuritis.

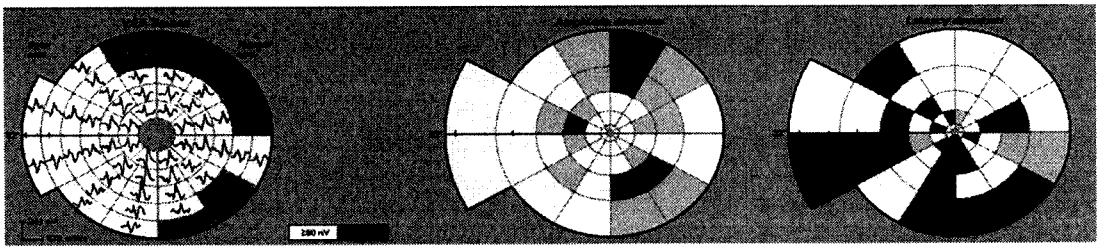
In a pilot study we have tested 10 patients from the onset of optic neuritis for a period of at least one year. There are initial amplitude losses and latency delays seen in the clinically affected eye. In 2 patients with a low risk of future multiple sclerosis (MS), ie normal cranial MRI, the latency changes recovered over 6-9 months, while the amplitude losses remained. Both of these patients had pale optic discs on examination consistent with optic atrophy. Other patients did not show the recovery of latency values.



a) HF Optic Neuritis - Acute stage – Humphrey grey scale



b) mVEP Optic Neuritis - Acute stage



c) mVEP Optic Neuritis - the same patient one month later – shows recovery of amplitudes but persisting latency delays

Patients with previous optic neuritis have also been tested. These patients all showed ongoing amplitude losses with variable latency recovery. Those patients with MS or a high risk for MS had ongoing amplitude and latency changes up to 6 years after the acute episode. Latency delays were longer in those patients with a diagnosis of clinically definite MS compared with those with a high risk, which were in turn longer than those with a low risk of MS. It may be that the degree of latency delay on mVEP can be used as a further predictor of those patients who are more likely to develop clinically definite MS.

We also expect the multifocal VEP will be able to localise the abnormality to a specific area of the visual field and thus demonstrate exacerbations of existing lesions and/or new areas of demyelination. Furthermore, the multifocal VEP may be shown to be a sensitive enough tool to detect possible remyelination, and would be helpful in evaluating future therapies aimed at this goal.

A study will be conducted using multifocal VEPs in optic neuritis subjects, and their responses will be tested during the recovery phase to establish the relationship between multifocal VEP responses and the demyelinating episode. A new algorithm for determining latency from multichannel input has been designed. This will enable us to determine localised conduction delays in individual parts of the optic nerve.

Use of head-mounted displays

Current mVEP recordings use a high resolution, large screen display (22 inch or larger), and subjects are required to sit close to the screen. The distance of the subject from the screen changes the area of field stimulated, and also changes the focal length and thus the required spectacle correction, so must be closely controlled during the recording. The CRT monitor also produces a large electromagnetic field which may affect the recordings when the subject is in close proximity to the screen. Recording is limited to one eye at the time.

To overcome these limitations the concept of using a head mounted display was explored. In a pilot study using the ProView 60 (Kaiser Electronics) we have established that the ObjectiVision system can be used in recording with virtual reality goggles, both in normal subjects and glaucoma (Patent - Graham SL et al "Method and apparatus for objective electrophysiological assessment of visual function" 2001, PCT/AU01/00423). Currently we are adapting a different model of VR Goggles to our current stimulus design, with the intention of conducting a formal trial in normals and glaucomas.

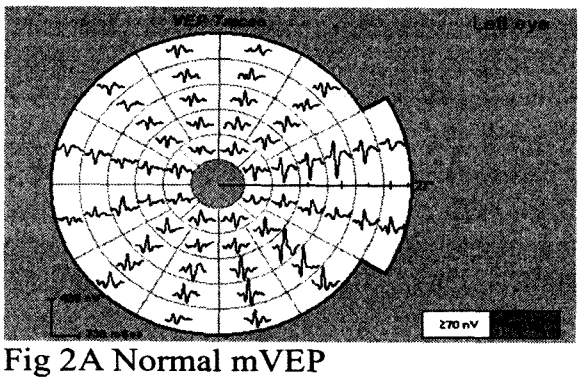


Fig 2A Normal mVEP

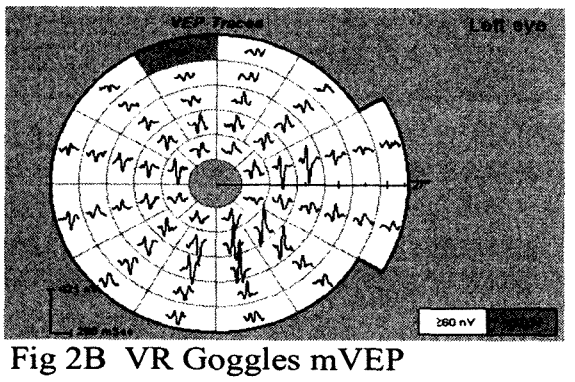


Fig 2B VR Goggles mVEP

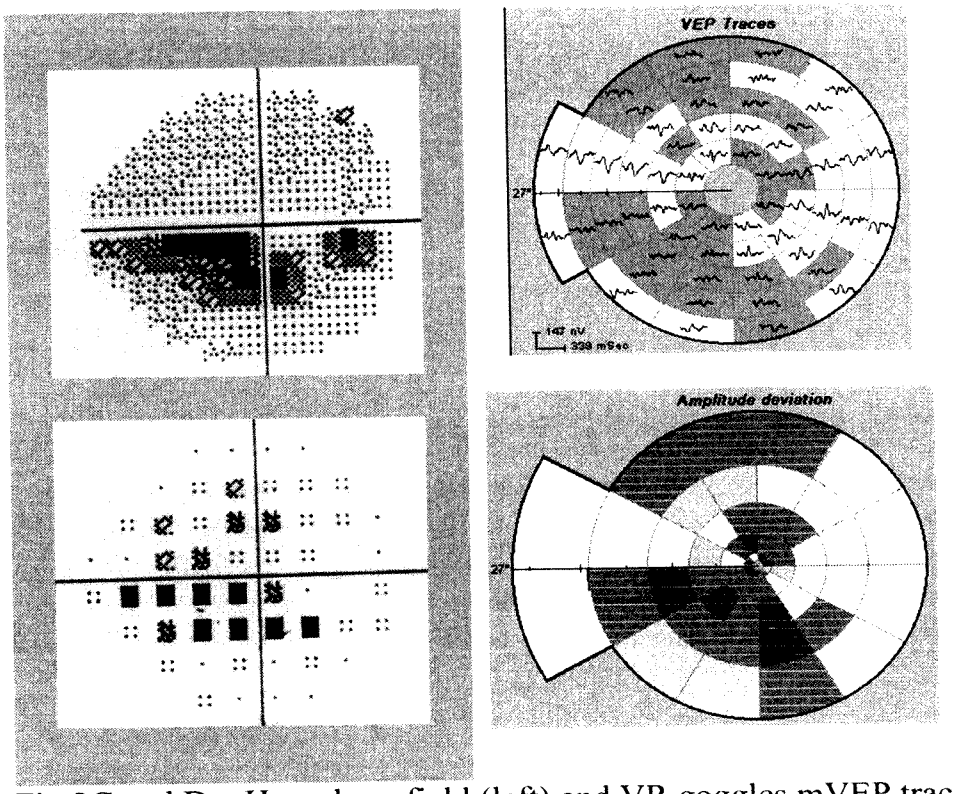


Fig 2C and D – Humphrey field (left) and VR goggles mVEP traces and amplitude deviation plot for a subject with an inferior arcuate scotoma and early superior defect.

The use of virtual reality goggles in these recording techniques provides significant advantages. It standardises the distance to the display, reducing problems of refraction, variable head position and thus area of field tested. It removes the problem of electromagnetic noise emanating from the screen when the subject sits close to the monitor. It reduces the space required by removing the need for a large monitor and it makes the test potentially portable. Virtual reality goggles have good patient acceptance, and both monocular or binocular recording can be performed. Simultaneous binocular recording can be achieved with the application of the spread spectrum technique to provide different pseudorandom stimulus patterns to the two eyes at the same time. The stimulus algorithm is divided into twice the number of segments and these can be distributed between the two eyes, still providing different stimulus sequences to each part of the field and with each subsequent run. The cross-correlations can derive VEP results from each eye independently. This has the advantage of shortening the test time significantly. It also standardises conditions of the recording such that the two eyes are recorded under the same conditions in terms of the subject's visual attention and extraneous noise levels. This aids in the reliability of direct comparisons between eyes of an individual.

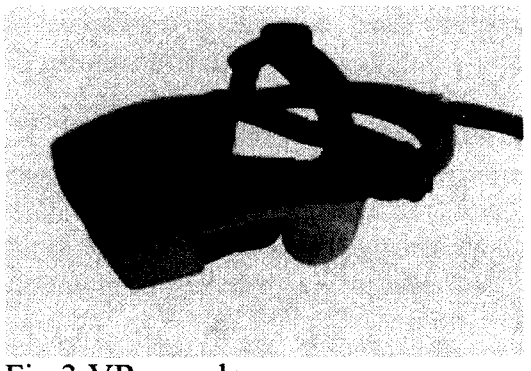


Fig 3 VR goggles

The prototype VR goggles-based system for objective perimetry will provide a unique method for assessing the human visual field. We anticipate that the successful application of VR goggles will improve signal-to-noise ratios. Binocular stimulation will halve test time and increase accuracy of asymmetry analysis. VR goggles will make the test portable, increasing access to patients in nursing homes and in remote areas.

Conclusions

Conventional forms of perimetry are dependent on subject responses and require a high level of cognitive ability to perform the test. This makes these tests challenging for individuals, particularly the elderly and children. The studies presented in this thesis establish the new concept of objective perimetry and have demonstrated for the first time that an objective visual field assessment can be performed and applied in clinical practice. The multifocal pattern visual evoked potential (mVEP) using a multichannel bipolar recording technique, shows a strong relationship between subjective visual field loss and the recorded cortical response. In addition, we found that between-eye asymmetry analysis was sensitive in the identification of early defects. We have tested over 800 subjects with glaucoma and found the test to be 95% sensitive and 94% specific.

There are several important principles for mVEP recording that were established by the studies. Firstly the electrode positioning over the occipital cortex is extremely important for signal detection. It is necessary to use multiple channels, with a minimum of a vertically and horizontally oriented pair of bipolar electrodes so that all underlying dipole orientations are detected. This explains in part why previous conventional VEP recordings were not reliable at detecting glaucoma. When averaging signals over larger areas of the cortex, care must be taken to avoid cancellation effects. For the bipolar mVEP sectoral averaging is the best method.

Secondly asymmetry analysis is a useful technique for detecting early changes. However this will not be reliable in cases where symmetric field loss occurs between the eyes, or for detecting damage in the less affected eye when one eye has advanced loss. It

is necessary to examine both the monocular amplitude deviation and the asymmetry plot in combination.

Thirdly the inter-subject variations in amplitude within the population can be reduced by applying a method of scaling based on underlying EEG levels. This also reduces sex differences between males and females, and seems to remove any age-related change as well. Without scaling the normals database is too broad to show statistically significant changes unless they are marked.

Field loss from other neurological disorders such as cortical infarcts or optic nerve compression by tumors can be demonstrated by the mVEP. It is important to note that there may be extra-striate lesions producing homonymous quadrant defects that will not be detected, as the V1 region is still intact.

One of the major advantages of mVEPs over subjective testing is that data on both signal speed (latency) as well as strength (amplitude) is recorded. Latency delays are not a significant feature of glaucoma, but in demyelinating diseases (MS and optic neuritis) there are significant delays, which tend to persist even when signal amplitude recovers. This is an important diagnostic feature and provides additional information that cannot be gleaned from subjective testing.

Newer stimulus techniques such as sparse stimulation or blue/yellow patterns have the potential to provide better signal to noise ratios at least in the central field, and possibly greater sensitivity to early glaucoma. Larger test zones are required for the blue/yellow so there is some loss of topographical data, but pilot studies are suggesting very good sensitivity. Further work needs to be done on the effects of age and cataract. For the black/white mVEP it does appear the cataract reduces the central amplitudes[236].

In terms of patient outcomes, the roles of objective perimetry in assessing the glaucoma patient are summarized in the Glaucoma Investigation Model patient flow chart in Chapter 8 (Fig.11). The mVEP in the majority of cases supports or helps rule out subjective field loss, it reassures the clinician and patient when perimetry results are inconsistent, variable or excessive changes are seen, and may help prevent over-treatment. These situations are frequently encountered in clinical practice due to the large number of patients who have trouble with perimetry. In some early or high risk glaucoma cases it may detect functional damage earlier enabling commencement of treatment at an earlier stage. Many cases with excessive field loss might otherwise be sent for diagnostic imaging with CT scan or MRI, since the clinician is uncomfortable accepting the disparity between structural and functional loss. More rarely, should both subjective and objective tests be out of proportion to disc changes then further pathology should be suspected (eg intracranial tumor) and CT/MRI should be considered.

The mVEP provides some significant advantages over subjective testing of the visual field. It presents results that remove the effects of patient indecision. It is very easy for

patients to perform first time and has a high level of patient acceptance. Unlike other electrophysiological tests such as the pattern ERG, the set-up is non-invasive.

The main limitation of the mVEP as a form of objective perimetry remains intra-individual reproducibility and noisy recordings which can lead to false positives. There is still a level of patient co-operation required together with technician experience to recognize noise and intervene during the recording. The latest versions of the software have included noise warnings and a noise index to flag alpha rhythm (patient losing concentration), low frequency and high frequency noise. However the reproducibility of individual test points is still only around 15%, with the outer rim points more variable. This limits the ability of the test to be used for sequential follow-up of patients. Further developments in mVEP technology should be directed towards improving signal to noise ratios and thereby both increasing sensitivity and reducing variability. Unfortunately there is still some patient co-operation required in its current format so it cannot be perceived as a purely objective test.

Finally, if the application of a head mounted display can be successfully developed then there may be further significant improvements in recording as a result. The opportunity also may arise for the placement of a preamplifier on the headset close to the electrodes which would also serve to reduce noise.

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