Structural and Functional Investigations of Vigabatrin Toxicity

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The purpose of this thesis was to investigate visual dysfunction arising from vigabatrin (VGB) toxicity: structural investigation utilising optical coherence tomography (OCT) and functional investigation using multifocal electrophysiology.

OCT of the retinal nerve fibre layer (RNFL) based upon a fixed diameter circle scan and enabling reference to the manufacturers' large proprietary normative database, revealed a specific finding associated with vigabatrin-attributed visual field loss (VAVFL); namely, a characteristic pattern of nasal quadrant attenuation with a normal temporal quadrant thickness. This was present in all 11 individuals (including 2 learning-disabled adults) with VAVFL. A further 4 of 16 (including 3 learning-disabled adults and three children) VGB-exposed individuals with normal visual fields (VGB-E) also manifested this pattern as did two of three individuals (one learning-disabled adult and two children) exposed to VGB but unable to undertake perimetry. The pattern was absent in all 13 individuals treated with non-gabaergic anti-epileptic drugs manifesting normal fields and in 9 normal children.

OCT is readily achievable in children as young as 3 years and in learning-disabled adults and should be essential for identifying VAVFL.

A re-analysis of RNFL thickness for quadrant/sector differences by OCT and by scanning laser ophthalmoscopy (Heidelberg Retinal Tomography (HRT)) on 13 individuals with VAVFL, 8 VGB-E and 21 normal individuals previously published (Wild et al., 2006) confirmed the abnormal nasal/normal temporal pattern of attenuation.

Four children, exposed to VGB in utero, from three mothers (two with VAVFL and nasal RNFL attenuation) yielded normal visual fields and RNFL thicknesses.

The amplitudes and implicit times of the mfERG waveform were normal in all 5 VAVFL and in 9 VGB-E, when compared to 13 normal individuals. This suggests that neither bipolar cell nor photoreceptor cell dysfunction, respectively, is implicated in VGB toxicity.

The mfVEP amplitudes were normal in all 5 VAVFL and in all 9 VGB-E, when compared to 16 normal individuals. The lack of abnormality may arise from the mismatch between cortical functional topography and the characteristics of VAVFL and the technical limitations associated with the monocular analysis of the mfVEP.

For My Parents

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STATEMENT 4: PREVIOUSLY APPROVED BAR ON ACCESS

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans **after expiry of a bar on access previously approved by the Graduate Development Committee.**

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Chapter 1: Vigabatrin In A Clinical Context

1.1. Clinical indications for the use of vigabatrin

Vigabatrin (VGB) was first licensed in the UK in 1989 as add-on therapy for partial-onset epilepsy. At that time, the drug was welcomed as a new treatment for refractory epilepsy and was quickly used far beyond the original licensing applications. VGB rapidly acquired a reputation as an effective, well-tolerated drug in competent adults. (Marson et al., 1996, Cramer et al., 1999) Prescription of VGB spread into paediatric use and was variably reported as effective in the majority of epilepsy types affecting this population. Particularly, it was hailed as the treatment of choice for West Syndrome. VGB was also readily prescribed for learning- disabled adults where the perceived advantage of minimal adverse effects was paramount particularly with respect to cognitive impairment.

The first report of vigabatrin-attributed visual field loss (VAVFL) appeared in 1997. (Eke et al., 1997) The characteristics of the field loss, a concentric constriction with nasal predominance and relative temporal sparing (Wild et al., 1999), are such that it is asymptomatic until the defect is advanced. (Hardus et al., 2000a) VAVFL is irreversible. (Johnson et al., 2000) Due to the predominantly asymptomatic nature of the field loss, screening for VAVFL with perimetry is therefore mandatory. However, a developmental age of 9 years is required for perimetry and many children and approximately 25% of adults are unable to appreciate the requirements of perimetry. (Wild et al., 1999)

As a consequence of the visual field loss, VGB lost its place in mainstream adult epilepsy and has never been licensed for use in the USA. VGB is still occasionally prescribed in learning-disabled individuals and is also used in paediatric practice; the development of guidelines for examination of the latter group anticipated the ongoing usage. (2000) Paradoxically, the patients who are receiving VGB, and who are considered to benefit the most from the drug, are the very patients who cannot undergo perimetry and cannot therefore be monitored for the development of VAVFL.

Many clinicians mourn the loss of VGB and others continue to prescribe it in restricted practice. However, is the argument for the use of VGB really that compelling?

A potential use of VGB is for addiction treatment of cocaine and amphetamine. The reviving commercial interest in VGB is likely to renew interest in the mechanism of the visual dysfunction. At present, there are many unanswered questions concerning the pathogenesis of VGB toxicity. Whilst it is conceivable that biomarkers could be developed to identify individuals who could safely be prescribed VGB, no such safety net currently exists.

1.1.1 The evidence for VGB efficacy in adults

Numerous trials have reported the efficacy of VGB in adults. (Gram et al., 1983, Rimmer and Richens, 1984, Gram et al., 1985, Loiseau et al., 1986, Browne et al., 1987, Sivenius et al., 1987, Tassinari et al., 1987, Cocito et al., 1989, Dam, 1989, Remy and Beaumont, 1989, Tartara et al., 1989, Sander et al., 1990) (Reynolds et al., 1991) The most robust information comes from short-term double blind add-on trials of VGB versus placebo in refractory epilepsy. The short-term add-on studies of VGB demonstrate reasonably consistent results with a 42- 49% reduction in the frequently quoted ≥ 50% seizure rate reduction in focal onset epilepsy. (Tassinari et al., 1987, Tartara et al., 1986, Loiseau et al., 1986)

The limitations of short-term studies in severe refractory epilepsy are well documented. (Leach and Brodie, 1995) However, due to ethical limitations and market driven reasons of time constraints, this study design is the most frequently undertaken and often provides the only available evidence base for new anti-epileptic drugs (AEDs).

In order to achieve a monotherapy license, any new drug must be shown to be either better than the standard drug, or equally efficacious *and* better tolerated. In the absence of direct comparative head-to-head trials of VGB, one approach is to compare separate trial data. There have been two main attempts to use the Cochrane meta-analyses to compare eight newly developed drugs with each other.

The first comparison of gabapentin, lamotrigine, tiagabine, topiramate, vigabatrin and

zonisamide found no significant difference between the six drugs in terms of the 50% seizure reduction rates. VGB did not emerge as significantly better tolerated than the other five drugs. (Marson et al., 1996) The second study compared available data from each trial to assess absolute success rates, based on a calculation of success of the antiepileptic drug minus the success of placebo. (Cramer et al., 1999) Trends emerged which favoured vigabatrin and levetiracetam versus lamotrigine, but placebo data were highly variable across the different studies, highlighting the difficulty of comparing results across studies. No clear differences emerged between the new antiepileptic drugs from either of these analyses. Prior to the SANAD study, (Marson et al., 2007a, Marson et al., 2007b) it might be reasonably claimed that in adults with partial-onset epilepsy there was equivalent evidence for the adjunctive use of VGB as for any other AED.

Two direct attempts were made to assess whether VGB could justifiably be used as a first line drug. One open label trial in adults with partial epilepsy reported very favourable results (57.7% seizure free after one month, and 39.8% seizure free during the fourth month) in patients with moderate epilepsy defined as a maximum of 7 seizures per month. (Arzimanoglou et al., 1997) Following this promising report, a double-blind monotherapy study randomising 459 patients to either CBZ or VGB confirmed that VGB was better tolerated than CBZ, but less effective, and thus should not be considered a first-line agent. (Chadwick, 1999)

In summary, notwithstanding recent data for the other newer AEDs, there is indeed

reasonable evidence for the efficacy of VGB as an adjunctive therapy but no good evidence to justify using it as monotherapy.

1.1.2 Vigabatrin: the evidence for efficacy in children with partial and generalised seizures

There is a dearth of high-quality data assessing VGB efficacy in children, and, in part, this arises from difficulty in obtaining ethical approval for paediatric trials.

Open-label monotherapy trial

A study of 42 children reported similar efficacy for VGB and CBZ monotherapies. (Gobbi et al., 1999) Children with cryptogenic partial epilepsy demonstrated seizure response rates of 82% and 100% in VGB and CBZ, respectively, whilst children with symptomatic partial epilepsy demonstrated seizure response rates of 50% and 55% respectively though, as in the adult monotherapy studies, VGB was slightly better tolerated.

Single Blind Placebo Add-On Trials

The first of two studies reported a dose-response relationship and recommended dose escalation in those who seemed to show a partial response after 1 month. (Arteaga et al., 1992) However, this study was limited to 16 children (12 of whom had either symptomatic or cryptogenic partial onset epilepsy, with only 4 having idiopathic generalised epilepsy) over a one-month period.

The second trial reported high rates of seizure reduction from 97 per month on placebo to 9 per month after 6 months of treatment with VGB. (Dalla Bernardina et al., 1995) At 7 months, 8 of the 46 study patients were seizure free on VGB, compared with none rendered seizure free during one month of placebo. Thirty-nine of 46 patients completed the study, again supporting high tolerability.

Open-label Add On Trials

Three trials reported varying success of VGB in open label trials. One multi-centre trial reported a significant reduction compared with baseline for both partial and generalised seizures, in a study that excluded patients with IGE or West syndrome. (Gherpelli et al., 1997) A second trial, running for 24 months, reported 23% and 9% seizure freedom in cryptogenic partial epilepsies and symptomatic partial epilepsies respectively, (Coppola et al., 1997) but found that VGB was ineffective in myocolonic epilepsies and in LGS. In patients with intractable epilepsy, the rate of seizure response (defined as 50 -75% reduction in seizures) over 2-24 months was 43%. (Wong, 1995)

VGB Withdrawal Study

A unique study assessing withdrawal from VGB used the number of patients remaining in the study as the primary endpoint.(Chiron et al., 1996) This measure reflects a combined perception of both efficacy and tolerability. 93% of patients

randomised to stay on VGB remained in the study, compared with 46% of those who were randomised to placebo.

Retrospective Studies

VGB was considered to be effective in 43 children for complex partial seizures with or without secondary generalisation. In contrast, children with atonic seizures and myoclonic epilepsies responded badly. (Gibbs et al., 1992) Further support for VGB came from 73 patients treated with VGB, including 12 on VGB monotherapy, where high rates of >90% seizure reduction were found (30 of 73, including 7 rendered seizure free). ((Prasad et al., 2001)) The cohort constituted predominantly symptomatic epilepsy (50 of 73) although no difference was found in seizure response according to epilepsy type. Interestingly, long-term follow-up study of VGB in partial epilepsies in children showed persistent efficacy in 40% of patients. (Uldall et al., 1995)

LTG and VGB showed similar rates of improved seizure control and similar rates of treatment maintenance in 109 children with a higher proportion with idiopathic generalised epilepsy (IGE); 8 of 20 children with IGE responded to VGB. (Schapel et al., 1997) Similar results for efficacy and tolerability for both VGB and LTG across children with partial and generalised patients have also been found. (Dimova and Korinthenberg, 1999)

However, a comparison of VGB and Lamotrigine (LTG) as add-on therapy in a case series review of 105 children (Belanger et al., 1998) found that VGB was more effective than lamotrigine in partial epilepsies; however LTG was more effective for generalised epilepsies. Nevertheless, LTG has been shown to be better retained in patients with either partial or generalised epilepsy than VGB due to perceptions of lack of efficacy of VGB. (McDonald et al., 2005)

A review (Schmidt and Bourgeois, 2000) of therapies for Lennox-Gastaut Syndrome does not recommend VGB, even as a third-line agent. Only one study ((Feucht and Brantner-Inthaler, 1994) has reported high rates of response of VGB add-on therapy to VALP monotherapy in patients with Lennox-Gastaut Syndrome.

In summary, the literature suggests that the efficacy of VGB in children follows a similar pattern to that in adults. There is a consensus that VGB is a well-tolerated drug, effective as an adjunctive therapy in partial onset epilepsies both in the short term and also in the long term. Evidence regarding efficacy in idiopathic generalised epilepsies is more limited and does not support VGB as being highly efficacious in this group. However, as in adults, there is no data to support the use of VGB as a monotherapy over CBZ.

1.1.2.1 Vigabatrin and Infantile Spasms (IS)

The effectiveness of VGB for infantile spasms (IS) has been much vaunted and currently VGB remains arguably the drug of first choice. (2000). However, the use of VGB over alternative treatments is not supported in the literature.

Systematic Reviews

A Cochrane review of the treatment of infantile spasms (Hancock et al., 2003) included 11 randomised controlled trials (RCTs) but concluded all studies demonstrated poor methodology to varying degrees, rendering comparison and interpretation difficult. No treatment was found to be more efficacious than any other. For VGB specifically, high-dose VGB seemed to be more effective than low-dose VGB in controlling IS.

A more recent systematic review similarly concluded that no long term therapy could be recommended over another. (Mackay et al., 2004) This review included 14 studies of VGB and IS and 7 studies assessing VGB in Tuberous Sclerosis (TS). Again, problems in methodology hampered interpretation and the authors' supported the prescription of VGB but advised that the evidence was of low grade only.

Riikonen (Riikonen, 2000) reviewed all published trial data despite differences in methodology and, in common with the other systematic reviews, failed to demonstrate

improved efficacy of one drug over another but considered that ACTH should be used ahead of VGB due to the risk of developing VAVFL.

Randomised Double Blind Controlled Study

In the only randomised placebo controlled study of VGB as first-line treatment of infantile spasms, which after 5 days became an open label extension for up to 24 weeks. (Appleton et al., 1999) found a significantly higher percentage reduction in spasms in the VGB group compared with the placebo group at the end of the double blind period (77.9% cf 25.9%). At the end of the open-label extension period, 42% of the original 36 children were spasm free on VGB monotherapy. Those with cerebral dysplasia were most responsive to VGB. Overall, VGB was well tolerated despite rapid escalation of dosage, with no withdrawals due to adverse events.

Single Blind Randomised Studies

Two RCTs comparing VGB with ACTH and Hydrocortisone, respectively, (Vigevano and Cilio, 1997) Chiron and Dulac, 2002) found a much more rapid response rate to VGB than to steroid and better tolerability to VGB. However, at 14 days and beyond, rates of response were similar across the various groups. VGB was more effective in cerebral dysplasia and other cerebral malformations compared to ACTH. (Vigevano and Cilio, 1997)

A single blind two-week trial of low-dose versus high-dose VGB, with an open label follow-up study of 142 children, found similar rates of early response to VGB (defined as 7 consecutive days spasm free in the first 14 days) and showed high-dose VGB yielded a faster time to respond than low-dose VGB. (Elterman et al., 2001) Efficacy was prolonged, with 65 % of patients remaining spasm free at 3 months.

Open Label Randomised Studies

In contrast, either of prednisolone or tetracosactide was found to be more effective than VGB in the open-label randomised trial UKISS (Lux et al., 2005) which, interestingly excluded patients with TS since it was felt that the evidence for VGB use was already conclusive.

The primary outcome in the UKISS study was cessation of spasms, but this was only measured on days 13 and 14, in contrast to the RCTs of Vigevano and associates (Vigevano and Cilio, 1997) and Chiron and associates (Chiron and Dulac, 2002), described in Section 1.1.2.1 above It is notable that whilst UKISS used comparable maximum dosages of VGB to the two RCTs, UKISS was unable to confirm in almost half the study subjects that the full dosing protocol had been followed. The starting dose of VGB was proportionately low, whilst the doses of both ACTH and prednisolone were proportionately high. The lack of perceived efficacy of VGB may have been due to the low dosage. The UKISS study stands alone in reporting hormonal treatments as more effective than VGB.

Retrospective studies

An open label trial found that only 2 of 8 children with IS showed an improvement on VGB. However, this was sustained, with 1 child remaining seizure free for the 24 months of study period, and the other maintaining a seizure reduction of >50% for 30 months. (Coppola et al., 1997)

A case series review (Belanger et al., 1998) showed that 12 of 23 children with IS were rendered seizure free on VGB, either as monotherapy or as adjunctive therapy to ACTH. No difference was found between children with symptomatic IS and children with cryptogenic IS.

A similar proportion of children with IS responded positively to VGB, including 2 with TS as the underlying cause, who became seizure free. The most common diagnosis amongst non-responders was hypoxic-encephalopathy. (Prasad et al., 2001) However, all these retrospective studies involved small numbers of patients, who exhibited mixed treatment methodologies and therefore the evidence must be considered to be low-grade.

1.1.2.2 Vigabatrin and Tuberous Sclerosis (TS)

Only three studies have investigated the efficacy of VGB in individuals with TS manifesting seizures other than IS. Numbers are, not surprisingly, small and are

underpowered to draw any firm conclusions; however, all report in favour of VGB. One retrospective study reported seizure reduction in three of five patients (Prasad et al., 2001) whilst an open label study reported seizure freedom in 2 of 4 children on therapy with VGB, and seizure reduction in a third child, and good seizure response to VGB in 5 of 8 adults with TS. (Laan, 2004) The VGB withdrawal study discussed in section 1.1.2 also reported good results in TS (Chiron et al., 1996) but all of these studies involve very small numbers,

1.1.3 Vigabatrin and Learning Disability

One of the perceived advantages of VGB is the relatively low rate of associated cognitive impairment, an important consideration in all patient groups, but of particular functional significance when treating people with pre-existing cognitive impairment.

The best evidence for the impact of VGB on cognitive function in competent adults is that provided by Dodrill. (Dodrill et al., 1995) In a randomised, double-blind, placebo-controlled, add-on study evaluating cognitive and Quality of Life (QoL) scores in adults with focal onset epilepsy, receiving appropriate doses of VGB and who were seizure responders, Dodrill and associates found similar cognitive and QoL scores at the end of treatment to those at baseline. Only one test, the Digit Cancellation Test showed any deterioration with escalating doses of VGB.

An earlier double blind randomised crossover study in 10 healthy volunteers assessed cognitive scores on a fixed dose of VGB of 2g/day; only one individual performed worse on VGB. (Thomas and Trimble, 1996)

In a notably larger cohort of 111 patients (53 on placebo, 58 VGB exposed), no difference was found in cognitive performance compared with placebo. (Bruni et al., 2000) and these findings were sustained after 12 months, although the group treated with VGB showed a trend towards improvement. (Guberman and Bruni, 2000)

A unique study of efficacy and cognitive performance in 36 learning-disabled adults treated with VGB, involved follow-up at 3 months, 2 years, and 5 years. Forty-two percent showed a >50% seizure reduction at 3 months, falling to 22% after 5 years. Those patients with partial seizures responded best to VGB. Psychological performance was maintained throughout the study period.

A single open label trial of add-on therapy of VGB in 22 adults with severe epilepsy and learning disability found a reduction in mean seizures of 49%, overall, compared with baseline. (Armour et al., 1992) No adults were withdrawn from VGB due to adverse effects. In keeping with the findings of (Pitkanen et al., 1993) and those reported in adults and children, a trend was seen towards better seizure reduction in partial compared with generalised seizures.

In conclusion, VGB is effective in the learning-disabled population, where efficacy varies depending on epilepsy type mirroring that seen in children and competent adults. Further, in the learning-disabled population, VGB does not impact upon cognitive function.

1.1.4 A New Clinical Indication for Vigabatrin

VCB has been studied as a potential short-term anti-addiction drug; specifically for use in cocaine and methamphetamine dependence. Even tobacco dependency has been cited as a potential future use for VGB. (Dewey et al., 1999) Initial reports that vigabatrin may be useful in addiction date back to 1998. (Dewey et al., 1998) Animal studies measuring *in vivo* concentrations of dopamine in the nucleus accumbens (DANA) of rats pre- and post- cocaine administration demonstrated, on average, an increase of 482%. The increase in DANA concentration was 365% following VGB administered 2 hours prior to cocaine intake. The attenuation of increased DANA levels was sustained in ongoing VGB administration, in a dose dependent manner. (Morgan and Dewey, 1998) Anticipatory cues associated with cocaine administration in rats also produce a 25% increase in DANA, which is abolished by VGB. (Gerasimov et al., 2001)

To date, only two studies have investigated VGB as an anti-addiction agent in humans. In the first, 30 individuals were recruited into a 9-week open-label safety study, which investigated vigabatrin in methamphetamine ('crystal meth') and/or

cocaine addiction. Only 18 individuals completed the study, of which 16 tested negative for stimulant drugs in urinary screening in the final 6 weeks. No long-term data regarding sustained abstinence are available. (Brodie et al., 2005) An attempt to assess ocular safety of VGB when used as an anti-addiction agent found no ocular involvement in 18 individuals exposed to 137g of cumulative VGB over 9 weeks. (Fechtner et al., 2006) Only 18 of 28 individuals completed the study, of which 16 tested negative in final urinary screening. Visual testing comprised visual acuity, and peripheral visual field investigation (Humphrey Field Analyzer Program 60-4) at baseline, weeks 1, 4, 8 and at 4 weeks or more post completion. No significant changes in visual acuity and visual field from baseline were reported.

Clearly, major uncertainties remain regarding both the efficacy and the safety of VGB in this setting. Given that VGB has a dose-dependent effect on DANA concentration, high levels of VGB might be required. No data is available regarding efficacy of VGB in the face of escalating intakes of cocaine or methamphetamine, and users may be able to overcome the inhibition with increased drug intake. Drug withdrawal programmes often last several months to years and this could result in considerable VGB dosing. The ocular safety data, therefore, represents an extremely idealised and minimal VGB exposure. Calls for licensing of VGB as an anti-addiction drug should be regarded as premature.

1.1.5 Summary and Conclusions

Epilepsy is currently the sole clinical indication for vigabatrin.

In adults, there is good evidence for its clinical efficacy as add-on treatment for focal epilepsy. The only study exploring its potential as first line monotherapy suggests that it is less effective than carbamazepine. There is no robust evidence for the efficacy of VGB in idiopathic generalised epilepsy in adults and there is even some suggestion that it may be less effective in this group.

In children, the data are limited, but sufficient to support VGB being effective and well-tolerated; as in adults, seizure control appears to be better in partial-onset compared with generalised seizures. The sole open-label monotherapy study comparing carbamazepine to VGB shows no difference in efficacy. Despite several studies using VGB for IS, three reviews have concluded that there is no definite advantage for VGB. One review advocates against VGB in IS despite current UK paediatric guidelines.

In learning-disabled adults, the limited data confirm the effectiveness of VGB as add-on therapy in focal-onset epilepsy and indicate that VGB is well-tolerated with no adverse effects on cognition.

1.2 Vigabatrin Attributed Visual Field Loss: Characteristics And Pathogenesis

The first report of VAVFL appeared in 1997 (Eke et al., 1997) with a case series of three individuals exposed to VGB for a minimum of 28 months. The field loss in each case was a bilateral constriction. There are now nearly 200 publications discussing VAVFL.

1.2.1 Topographic Characteristics Of The Field Defect

The characteristics of the field defect associated with VGB (Lawden et al., 1999, Wild et al., 1999) are now well accepted. (Daneshvar et al., 1999) The field loss is a bilateral 'concentric' constriction which preferentially affects the nasal field, both in terms of area and depth, resulting in a characteristic pattern of binasal attenuation which, within the central field (i.e. within 30° from fixation) manifests by static threshold perimetry as a steep-sided binasal annulus extending vertically across the horizontal midline and also centripetally. Initially, the temporal field is spared to varying extents, but in advanced cases, becomes concentric within the central field.

The propensity for nasal visual field loss is more apparent by static perimetry than by kinetic perimetry. (Lawden et al., 1999, Wild et al., 1999, Wild et al., 2007) Only one study has attempted to quantify the nasal preferential loss. Patients were assessed with the 120 point screening test protocol of the HFA. The visual field loss in the nasal

hemifield was more severe than in the temporal hemifield in 75% of 21 individuals with VAVFL. (Manuchehri et al., 2000)

1.2.1.1 Is this purely a peripheral disorder?

Whilst the visual field loss is predominantly peripheral, there is both structural and functional evidence of retinal dysfunction. Central retinal abnormalities have been observed on ophthalmoscopy including reports of retinopathy (Krauss et al., 1998, Beran et al., 1996) along with optic nerve pallor (Eke et al., 1997, Lawden et al., 1999, Crofts et al., 1997). The first report of reduced peripapillary nerve fibre layer was in 1999, (Miller et al., 1999) and since that report there has been growing evidence to confirm this as an important finding even in early VAVFL (Wild et al., 2006, Buncic et al., 2004).

Central clinical abnormalities have been identified including subtle colour-vision defects and irregularities of the macula reflex. (Krauss et al., 1998) Similarly, abnormalities in colour vision have also been reported along with reduced contrast sensitivity by (Nousiainen et al., 2000) although, confusingly, these abnormalities have also been reported in Carbamazepine-exposed individuals. Manuchehri (Manuchehri et al., 2000) also found a higher rate of incorrectly read Ishihara pseudo-isochromatic plates in VAVFL compared with CBZ-exposed controls, but were unable to demonstrate evidence of reduced visual acuity. One case of severely impaired visual acuity and 5 cases of impaired colour vision were identified in a

group of 22 VGB exposed individuals, which, it was felt, was attributable to VGB. However, the proportion of these cases with VAVFL is not clear. (Paul et al., 2001)

1.2.2 The Asymptomatic Nature of VAVFL

The characteristics of the field loss in VAVFL are such that the patient is unaware of the field loss until the defect is advanced. The asymptomatic nature in the early stages is most likely to arise from the normal or near normal visual acuity associated with the relative sparing of the temporal field compensating for the nasal loss in the contralateral eye. (Wild et al., 1999)

Most authors agree that VAVFL changes are asymptomatic.(Lawden et al., 1999, Eke et al., 1997, Wild et al., 1999) However, one study (Schmidt et al., 2004) described a positive predictive correlation between the response to two questions on a questionnaire relating to vision and the presence of VAVFL. Nevertheless, the severity of the field loss was not described.

1.2.3 The Scale of the Problem

It is difficult to determine accurately the prevalence estimate of VAVFL. This is due to a number of factors, including the sensitivity and specificity of the given visual field protocol to identify VAVFL; the performance of the patient during the visual field examination; the experience of the clinician interpreting the result of the

examination and the sampling bias present in the given cohort with particular reference to the duration, daily and cumulative dose of VGB; and the small numbers of patients associated with many cohorts. Furthermore, approximately 25% of adult patients exposed to VGB are unable to cooperate with the demands of perimetry. (Wild et al., 1999)

The highest estimate of prevalence of VAVFL is 90% derived from a cohort of 20 individuals. (Besch et al., 2002) A further 9 studies yield a wide range of prevalence estimates from 17 to 73%. (Daneshvar et al., 1999, Arndt et al., 1999, Hardus et al., 2000c, Gross-Tsur et al., 2000, Kalviainen et al., 1999, Lawden et al., 1999, Miller et al., 1999, Wild et al., 1999, Wohlrab et al., 1999, Rao et al., 1998) A pooled analysis of 528 patients from all available studies resulted in a prevalence estimate of 32% (95 % CI 28% -36%). (Kalviainen and Nousiainen, 2001)

The prevalence of VAVFL in children and adolescents is generally lower than that reported in adults. (Vanhatalo et al., 2002, Wild et al., 2007) (You et al., 2006) The lower prevalence in children may reflect increased retinal plasticity, although this is unsubstantiated, but may also reflect difficulties in the visual field examination of children. Indeed, three studies have shown prevalence estimates in children that are generally higher than those found in adult cohorts. One study (Wohlrab et al., 1999) reported a prevalence estimate of 42% for asymptomatic VAVFL in a paediatric group, however, only 12 of 153 could undertake the visual filed examination. The second (Luchetti et al., 2000) and third studies (Gross-Tsur et al., 2000) reported

VAVFL in 8 of 13 children (62%), and in 11 of 17 children (65%) respectively. These latter two prevalence estimates may also reflect difficulties associated with the visual field examination of children.

1.2.4 Is VAVFL Stable?

No Improvement In VAVFL Following Withdrawal From VGB

Despite some reports to the contrary (Versino and Veggiotti, 1999, Krakow et al., 2000, Giordano et al., 2000, Fledelius, 2003), it is clear that VAVFL persists after withdrawal of VGB.(Johnson et al., 2000, Kjellstrom et al., 2008a, Paul et al., 2001)

The most frequently cited report of reversibility was that of an eleven-year-old child with rapidly improving fields after VGB withdrawal.(Versino and Veggiotti, 1999) Further paediatric cases followed amid suggestions that reversibility of VAVFL represented physiological differences and capacity for retinal plasticity in children. The more likely explanation is that of improved compliance with the visual field examination with age. Indeed, improved visual field performance in paediatric patients exposed to VGB is well documented. (Vanhatalo et al., 2002) These cases of apparent reversibility exemplify the limitations of perimetry, particularly in children. The non-reversibility of VAVFL, following withdrawal, was confirmed in 8 paediatric patients. (Luchetti et al., 2000)

Similarly, two adults with VAVFL exhibited an apparent improvement in the visual field after withdrawal of the drug.(Krakow et al., 2000) ERG abnormalities were cited as supportive evidence but, tellingly, the most striking ERG abnormality was that of reduced or absent oscillatory potentials; these have subsequently been shown to be associated with vigabatrin therapy rather than vigabatrin toxicity. (A fuller discussion of electrophysiology in Vigabatrin is contained in Section 1.3.3). Again, the most plausible explanation for the apparent improvement was that of the perimetric learning effect. (Wild et al., 1999)

The only study involving substantive numbers of patients to report improvement in VAVFL following withdrawal of VGB involved 26 patients with a mean follow-up of 12 months post withdrawal. (Fledelius, 2003) In general, the illustrated fields prior to withdrawal were of poor quality and it is likely that the apparent improvement can again be attributed to the perimetric learning effect. Numerous studies have subsequently confirmed that VAVFL is non-reversible.(Wilson and Brodie, 1997, Paul et al., 2001, Nousiainen et al., 2001, Newman et al., 2002, Kjellstrom et al., 2008b)

No Worsening Of VAVFL Following Withdrawal Of VGB

In addition, VAVFL also does not seemingly progress following withdrawal from VGB. (Nousiainen et al., 2001, Newman et al., 2002)

On Continued VGB

The progressive nature, from detection, of VAVFL on continued therapy with VGB is widely clinically accepted but refuted by one research group. Two studies from one centre assert that VAVFL either does not deteriorate at all (Paul et al., 2001) or progresses in a small number of individuals. (Best and Acheson, 2005) In contrast, and in keeping with most clinicians' findings, two studies from a Netherlands group (Hardus et al., 2000b, Hardus et al., 2003) report progression.

Visual stability was reported in 15 patients on continued VGB therapy over a 12-month period. (Paul et al., 2001) However, seven of the initial 22 patients withdrew from VGB either due to poor seizure control or concerns about visual progression, and therefore these patients were excluded from the analysis. The second study reported stability of VAVFL in 15 of 16 individuals who remained on VGB over a period of 18 – 43 months. (Best and Acheson, 2005) All had received vigabatrin for at least 5 years. The remaining patient demonstrated unequivocal deterioration of the VAVFL and discontinued treatment. The time periods over which potential progression has been studied may be insufficient to identify progression of VAVFL.

The group reporting progression from detection of VAVFL (Hardus et al., 2000b, Hardus et al., 2003) found that all 11 individuals remaining on VGB showed clear progressive loss with continuing medication over 37 – 47 months.

1.2.5 The Natural History of Vigabatrin Attributed Visual Dysfunction

Various factors, including smoking, age and male gender, have been suggested to increase the risk of developing VAVFL. Male preponderance is generally considered to be the major risk factor (Wild et al., 2007, Wild et al., 1999) (Hardus et al., 2000b, Hardus et al., 2001, Kalviainen and Nousiainen, 2001). In contrast, Manuchehri did not report any significant correlation with male gender in 20 cases of VAVFL and 11 controls. (Manuchehri et al., 2000)

Age has been identified as a risk factor for VAVFL by univariate analysis in some studies (Wild et al., 2007, Schmitz et al., 2002 but not in others {Manuchehri, 2000) although many have not specifically addressed the issue of age. The potential association with increasing age is likely to reflect a vascular component in the pathogenesis of VAVFL.

1.2.6 The Relationship Between Dosing and VAVFL

The evidence regarding the dose-responsive nature of VAVFL is equivocal. The majority of studies have shown a relationship between cumulative doses and/or dose duration and the development of VAVFL. ((Arndt et al., 1999, Daneshvar et al., 1999, Eke et al., 1997, Krauss et al., 1998, Lawden et al., 1999, Mackenzie and Klistorner,

1998, Vanhatalo et al., 1999, Wilson and Brodie, 1997) However, other studies have reported contrary findings. (Best and Acheson, 2005, Paul et al., 2001, Wild et al., 1999)

Lawden and associates reported a mean cumulative dose of 4.4kg for those with VAVFL compared to 1.7kg in those exposed to VGB but with normal fields. (Lawden et al., 1999) The severity of VAVFL is moderately correlated with cumulative dose 0.525 (p=0.002) (Manuchehri et al., 2000) and with duration. (Hardus et al., 2000c) In the latter study, which involved a cohort of 157 patients, of whom 118 had been exposed to VGB, a significant correlation was present between the severity of VAVFL and dose duration, with more extensive defects seen in those exposed for 2-4 years, or 4-6 years, as compared to 0-2 years. Similarly, a moderate correlation was found between the extent of VAVFL and duration of exposure. (Toggweiler and Wieser, 2001) A recent attempt to quantify severity of visual field loss in VGB examined the relationship between maximum daily dosing, cumulative dose and dose duration with visual parameters.(Conway et al., 2008) Maximum daily dosing was found to be the single most reliable indicator of development of VGB.

Some studies have failed to demonstrate a definite relationship with VGB dosing and VAVFL ((Wild et al., 1999, Kalviainen et al., 1999). In the former study, no significant difference was found in occurrence of VAVFL in those treated for greater than 4 years compared to those treated for less than 4 years. Kälviäinen and associates were also unable to demonstrate a relationship between dose duration or

cumulative dose and VAVFL, despite a robust prospective study assessing VGB monotherapy of 32 patients over a mean duration of 69 months. It is possible however, that the prospective nature limited the duration and also the higher ranges of cumulative dose.

In the largest study to date, involving 563 patients of whom 421 were exposed to VGB, and based upon the 432 individuals able to produce a reliable outcome, the presence of VAVFL was associated with increasing duration (odds ratio 14.2; 95% CI 5.0 to 40.5) and increasing mean dose (odds ratio 8.5; 95% CI 2.2 to 33.2). (Wild et al., 2007)

1.2.7 The Development of Paediatric Guidelines

Appleton (Appleton, 1998) published a consensus guideline from a paediatric advisory group addressing the prescription of vigabatrin in children. The recommendations included 6-12 monthly visual field examination in children with a cognitive age of greater than 9 years. No technique was recommended for children with a cognitive age of less than 9 years. The guidelines counselled against widespread withdrawal of the drug, and advised an individual risk/benefit analysis. It was advocated that VGB remain the drug of first choice in children with seizures caused by TS and the second or third choice in children with epilepsy due to symptomatic or cryptogenic partial epilepsies.

The guidelines were revised in (2000) and again advised visual field examination which adhered to a specific protocol. It was accepted that electroretinography performed according to International Society for the Clinical Electrophysiology of Vision (ISCEV) standards would be useful in children. Vigabatrin was once again named as the drug of choice in children with IS.

1.2.8 Co-Medication

Some studies have implicated other AEDs in the pathogenesis of VAVFL. (Eke et al., 1997, Wild et al., 1999). However, the large number of potential polytherapies combined with the small cohorts makes attribution difficult. (Hardus et al., 2001) The issue is further confounded by isolated reports in the literature of visual field loss seemingly attributable to a given AED.

Non GABA Drugs

CBZ has been considered to be associated with visual field loss. (Leach, 1998, Kalviainen et al., 1999) However, this finding has not been confirmed in subsequent studies. (Wild et al., 2007)

GABA Drugs

Valproate has been suggested to potentiate an increased prevalence of VAVFL. This theory is plausible given the mild GABA-ergic action of valproate.

Arndt (Arndt et al., 1999) reported that polytherapy of VGB and valproate (VALP) was associated with the two most severe cases of VAVFL in a cohort of 52, where severity of visual field loss was also worse as a while in the VGB/VALP group compared with a VGB/CBZ group. Wild demonstrated attenuated retinal nerve fibre layer (RNFL) thickness in individuals with VAVFL. The cohort exposed to VGB and Valproate had relatively thinner RNFL. (Wild et al., 2006) Valproate, alone, and in appropriate dosing, does not cause detectable abnormalities of retinal dysfunction (Ozkul et al., 2002) (Wild et al., 2006)

Progabide was implicated in a single case report as causing 'tunnel vision' in an individual receiving progabide and Phenobarbital. (Baulac, 1998) Interestingly, replacement with valproic acid led to an improvement in the fields, which raises the possibility of an artefact due to the learning effect, rather than a true improvement in the field.

In general, it is felt that Tiagabine does not result in visual field loss, when used either as monotherapy (Collins, 1998, Kalviainen, 1999) or as adjunctive therapy. (Lawden, 2003, Krauss et al., 2003) However, one single case report described apparent visual field defect attributable to tiagabine adjunctive therapy in an individual with bipolar disorder.(Kaufman et al., 2001) The field returned to normal on withdrawal of the drug, suggesting, once again, an artefact arising from the perimetric learning effect.

1.2.9 Electrophysiology and Imaging In Vigabatrin Attributed Visual Dysfunction

1.2.9.1 Electroretinogram (ERG)

The most widely used electrophysiological technique in the investigation of VGB toxicity is the ERG. Initially, the whole-field ERG was found to be normal in patients with VAVFL (Blackwell et al., 1997, Gross-Tsur et al., 2002, Harding, 1997, Lawden et al., 1999) suggesting that such ERGs are a relatively insensitive tool for the detection of VAVFL. However, the consensus opinion is that the photopic ERG exhibits a reduction in amplitude suggesting dysfunction of the cone receptor pathway and are irreversible after withdrawal (Arndt et al., 1999, Krauss et al., 1998, Coupland et al., 2001, Sills et al., 2001). Occasional reports of reduced scotopic b-waves in ERG implicate rod pathway dysfunction (Daneshvar et al., 1999, Coupland et al., 2001) and may either be a treatment effect, or more likely reflect the end stage of VGB toxicity with catastrophic retinal destruction.

The 30 Hz flicker ERG has been suggested as the most effective electrophysiological tool for the detection of VGB dysfunction. Harding and associates (Harding et al., 2000) reported that the 30Hz flicker response predicted VAVFL with 100% sensitivity and 75% specificity. However, at 100% sensitivity, Brigell and colleagues were only able to demonstrate a specificity of 50% for the same measure. (Brigell, 2000) Reduced photopic oscillatory potentials were initially reported to be indicative of VGB toxicity (Krauss et al., 1998) but this reduction has since been demonstrated

to be a treatment effect. (Harding et al., 1998, Duckett, 1998, Westall et al., 2003)

Deterioration in the photopic ERG has been used as a measure for monitoring the progression of VGB dysfunction. (Brigell, 2000) Eleven of 14 patients with VAVFL showed progression of the photopic ERG over time. The sensitivity for progressive VAVFL was 78%. However, the specificity was low as 23 of 46 patients without VAVFL also showed such ERG changes. It is uncertain whether the reduced photopic ERG reflects treatment effects or is a marker for VGB dysfunction preceding overt clinical change.

1.2.9.2 Multi-Focal Electroretinogram (mfERG)

There have been few studies involving the mfERG in VGB-exposed individuals. Two patients with reduced peripheral amplitudes compared with central responses and no delay in implicit times were reported. (Lawden et al., 1999) An abnormal mfERG responses was also found in 2 patients; a reduction was present in the amplitude of summated action potentials, particularly those of what was termed the 'b wave', and were more pronounced peripherally. (Mackenzie and Klistorner, 1998) Abnormal mfERGs were also found in 6 of 12 patients with VAVFL, again with reduced amplitudes in the periphery, although there was no congruency with the visual field loss. (Ponjavic and Andreasson, 2001) However, a normal mfERG was present in one patient with VAVFL , who also exhibited abnormal full-field ERGs. (Reuther, 1998)

The wide-field mfERG has been shown to exhibit a reduction in the peripheral amplitude response of particularly P1, compared to the central amplitude response in patients with VAVFL. (McDonagh et al., 2003) However, access to this application has not been made commercially available and the technique has not been investigated by other groups. An abstract from the same group found a delay in the P1 implicit time in the peripheral response.

1.2.9.3 Electro-oculogram (EOG)

A reduced Arden Index (AI) was found in 2 of the 3 patients comprising the original case series of VAVFL described by (Eke et al., 1997).However, following withdrawal of VGB, these patients exhibited an AI within the normal range. (Harding, 1997) The improvement of the AI following withdrawal of VGB has since been confirmed. (Harding et al., 1998) and indicates a metabolic effect of VGB on the retinal pigment epithelium and the retinal pigment epithelial-outer segment complex. (Coupland et al., 2001, Comaish et al., 2002, Lawden et al., 1999, Arndt et al., 1999)

1.2.9.4 Visual Evoked Potential (VEP)

A majority of studies report a normal VEP in adults with VAVFL. (Eke et al., 1997, Lawden et al., 1999, Liegeois-Chauvel et al., 1989, Mauguiere et al., 1997, Reuther, 1998, Wilson and Brodie, 1997) and in children with VAVFL. (Uldall et al., 1995)

However, an abnormal VEP was found in 1 of 4 VGB exposed patients (Krauss et al., 1998), and in 22% of 32 patients exposed to VGB (including the 4 previously described by Krauss and associates). (Miller et al., 1999) A similar prevalence of abnormality has been found in a further adult cohort (30%), the majority of whom exhibited advanced VAVFL (Daneshvar et al., 1999) and also in children (33%). (Gross-Tsur et al., 2000) The reduction in the VEP reflects the central dominance of the traditional summed VEP responses and it can be postulated that mfVEP technology may identify VGB toxicity more peripherally.

1.2.9.5 Fundal Abnormalities Associated with VAVFL

The retinal abnormalities associated with VAVFL, visible by fundoscopy, if present, are subtle and include retinal nerve fibre layer (RNFL) attenuation, (Miller et al., 1999, Buncic et al., 2004, Frisen and Malmgren, 2003) abnormalities of the macula (Krauss et al., 1998) including epi-retinal membrane formation,(Krauss et al., 1998, Buncic et al., 2004) peripheral vessel irregularity (Krauss et al., 1998, Wild et al., 1999) and peripheral pigmentary disturbances.(Lawden et al., 1999, Wild et al., 1999) The optic nerve head exhibits a characteristic 'inverse' or nasal atrophy. (Buncic et al., 2004, Frisen and Malmgren, 2003) It seems likely that these manifestations, particularly that of the optic atrophy, are late presentations of VAVFL.

The first case report, by optical coherence tomography (OCT) of an attenuated RFNL layer in VAVFL was that of (Choi and Kim, 2004). An abnormally attenuated RNFL

associated with VAVFL as measured by OCT (fast RNFL 3.4) in at least one quadrant was described in a further 12 eyes (75%) of a case series comprising a mixed-age cohort of 8 individuals, and outside of normal limits in at least 2 quadrants in 9 eyes (56.3%). Despite advanced VAVFL, no eye showed reduced RNFL thickness in the temporal quadrant. In each case, the visual field was congruent with the corresponding RNFL thickness. (Rebolleda et al., 2005)

In a case-controlled study (Wild et al., 2006), which was accepted for publication prior to publication of the commentary by Rebolleda and associates, an attenuated average retinal nerve fibre layer (RNFL) was reported in all 13 adults with VAVFL. Of the 8 individuals who were exposed to VGB but with normal fields, 3 exhibited an average RNFL thickness, which lay just beyond the 95% confidence interval. This latter finding may reflect either a precursor of functional abnormality, or may be an artefact due to the lack of statistical precision of the 95% confidence interval, which was derived from 20 age matched normal individuals. All but two of the 14 non-GABAergic controls and all 7 of the valproate controls exhibited an average RNFL thickness within the normal range.

An attenuated RNFL thickness associated with VAVFL has also been described in a single case report using scanning laser ophthalmoscopy (SLO). (Viestenz et al., 2003) This technique was also used in the case-control study of (Wild et al., 2006) although SLO exhibited less sensitivity (77%) than OCT for the identification of VAVFL. A third imaging modality, namely nerve fibre layer polarimetry, has also confirmed the

association between an attenuated RNFL thickness and VAVFL: all 8 individuals manifested this finding. (Durnian and Clearkin, 2007)

1.2.10 Pathogenesis

A consensus view of the findings from visual electrophysiology implicates retinal dysfunction, possibly Amacrine and/or Muller cell dysfunction. The conclusion from the limited number of studies involving retinal imaging, namely an attenuated RNFL layer, is of course, compatible with retinal dysfunction. Post-mortem examination of the retinae of a patient with advanced VAVFL found profound atrophy of the peripheral retina including ganglion cell and nerve fibre layer loss. (Ravindran et al., 2001).

A VGB dose-dependent disruption of the photoreceptor layer of the albino rat was demonstrated prior to VGB licensing. (Butler et al., 1987) Microvacuolation attributable to VGB was also found prior to licensing of the drug in beagle and monkey brain cortex. (John et al., 1987) Subsequent work in the rat showed that VGB crosses the blood-retinal barrier and accumulates at a concentration of five-fold compared to that measured in the brain.{Sills, 2001} Interestingly, a six-fold increase in GABA concentration has been found in the rat retina following VGB. (Neal et al., 1989) VGB toxicity is seemingly light dependent: a dose-dependent relationship between retinal damage and VGB has been found in the albino rat at 20,000 lux in the albino rat but not in the dark. However, GABA accumulation plus light does not

result in retinal damage. (Izumi et al., 2004) (Izumi et al., 2004)

Irreversible reduction in the photopic ERG, the flicker response and the OPs arising from VGB is associated with peripheral disorganisation of the outer retina in albino rat, in particular cone photoreceptor damage. (Duboc et al., 2004)

It is clear that the mechanism of VGB toxicity cannot be derived from electrophysiology or ocular imaging and has not yet been elucidated from animal toxicology studies.

1.2.11 Conclusion

VGB unequivocally causes irreversible visual field loss, which is initially asymptomatic. The severity of the field loss seems to vary between individuals. The principal risk factors for the development of VAVFL are male gender, mean dose and dose duration of VGB. Fundal abnormalities in VAVFL are subtle, if present at all. The findings from visual electrophysiology and retinal imaging studies are indicative of a retinal location for VGB toxicity. However, the precise pathogenesis of VGB visual dysfunction is not known.

1.3 Assessing VGB Toxicity in Children and Learning Disabled Adults: General Considerations

Assessing VGB toxicity in children and learning disabled adults requires successful application of perimetry and electrophysiology. Traditionally, perimetry, in particular, has not been undertaken in children and learning disabled adults.

Perimetry: Challenges In Children And Learning Disabled Adults

It is generally considered that individuals require a developmental age of nine years in order to reliably perform perimetry. (Wild et al., 1999) Other factors that may affect ability to undertake perimetry include interest, levels of alertness, and fatigue.

In infants and children, clinical assessment of the visual field is often overlooked due to perceived difficulty. However, gross defects such as hemianopia or quandrantanopia may be apparent upon careful clinical observation. The usual approach is to look for a shift in the individual's fixation from an object of interest in the central field to a newly introduced object in the peripheral field. A standardised method for use in infants and children has been described. (Mohn et al., 1988) It should be noted that the visual field of newborns extends only to approximately 30^0, which then expands over 12 to 15 months to the extent of that in adults. (Suchoff, 1979)

In learning-disabled adults, a wide spectrum of ability is encountered; traditional confrontation assessment of the visual field may be possible, but in those individuals who do not possess language skills, the only clinical assessment involves watching for a shift in fixation towards an object induced into the visual field. (Mohn et al., 1988)

1.3.3 Electrophysiological techniques currently used in children and learning-disabled adults

Given the constraints in assessing the visual field of children and learning-disabled adults, attempts to quantify objectively VGB-attributed visual dysfunction have been made using electrophysiological techniques, namely Visual Evoked Potentials (VEPs), Electroretinograms (ERG) and the electro-oculogram (EOG).

Applications of The Visual Evoked Potential (VEP) In Children And Learning-Disabled Adults

VEP responses reach maturation at between four to six months of age and therefore represent a useful reference point for assessing vision, even in infants. (Brecelj, 2003) VEPs have been used to assess vision in pre-verbal children and in patients with developmental delay. (Spencer and Harding, 2003) Standard methods of recording ensure consistent results. A comprehensive account of VEP in ocular and neurological conditions in children is that of Westall and colleagues. (Westall et al., 2000)

The VEP has a wide range of applications in paediatric practice, but is under-utilised in learning-disabled patients (Tables 1.3.1 and 1.3.2). The VEP is of particular benefit in neurological disease and in those exhibiting developmental delay. However, in the presence of optic nerve disease, the VEP is likely to be of small amplitude and to be delayed and therefore, in such cases, will be limited as a technique in monitoring any visual dysfunction posteriorly to the optic nerve.

Applications of The Electroretinogram In Children And Learning-Disabled Adults

The ERG represents a mass response from the retina and as such is useful in assessing retinal disorders and in localising the site of the visual dysfunction. However, ERG recording is relatively time consuming and invasive. In children less than 5 years of age, recording of the ERG typically requires the use of a contact lens electrode and sedation. (Fulton et al., 2006)

There are five types of ERG responses recommended for the standard ERG examination. (Marmor et al., 2008) The ERG can distinguish between outer, middle or inner retinal location dependent upon the type(s) of ERG being employed. ERG responses do not maturate to those present in adults until 3-5 years of age and age-matched reference values must be used for children less than 5 years of age in order to avoid over-diagnosis of retinal dysfunction. (Laget et al., 1984) The ERG remains the preferred method for assessing inherited retinal disorders and dystrophies (Table 1.3.2). In cerebral causes of visual failure, the ERG is normal. (Laget et al., 1984) The

30-Hz flicker ERG has been used in infants to identity VGB toxicity, and possibly to predict the development of VGB toxicity. (Westall et al., 2002)

The Multifocal ERG In Children And Learning-Disabled Adults

The mfERG has been successfully applied in those unable to perform perimetry (Hood et al., 2003a) and has been shown to have good repeatability (Mazinani et al., 2007) making it an appropriate technique for monitoring disease progression. A summary of the potential utility of the mfERG is given in Table 5. A detailed discussion of mfERG in VGB toxicity was given in section 1.5.1.

Multi-focal VEP In Children And Learning-Disabled Adults

The mfVEP has been widely used in adults to study diseases both of the ganglion cells and the optic nerve, including optic neuritis and multiple sclerosis (Grover et al., 2008, Klistorner et al., 2008) and glaucoma. (Hood and Greenstein, 2003, Grippo et al., 2006) This technology has already been successfully applied in those unable to perform perimetry (Hood et al., 2003b).

There are a number of potential clinical applications of mfVEP (Table 1.3.3). However, many protocols are time-consuming requiring in the region of half an hour. (Hood et al., 2003b) Fortunately, it is seemingly possible to achieve useful responses from as few as 4 recording cycles, with a recording time of a few minutes. (Hood et al., 2007)

Field-specific VEPs

A novel use of the standard VEP (the field-specific VEP), which is applicable to children older than approximately 3 years of age, has been described. (Harding et al., 2002b) This technique enables recording of separate central and peripheral responses by presenting a stimulus with both a central and peripheral stimulus of reversing checks that increase in size with eccentricity. The central and peripheral checks are separated by a blank annulus. The checks reverse at different rates, thus allowing identification of peripheral and central responses. In conditions where central vision is spared, such as in vigabatrin-attributed visual field loss (VAVFL), standard VEP responses remain normal. The recording of an attenuated or absent peripheral response in the presence of a normal central response is indicative of peripheral visual field loss.

In a paediatric study assessing VGB toxicity, 35 of 39 children (mean age 12.2 years) were able to undertake the field-specific VEP, whereas only 12 children were able to undergo perimetry. (Harding et al., 2002b) The field-specific VEP identified 3 of 4 children with VAVFL identified by perimetry (75% sensitivity) and correctly designated 7 of 8 children with normal fields by perimetry (87.5% specificity).

Retinal Imaging

A detailed discussion of retinal imaging in relation to VGB toxicity was given in section 1.2.12. Estimation of RNFL thickness has been quantified in children using OCT (Rebolleda et al., 2005) and SLO (Lundvall Nilsson A.E., 2007) where children as young as 6 years of age have been assessed. In particular, OCT has been shown to identify VGB toxicity in children.(Rebolleda et al., 2005)

1.3.5 Conclusion

The outcome of perimetry in children and learning-disabled adults is dependent upon developmental age, as well as alertness and concentration. It is generally accepted that perimetry is not possible in individuals exhibiting a developmental age of less than 9 years.

ERG identifies VGB toxicity in infants, although sedation is required. However, both mfVEP and mfERG remain untried in children and learning disabled adults, and this may reflect restricted access to multifocal technology.

Estimation of RNFL thickness is possible in children with a chronological age of at least 6 years and, in older children at least, identifies VGB toxicity.

Table 1.3.1 Current Uses of VEP

Clinical Application	Characteristics of VEP
Visual Acuity	Decreasing check size until VEP responses not discernible correlate with acuity
Albinism	Flash VEP asymmetry comparing right and left eyes due to abnormal decussation at the chiasm
Cerebral visual impairment	Prognostic information; poor VEP responses correlate with poor visual outcome
Delayed Visual Maturation	Progressive improvement in latency and amplitude
Ocular Motor Apraxia	Normal age appropriate pattern VEP responses prior to developing head thrust

Table 1.3.2 Current Applications of ERG

Inherited retinal degenerations and dystrophies e.g. retinitis pigmentosa (RP)
Lipopigment storage diseases e.g. Batten disease
Mucopolysaccharidoses
Inflammatory retinopathy incl. Cogan's syndrome
Toxic retinopathies
RP-like dystrophies, e.g. Usher

Table 1.3.3 Potential Clinical Applications of mfVEP

Clinical application	Clinical conditions
Detecting visual field loss in subjects unable to perform perimetry	Toxic (e.g. vigabatrin) Structural (e.g. post trauma, space occupying lesions) Raised intracranial pressure (e.g. obstructive hydrocephalus, idiopathic intracranial hypertension or IIH)
Monitoring disease progression	Optic neuritis, glaucoma, retinitis pigmentosa, IIH

Table 1.3.4 Potential Clinical Applications of mfERG

Clinical Conditions
Inflammatory retinopathy
Toxic retinopathy e.g. vigabatrin
Malignant retinopathy e.g. melanoma
Glaucoma
Retinal dystrophies e.g. RP, Usher, Biedl

Chapter 2: Rationale For Research

2.1 Clinical Reason For Study

The Welsh Epilepsy Unit at the University Hospital of Wales was a centre, during the 1980s, for the Phase III trials of VGB. Following licencing of VGB, a large number of patients attending the Welsh Epilepsy Unit were treated with the drug. As a consequence, the local population has a far higher prevalence of patients with exposure to VGB than that of most, if not all, epilepsy services across the UK and worldwide.

The proportionately large numbers of patients exposed to VGB attracted research interest, and generated the acquisition of concomittant expertise, in the problem of vigabatrin-attributed visual dysfunction. My clinical neurology supervisor (PEMS) had recognized, at an early stage, the potential scale of the problem of VAVFL, had been rigorous in implementing a regular visual screening programme for patients exposed to VGB under his care at the University Hospital of Wales and has subsequently developed great awareness and experience of the clinical management of patients exhibiting VAVFL. My vision science supervisor (JMW), whilst in Birmingham, had acquired a reputation as an acknowledged international expert in the interpretation of VAVFL, and a productive collaboration between these two individuals was instituted on his appointment in Cardiff. A postgraduate student, Catherine Robson, had obtained her PhD in 2007 for a thesis which involved the investigation of VAVFL in patients attending the Welsh Epilpesy

Unit (Chapter 3.1). The research reported in this thesis extends the previous research undertaken by the groups in Birmingham and Cardiff and is concerned with the identification of vigabatrin-attributed dysfunction in particular sub-groups of patients treated with VGB.

In the current context of a relative plethora of available anti-epileptic drugs, vigabatrin might appear to be a dinosaur: once suited to its environment but now categorically shown to be not fit for purpose due to a major flaw namely irreversible visual field loss. A pragmatic viewpoint is that the prescription of VGB is no longer justified, under any circumstances. However, when dealing with complex clinical issues, this hard-and-fast rule becomes less easy to apply, particularly when dealing with a patient with catastrophic epilepsy.

VGB, somewhat controversially, is still recommended as a first-line treatment for IS in West Syndrome (both with and without a causative diganosis of TS. (Appleton, 1998, Lux et al., 2001) Although the evidence for VGB as a superior agent to hormonal methods of treating IS is lacking, (Hancock et al., 2001) it is clear that VGB is nonetheless a highly effective agent, and some children respond better to VGB than to ACTH, or are better served by VGB because of the adverse effects associated with hormonal treatment. Therefore, it is immediately apparent that simply removing VGB from the prescribing inventory is not an appropriate response and will not best serve the

epilepsy commnunity as a whole.

Learning-disabled adults are a particularly vulnerable group often living in delicately balanced social cirumstances. Altering an effective anti-epileptic regimen, in this case VGB, because of an unquantified risk of developing visual field loss, often proves to be an unpalatable option for all concerned. This, coupled with the relative lack of an effect on cognitive function of VGB compared to other anti-epileptic drugs results in high rates of retention of VGB in this group. (Dodrill et al., 1995)

Clinical pilot studies assesssing the utility of VGB as an anti-addiction drug for cocaine and crystal methamphetamine (with potential uses for alcohol and even smoking being suggested) have once again brought VGB to the fore (Brodie, 2005, Brodie et al., 2003, Fechtner et al., 2006). Whilst the planned short-term restricted use of VGB may reduce the risk of VAVFL in a given population, no individual predictive markers yet exist, and no definitive marker preceding irreversible damage has yet been determined.

Given the limitations of the visual field examination in patients with epilepsy and, in particular, those exposed to VGB (approximately 25-30% of the latter patients are unable to comply with the requirements of perimetry) and, also, armed with the knowledge that vigabatrin induces retinal damage; it seemed appropriate to utilise novel techniques

which, a priori, seemed applicable to the study of vigabatrin associated visual dysfunction at a functional and structural level. Given the current set of circumstances, the pressing clinical question is how best to address the question of the surveillance of visual dysfunction in those individuals where VGB remains the only therapeutic intervention – namely, children and learning disabled adults. These populations are currently disadvantaged, largely because perimetry requires a developmental age of approximately 9 years old and a reasonable degree of sustained alertness.

The research topic for this thesis continues the study of a technique that showed potential for identifying vigabatrin-attributed visual dysfunction in those incapable of perimetry namely, measurement of retinal nerve fibre layer (RNFL) thickness by optical coherence tomography. This previous research had suggested the possibility of the identification of damage to the RNFL that preceded clinical loss; a lofty ambition, but, clinically, a far more useful one. In addition, the research topic for this thesis also involves the investigation of the suitability of emerging multifocal visual electrophysiological techniques for the identification of vigabtrin-attributed visual dysfunction.

2.1.1 Previous Work

As part of her PhD thesis, Catherine Robson had, in particular, shown in a prospective cross sectional obervational study (Wild et al., 2006) that attenuation of the RNFL thickness, as estimated by optical coherence technology (OCT) and by scanning laser

ophthalmoscopy (SLO), was a reliable biomarker for VAVFL, particularly that recorded by OCT. As described previously (Chapter 1.2.12.5.) such a finding was consistent with two previous single-patient case studies of VAVFL using SLO (Viestenz et al., 2003) and OCT (Choi and Kim, 2004) and a commentary on the RNFL thickness, derived by OCT, in 13 individuals exposed to VGB. (Rebolleda et al., 2005) The latter was published after our group's manuscript had been accepted for publication. Subsequently, an attenuated RNFL associated with VAVFL was also found by a third imaging modality, namely scanning laser polarimetry (SLP). (Durnian and Clearkin, 2007) The attenutaed RNFL was also consistent with the post-mortem appearance of the retinae of an individual with advanced VAVFL. (Ravindran et al., 2001)

Interestingly, a characteristic clinical observation, labelled 'inverse atrophy', had been coined to highlight the differential pattern of optic nerve head atrophy present with VAVFL, compared to that of the normal manifestation of optic neuropathy. (Buncic et al., 2004) The retinal nerve fibre layer exhibited an atrophy which spared the temporal regions of the retina and optic nerve head, whilst showing attenuated nasal, superior and inferior sectors of the optic nerve head. This characteristic pattern of atrophy had also been identified in digitally-enhanced ocular fundus photography. (Frisen and Malmgren, 2003) The inverse pattern of atrophy had not been reported in any other ocular condition. The possibility of identifying this pattern using OCT, potentially and specifically manifested as a nasal sector attenuation at the optic nerve head would obviate the need for fundoscopy, with its inherent difficutlies and limitations, and would greatly enhance

the ability of diagnostic imaging in the identification of VAVFL.

The notion of structural change pre-dating visual field loss is well-established in glaucoma, (Caprioli et al., 2006) and is seemingly predictive of the development of multiple sclerosis. (Sepulcre et al., 2007) Therefore, it was felt that attenuation of the RNFL layer potentially and specifically manifested as a nasal sector attenuation at the optic nerve head, might be a precursor of VGB-attributed functional damage. The possibility of nasal atrophy in eyes of individuals exposed to VGB manifesting normal visual fields was of particular interest. Notwithstanding the latter, and of paramount importance, was whether the OCT could be applied to children below a chronological age of 6 years, and to learning-disabled adults.

Thus, if a nasal sector optic nerve head RNFL abnormality could be identified as a specific finding to VAVFL, and OCT could be applied to children and learning-disabled adults, then such findings would be of major importance in the detection and monitoring of VGB visual dysfunction.

2.2.2 Multifocal Electrophysiology in VGB Visual Dysfunction

Multifocal visual electrophysiology has not been widely applied to VGB visual

dysfunction. The development of this technology affords the potential to investigate regional change that standard electroretinography (ERG) and visual evoked potential (VEP) recording cannot provide. Furthermore, the mass response of each of these modalities, it is argued, may obscure the identification/presence of local abnormalities. (Poloschek and Sutter, 2002, Hood et al., 2003a) Given that VAVFL is, initially at least, a focal/ localised disease, predominantly affecting the nasal visual field and exhibiting varying degrees of sparing of the temporal field, and latterly producing a concentric defect, it was felt that these techniques seemed appealing.

It is noteworthy that, amid the conflicting human and animal electropysiological, structural and epidemiology reports, there is no clear theory underpinning the pathogenesis of VAVFL. One possible advantage of examining patients with both mfVEP and mfERG, might be the insight that could be afforded into cellular locations of vigabatrin toxicity. This combination has been considered to be clinically useful in a variety of clinical scenarios, including non-organic visual loss. (Renner et al., 2005)

Multifocal ERGs theoretically reflect integrity anywhere within the retina; in practice however, much of the electrical response is dominated by the bipolar cells, with the photoreceptors impacting on implicit times and the ganglion cells exerting subtle effects on the shape of the P1 waveform. (Hood et al., 2002) However, the mfVEP reflects integrity anywhere from the ganglion cell through the optic nerve, chiasm, tracts, and

visual cortical areas. Amidst this wealth of representation, it is postulated that cortical changes may dominate. (Hood, 2004)

In addition, the relatively short, and potentially interrupted examination time, with sequences requiring attention and fixation for as little as 15 seconds at a time, for a total recording time of 7 minutes, suggested that the multifocal techniques might be applicable to young children and learning-disabled adults.

2.3 Aims of the Thesis

The aim of the thesis was therefore two-fold. Firstly, to investigate the suitability, in competent adults, of RNFL measurement by OCT, referenced to a commercially available normative database, as a measure of VGB dysfunction. Secondly, to investigate the potential of mfERG and mfVEP, and if positive, RNFL measurement by OCT for the investigation of VAVFL in children and learning-disabled adults.

2.3 General Methodology

The development of an objective and rapid technique for the assessment of VGB dysfunction, with high sensitivity and specificity, would enable safe continued presciption of VGB as determined by clinical need. The fervent hope was that the

structural or electrophysiological techniques would reveal changes that pre-date irreversible visual loss. The ideal outcome of the study was the development of a clinically useful tool - one that is readily performed clinically - rather than a research tool, per se. Such a finding would enable VGB to be re-introduced into mainstream epilepsy practice.

The three techniques required validation in individuals able to perform static perimetry; perimetry is the only means of quantifying visual field loss in VGB toxicity and as such, must be considered the gold standard. As would be expected, the validation group largely comprised competent adults, although a few children and learning disabled adults proved able to undertake static perimetry.

Participants who volunteered to take part in the studies were drawn from patients attending either the tertiary epilepsy clinics or the paediatric neurology clinics at the University Hospital of Wales. The corresponding caring physician initially approached all potential participants and requested permission from the potential participant to provide me with their name and contact details.

It was anticipated that limited numbers of individuals with exposure to VGB would result in a corresponding limited number of children and to a lesser extent, learning-disabled

adults being enrolled in the study. Therefore, for pragmatic reasons of time constraint, the three investigative techniques were simultaneously applied to the competent adults, children and learning-disabled adults. Ethical approval had been obtained from South East Wales Ethical Committee for all the studies described in this thesis. In addition the studies were approved by the Research and Development Office of the Cardiff and Vale NHS Trust.

The investigation of RNFL thickness soon became the focus for the majority of my work, as this seemed by far the most promising tool.

2.4 Study Logistics And Limitations

A number of logistical difficulties were encountered with this study, as is often the case in studies driven by clinical need. The major unknown associated with the development of the thesis was that it was not possible to predict whether the study participants (particularly children and learning-disabled adults) would be able to cooperate with the three investigative techniques.

Considerable time was invested in applying the techniques to competent adults in order to minimise operator error and to gain a clear idea of the intrinsic difficulties associated with the study cohort. There was a major time requirement involved in acquiring

sufficient expertise in electrophysiology. Early on in the development of the thesis, a visit was made to Professor Colin Barber (Consultant Electrophysiologist at the Queens Medical Centre, Nottingham) to acquire expertise in multifocal electrophysiology recording. He and his unit provided a useful link for ongoing advice and assistance. The author also attended the practical course on visual electrophysiology recording preceding the annual meeting of ISCEV in 2005. The experience gained from this course was invaluable, but it was felt that considerable practice was required prior to commencing the study. Approximately 240 hours for mfERG, and 180 hours for mfVEP, were spent acquiring traces from normal individuals prior to undertaking the various studies.

There was a particular difficulty with the mfERG data. It emerged (see chapter 5) that a software upgrade of the VERIS system had resulted in an additive shift of approximately 7 milliseconds in the P1 implicit time. It was uncertain whether this shift was uniform, within and between participants and whether or not it was possible to predict what effect, if any, the software change exerted on the amplitude. This anomaly was noticed at the initial data analysis, and necessitated discarding of the entire normative database (23 individuals) and the acquisition of a new, temporally correct database.

The other major logistical problem with the development of the thesis related to the requirement for reliable normative databases. Whilst robust normative databases exist for the Humphrey Visual Field Analyzer and for the StratusOCT systems, databases for the

mfVEP and mfERG had to be acquired. The local ethical committee felt that the ethical constraints involved in exposing healthy children to invasive testing with mfERG, which requires the pupil to be dilated and the use of a DTL fibre (with a consequent small risk of corneal abrasion) outweighed the benefits of establishing an electrophysiology dataset for children.

A further difficulty was the access to the OCT. The OCT is housed in the Media Resources department at the University Hospital of Wales, in a room with general photography requirements. Staff of the department performs NHS clinical OCT scans, and clinical requests are prioritised over research use. This resulted in long delays (over an hour on several occasions) for some research participants, and led to individuals withdrawing from the study, or simply refusing to wait or to return for non-completed imaging studies. Out of hours access is not routinely available and this proved particularly problematic for testing children.

I am indebted to my colleagues for their prompt referral of suitable patients particularly my supervisor (PEMS) and the other members of the Epilepsy Unit, but also my Paediatric Neurology colleagues, Frances Gibbon and Johann te Water Naude. Agreement to participate in the study was approximately 70% of those individuals referred to me. Where possible, study visits were arranged for participants on days on which they had pre-existing or required clinical appointments. Unfortunately, the mean

rate of non-attendance (38%) was disappointing, but understandable given the complex problems faced by the participants. In addition, illness often forced cancellation or unforeseen non-attendance.

Some individuals were either unable or failed, to attend for examination with all the various investigative modalities, even after re-booking of the failed appointments. Other individuals were unable to cooperate with one or more of the investigative modalities. Such failed appointments and/ or an inability to undertake an investigative modality necessitated the recruitment of additional patients, resulting in delays in data collection and yielding different datasets for the various studies, making comparison across studies difficult.

Despite these challenges, a total of 104 individuals attended for testing in at least one of the investigative modalities. The number of appointments totalled 395 and, on average, each appointment lasted one and a half hours. Thus, over 590 hours were spent in data collection, which yielded approximately 180 visual fields, 164 OCT measurements, 108 mfERG trace arrays and 125 mfVEPs trace arrays. Following the software shift with the mfERG, 15 individuals were unavailable for repeat testing and so were not included in the subsequent analysis of the mfERG, but were included in the analysis of the mfVEP.

Chapter 3: Utility of Retinal Nerve Fibre Layer Thickness as Measured by OCT in VGB-Attributed Visual Dysfunction

3.1.1 OCT: Introduction

OCT is a non-invasive imaging technique, which provides high-resolution cross-sectional images of the tissue in question. The technique first became commercially available for imaging the ocular fundus in the mid-1990s.

The current commercially available instruments utilise conventional standard interferometry. Advances on this technique include ultra high-resolution, adaptive optics and Fourier domain analysis in order to improve resolution. (Drexler and Fujimoto, 2008)

OCT has been used in a variety of clinical contexts including macular, vitreo-retinal, optic nerve head and retinal nerve fibre layer disorders. Measurement of the RNFL is usually provided by circular scans centred on the optic nerve head, generating, in the case of the Stratus OCT, a RNFL thickness regional map over 12 sectors of the optic disc. RNFL thickness attenuation has been detected by OCT in glaucoma and in ocular hypertension (Zangwill et al., 2000), where it seemingly precedes functional

visual loss. (Caprioli et al., 2006) Of increasing interest in neurological disorders, RNFL thickness correlates with axonal loss as measured by brain MRI in multiple sclerosis, and this holds true for clinically unaffected eyes, as well as eyes with documented optic neuritis. Temporal RNFL attenuation may predict the development of relapses and deterioration in multiple sclerosis. (Sepulcre et al., 2007)

An attenuated RNFL has been reported in VAVFL. (Wild et al., 2006) (Choi and Kim, 2004, Rebolleda et al., 2005, Wild et al., 2006) Wild and associates (Wild et al., 2006) also showed an attenuated mean RNFL in some individuals exposed to VGB but manifesting normal visual fields, suggesting that RNFL changes may also predate functional visual loss in VAVFL.

3.1.2 OCT: Principles, Acquisition and Interpretation

The Optical Coherence Tomography (OCT) is analogous to ultrasound, in that the image is based on differing reflecting properties of the tissues examined. However, the OCT image is created by reflection of light rather than sound. The intensity of the reflected light from the retina will vary depending upon the structure from which it has been reflected. (Chauhan and Marshall, 1999) In order to determine differential reflected light properties, the principle of interferometry is applied. Interferometry describes the superimposition of two or more waves. Measurement of the output wave is dependent upon the phase differences between the input waves (Figure 3.1.1). The Michelsen interferometer is widely applied in the commercially available OCT

instruments (Figure 3.1.2) and essentially comprises a coherent light source, a detector, two mirrors and one beam splitter.

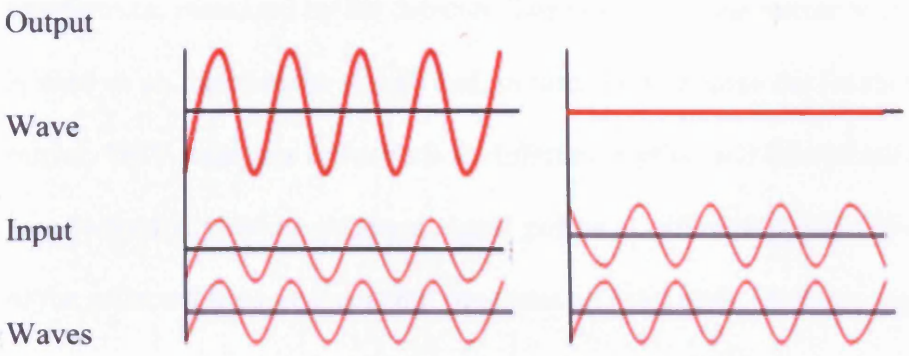


Figure 3.1.1 The left-hand diagram illustrates constructive interference; two waves with coincident phase add to produce a larger amplitude output wave. The right-hand diagram illustrates destructive interference; two waves in opposite phase, and if of equal amplitude, cancel each other out.

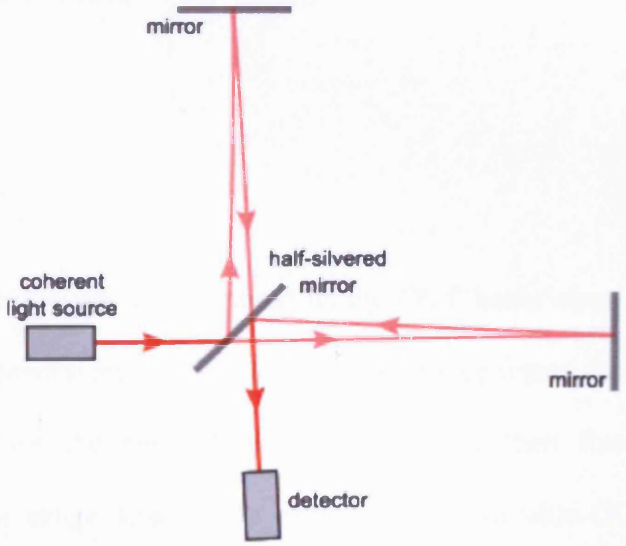
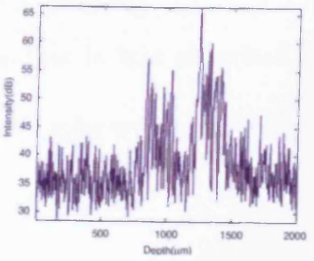


Figure 3.1.2 The Michelson Interferometer

The emitted light is split by the beam-splitter, one becoming the reference beam and the other, the test beam. The position of the mirror(s) may be altered, which change the resultant distance (path length) travelled by the light and creates a phase interference, measured by the detector. The position of the mirror in the reference arm is used as an interference signal, and, in turn, to determine the location of the sample mirror. OCT measures reflections at different depths, and the reflection mirror scans longitudinally, yielding different signal pulses at different depths. Plotting amplitude of the reflected light versus depth generates an axial scan. (A-Scan, Figure 3.1.3).

Figure 3.1.3 A-Scan of the retina.



The amplitude of the reflected light is plotted against depth (ie the position of the tissue acting as a reflector).

Multiple A-scans are acquired as the OCT beam scans transversely across a tissue. Digital processing and digital smoothing techniques then align these images in order to improve the signal-to-noise ratio; these then form a two-dimensional cross-sectional image. One of the commercially available OCT systems, and that used in this thesis, the Stratus OCT, acquires 400 A-scans per second, totalling 512 sequentially obtained A-Scans in 1.3 seconds, and generates an axial resolution of 9-

10 microns. Numerous scan modes are available; primarily these are composed of circles or straight lines.

Standard interferometry applied by the Michelson interferometer measures interference in metres (fit for purpose measuring cosmic ether); clearly retinal imaging demands interferometry distances of micrometres. The vast shortening of the measurable distance is achieved by altering the type of light source. Conventional OCT uses a low-coherence light source. The axial resolution depends upon the wavelength and bandwidth of the incident light (Jaffe and Caprioli, 2004). In imaging the retina, shorter wavelength light (800nm) is used as this is less absorbed by the high-water content structure of the cornea and vitreous. Light with a broad spectral bandwidth produces a shorter coherence beam, which maximises the optical path length mismatch between the reference and sample mirror. A super-luminescent diode (SLD) is commonly used to generate a uniform, low-coherence light source centred at a wavelength of 830 nm. (Schuman et al., 1995). The Stratus OCT achieves higher axial resolution than the forerunners (OCT1 and OCT2) due to better equality between the optical dispersions in the reference pathway and the sample pathway (Jaffe and Caprioli, 2004). Transverse resolution is essentially independent of light source wavelength and is limited by pupillary aperture and optical quality of the eye to approximately 10μm (Drexler, 2004).

New advances in OCT technology (ultra-high resolution) utilise an ultrabroad-bandwidth femtosecond laser technology, which reduces axial resolution to 2-3μm.

(Drexler et al., 2003) To date, this technology has been expensive and hence, has been restricted to research applications. Recently, multiplexed SLDs have been combined to synthesize a broadband spectrum, which has the potential for commercial development, being cheaper than femtosecond technology. (Unterhuber et al., 2004) However, the multiplexed SLD system generates a peak emission wavelength of 900nm, overlapping with water absorption at 980nm, and hence limiting the resolution. Adler et al., 2004, Ko et al., 2005) At the time of writing, the newest comnmercial instruments use a single SLD and produce axial image resolutions of 5-8μm.

3.1.3 OCT: Retinal Nerve Fibre Layer Detection

Retinal thickness is calculated as the distance between the vitreo-retinal interface and the anterior boundary of the reflectance layer (traditionally labelled red in the pseudo-coloured bands) corresponding to the Retinal Pigment Epithelium (RPE) which is the choriocapillaris. (Schuman et al., 1996) Technical difficulties in identifying the appropriate boundaries are more common at the posterior boundary.

RNFL thickness is defined by the distance bounded by the first reflection off the retinal surface - one of the most consistent sources of reflectivity, (Chauhan and Marshall, 1999) and the RNFL posterior boundary. This latter boundary is an arbitrary definition based on the change in the refractive index in the retinal tissue, which is believed to indicate a change in the structure i.e. a movement from the RNFL

into the more posterior layers. This definition for the boundary is not yet fully accepted. (Jaffe and Caprioli, 2004)

3.1.4 OCT Relationship between RNFL and Perimetry

The topographic relationship between the retinal nerve fibre layer and the visual field has been described, based upon retinal ganglion cell (RGC) loss in glaucoma. (Figure 3.1.4). However, the number, separation and positioning of the stimulus locations in the clinically utilised visual field protocols are not ideal for investigating the relationship between reduction in the visual field and attenuation of the RNFL. For example, only one-sixth of the available stimulus locations correspond to the region in which those RGC axons in the nasal half of the retina enter the optic nerve head. Various topographical relationships between the given stimulus location and the corresponding RGC axon entry into the optic nerve head have been described. (Magacho et al., 2005, Boland et al., 2008, Garway-Heath et al., 2000)

3.2 The Application Of Imaging Techniques In VAVFL

Ophthalmoscopy, fundus photography and other imaging modalities have been scrutinised in the hope of identifying changes predictive or diagnostic of VAVFL (Frisen and Malmgren, 2003, Buncic et al., 2004, Wild et al., 2007). The fundal abnormalities associated with VAVFL are subtle when viewed by fundoscopy. The field loss can occur in the presence of a seemingly normal retina and/or normal optic nerve head. (Kalviainen et al., 1999, Newman et al., 2002) However, it can also

occur with optic nerve atrophy (Daneshvar et al., 1999, Wild et al., 1999, Frisen and Malmgren, 2003, Buncic et al., 2004, Harding et al., 2002a) with or without one or more of a variety of retinal abnormalities including surface wrinkling retinopathy (Krauss et al., 1998, Buncic et al., 2004), peripheral retinal arterial narrowing, (Krauss et al., 1998, Wild et al., 1999), peripheral retinal hypopigmentation (Lawden et al., 1999), irregular sheen at the macula (Krauss et al., 1998) and thinning of the retinal nerve fibre layer (Miller et al., 1999, Frisen and Malmgren, 2003, Buncic et al., 2004). The attenuation of the retinal nerve fibre layer, both by fundoscopy (Buncic et al., 2004) and by image enhancement of fundus photographs (Frisen and Malmgren, 2003), can show a nasal predilection which can frequently be associated with corresponding secondary nasal optic atrophy.

The subtlety and variation of the associated optic nerve head and retinal abnormalities, nevertheless, precludes the use of fundal examination by ophthalmoscopy as a marker of VAVFL field loss. Visual electrophysiology has identified markers of VAVFL field loss, particularly the 30Hz flicker electroretinogram (ERG) (Harding et al., 2000, Coupland et al., 2001); however, no one stand-alone ERG criterion possesses a clinically acceptable sensitivity and specificity.

Measurement of the retinal nerve fibre layer thickness, using either scanning laser ophthalmoscopy, (Viestenz et al., 2003, Wild et al., 2006) OCT (Choi and Kim, 2004) (Rebolleda et al., 2005) (Wild et al., 2006) or nerve fibre layer polarimetry (Viestenz et al., 2003, Durnian and Clearkin, 2007) shows considerable potential as a marker for VGB toxicity. However, such potential is based upon case histories (Viestenz et al.,

2003, Choi and Kim, 2004), retrospective case analysis (Rebolleda et al., 2005) or uncontrolled studies of small numbers of individuals with field loss (Durnian and Clearkin, 2007). Only one case-controlled prospective study has been undertaken (Wild et al., 2006).

The case-controlled prospective study from the Cardiff group (Wild et al., 2006) measured retinal nerve fibre layer thickness using OCT with the StratusOCT and the Proportional Circle Scan set at a scan radius corresponding to the vertical diameter of the individual optic nerve head. At 100% specificity, based upon the 95% confidence limits derived from 20 age-matched normal individuals, the mean of the retinal nerve fibre layer thickness over the circular scan yielded 100% sensitivity for 13 individuals with VAVFL visual field loss. However, 3 of 8 individuals exposed to VGB but with normal fields and 2 of 14 individuals receiving carbamazepine monotherapy (a non-GABAergic antiepileptic drug) and exhibiting normal fields also manifested mean retinal nerve fibre layer thicknesses outside the apparent normal range. The former raises the possibility of an earlier manifestation of vigabatrin toxicity than visual field loss, whilst the latter questions the validity of the confidence intervals.

The Proportional Circle Scan utilises a scan diameter based upon a function of the vertical diameter of the individual optic nerve head. This is in contrast to the more commonly used alternative; a fixed scan radius which does not account for variation in the optic nerve head size. The former has the advantage of overcoming the between-individual differences in the topographical variation of the normal nerve fibre layer thickness inherent with the use of a fixed scan radius and arising from between-individual variations in the size of the optic nerve head. However, only the fixed scan radius protocol benefits from the manufacturer's

standardised and substantive generic database of normal values contained within the instrument software and which is available to all users. Thus, there is a pressing need to validate the previous findings of retinal nerve fibre layer attenuation, obtained with the Proportionate Circle scan and the small proprietary database, (Wild et al., 2006) against the fixed scan radius and the corresponding generic database of normal values, as a marker of VGB toxicity, particularly nasally.

3.2.1 *Aims*

The purpose of the study, therefore, was two-fold. Firstly, to confirm the validity of retinal nerve fibre attenuation measured by OCT as a marker of VGB toxicity with particular reference to a standardised set of generic normal values (i.e. those of the 3.4 RNFL thickness protocol of the StratusOCT). Secondly, to determine whether retinal nerve fibre attenuation within the nasal quadrant is a more sensitive marker of VGB toxicity than that for the remaining quadrants.

3.2.2.2 *Methods*

The study utilised a cross-sectional, prospective, observational design.

3.2.2.1 Cohorts

The cohort comprised 3 groups of individuals with focal onset epilepsy. Group 1 comprised 11 individuals (4 males and 7 females; mean age 41.5 years, SD 11.1) exposed to VGB and who manifested VAVFL visual field loss. Group II comprised

16 individuals (3 males and 13 females; mean age 38.3 years, SD 16.1) exposed to VGB and who manifested normal visual fields. This latter group included 3 learning disabled adults and 3 adolescents aged 13, 13, and 15 years, respectively. Four individuals were receiving VGB at the time of the study, one in Group I and three in Group II. Four of the individuals in Group I and four in Group II had taken part in the previous study of retinal nerve fibre layer thickness using the Proportionate Circle scan (Wild et al., 2006). Group III comprised 13 individuals (4 males and 9 females; mean age 47.8 years SD 14.2) with no exposure to VGB or other GABA-ergic anti-epileptic drugs and who were currently being treated with one or more non-GABA-ergic anti-epileptic drugs, primarily carbamazepine. Twelve of the thirteen individuals in Group III were drawn from the corresponding group (Group IV) described by Wild et al (Wild et al., 2006). The individuals in Group III provided a match in terms of age and of gender to the VGB-exposed individuals as far as possible.

The eight individuals exposed to VGB and the 12 individuals exposed to non-GABAergic AEDs were selected so as to provide some indication as to the consistency in the RNFL thickness measured by the two differing scan protocols.

The adults were recruited from the Welsh Epilepsy Unit, University Hospital of Wales, Cardiff, and the adolescents from the Paediatric Neurology and Adolescent services at the University Hospital of Wales, Cardiff. All individuals conformed to rigid inclusion criteria in each eye including a distance refractive error of less than or

equal to 5 dioptres mean sphere and less than 2.5 dioptres cylinder; open angles, clear ocular media; no fundal or optic nerve head abnormalities characteristic of known disease other than VGB toxicity; no previous ocular surgery or trauma; no history of diabetes mellitus and no family history of glaucoma. All individuals exhibited a visual acuity of 6/9 or better in each eye.

The individuals exposed to VGB attended for two visits. Visual field examination of the right eye was undertaken at one visit and retinal nerve fibre layer imaging of the same eye at a second visit. The order of the perimetry and imaging visits was randomised between individuals. All individuals in the non-GABAergic epilepsy control group (Group III) exhibited normal fields and attended for retinal imaging of the right eye only.

3.2.2.2 Perimetry

Perimetry comprised two examinations: Three-Zone Age-Corrected suprathreshold perimetry undertaken with the Full Field 135 Screening Test followed by threshold perimetry undertaken with Program 30-2 and the FASTPAC strategy of the Humphrey Field Analyzer 750 (Carl Zeiss Meditec, Dublin, CA). Distance refraction corrected, where appropriate, for the viewing distance of the perimeter bowl was utilised for examination of the central field. No correction was utilised for examination of the peripheral field (i.e. that beyond 30° eccentricity). Individuals were given frequent rest periods, both throughout and between perimetric examinations, and occasionally required more than one visit to

provide a conclusive visual field outcome.

All 27 individuals exposed to VGB were able to undertake suprathreshold perimetry and all exhibited incorrect responses to each of the three types of catch trials within the normal range. Two adolescents could not perform threshold perimetry. For threshold perimetry, one individual, exposed to VGB but with normal fields, exhibited incorrect-responses to the fixation loss catch trials which were outside of the normal range; and two individuals, both with VGB-attributed visual field loss, exhibited incorrect-responses to the false-negative catch trials which were outside of the normal range, one of whom also exhibited an abnormal fixation loss rate.

3.2.2.3 Imaging

Retinal nerve fibre layer thickness was undertaken using OCT with the Stratus OCT (Carl Zeiss, Meditec, Dublin, CA) and the 3.4 RNFL thickness protocol. This approach undertakes 512 sequentially obtained A-scans in 1.3 seconds along a circle 3.4mm in diameter positioned at the centre of the optic nerve head. The contralateral eye was occluded and individuals viewed the internal fixation target. The z-offset and polarisation were obtained before each scan. Three scans were obtained and the mean of the three scans calculated by the instrument software. All scans exhibited a signal to noise ratio of greater than 25dB, and at least 90% good quality A-scans. The mean image was analysed by Stratus OCT software Version 3.0. Additionally a single representative image was analysed by Stratus OCT software Version 3.0 to produce a

RNFL Thickness Average Analysis Report.

3.2.2.4 Analysis

The visual fields for each individual were evaluated masked to the Group status and to the results for the retinal nerve fibre layer thickness by one of the supervisors of the thesis (JMW) who has 25 years of experience of interpreting the results of automated perimetry and 10 years of experience in interpreting VGB-attributed visual field loss. VAVFL was defined before the onset of the study as a bilateral, symmetric, 'concentric', steeply sided absolute defect in the peripheral field recorded by two-level, three-zone suprathreshold perimetry, consistent with the field loss in the central field recorded by threshold perimetry and with an appearance characteristic of that attributable to VGB. In mild to moderate cases, the field loss characteristic of VGB in the central field extends, to varying amounts, in an annulus above and below the horizontal midline at the nasal extremities of the central field and centripetally with varying amounts of sparing of the temporal field. In the most severe cases, the defect is completely concentric within the central field.

The retinal nerve fibre layer thickness for each individual was analysed in terms of the absolute values of thickness displayed in the RNFL Thickness Average Analysis Report – Version 3.0 (i.e. the average thickness of all 4 oblique quadrants and the thickness for each individual oblique quadrant) and in terms of the corresponding percentile ($\leq 1^{st}$, $\leq 5^{th}$ $\leq$100th percentile) of the result within the normal population. Descriptive statistics of the magnitudes of the retinal nerve fibre layer were used as

appropriate. The StratusOCT software does not contain a database for children although children are considered to exhibit comparable RNFL thicknesses with adults; the adolescents in this study were rated against normal values for an 18-year-old.

The study followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from each individual after explanation had been given of the nature and possible consequences of the study. In the case of the adolescents and the learning disabled adults, written informed consent was obtained from the parent or legal guardian, as appropriate. The study had approval from the local institutional review board.

3.2.2 Results

The summary measures (Group Mean, SD and Range) of the demographical characteristics for each of the three Groups are given in Table 3.2.1.

The summary measures (Group Mean, SD and Range) for each of the three Groups of the retinal nerve fibre layer thickness, as a function of quadrant, is given in Table 3.2.2.

The frequency, across individuals, of the magnitude of the percentile (≤1st, ≤5th,

$\leq 100^{th}$) of the measured value of the retinal nerve fibre layer thickness for each quadrant in each of the Three Groups is given in Table 3.2.3.

All 11 individuals with VAVFL visual field loss (Group I) exhibited an abnormally attenuated retinal nerve fibre layer (i.e. $\leq$1st or $\leq 5^{th}$ percentile) when averaged across the four quadrants. The 11 individuals also manifested an abnormal nerve fibre layer thickness in the nasal quadrant; seven also exhibited an abnormal thinning in the superior and/or inferior quadrants. Strikingly, all 11 individuals exhibited a normal retinal nerve fibre layer thickness for the temporal quadrant. An example of the nasal attenuation and temporal sparing, together with the appearance of the field loss in the central field is given in Figure 3.2.1.

Twelve of the 16 individuals in Group II (i.e. those exposed to VGB but with normal visual fields) exhibited a normal retinal nerve fibre thickness when averaged across the four quadrants and also for each individual quadrant. The remaining four individuals all exhibited abnormally attenuated average and nasal retinal nerve fibre layer thicknesses in the presence of a normal temporal nerve fibre layer. Two of these 4 individuals exhibited additional attenuation in the inferior and superior quadrants respectively.

Ten of the 13 individuals exposed to non-GABAergic anti-epileptic drugs exhibited a normal retinal nerve fibre thickness when averaged across the four quadrants and also

for each individual quadrant. However, three of the 13 individuals each exhibited a normal average thickness but an abnormal thickness in one of the superior, inferior, or temporal quadrants, respectively. The retinal nerve fibre layer thickness in the nasal quadrant was normal for all 13 individuals.

The cumulative dose of VGB exhibited a moderate correlation ($r = -0.65$, $p \leq 0.01$) when taken across a set of individuals with the retinal nerve fibre layer thickness in the nasal quadrant (Figure 3.2.2 and Table 3.2.4). The magnitude of the correlation is limited by the floor effect of the retinal nerve fibre thickness measurement at approximately 35–40μm which presumably arises from glial cell hypertrophy (Harwerth et al., 2007) replacing the nerve fibre layer and also from the finite thickness of the internal limiting membrane.

Group (Total)	Gender		Mean Age (yrs) (SD)	Mean Duration of Epilepsy (yrs) (SD)	Mean Duration of Vigabatrin (yrs) (SD)	Cumulative dose of Vigabatrin (kg) (SD)
	Male	Female				
I (11)	4	7	41.5 (11.1)	25.0 (6.0)	8.8 (2.3)	9.8 (2.7)
II (16)	3	13	38.3 (16.1)	20.1 (8.4)	7.6 (2.8)	6.8 (2.6)
III (13)	4	9	47.7 (14.2)	22.7 (11.4)	0	0

Table 3.2.1 The summary measures (Group Mean, standard deviation [SD]) of the demographical characteristics for each of the three Groups (Group I, individuals exhibiting VAVFL visual field loss; Group II, individuals exposed to VGB but with normal visual fields; Group III, individuals receiving non-GABA-ergic anti-epileptic drug therapy).

Retinal Nerve Fibre Layer Thickness (microns) by Quadrant (Mean, SD)					
Group (Total)	Average	Nasal	Superior	Temporal	Inferior
I (11)	**64.0 (7.64)**	**37.1 (4.81)**	**73.9 (15.4)**	**68.45(9.71)**	**76.2 (19.39)**
II (16)	**91.06 (11.92)**	**62.0 (14.47)**	**98.62 (21.63)**	**79.94 (16.11)**	**103.6 (17.00)**
III (13)	**93.23 (11.02)**	**74.8 (15.1)**	**107.0 (20.36)**	**71.38 (13.87)**	**117.85 (17.34)**

Table 3.2.2 The summary measures of the Retinal Nerve Fibre Layer thickness (Group Mean, SD) for each quadrant for each of the three Groups (Group I, individuals exhibiting VAVFL visual field loss; Group II, individuals exposed to VGB but with normal visual fields; Group III, individuals receiving non-GABA-ergic anti-epileptic drug therapy).

	Average RNFL			Nasal RNFL			Superior RNFL			Temporal RNFL			Inferior RNFL		
roup	N	<5 %	<1%	N	<5%	<1%	N	<5%	<1%	N	<5%	<1%	N	<5%	<1 %
=11)	0	3	8	0	3	8	4	5	2	11	0	0	3	5	3
=16)	12	4	0	12	4	0	15	1	0	16	0	0	15	1	0
I =13)	13	0	0	13	0	0	11	2	0	12	1	0	12	1	0

Table 3.2.3. The frequency, across individuals, of the magnitude of the percentile (≤1st, ≤5th) of the measured value of the Retinal Nerve Fibre Layer Thickness for each quadrant in each of the three groups. N indicates a normal value i.e. greater than the 5th percentile.

Group	Average		Nasal		Superior		Temporal		Inferior	
	r value	p value	r value	p value	r value	p value	r value	p value	r value	p value
I (n=11)	-0.216	0.262	-0.495	0.061	0.064	0.426	0.186	0.292	-0.464	0.075
II (n=16)	0.029	0.458	-0.120	0.331	0.318	0.115	-0.130	0.480	0.109	0.343

Table 3.2.4 The correlative values of the Retinal Nerve Fibre Layer Thickness versus cumulative dose for each quadrant in both of the VGB exposed groups.

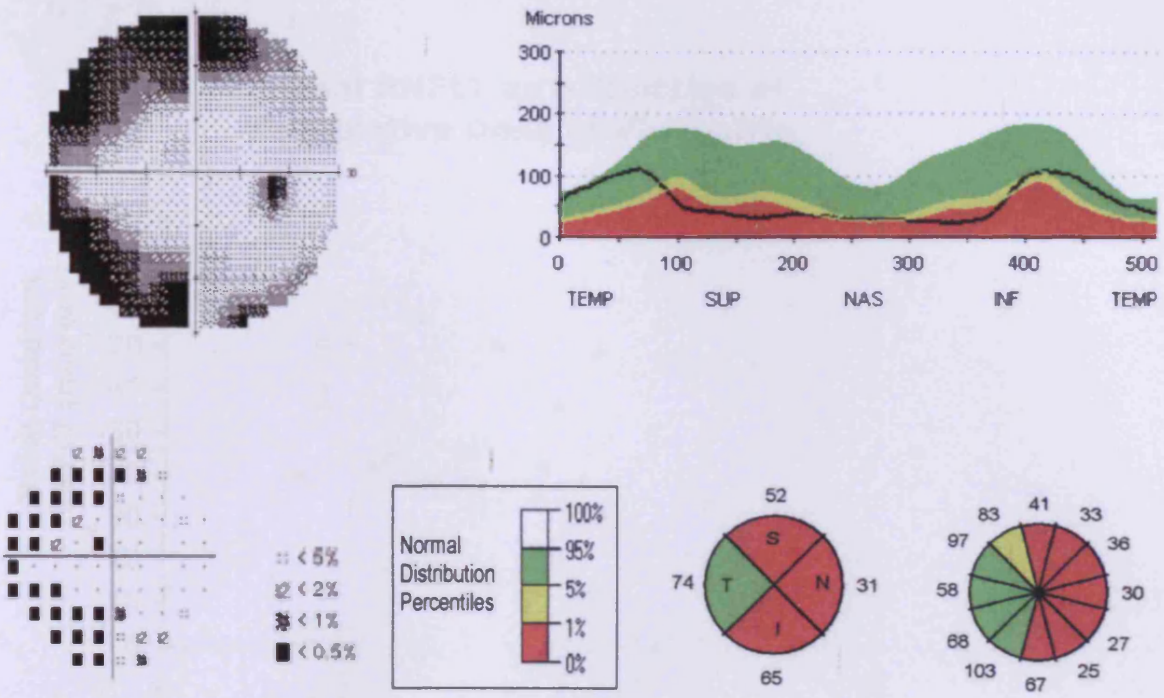


Figure 3.2.1. The grey scale (top left) and the Pattern Deviation probability map (bottom left) recorded for the right eye with Program 30-2 and the FASTPAC algorithm of the Humphrey Field Analyzer exhibiting VGB-attributed visual field loss and the corresponding retinal nerve fibre layer attenuation illustrated in terms of the height profile (top right) and the quadrant and sector distributions (bottom right) relative to the database of normal values.

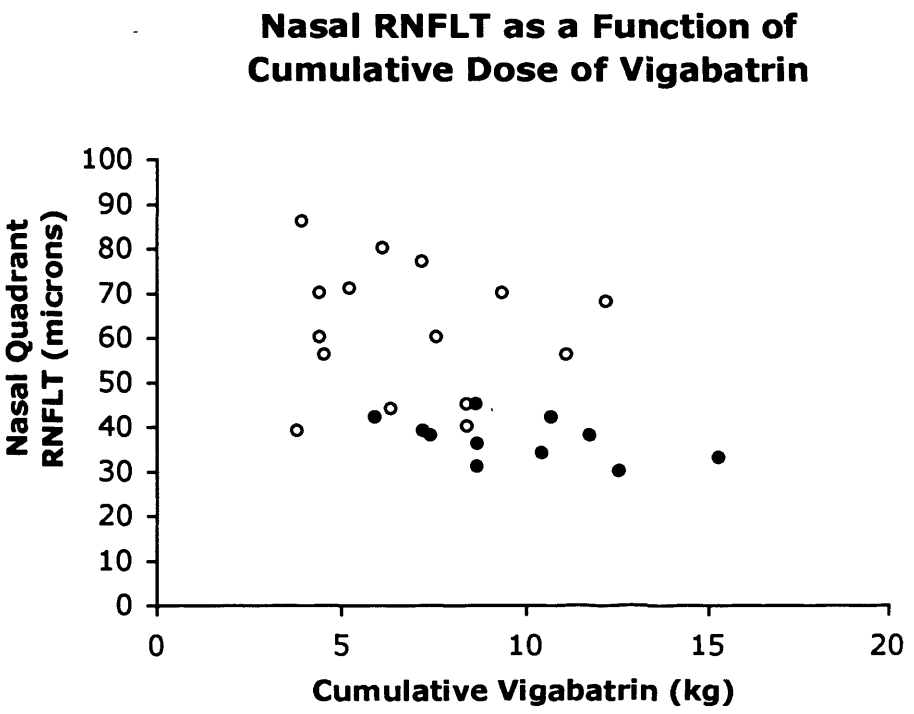


Figure 3.2.2. Nasal retinal nerve fibre layer thickness (μm) as a function of cumulative dose of VGB (kg) for the 16 patients exposed to VGB but with a normal visual field (open circles) and for the 11 patients manifesting VGB–attributed visual field loss.

3.2.4 Discussion

This study confirms that attenuation of the retinal nerve fibre layer thickness, relative to the manufacturer's generic database of normal values, is associated with VGB toxicity. Moreover, it shows that the toxicity is particularly associated with retinal nerve fibre layer thinning in the nasal quadrant and with preservation in the temporal quadrant.

The predilection for attenuation of the retinal nerve fibre layer in the nasal quadrant with temporal quadrant sparing is in agreement with a characteristic pattern of peripheral retinal nerve fibre layer atrophy, sparing of the central region, and corresponding secondary optic atrophy which has been found in some individuals with VGB toxicity and labelled, variously, as 'C-shaped' or 'temporal sparing atrophy' (Frisen and Malmgren, 2003) or 'inverse atrophy' (Buncic et al., 2004). Such a pattern of optic atrophy is distinct from that of acquired and congenital optic neuropathies. However, the inverse retinal nerve fibre atrophy of VGB toxicity is seemingly difficult to recognize using fundoscopy, alone (Buncic et al., 2004) and, when visible, probably indicates an advanced stage of atrophy. Recognition can also be further confounded by the presence of co-existing optic nerve hypoplasia in some patients treated with VGB. Temporal quadrant nerve fibre layer attenuation is likely to be present only when the field loss is concentric within the central field.

The attenuation of the nasal quadrant retinal nerve fibre layer and the preservation of

temporal quadrant nerve fibre layer is entirely compatible with the characteristics of the field loss attributable to VGB, i.e. a concentric constriction which in the mild to moderate stages exhibits a nasal predominance and a relative sparing of the temporal field. The retinotopic correspondence with the visual field is such that the retinal nerve fibres in the unaffected temporal optic nerve head quadrant originate from the papillomacular bundle and from the fovea. (Figure 3.2.3) The visual field in the region that corresponds to the temporal quadrant of the optic nerve head is typically unaffected by VGB and remains normal even in the most advanced stage of field loss. The fibres corresponding to those locations which exhibit nasal VGB-attributed visual field loss enter the optic nerve head immediately either side of the superior pole (but with a slightly greater preponderance superior-nasally) and nasally to the inferior pole (Garway-Heath et al., 2000). The attenuated nerve fibre layer in the nasal quadrant accounts for the temporal field loss. The mechanism of retinal toxicity is unknown, and the aetiological agent may be VGB itself, or the resulting elevated level of GABA within the retina, or a combination thereof. The presence and pattern of nerve fibre layer atrophy may represent either primary or secondary soma, or primary or secondary fibre, damage.

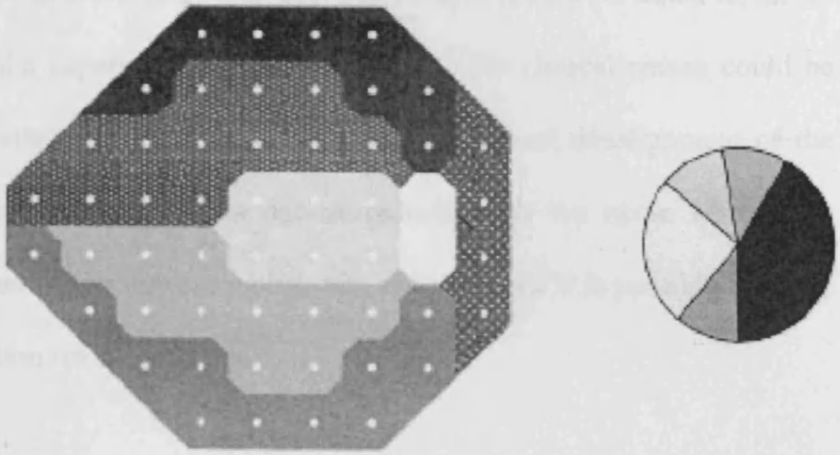


Figure 3.2.3 Association of regions of the visual field and sectors of the optic disc.

The retinotopic correspondence with the visual field derived by Program 24-2 (left- the visual field for a right eye, right- the optic nerve head for the right eye).

The predilection for the nasal quadrant nerve fibre layer thinning in VGB toxicity measured here by OCT is also compatible with that found by nerve fibre layer polarimetry (Durnian and Clearkin, 2007).

All 13 patients treated with non-GABA-ergic drugs manifested a normal nerve fibre layer averaged across the quadrants. Such a finding is in agreement with the presence of a normal retinal nerve fibre layer thickness in patients treated with the non-GABA-ergic anti-epileptic drug carbamazepine and with the mildly GABA-ergic drug sodium valproate (Lobefalo et al., 2006) (Wild et al., 2006). However, 3 of the 13

individuals manifested an abnormally thin nerve fibre layer (below, or equal to, the 5th percentile) in either the superior or inferior quadrants. No clinical reason could be found for this mild attenuation. However, inadvertent vertical misalignment of the patient/scan circle can result in an apparent reduction in the nerve fibre layer thickness in the region of the corresponding vertical pole and it is possible that this might be the explanation for the findings.

Re-analysis of the cohort examined by Catherine Robson with proportional circle OCT scans confirmed the same pattern of inverse atrophy, with nasal quadrant RNFL atrophy specific to VGB toxicity. Whilst there was good quantitative agreement between the results for each individual sector between the 3.4RNFL scan and the proportional circle scan, the latter technique designated apparent temporal RNFL attenuation was in 50% of the non-VGB-exposed epilepsy controls, and 40% of individuals with VAVFL. It is likely that such results emanate from inappropriate confidence intervals arising from the small sample size of 20 normal individuals. It should be noted that the normative database for the 3.4RNFL scan comprises approximately 450 individuals.

Three of the 16 individuals exposed to VGB but exhibiting normal visual fields exhibited abnormally attenuated average and nasal quadrant retinal nerve fibre layer thicknesses in the presence of a normal temporal quadrant thickness. This pattern of nerve fibre layer thinning is identical to that encountered in the individuals with VAVFL visual field loss and suggests that measurement of retinal nerve fibre layer

thickness, at least by OCT, is a more sensitive measure of VGB toxicity than perimetry. The finding is not surprising given that structural abnormality manifests before functional abnormality in, for example, open angle glaucoma (Caprioli et al., 2006) and multiple sclerosis (Sepulcre et al., 2007). The association between increased nasal quadrant nerve fibre layer attenuation and increasing cumulative VGB dosing adds further support to this hypothesis.

Based upon the various findings, it would seem that an attenuated retinal nerve fibre layer, measured by OCT, in at least the nasal quadrant combined with a normal nerve fibre layer in the temporal quadrant is a highly sensitive and specific indicator of vigabatrin-attributed visual field loss. Due to the small numbers of individuals exposed to VGB managed at any one centre, including the cohort utilised for this thesis, it is difficult to estimate accurately the magnitude of the sensitivity and specificity. More precise estimates of the magnitudes will only become available as a consequence of pooling experiences between centres.

In summary, the presence of nasal quadrant retinal nerve fibre layer attenuation determined by OCT using the 3.4 RNFL thickness protocol possesses clinically excellent sensitivity and specificity as a marker of VGB toxicity. The technique should be used as a baseline measure to augment perimetry in all patients prior to commencing VGB therapy either for epilepsy or for substance abuse (Fechtner et al., 2006). It should also be introduced into the examination routine of those patients already exposed to VGB and should become the technique of choice for learning

disabled adults. It should be used wherever possible in children; however, for optimum interpretation of the findings, in children normal values will be required for the generic database. The development of nasal retinal nerve fibre layer attenuation should be adopted a clinical indicator for withdrawal of VGB.

3.3. RNFL Thickness as Measured by Proportional Circle Scan

3.3.1 Aim

The RNFL data of Wild et al (Wild et al., 2006) derived by StratusOCT was reanalysed for the presence of an attenuated RNFL in the nasal optic nerve head quadrant as a specific indicator for VAVFL, and for the level of agreement of the RNFL thickness.

3.3.2 Methods

The raw data from the original study cohort was analysed with respect to individual quadrant RNFL thickness within an individual, within each of the groups. Quadrant attenuation was determined by 90% confidence intervals derived from the normal control cohort. Group I comprised 13 patients with epilepsy of varying aetiology previously, or at the time of the original study, exposed to VGB who manifested VAVFL field loss. Group II comprised 8 patients with epilepsy previously, or at the time of the original study, exposed to VGB who manifested normal visual fields. Two additional patients exposed to VGB exhibited equivocal visual fields and therefore

could not be categorised into either Group I or II. Group III comprised 14 patients with epilepsy who had never been exposed to VGB and who, at the time of the original study, were receiving carbamazepine monotherapy. Group IV comprised 20 clinically normal individuals who did not have epilepsy and who had not previously been exposed to anti-epileptic drugs. A further group of patients (Group V) patients had received valproate monotherapy and served as a post hoc control

All patients had been recruited from the Welsh Epilepsy Unit, University Hospital of Wales, Cardiff and the normal individuals from the University Hospital of Wales and from the Eye Clinic, Cardiff School of Optometry and Vision Sciences, Cardiff University. The participants had been matched as closely as possible in age within- and between- the respective groups. As far as possible, patients had been matched for age at onset, and duration, of epilepsy.

All participants had previously undergone ocular examination and conformed in each eye to rigid inclusion criteria including a distance refractive error less than or equal to 5 dioptres mean sphere and less than 2.5 dioptres cylinder; open angles, clear ocular media; no optic nerve head or fundal abnormalities characteristic of known disease; no previous ocular surgery or trauma; no history of diabetes mellitus and no family history of glaucoma. All participants had manifested a visual acuity of 20/30 or better in each eye and an intraocular pressure of 21mmHg or less.

The participants had attended for two further visits. At one visit, they had undergone visual field examination of the right eye; at the other visit they had undergone retinal imaging in the same eye. The order of the imaging and perimetry visits had been randomised between individuals.

Perimetry methodology was performed as detailed above.

3.3.3.1 Imaging

Each participant had firstly undergone the Fast Optic Disc scan, centred upon the optic disc, from which the vertical diameter of the optic nerve head had been obtained. The participants had undergone three separate 360° circular scans, centred upon the optic disc, using the Proportional Circle Scan incorporating a scan radius which corresponded to the vertical diameter of the optic nerve head, thereby accounting for between-subject differences in the size of the optic nerve head.

The contralateral eye had been occluded and participants had fixated on the internal fixation target. The z-offset and polarisation had been optimised before each Proportionate Circle and Macular Thickness scan had been acquired. All scans had exhibited the requirements of a signal to noise ratio greater than 25dB and at least 90% good quality A-scans. The right eye of each participant had been dilated with 0.5% tropicamide prior to imaging in order to ensure a minimum pupil diameter of 5mm.

3.3.3 Agreement Between RNFL Thickness Derived By The Fixed Radius Scan And Proportional Circle Protocols.

Eight VGB-exposed individuals, and twelve individuals from Group 3 were common to both the current and the original study and had, therefore, undergone both modalities of OCT optic nerve head imaging. These 20 individuals were utilised to investigate agreement between the RNFL thicknesses derived by the two scan protocols.

3.3.3.2 Analysis

The RNFL quadrant thicknesses for individual participants in each group of the original study were compared against the lower 90 % confidence limit calculated from the individuals in Group IV (the normal controls) with particular emphasis on the normality of the nasal optic nerve head quadrant thickness. The level of agreement between the two scan protocols for the 20 individuals common to both studies was illustrated graphically (Bland and Altman, 1986) for both the average thickness (of all four quadrants) and for the thickness of each individual quadrant.

3.3.4 Results

The summary measures (Group Mean, SD and Range) of the demographical characteristics for each of the five Groups are given in Table 3.3.1.

The summary measures (Group Mean, SD and Range) for each of the five Groups of

the retinal nerve fibre layer thickness, as a function of quadrant, are given in Table 3.3.2.

The frequency, across individuals, of the measured RNFL thickness lying beyond the lower 90% confidence interval for each quadrant in each of the five Groups is given in Table 3.3.3.

The outcome of the proportional circle scan is tabulated below for both the average of all four quadrants, (Table 3.4.1) and for each individual quadrant. The individual quadrant data is also displayed in boxplots for each of the individual quadrants, and compared with the lower limit for 95% confidence intervals for the individual quadrant.

All 13 individuals with VAVFL (Group I) demonstrated an attenuated nasal quadrant RNFL, and 6 of 10 VGB-exposed individuals manifesting normal fields also demonstrated an attenuated RNFL thickness. However, 7 of 20 individuals with epilepsy (Group II) also demonstrated an attenuated nasal quadrant RNFL thickness based on the same criterion, compared with 7 of 22 normal control individuals.

The superior and inferior quadrants were also more attenuated in the VAVFL group compared with the other groups. For the superior quadrant, 11 of 13 individuals with

VAVFL demonstrated superior quadrant thickness below the lower 95% confidence limit. This compares with, attenuated superior quadrant rates of 6 of 11 in group II, 5 of 20 in group III, and 5 of 22 in group IV.

Inferior quadrant attenuation occurred in 11 of 13 participants with VAVFL, compared with 6 of 10 individuals in Group II, although 12 of 20 individuals in Group III also demonstrated an attenuated RNFL value, as did 6 of 22 individuals in Group IV.

Strikingly, however, temporal quadrant attenuation was evident in only 6 of 13 individuals with VAVFL, compared with 3 of 11 in group II, and a high rate of attenuation in the internal epilepsy group, with 10 of 20 demonstrating attenuation, and a background rate of 6 of 22 in group IV, the normal controls. Temporal quadrant attenuation therefore, was no more likely in VAVFL than in the other groups.

When the results from the Proportional Circle scan were re-analysed, one of the two individuals exposed to VGB who were unable to produce conclusive visual field results, demonstrated nasal RNFL attenuation, only, and one demonstrated a normal RNFL throughout each of the four quadrants.

Table 3.3.2 Summary measures for RNFLT for average and quadrant values as measured by proportionate circle scan for all groups.

Retinal Nerve Fibre Layer Thickness (microns) of Average and all Quadrants Mean(SD)					
Group (Total)	Average	Nasal	Superior	Temporal	Inferior
I (13)	65.6 (11.95)	41.61 (9.98)	78.46 (22.28)	61.54 (8.99)	76.15(18.23)
II (10)	94.4 (16.88)	68.4 (25.80)	109.1 (22.72)	76.1 (13.95)	118.6 (19.86)
III (20)	100.1 (14.50)	80.05 (14.42)	121.0 (25.60)	71.85 (17.84)	125.1 (19.73)
IV (22)	111.1 (11.10)	89.77 (18.70)	128.86 (16.80)	82.5 (17.25)	139.81 (20.71)

3.3.4.1 Agreement between Proportional Circle and Fixed Radius Scan RNFL Data

Mean differences between the two methods were lowest for the average of all four quadrants, and highest for the superior and inferior quadrants (Table 3.5.1). Bland Altman plots, with 2 standard deviation limits inserted, demonstrate an acceptable agreement between the two methodologies. This held true across the groups, and was not affected by wide variation in measured values. Only one individual in each of the temporal and superior quadrants fell outside the 2SD limits – this individual was not common to both.

Group (Number of individuals)	Mean difference Average RNFL (SD)	Mean difference Nasal RNFL (SD)	Mean difference Superior RNFL (SD)	Mean difference Temporal RNFL (SD)	Mean difference Inferior RNFL (SD)
VAVFL (4)	-6.25 (6.18)	-2.50 (10.97)	-5.75 (7.93)	-7.75 (10.60)	-15.25 (10.53)
VGB – Exposed (4)	2.25 (17.3)	6.25 (21.75)	12.75 (42.75)	-3.75 (10.40)	10.75 (23.78)
Epilepsy Controls (12)	7.9 (9.65)	8.67 (18.10)	14.50 (21.47)	0.08 (14.46)	8.17 (14.49)
Total (20)	3.95 (11.83)	5.95 (17.41)	10.10 (25.14)	-2.25 (12.89)	4 (18.10

Table 3.3.5.1 Difference between Proportional Circle and 3.4RNFL thickness values for average and individual quadrants.

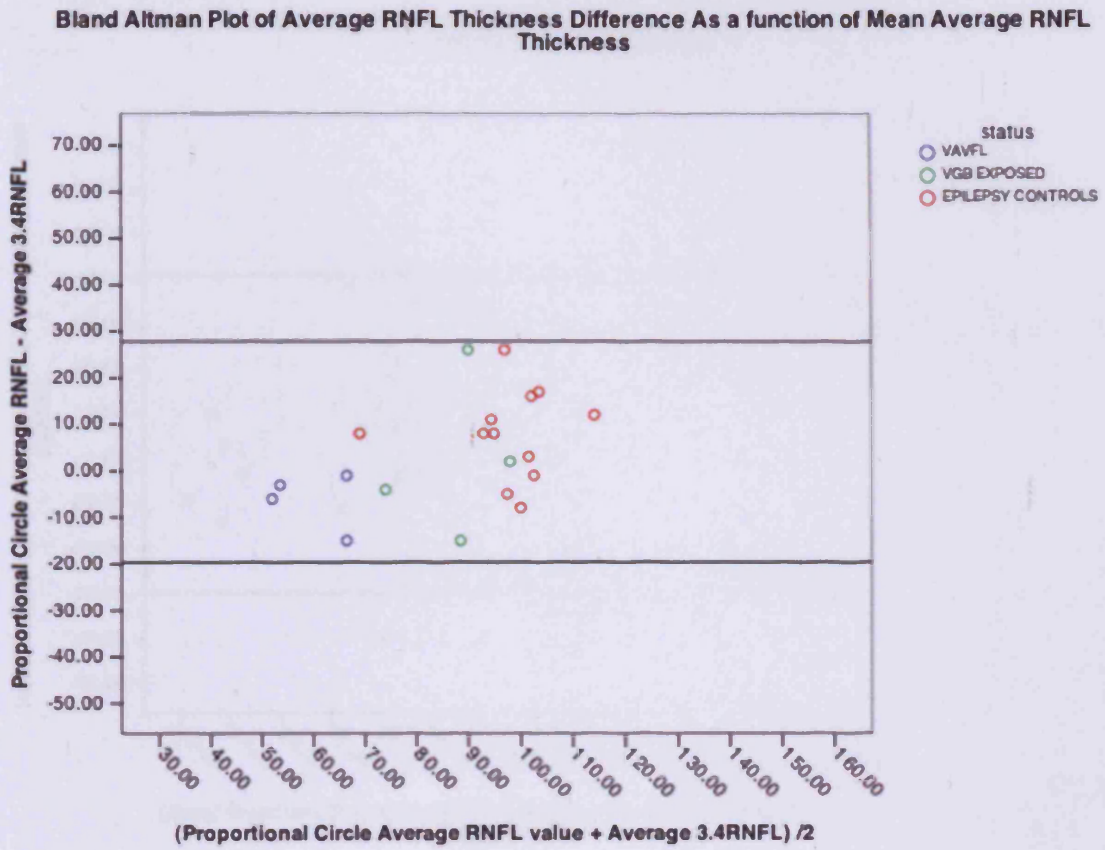


Figure 3.3.5.1 Bland Altman Plot of Average RNFL Thickness.

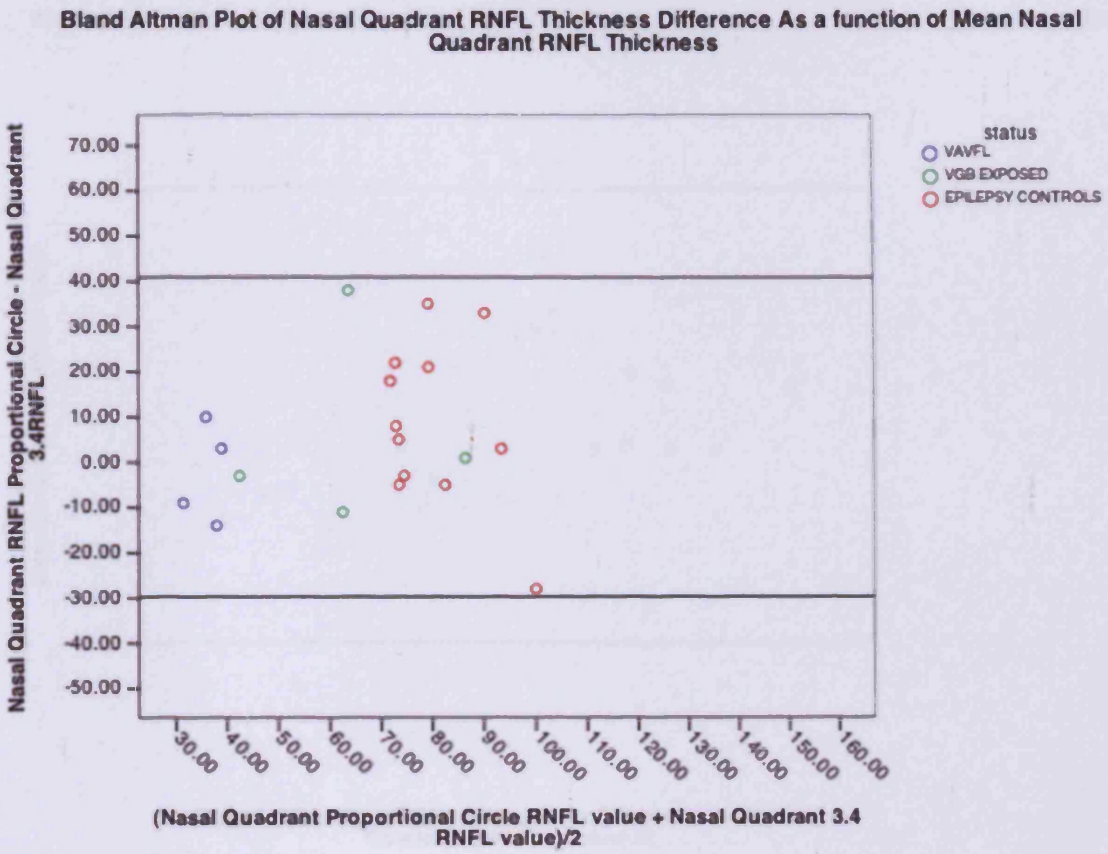


Figure 3.3.5.2 Bland Altman Plot of Nasal Quadrant RNFL Thickness

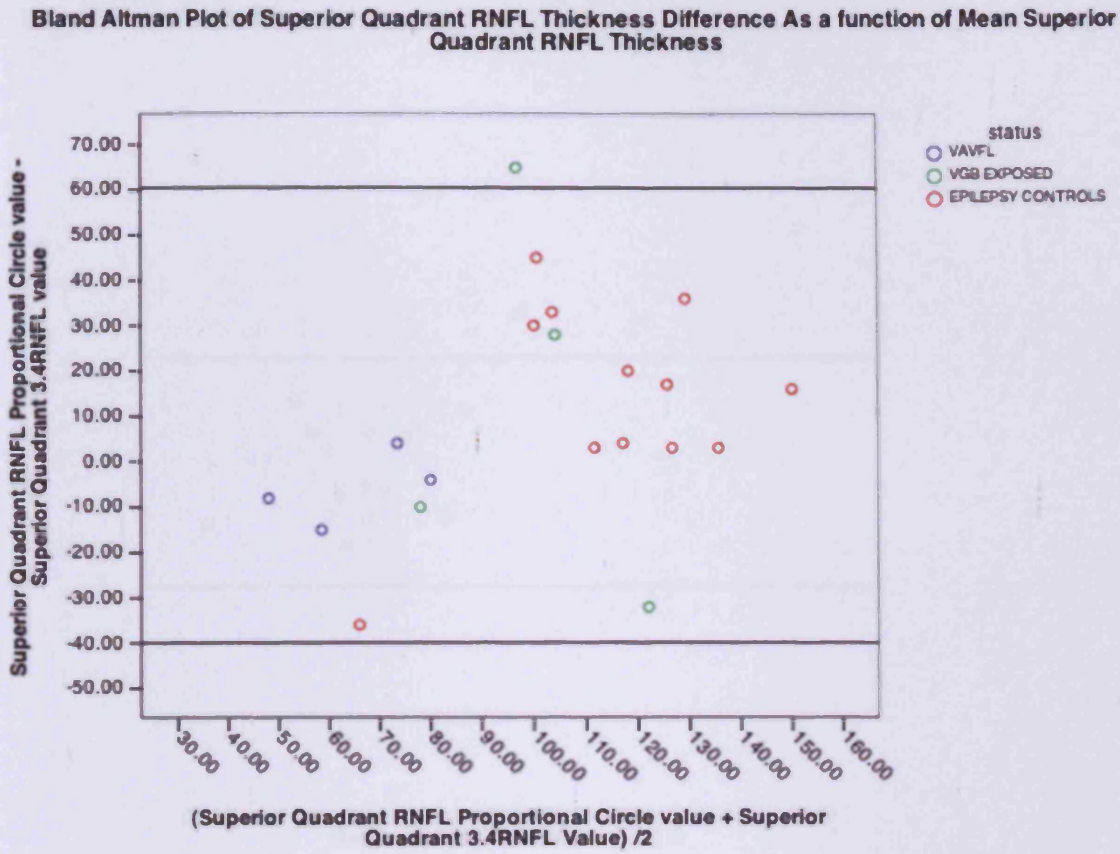


Figure 3.3.5.3 Bland Altman Plot of Superior Quadrant RNFL Thickness

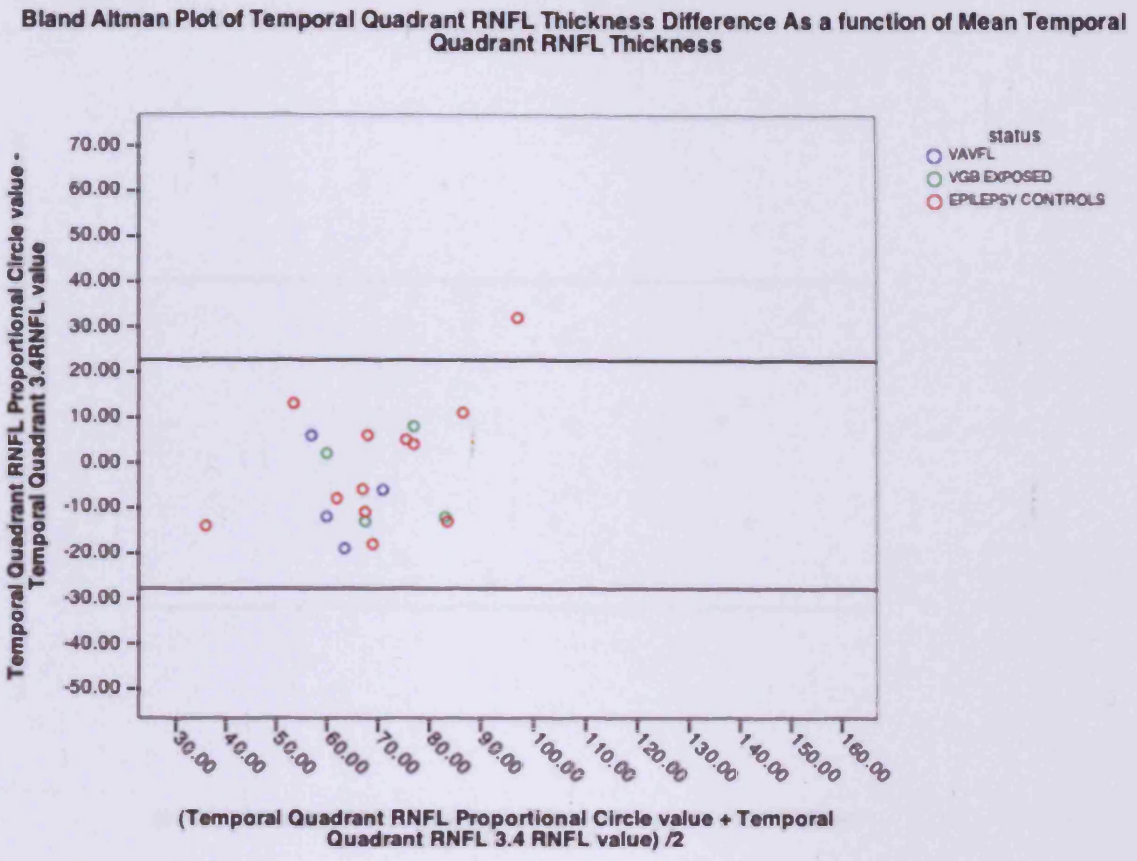


Figure 3.3.5.4 Bland Altman Plot of Temporal Quadrant RNFL Thickness

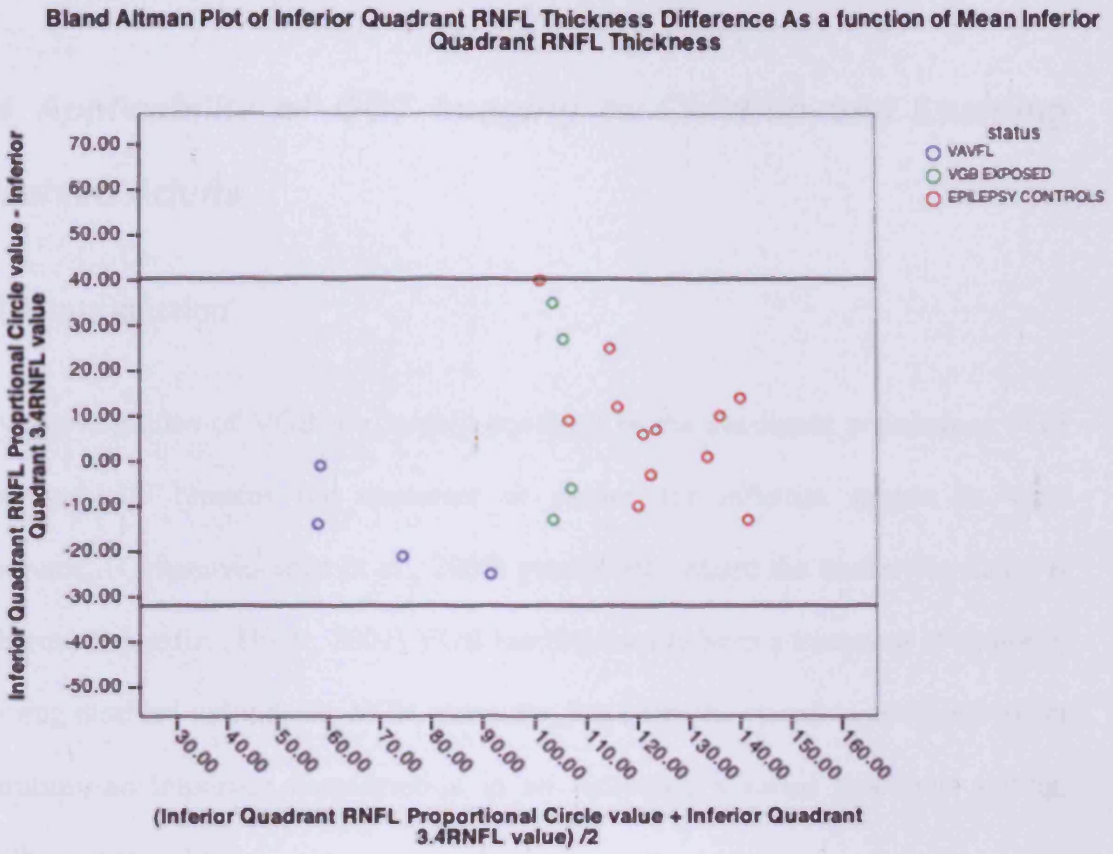


Figure 3.3.5.5 Bland Altman Plot of Inferior Quadrant RNFL Thickness

3.4 Applicability of OCT Imaging to Children and Learning Disabled Adults

3.4.1 Introduction

New prescriptions of VGB are largely confined to the paediatric population; VGB controversially remains the treatment of choice for infantile spasms in West syndrome, (Cvitanovic-Sojat et al., 2005) particularly where the underlying cause is Tuberous Sclerosis. (Thiele, 2004) VGB has historically been a treatment of choice in learning disabled individuals. VGB, uniquely, has been shown not to adversely affect cognition, an important consideration in an individual's social functional setting. (Gillham et al., 1993)

Perimetry requires a developmental age of approximately 9 years, thereby excluding many children and approximately 20% of all adults with epilepsy. We have shown that RNFL thickness is a reliable marker of VGB toxicity in competent adults. If RFNL thickness were to be adopted in these populations, it requires to be shown to both applicable and valid in these populations.

3.4.2 Aims

The aims of this study were two-fold; firstly to investigate whether OCT is applicable

in children and learning disabled adults, and secondly, to investigate whether OCT identifies VAVFL in these populations.

3.4.3 Methods

The original study cohort described above (see 3.2.2) included 5 learning disabled adults, and 3 children, who were capable of undergoing perimetry and underwent, in a random order: Humphrey Field Analyzer (HFA) Three Zone 135 Point Screening Field; HFA Program 30-2 with the FASTPAC strategy; and standard 3.4 RNFL estimation at the optic nerve head using the Stratus OCT III. 3 of the children and 5 of the adults were not able to undertake the FASTPAC strategy. A further 2 VGB-exposed children and a further learning disabled adult were not able to comply with perimetry and underwent OCT only. Four more children had been exposed to VGB in utero, (see chapter 4.1) and also underwent the same provided 135 screening fields and OCT. A further 9 healthy children (aged 3 to 16 years) underwent OCT, only, as normal controls. Thus, the cohort totaled 9 children and 6 learning disabled adults (Table 3.4.3).

3.4.4 Results

3.4.4.1Completion Rates

OCT was successfully performed in all 18 children and 5 of 6 LDAs. The sixth individual exhibited congenital nystagmus and bilateral congenital VIth nerve palsies,

with VAVFL. Consequently, he was unable to locate the internal fixation target or to fixate for the required acquisition time.

3.4.4.2 Mean RNFL Thickness

Of the applicability cohort, only 8 VGB-exposed individuals had completed perimetry. Of these, two had VAVFL as identified by perimetry. An attenuated RNFL (<95% CI, as defined by STRATUS III normative database) identified the two cases of VAVFL identified by perimetry. Of the 6 remaining VGB-exposed individuals with perimetry, all showed normal mean RNFL thickness.

3.4.4.3 Nasal RNFL Thickness

Nasal RNFL thickness was attenuated in both cases of VAVFL. A further two VGB-exposed individuals manifesting normal visual fields showed an attenuated nasal quadrant RNFL thickness.

3.4.4.4 Individuals Unable to Cooperate with Perimetry

Of the three VGB-exposed individuals unable to cooperate with perimetry, one demonstrated attenuated retinal nerve fibre layer thickness in both nasal quadrant and mean average values. The remaining two showed normal RNFL thickness.

3.5 Retinal Nerve Fibre Layer Thickness in Healthy Children

3.5.1 Introduction

Retinal Nerve Fibre Layer Thickness (RNFLT) in children is considered to equal that of adults. In support of this theory, RNFLT as measured by Scanning Laser Polarimetry, in 143 children indeed equals that of adults, (Lundvall Nilsson, 2007). However, the in-built normative database for StratusOCT does not extend below the age of 18 years, and as OCT relies on different methodology for estimating RNFLT, may yield different results in children.

3.5.2 Aims

To determine whether children have comparable mean and quadrant RNFLT values, as estimated by OCT as adults.

3.5.3 Methods

Nine healthy children were recruited (1 male, 8 females) ranging in age from 3 years to 16 years. All individuals conformed to rigid inclusion criteria in each eye including a distance refractive error of less than or equal to 5 dioptres mean sphere and less than 2.5 dioptres cylinder; open angles, clear ocular media; no fundal or optic nerve head abnormalities characteristic of known disease; no previous ocular surgery or trauma; no history of diabetes mellitus and no family history of glaucoma. All individuals

exhibited a visual acuity of 6/9 or better in each eye.

Retinal nerve fibre layer thickness was undertaken using OCT with the Stratus OCT (Carl Zeiss, Meditec, Dublin, CA) and the 3.4 RNFL thickness protocol. This approach undertakes 512 sequentially obtained A-scans in 1.3 seconds along a circle 3.4mm in diameter positioned at the centre of the optic nerve head. The contralateral eye was occluded and individuals viewed the internal fixation target. The z-offset and polarisation were obtained before each scan. An attempt was made to measure RNFLT in both eyes, but only the right eye was imaged for the two youngest children (aged 3 years and 4 years respectively). The mean image was analysed by Stratus OCT software Version 3.0.

3.5.3.1 Analysis

The retinal nerve fibre layer thickness for each individual was analysed in terms of the average values thickness of all 4 oblique quadrants (average RNFLT) and the thickness for each individual oblique quadrant. In all but one case, three measurements were taken, and the mean of those three were used for comparative analysis. In the case of one individual (aged 3 years) only two records were obtained, and again the mean value was used for analysis. The two measures showed good agreement.

3.5.4 Results

Mean and standard deviation values were derived for average and quadrant RNFLT value (Table 3.5.1). Whilst mean values for both average and quadrant fell within normal young adult values, quadrant values varied more than mean average, particularly temporal and inferior values.

Values plotted against age were prepared for the average RNFLT and also for the quadrant values. Temporal RNFLT values correlated negatively with increasing age (Pearson correlation = -0.792, $p \leq 0.05$), whilst inferior quadrant RNFLT values showed a positive correlation (Pearson correlation = 0.97, $p \leq 0.01$).

It was apparent from analysis of the raw data values that the under 6-year-old individuals form an outlying group in the quadrant values. The characteristics of the regression slopes are tabulated in Table 3.5.2 for all the control individuals both with and without the under 6-year-olds. The regression analyses as a function of age for the over 6-year-olds are displayed graphically (Figures 3.5.1-3.5.5.).

Age (years)	Total number	Average RNFLT mean value (u) (SD) [Confidence Interval]	Nasal RNFLT mean value (u) (SD) [Confidence Interval]	Superior RNFLT mean value (u) (SD) [Confidence Interval]	Temporal RNFLT mean value (u) (SD) [Confidence Interval]	Inferior RNFLT mean value (u) (SD) [Confidence Interval]
3	1	125	145	121	157	72
4	1	109	100	170	96	64
5	1	100	78	144	111	64
6	1	112	84	148	136	76
12	2	114.5 (7.78)	67 (2.83)	152.5 (7.78)	93 (16.97)	132 (5.66)
15	2	105 (7.07)	82 (1.41)	120.5 (17.67)	83.5 (9.19)	143.5 (16.26)
16	1	113	104	136	64	150
Total	9	110.9 (8.16) [104.62 – 117.17]	89.89 (24.23) [71.23 – 108.51]	140.56 (18.71) [126.18 – 154.95]	101.89 (29.50) [79.21 – 154.95]	108.56 (38.6) [78.89 – 138.23]

Table 3.5.1 Table detailing the average and quadrant RNFLT values categorized by age.

	Cohort Inclusive Of <6 Years Of Age		Cohort Exclusive Of <6 Years Of Age	
	Regression Coefficient	R Square	Regression Coefficient	R Square
Average	-.64	.31	-.56	.32
Nasal Quadrant	-.39	.15	-.15	.22
Superior Quadrant	-.68	.50	-.59	.34
Temporal Quadrant	-.50	.27	-.13	.18
Inferior Quadrant	-.17	.03	-.64	.40

Table 3.5.2 Regression coefficient and R Square values for the normal control cohorts inclusive and exclusive of the <6 year-olds respectively

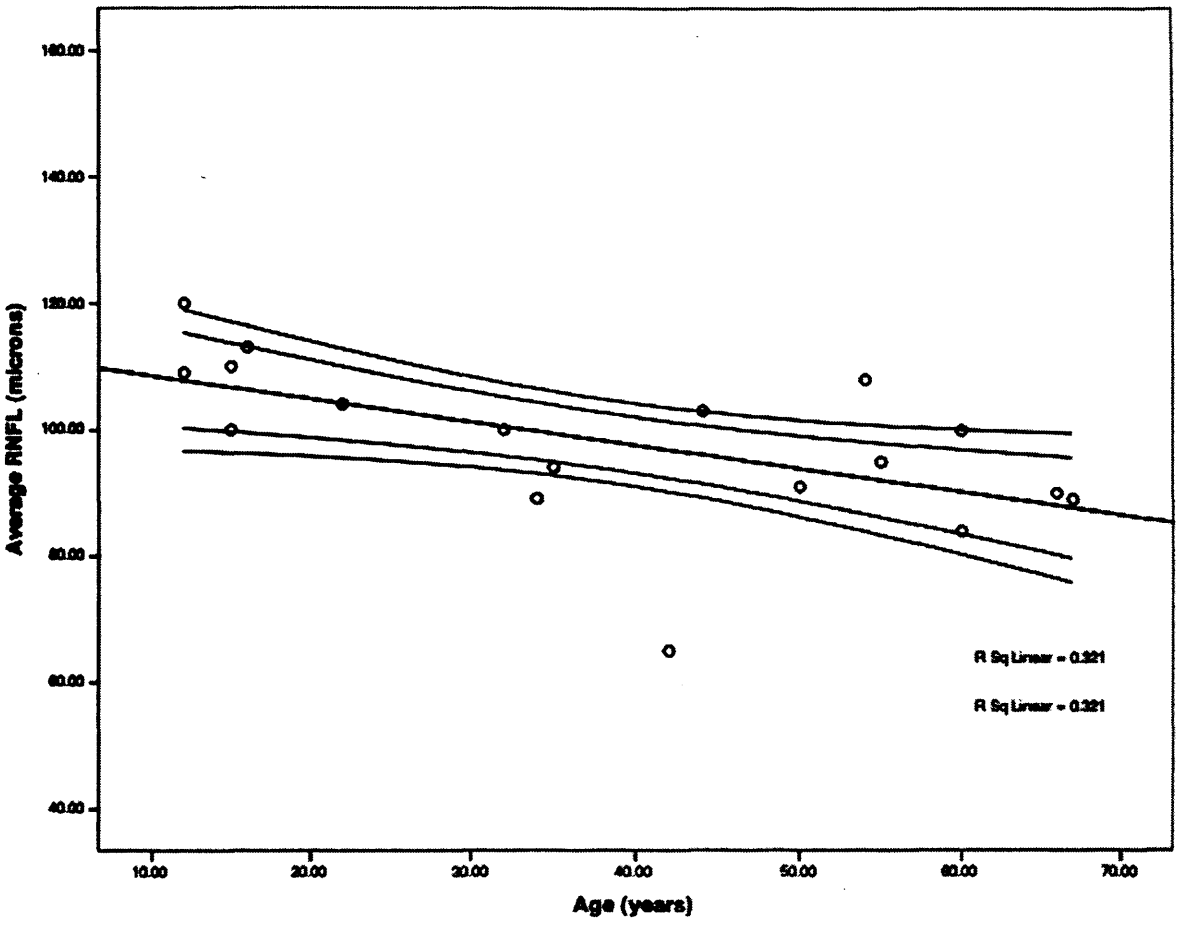


Figure 3.5.1 Average RNFL plotted against age for the normal cohort over the age of 6 years. Confidence Intervals are displayed for the 95th and 90th percentile.

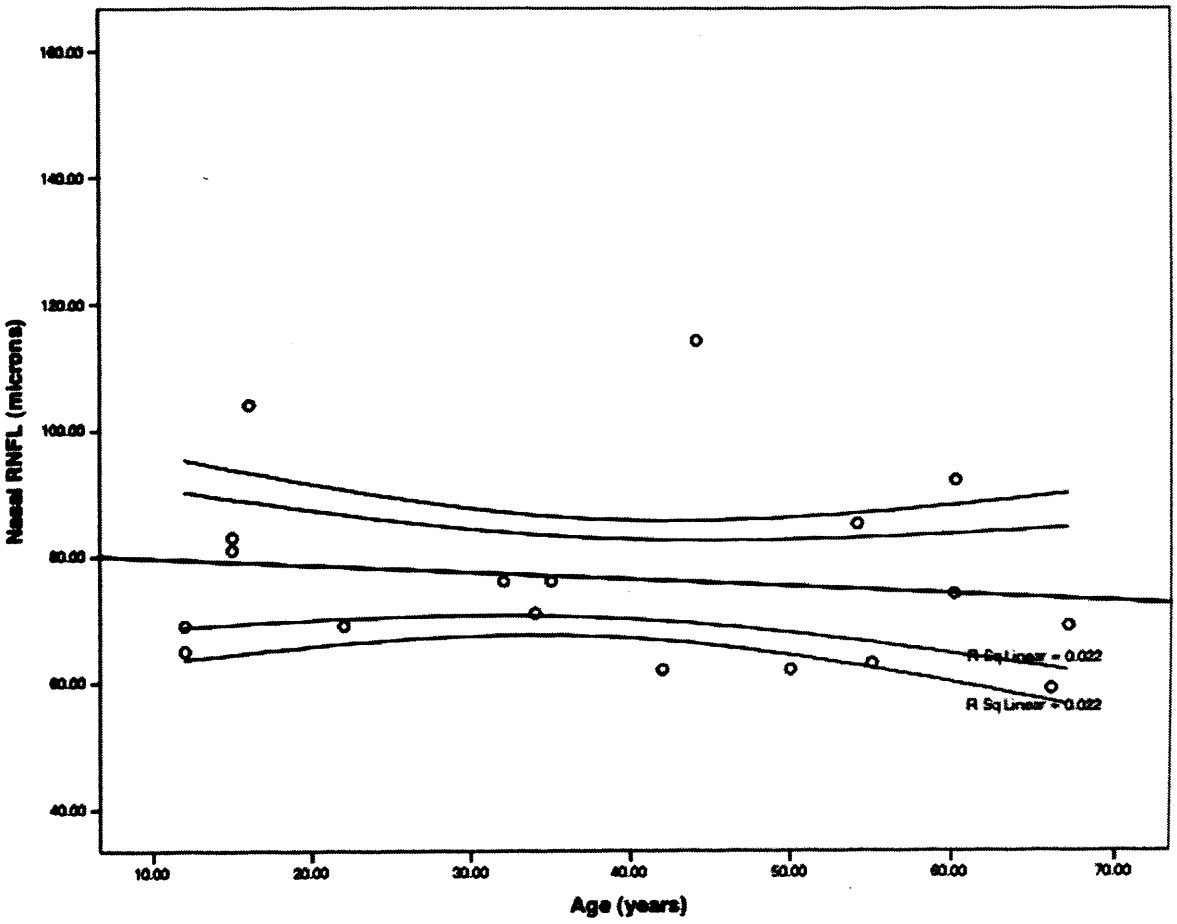


Figure 3.5.2 Nasal quadrant RNFL plotted against age for the normal cohort over the age of 6 years. Confidence Intervals are displayed for the 95th and 90th percentile.

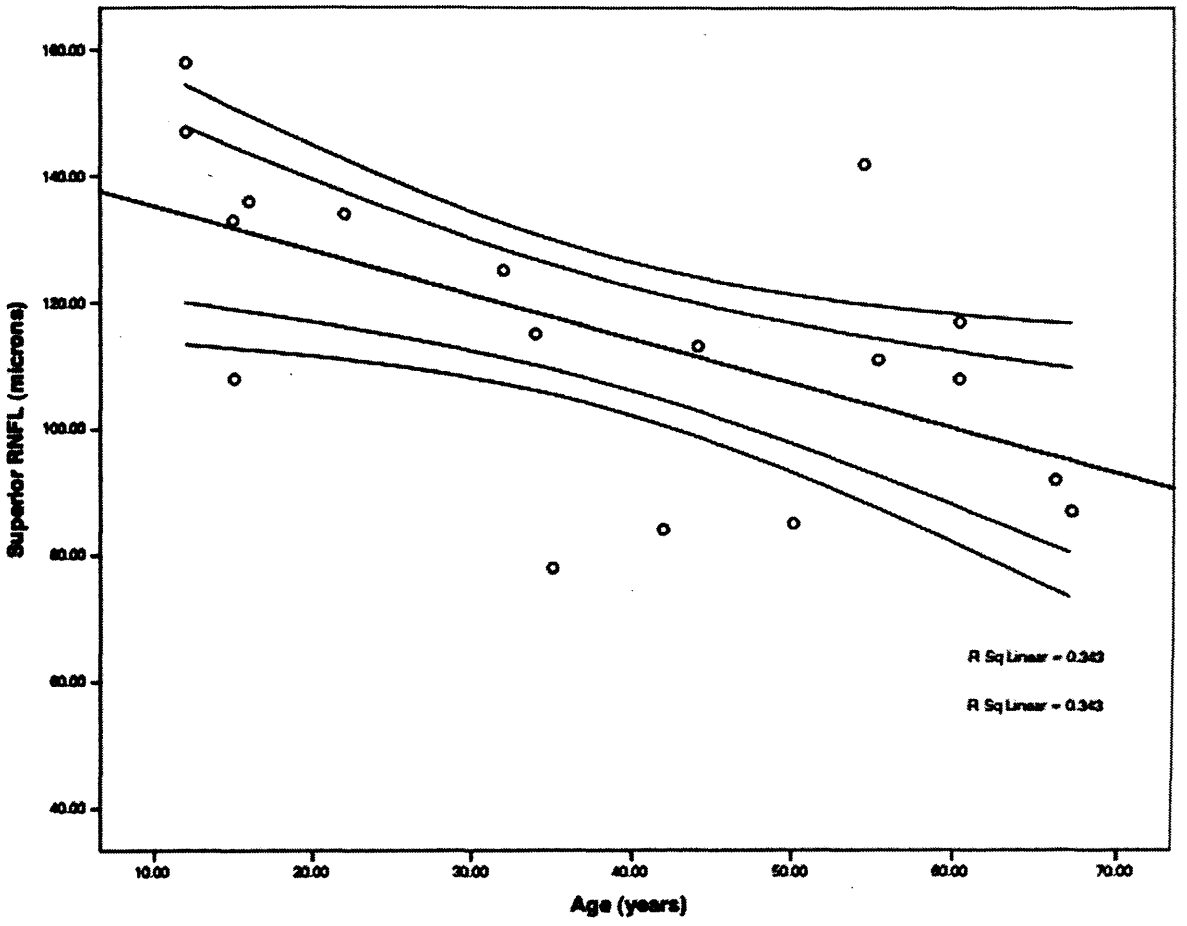


Figure 3.5.3 Superior quadrant RNFL plotted against age for the normal cohort over the age of 6 years. Confidence Intervals are displayed for the 95th and 90th percentile

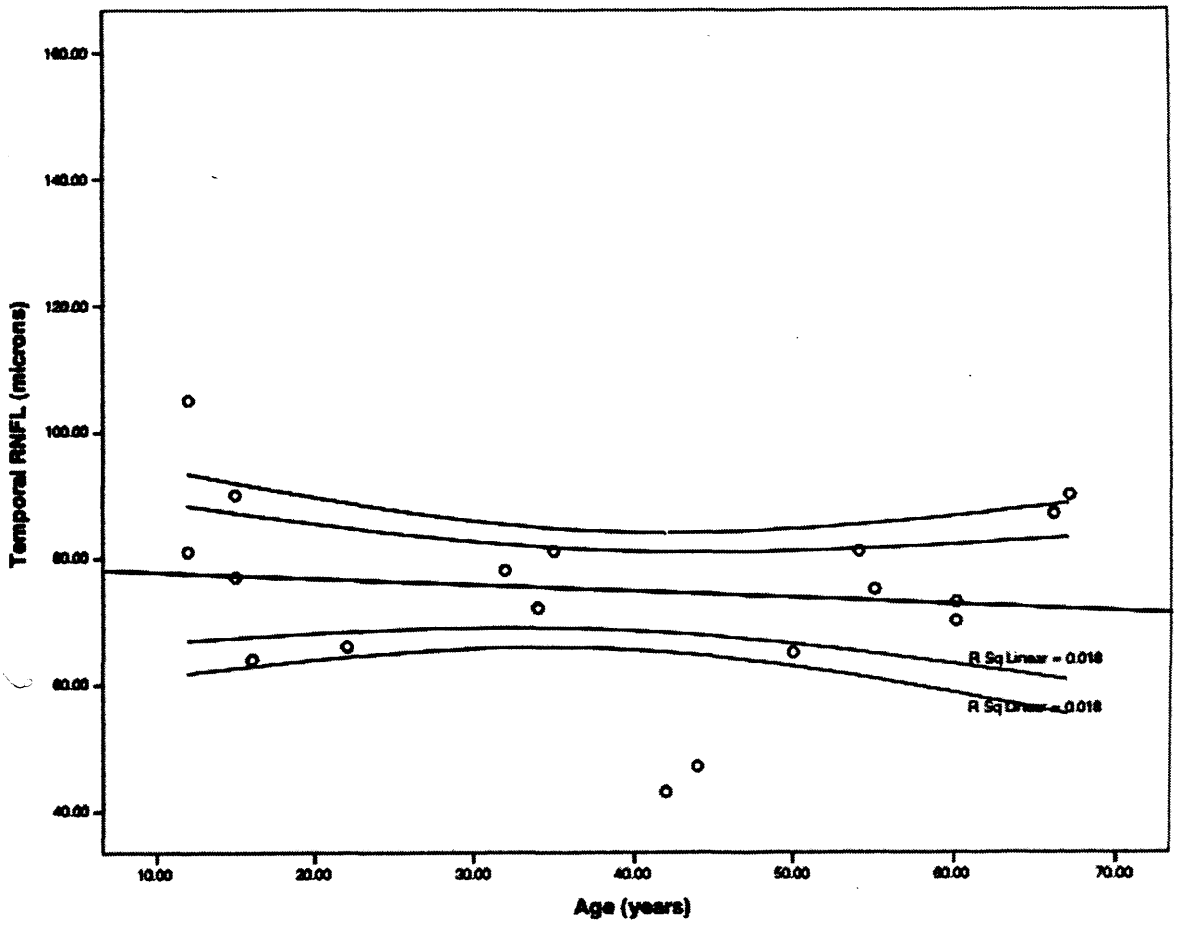


Figure 3.5.4 Temporal quadrant RNFL plotted against age for the normal cohort over the age of 6 years. Confidence Intervals are displayed for the 95th and 90th percentile

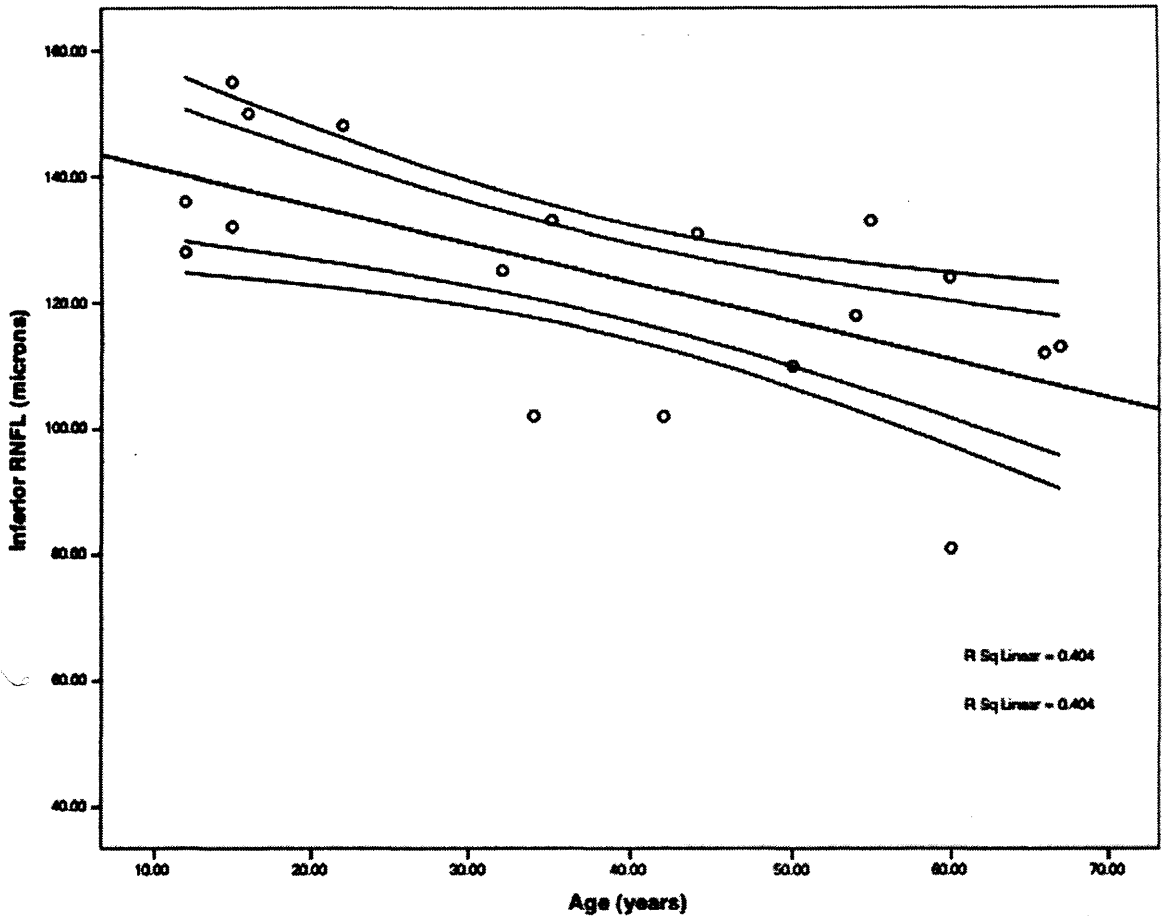


Figure 3.5.5 Inferior quadrant RNFL plotted against age for the normal cohort over the age of 6 years. Confidence Intervals are displayed for the 95th and 90th percentile

3.6 Discussion

3.6.2 RNFLT: Applicability to Children and Learning Disabled Adults

OCT is a sensitive and applicable tool for identifying VAVFL in children and LDAs. All 18 children provided good quality images on OCT. As in adults, an attenuated nasal quadrant RNFL was associated with VAVFL. An attenuated nasal quadrant RNFL thickness was present in one of three individuals unable to cooperate with perimetry.

3.6.3 RNFLT: Relatedness of Adult Normative Data with reference to Healthy Children

The mean RNFLT values for both average and quadrant values generated by each of the healthy children fell within the 95% confidence interval for 18 year-olds as determined by the StratusOCT generic normative database. However, for optimum interpretation of OCT in children an age-corrected normative database will be required.

The generic database for the Stratus OCT is age-matched allowing for an attenuation in RNFL with increasing age. Unsurprisingly, regression analyses for this data also shows the expected decline in RNFL in both mean and quadrant values.

Adult data from a large Asian cohort also displays the same trend, though notably they reported a slower rate of decline, but with much wider minima and maxima

values and hence, much larger confidence intervals. (Parikh et al., 2007) A larger cohort in the data presented in this thesis would, of course have likely yielded tighter correlations.

The likely explanation for the outlying values produced by the under 6-year-olds lies in pragmatic technical sizing difficulties with the OCT machine relative to both the head rest and the table, resulting in axis distortion. This, coupled with proportionately worse variation in inferior and superior quadrant values in the case of inaccurate fixation, (Harwerth et al., 2007) probably explain the positive correlation seen with inferior quadrant values, and increasing age. There is no theoretical reason to suspect any limitations in measuring infant data with the OCT technology.

3.7 Conclusions

In summary, the presence of nasal quadrant retinal nerve fibre layer attenuation determined by OCT using the 3.4 RNFL thickness protocol possesses clinically excellent sensitivity and specificity as a marker of VGB toxicity. The technique should be used as a baseline measure to augment perimetry in all patients prior to commencing VGB therapy either for epilepsy or for substance abuse (Fechtner et al., 2006). It should also be introduced into the examination routine of those patients already exposed to VGB and should become the technique of choice for learning disabled adults. It should be used wherever possible in children; however, for optimum interpretation of the findings, in children normal values will be required for

the generic database. The development of nasal retinal nerve fibre layer attenuation should be adopted a clinical indicator for withdrawal of VGB.

Longitudinal assessment of current paediatric usage of VGB, and of putative short-term use as an anti-addiction drug, should incorporate OCT (which represents a breakthrough for monitoring such cases).

OCT identifies abnormality of the retinal nerve fibre layer, which is associated with VAVFL. This finding confirms that of Wild et al (Wild et al., 2006) using a different methodology and a predominantly new dataset. The mean RNFL value is attenuated because of nasal quadrant RNFL attenuation. The nasal quadrant is attenuated in 100% of cases of VAVFL.

Nasal quadrant RNFL attenuation occurred in VGB-exposed individuals manifesting normal visual fields; this probably represents structural change prior to irreversible visual loss. There is a precedent for structural change in RNFL as measured by OCT prior to functional visual loss in glaucoma (Caprioli et al., 2006) and recently multiple sclerosis.(Sepulcre et al., 2007) Further supporting evidence for this includes the positive relationship between nasal quadrant RNFL attenuation and cumulative VGB dose and duration.

Nasal quadrant RNFL attenuation was absent in all epilepsy controls, and in all normal individuals, confirming that nasal RNFL attenuation cannot be viewed as a normal finding. In contrast, temporal quadrant RNFL attenuation is seen in Optic Neuritis, (Sepulcre et al., 2007) and other optic neuropathies. Thus, nasal quadrant RFNL attenuation is a more accurate measure of VAVFL, and is a better predictor of VAVFL than mean RNFLT, given the variability of temporal RNFLT, and the contribution that makes to the mean RNFL value.

OCT is applicable to children and learning disabled adults, with high completion rates in this group. Development of normative databases for children would improve reliability of quadrant values, whilst mean RNFL values fall within that expected in 18 year-olds. Nasal quadrant attenuation adjusted for 18 year-old values identified VAVFL in this group.

Chapter 4

4.1 HRT Measures In VGB Toxicity

4.1.1 Introduction

As described in Chapter 3, previous work from our group reported the utility of both OCT and scanning laser ophthalmoscopy (SLO), using the Heidelberg Retinal Tomograph (HRT) II, for identifying VGB toxicity. (Wild et al., 2006) As also described in Chapter 3, this earlier work did not analyse the results in terms of the topographical (ie segmental) distribution of RNFL thickness. The reanalysis of the OCT segmental results (using the variable diameter scan) was discussed in Section 3.3.

It was also considered appropriate, in the context of the differential atrophy of the disc in patients exposed to VGB identified by OCT in this thesis, to reanalyse the findings obtained with the HRT with particular respect to the segmental distribution of neuroretinal rim thickness and to the other descriptive parameters of the optic nerve head.

A single case report using the HRT in a patient with VAVFL described a reduced mean RNFL thickness (Viestenz et al., 2003); however, no specific segmental analysis was undertaken.

HRT yields a variety of descriptive parameters of the optic nerve head; many of these are

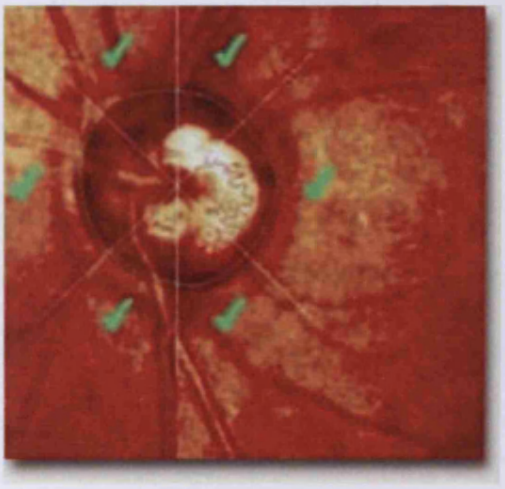
dependent upon the reference plane used to distinguish the neuroretinal rim from the cup. Measures that are independent of the reference plane include the Disc Area, Cup Shape and Height Variation in Contour. The standard reference plane is set as a default at 50μm below the contour line at the temporal disc margin, at the location of the papillomacular bundle. (Burk et al., 2000). At the optic nerve head, structures below this plane are ascribed to the cup whilst those above the reference plane are ascribed to the neuroretinal rim (NRR). The location of this reference plane (SRP) was deliberately selected based upon the stability of the papillomacular bundle in glaucoma. (Burk et al., 2000)

'Mean retinal nerve fibre layer thickness' measures are derived by the HRT as the difference between the reference plane and the retinal surface height profile along a contour line drawn by the user. It must be understood that, in contrast to the OCT, which defines the RNFL by boundaries, no such anatomical boundaries are identified by the HRT. The reference plane is an arbitrary fixed depth that is uniformly applied to each image and thus will be subject to inter-individual variation in normal retinal thickness. Similarly, the retinal surface height profile is contour line dependent and therefore subject to further variation. Thus, the parameter of 'mean RNFL thickness', whilst a quantitative value, is merely a marker for RNFL thickness rather than a true estimate of RNFL. A segmental measure of RNFL thickness is not available when the option for such an approach is not pre-defined at the time of the examination. Therefore, sector NRR area values were analysed with particular reference to the presence and frequency of inverse optic nerve head atrophy (Frisen and Malmgren, 2003, Buncic et al., 2004). The pattern of atrophy in VGB toxicity as described in Chapter 3 spares the temporal oblique quadrant of the disc, instead affecting the nasal, superior and inferior oblique quadrants of the disc and retina.

This "inverse optic nerve head atrophy" is in contrast to the temporal disc atrophy seen in primary and secondary optic nerve pathologies.

The manufacturer's generic database of normal values for the various HRT optic nerve head parameters is contained within the software package known as the Moorfield Regression Analysis (MRA). The database derives confidence limits (95%, 99% and 99.9%) for the neuroretinal rim area, taking into consideration the covariance between the NRR and optic nerve head. (Wollstein et al., 1998) Confidence limits are available for a global NRR measure, and also for each of six sectors of variable width/area. The sectors do not correspond to those of the OCT (Figure 4.1.1.).

Figure 4.1.1 The differences in sectors between the Stratus OCT (Left) and the HRT II (Right) illustrated from the respective printouts.



4.1.2 Methods

The group comprised 13 individuals with VAVFL, 8 VGB-exposed individuals manifesting normal visual fields, 13 patients with epilepsy exposed to alternative anti-epileptic drugs, and 21 healthy individuals who served as normal controls.

All patients underwent the same examination routine as that described in section 3.3. Briefly, all patients had been recruited from the Welsh Epilepsy Unit, University Hospital of Wales, Cardiff and the normal individuals from the University Hospital of Wales and from the Eye Clinic, Cardiff School of Optometry and Vision Sciences, Cardiff University and had been matched as closely as possible in age within- and between- the respective groups. All patients were matched as far as possible, for age at onset, and duration, of epilepsy.

The inclusion criteria in each eye were a distance refractive error less than or equal to 5 dioptres mean sphere and less than 2.5 dioptres cylinder; a distance visual acuity of 20/30 or better in each eye; an intraocular pressure of 21mmHg or less, uncorrected for corneal thickness; open angles; clear ocular media; no optic nerve head or fundal abnormalities characteristic of known disease; no previous ocular surgery or trauma; no history of diabetes mellitus and no family history of glaucoma.

The participants had attended for two visits. At one visit, the visual field examination was undertaken, as detailed in Section 3.3, for the right eye only; at the other visit retinal imaging was undertaken in the same eye. The order of the imaging and perimetry visits was randomised

between individuals.

4.1.2.1 Imaging

The central corneal radius had been determined, prior to imaging, using a Bausch and Lomb keratometer (Bausch and Lomb, Rochester, NY) in order to correct the images for ocular magnification. Three separate scans of the optic nerve head, and the immediate surrounding retina, had been automatically obtained by the HRT software which had then computed the mean of the three scans to form the output topography image and the SD of the mean to ascertain the quality of the resulting mean image. The field size was 15°x15°. The participants had fixated on the internal fixation target. The SD of the mean was ≤10μm in 9 of the 55 participants, between 11μm and 20μm in 36, and between 21 and 30μm in 9 participants and 57μm in the remaining participant (who was from Group III). The contour line had been drawn by IAC, a senior ophthalmologist trained to fellowship standard in glaucoma and highly experienced in optic nerve head assessment and in the drawing of the contour line with the HRT, who was masked to the purpose and design of the study. The images had been analysed by software version 1.6.

4.1.2.2 Analysis

The HRT standard printout including the Moorfields Regression Analysis was produced for each individual.

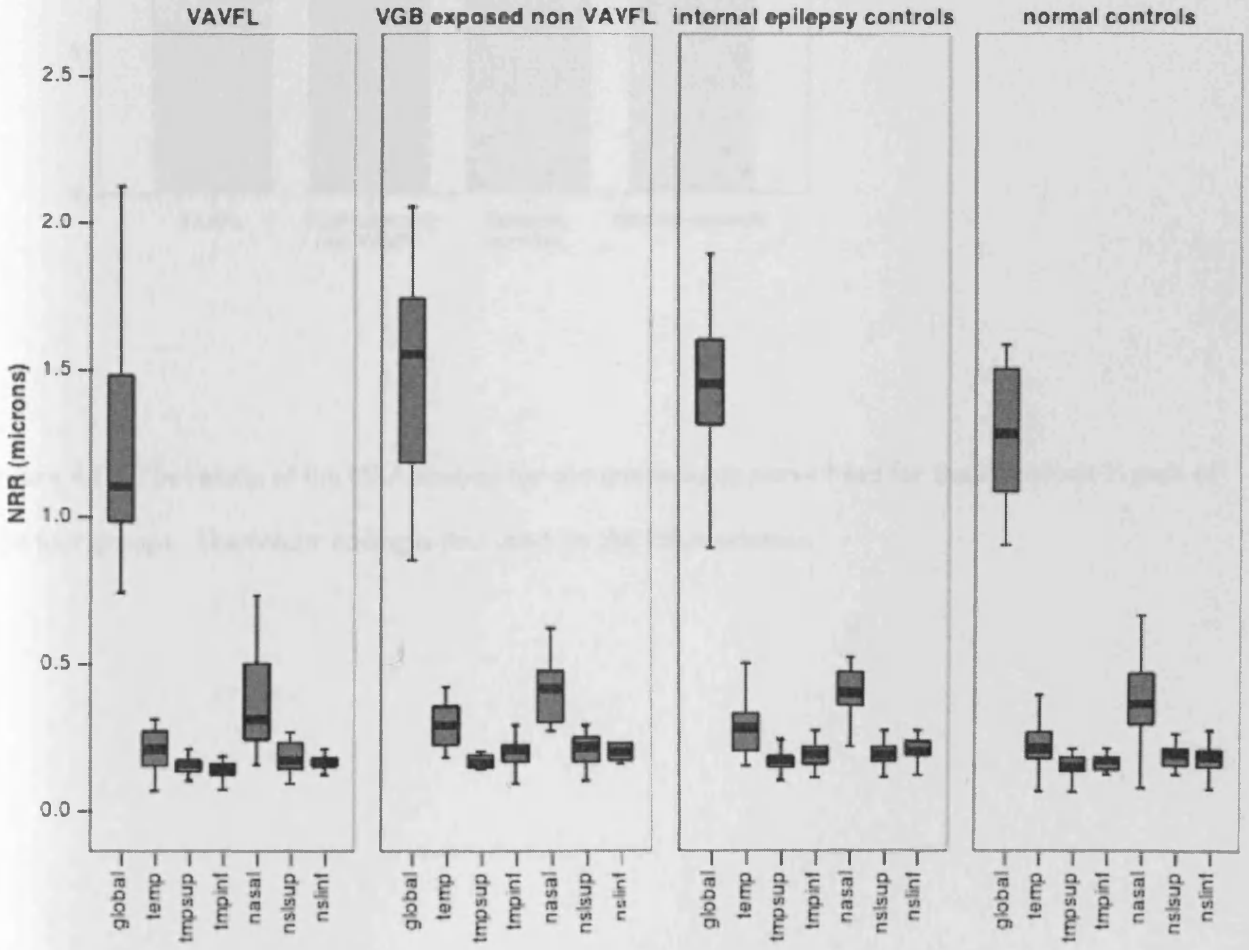
4.1.3 Results

The box and whisker plots for the NRR sector values for each of the four groups is presented in Figure 4.1.1. The analysis does not identify any segmental differences in the NRR sector areas. Some eight data points were excluded as the data as measured by the HRT was clearly inaccurate and yielded illogical quantities likely representing misreading of the raw data by the MRA.

The MRA analysis by sector defined all the normal individuals as 'normal'. (Figure 4.1.2) Three of the 21 patients with epilepsy with no exposure to VGB, were designated as 'borderline' in the superior nasal sector (Table 4.1.1). However, all 3 manifested normal visual fields and yielded normal ophthalmological findings. Six of the 13 individuals with VAVFL exhibited normal NRR areas in all 6 sectors. Of the remaining seven individuals, four were identified as 'outside normal limits', with three identified as 'borderline'. Of these latter four, all exhibited a reduced NRR area in one or more of the nasal sectors together with a normal temporal sector. Of the three individuals with VAVFL designated as borderline, the possible reduction in NRR areas was greatest in one or more of the various nasal sectors; the temporal sectors were all normal. One individual exposed to VGB but with normal visual fields exhibited similar reduction in the NRR area in the nasal sectors, combined with a normal temporal sector, to those with confirmed field loss.

Those individuals with VAVFL identified as abnormal by MRA also demonstrated attenuated mean and nasal RFNL by proportionate circle RNFL imaging with OCT and all had moderate to severe VAVFL.

Figure 4.1.1 Box and whisker plots for NRR area by sector. Left patients with VAVFL, middle left, patients exposed to VGB but with normal fields, middle right, patients with epilepsy with no exposure to VGB, right, normal individuals. The solid line within the box represents the 50th percentile, the extremities of the box the 15th and 85th percentiles respectively and the whiskers the highest and lowest values in the distribution.



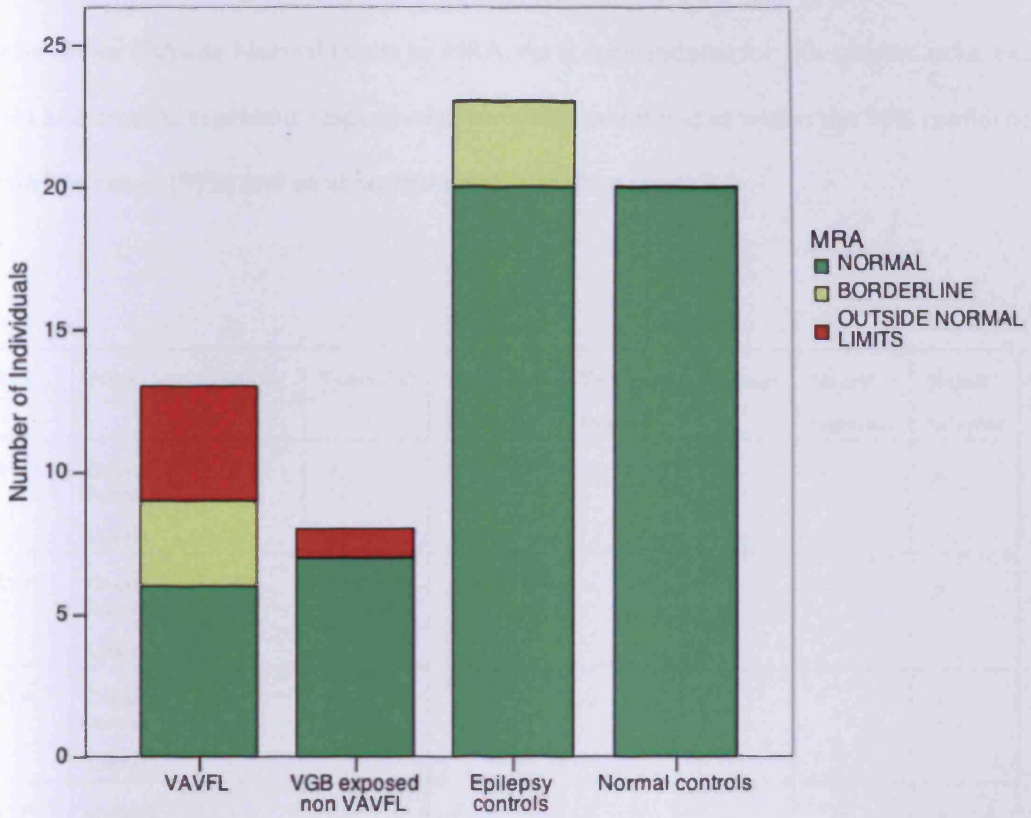


Figure 4.1.2 The results of the MRA analysis for the entire optic nerve head for the individuals in each of the four groups. The colour coding is that used on the MRA printout.

Table 4.1.2 Pattern of abnormality for the Neuroretinal Rim Area for those individuals deemed as Borderline or Outside Normal Limits by MRA. As is conventional for this system, ticks, exclamation marks and crosses represent respectively, normality as defined as within the 95% confidence limits, a borderline result (99%) and an abnormal result (99.9% percentile).

Status	MRA	Global	Temporal	Temporal/ Superior	Temporal/ Inferior	Nasal	Nasal/ Superior	Nasal/ Inferior
VAVFL	Outside Normal Limits	x	√	!	x	x	x	x
VAVFL	Outside Normal Limits	√	√	√	√	!	√	x
VAVFL	Outside Normal Limits	√	√	√	x	!	√	x
VAVFL	Outside Normal Limits	!	√	√	√	x	!	!
VAVFL	Borderline	!	√	√	√	!	!	!
VAVFL	Borderline	√	√	√	!	√	√	!
VAVFL	Borderline	√	√	√	√	√	!	√
VGB exposed Non-VAVFL	Outside Normal Limits	x	√	X	!	x	x	!
Epilepsy Control	Borderline	√	√	√	√	√	!	√
Epilepsy Control	Borderline	!	√	√	√	√	!	!
Epilepsy Control	Borderline	√	√	√	√	√	!	√

4.1.4 Discussion

Inverse optic nerve head atrophy defined as a reduction in the nasal NRR area beyond normal limits was present in 7 of 13 individuals with VAVFL, and appeared to be present in one individual exposed to VGB but with seemingly normal visual fields. The latter case is plausible in that this patient also exhibited an abnormal nasal quadrant RNFL thickness together with a normal RNFL thickness in the remaining three quadrants (Section 3.3). In addition, as was also discussed in Chapter 3, structural changes are identified in glaucoma prior to the development of functional loss, (Caprioli et al., 2006) and this precedent appears to hold true for VGB toxicity.

The sensitivity of the inverse optic atrophy as an indicator of VGB toxicity is clearly not as good as that demonstrated by measurement of the RNFL. The specificity of the presence of inverse optic nerve head atrophy would seem to be worse than that obtained for the measurement of the RNFL (by OCT) since three individuals with epilepsy and no VGB exposure demonstrated both inverse optic nerve head abnormality. However, it should be noted that these three individuals also exhibited abnormalities in sectors other than the nasal region in RNFL thickness measurement by OCT.

Although the MRA was able to identify VAVFL in 7 of 13 individuals, the nasal thinning was clinically less obvious than that for the RNFL attenuation by OCT.

The poorer sensitivity of the HRT to detect inverse optic nerve head atrophy manifested in terms of reduction in the NRR area compared to the OCT manifested in terms of attenuation of retinal

nerve fibre layer attenuation can be explained by the resolution necessary to identify abnormality in the structure under measurement. The NRR comprises a confined area where nerve fibres from all areas of the retina coalesce and merge with blood vessels prior to entering the optic nerve. The NRR sector measure is therefore, less likely to identify subtle attenuation in RNFL.

4.1.5 Conclusion

It would seem that the presence of inverse nasal optic atrophy as defined by a reduction in the NRR area is not as sensitive a marker of VGB toxicity than measurement of the RNFL by sectoral discrimination by OCT.

In addition, the presence of inverse nasal optic atrophy as defined by a reduction in the NRR area, under the resolution of the current commercially available HRT, is only associated with moderate to advanced cases of VAVFL.

4.2 Placental transfer of VGB: no indication of visual field loss

4.2.1 Introduction

It can be surmised that, worldwide, a substantial number of women of childbearing age will have received VGB. However, uncertainty persists regarding the potential for visual dysfunction in individuals exposed *in utero* to VGB. To our knowledge, only one report has described the outcome of systematic visual field examination of prenatally exposed individuals; two children, unrelated to each other, each produced inconclusive visual field examinations (Sorri et al., 2005). Many children exposed to VGB, prenatally, are reaching the age where complete ophthalmological examination is possible.

4.2.2 Aim

The aim of this case series was to assess whether VGB-attributed visual field loss was present in children with placental transfer of VGB.

4.2.3 Methods

Three families (4 children) were identified in which each mother completed one or more successful pregnancies whilst receiving VGB for refractory partial epilepsy. The three

mothers were aged 44, 39, and 39 years. The duration of the refractory partial epilepsy was 26, 17 and 24 years and the duration of VGB exposure 8.5, 9.75 and 6.7 years, respectively. The cumulative VGB dose and mean daily dose were 8.75, 10.5, and 7.32 kg, and 2.82g, 2.74g and 2.99g respectively. Two mothers (M2 and M3) had taken one other anti-epileptic drug (carbamazepine in both cases) within the conception period and this was continued throughout pregnancy in mother M3.

Three of the four children were female. The ages of the children were 6, 10, 8 and 15 years, respectively. All four children were born at term and were exclusively formula fed. VGB placental transfer, which may reach 100% (Tran et al., 1998, Abdulrazzaq et al., 2001), therefore represented the only mechanism of VGB exposure. Cumulative VGB gestational dosage was recorded and all three mothers reported compliance with medication before and during pregnancy. The estimated *in utero* exposure to VGB using area under the curve estimates for fetal growth is shown in Table 4.2.1.

4.2.3.1 Perimetry

Each of the mothers underwent perimetry of the full and central field with the Humphrey Field Analyzer [Carl Zeiss Meditec, Dublin CA]) as recommended by the Marketing Authorization Holder. (Pharma, 2001) Reliable outcomes to the visual field examination, in terms of incorrect responses to the false negative, false positive and fixation-loss catch-trials,

were obtained in all three mothers. Each of the children underwent two-zone suprathreshold perimetry of the full field in an identical manner to that of their mother. Reliable results were obtained in all four children.

4.2.3.2 Imaging with OCT

The RNFL thickness was determined for each mother and child using the RNFL Thickness 3.4 Protocol of the StratusOCT (Carl Zeiss Meditech, Dublin, CA). All scans exhibited the requirements of a signal to noise ratio greater than 25dB and at least 90% good quality A-scans. The study had approval from the South East Wales Ethics Committee.

4.2.4 Results

The three mothers and the four children were visually asymptomatic, and the visual acuity and fundoscopy, through dilated pupils, were normal in each individual.

Two of the three mothers each exhibited VAVFL for both types of visual field examination and an abnormally attenuated RNFL thickness. (Figure 4.2.1) The third mother exhibited a left upper temporal partial quadrantanopia, secondary to an anterior temporal lobectomy, and a normal RNFL thickness. Multifocal ERGs were within normal range for all mothers for both eyes.

All four children manifested normal visual fields. The mean and nasal quadrant RNFL thickness for each child was well within the normal range for adults (the StratusOCT software does not contain a database for children although children are considered to exhibit comparable RNFL thicknesses to adults). Results are contained in Figure 4.2.2.

Figure 4.2.1 Visual fields are presented for Mother 1. The grayscale depictions for left and right eyes are situated at the top, whilst pattern and total standard deviation responses are situated middle and lower respectively. Bilateral VAVFL, with predominantly nasal visual field loss is demonstrated.

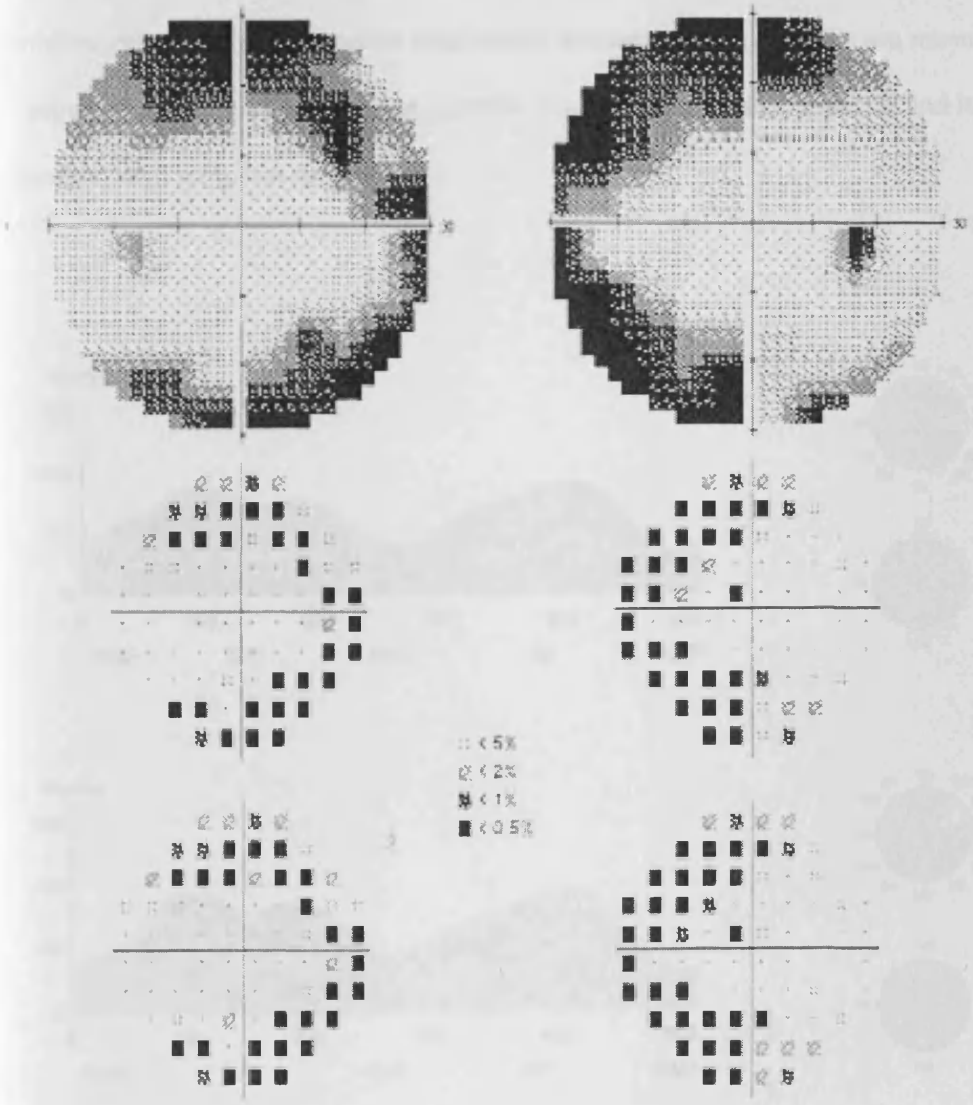
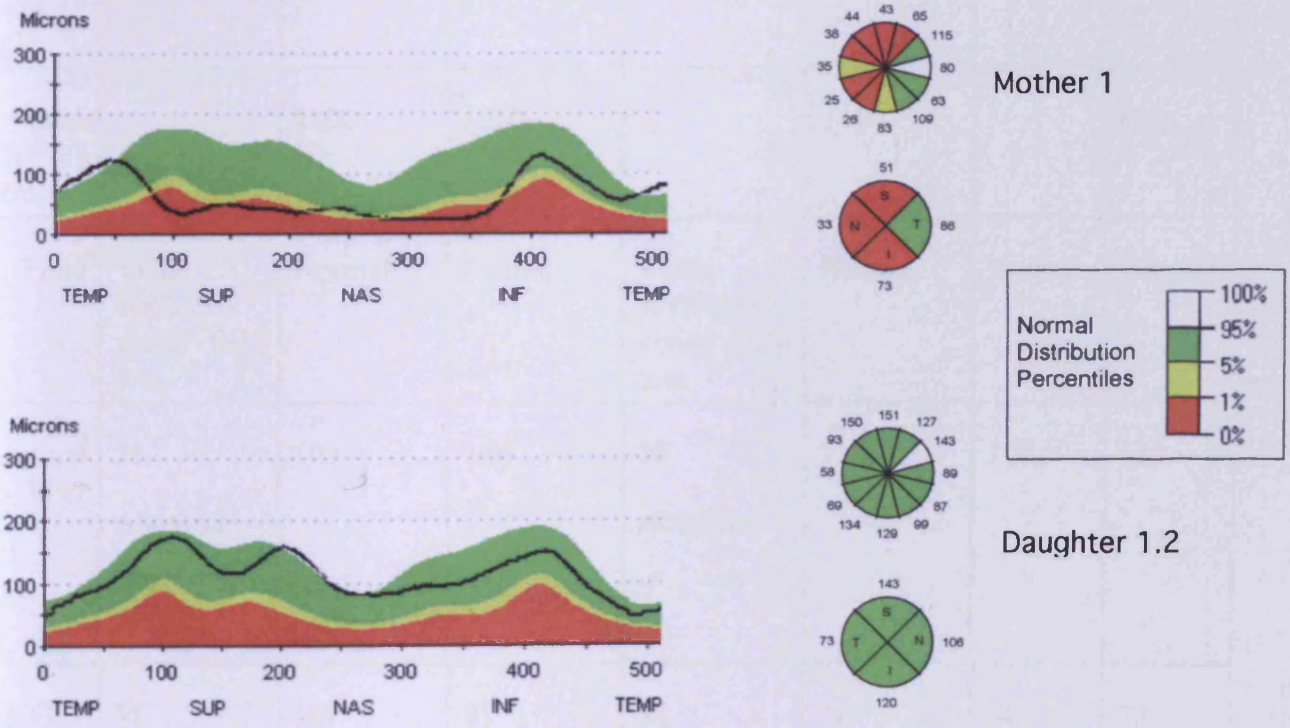


Figure 4.2.2 The RNFL thickness is demonstrated for Mother 1 (top) and one of her daughters (bottom). The letters N, S, T, and I represent oblique quadrants nasal, superior, temporal and inferior respectively. The normal distribution percentiles demonstrate the normal average and sector responses for the daughter, and in contrast the attenuated nasal, superior and inferior RNFL of the mother, with temporal sparing.



	Family 1			Family 2		Family 3	
	Mother 1	Daughter 1.1	Daughter 1.2	Mother 2	Daughter 2.1	Mother 3	Son 3.1
ge at ssessment rs)	44	6	10	42	9	43	15
GB estational ose (kg)	0.560	0.560	0.560	0.662	0.662	0.287	0.287
GB dose g/kg/day quivalent		1200	1200		1410		600
isual Field tatus	VGB-attributed visual field loss	Normal	Normal	VGB-attributed visual field loss	Normal	Normal	Normal
lean retinal erve fiber yer ickness RNFLT) m)	55 Abnormal	109	106	70 Abnormal	110	104	115
asal RNFLT m)	31	102	81	34	71	74	76

Table 4.2.1 VGB in utero. The table details the demographics and results for the three families. For the RNFL thickness, only abnormal results are labelled as such. All others are deemed to be within normal limits. Nasal RNFLT refers to nasal quadrant values.

4.2.5 Discussion

This is the first report of definitively normal visual fields in children exposed prenatally to VGB across a range of placental doses. The four children exposed prenatally to VGB manifested normal visual fields and a normal RNFL thickness.

Infants treated with VGB for infantile spasms normally receive dosing regimens of 100–150 mg/kg/day. It is noteworthy that the estimates of the maximum fetal daily dosing of the four children were up to ten times this amount. However, infants exposed to VGB after 6 months of age are approximately 2.5 times more likely to exhibit VGB toxicity compared with those exposed before 6 months of age (Westall, 2007) suggesting a possible physiological immaturity effect. A possible explanation for the lack of visual dysfunction following high *in utero* exposure may reflect the lack of placental metabolism of VGB, suggested by equal amounts of active and inactive enantiomers, in contrast to the maternal excess of active enantiomer (Challier et al., 1992). The pathogenesis of retinal toxicity in VGB is still not understood. It is speculative, but plausible, that the mechanism of toxicity requires VGB metabolites, which may be absent in utero and relatively under-produced in neonates. All children were exposed via placental transfer, alone. No definite assertion can be made regarding the safety of maternal breastfeeding whilst on VGB.

In terms of possible teratogenicity of VGB, the findings are clinically reassuring, and, if representative, obviate the need to identify and then examine ophthalmologically, children

exposed to VGB *in utero*. Furthermore, for the few remaining women of childbearing age still receiving VGB, the findings can aid informed discussion about potential visual aspects of VGB teratogenicity. The latter should also be placed in the context of possible unplanned pregnancy in women treated with VGB as an anti-addiction drug. (Fechtner et al., 2006)

4.3 VGB-Attributed Visual Field Loss In Uniquely Low Cumulative Dosing: Implications For Potential Use In Anti-Addiction Therapy.

4.3.1 Introduction

VGB offers potential as an anti-addiction therapy for misuse of stimulant drugs. The increase in brain extracellular dopamine in substance misuse is one of the markers and mediators of drug 'highs' (Gerasimov et al., 2001) and can be attenuated by elevation of GABA. The efficacy and visual safety of VGB as an anti-addiction therapy, has recently been investigated in an open-label study, (Fechtner et al., 2006) and is currently undergoing Phase II trials. In the trial cited above, VGB was prescribed in a short-term (9 weeks) low daily dosing and low cumulative dosing (0.137 kg) regime with a consequently lower risk of visual field loss.

4.3.2 Case Report

A 34-year-old man with epilepsy with no previous visual problems developed asymptomatic visual field loss during an estimated maximum cumulative dose of 0.15 kg of VGB i.e. similar to that advocated for anti-addictive therapy.

VGB had been prescribed as an adjunctive treatment to carbamazepine for intractable

temporal lobe epilepsy. He had previously taken sodium valproate but this was discontinued due to a perceived lack of efficacy. Initial titration of VGB was attempted up to 500mg twice daily over 8 weeks. Unfortunately this regimen was not well tolerated, and he returned reporting non-compliance and rapid unsupervised withdrawal after some 3 weeks. A further attempt, involving slow titration of 250 mg increments of VGB per month, proved unsuccessful and his clinician finally withdrew VGB some 8 months later, citing adverse effects and a perceived lack of efficacy. However, the patient asserted that he had taken the drug irregularly, and had discontinued use several weeks earlier. He remained visually asymptomatic and was called for routine visual assessment, including perimetry, some 14 months later (Figure 4.3.1). Perimetry was undertaken according to the techniques recommended by the marketing authorisation holder for VGB, namely two-zone (three level) age-corrected suprathreshold perimetry of the full field and threshold perimetry of the central field. The results clearly indicated a bilateral symmetrical concentric constriction with some sparing of the temporal field, typical of late-stage VAVFL. The individual had not been aware of visual field loss prior to the visual field examination and did not demonstrate any features consistent with functional visual loss. In addition, the field loss was remarkably consistent across the two types of perimetry, which necessitate two fundamentally different requirements in response.

4.3.3 Discussion

The use of VGB as a potential anti-addiction agent is predicated upon a short-term, low cumulative dose schedule and is therefore designed to minimise the risk of visual field

loss. Unfortunately, there are large inter-individual differences at which individuals manifest VAVFL following VGB. The dosing regimen is based upon an exposure of ≤10% of that considered to result in VAVFL. However, the original study investigating relationships between VGB dosing and visual field loss reported only a modest correlation between visual field defects and cumulative dose ingested (spearman correlation coefficient = 0.506, p = 0.09). (Manuchehri et al., 2000)

In the pilot addiction study, VAVFL did not occur in 18 individuals with methamphetamine or cocaine addiction who received a cumulative VGB dose of 0.137 kg, over 9 weeks. (Fechtner et al., 2006) It is notable that three weeks of the therapy schedule comprised 3.0 g/day of VGB (compared with typical adult epilepsy doses ranging from 0.5 to 3.0 g). A high mean daily dosing is known to carry an increased risk of VAVFL. (Wild et al., 2007)

Pragmatically, VGB prescription in addiction is likely to require higher doses than have been proposed. It may be possible to overcome the attenuation of dopamine increase in 'highs' with escalated substance dosing. To date, I am not aware of any research that investigates this issue. In a clinical context, higher baseline substance doses might respond more effectively to increased doses of VGB. Relapse may further obviate repeat prescription, further increasing the cumulative dose.

Our case demonstrates that VGB dosing within the proposed range for anti- addiction can cause irreversible visual field loss in susceptible individuals. Screening individuals

exposed to VGB with perimetry merely serves to document the onset of irreversible visual field loss. Currently, there are no available definitive predictors of individual risk factors for the development of VAVFL. An alternative biomarker for the development of VAVFL is an attenuated retinal nerve fibre layer thickness (Wild et al., 2006) and in particular, nasal quadrant attenuation (Chapter 3). Nasal quadrant attenuation may precede visual field loss, and in the absence of definitive trials (which would be ethically unsound), screening of the retinal nerve fibre layer thickness, with particular reference to the nasal quadrant might be adopted.

This case report supports the notion that there is no 'safe' dosing regimen for VGB for any given individual. Any anti-addiction protocol would need to be carefully discussed with the individual concerned, particularly as visual field loss might result in revocation of driving privileges. Anecdotally, I perceive that clinical use of VGB in epilepsy is once again increasing, possibly driven by the request by the Market Holders of VGB (Ovation) to licence VGB for partial-onset epilepsy in the US. The discussion here is clearly relevant in both settings, but individual contexts (e.g. no possibility of driving) might facilitate use of VGB with more ready acceptance of the visual risks.

Figure 4.3.1 This Visual Field Print-Out From The Full Field 135 Screening Test And The Three Zone Age-Corrected Strategy (Top) And Of Program 30-2 Using The FASTPAC Strategy, Exhibits VGB Attributed Visual Field Loss.

Page 159

Eye: Left

ID: DOB: 13-08-1968

Full Field 135 Screening Test

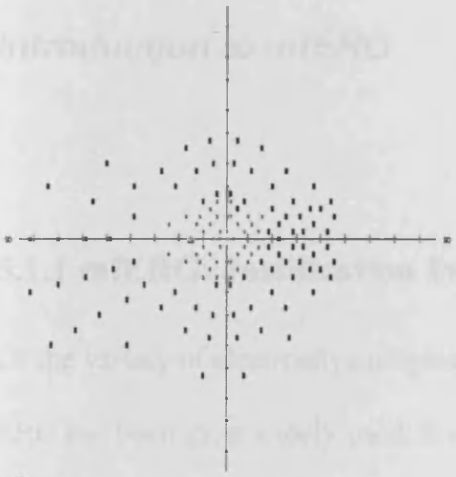
Fixation Monitor: Gaze/Blindspot
Fixation Target: Central
Fixation Losses: C/26
False POS Errors: 0/27
False NEG Errors: 0/11
Test Duration: 12:28

Stimulus: III, White
Background: 31.5 ASB
Strategy: Three Zone
Test Mode: Age Corrected

Pupil Diameter: 5.4 mm
Visual Acuity:
RX: +1.00 DS DC X

Date: 04-02-2003
Time: 12:09
Age: 34

Central Reference: 34 dB
Peripheral Reference: 34 dB



Seen: 31/135
Defect: 16/135
Not Seen: 86/135
Blindspot

Eye: Right

ID: DOB: 13-08-1968

Full Field 135 Screening Test

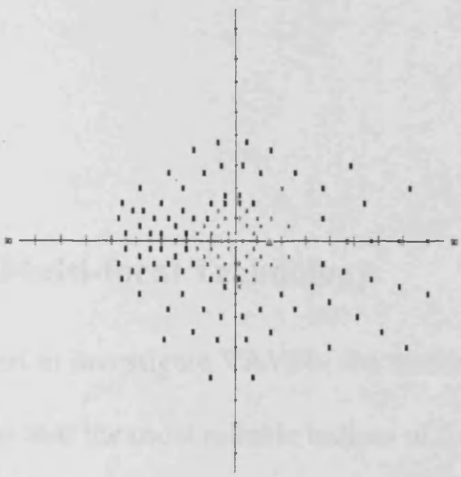
Fixation Monitor: Gaze/Blindspot
Fixation Target: Central
Fixation Losses: C/25
False POS Errors: 0/25
False NEG Errors: 2/11
Test Duration: 12:50

Stimulus: III, White
Background: 31.5 ASB
Strategy: Three Zone
Test Mode: Age Corrected

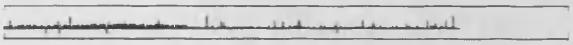
Pupil Diameter: 6.3 mm
Visual Acuity:
RX: +1.00 DS DC X

Date: 04-02-2003
Time: 11:18
Age: 34

Central Reference: 34 dB
Peripheral Reference: 34 dB



Seen: 34/135
Defect: 11/135
Not Seen: 90/135
Blindspot



Single Field Analysis

Eye: Left

ID: DOB: 13-08-1968

Central 30-2 Threshold Test

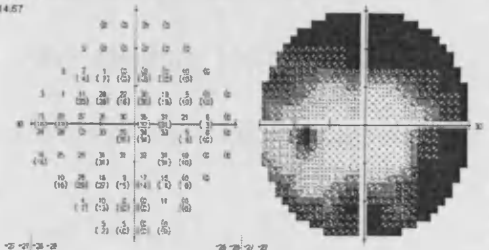
Fixation Monitor: Gaze/Blindspot
Fixation Target: Central
Fixation Losses: 4/24
False POS Errors: 0/17
False NEG Errors: 0/12
Test Duration: 14:57

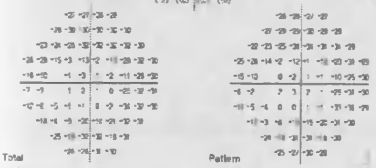
Fovea: OFF

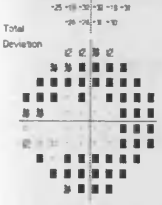
Stimulus: III, White
Background: 31.5 ASB
Strategy: FASTPAC

Pupil Diameter: 7.0 mm
Visual Acuity:
RX: +1.00 DS DC X

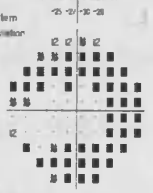
Date: 13-03-2003
Time: 15:52
Age: 34







Total Deviation



Pattern Deviation

MD -16.85 dB P < 0.5%
PSD 14.22 dB P < 0.5%
SF 1.70 dB
CPSD 14.09 dB P < 0.5%

Single Field Analysis

Eye: Right

ID: DOB: 13-08-1968

Central 30-2 Threshold Test

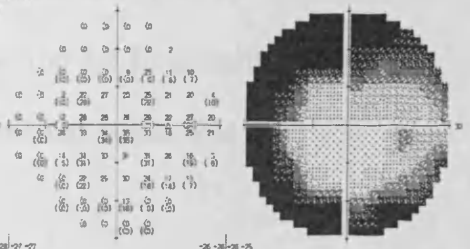
Fixation Monitor: Gaze/Blindspot
Fixation Target: Central
Fixation Losses: 4/23
False POS Errors: C/15
False NEG Errors: 5/15 xx
Test Duration: 14:03

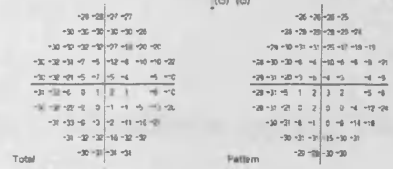
Fovea: OFF

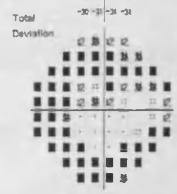
Stimulus: III, White
Background: 31.5 ASB
Strategy: FASTPAC

Pupil Diameter: 7.3 mm
Visual Acuity:
RX: +1.00 DS DC X

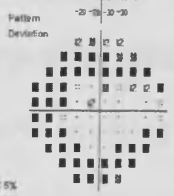
Date: 13-03-2003
Time: 14:56
Age: 34







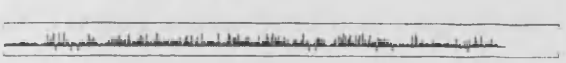
Total Deviation



Pattern Deviation

*** Low Test Reliability ***

MD -16.16 dB P < 0.5%
PSD 14.01 dB P < 0.5%
SF 6.13 dB P < 1%
CPSD 12.17 dB P < 0.5%



Chapter 5: The mfERG In VGB Toxicity

5.1 The Application Of Multifocal Technology In VAVFL: Introduction to mfERG

5.1.1 mfERG: Justification for using Multi-focal Technology

Of the variety of electrophysiological tools used to investigate VAVFL, the standard ERG has been most widely used. It seems clear that the most reliable indices of the standard ERG as markers of VAVFL include reduced photopic b-wave amplitudes (Arndt et al., 1999, Coupland et al., 2001, Krauss et al., 1998, Sills et al., 2001) and a reduced 30Hz flicker ERG. (Harding and Wild et al. 2000) Despite this, several studies have reported normal ERGs in VAVFL. (Blackwell et al., 1997, Gross-Tsur et al., 2002, Harding, 1997, Lawden et al., 1999). Section 1.2.12 contains a fuller discussion of electrophysiology changes in VGB related visual field loss.

However, there are major pragmatic technical difficulties in applying standard ERG to children and learning disabled populations. Young children often require sedation to undertake ERG, and the long testing time is labour intensive requiring several visits for many individuals, particularly children and learning disabled adults. The use of contact lens electrodes used frequently in ERG testing is associated with a significant risk of corneal abrasion. Thus, standard ERG requires a major time commitment (with the inherent cost therein), plus a willingness and investment in cooperation and carries

clinical risk of sedation and abrasion, making it a poor tool for screening or monitoring paediatric populations. Certainly, in the UK, with relative paucity of funding and strict ethical constraints governing screening investigations and consent issues for children and adults deemed unsuitable to provide consent, the current standard ERG is not a viable option.

The mfERG provides a topographical regional response designed to identify local or patchy disease processes. This contrasts with standard ERG, which is a mass response and local variations (typical in retinopathies of all kinds) are lost.

Whilst the mfERG is technically challenging, it is faster and better tolerated than its standard counterpart. We use DTL fibre as the electrode rather than a glass contact lens and find that it is exceptionally well tolerated. Once set-up, useful information can be derived from just 7 minutes of recording time, split into 15 or 30 second intervals, and if necessary, this time can be halved.

5.1.2mfERG: Introduction In Context of Standard Electrophysiology

The standard ERG is a mass response to a single flash of light: this is recorded under ISCEV guidelines in photopic and scotopic conditions to generate both a rod and cone driven response. (Marmor et al., 2008) The ERG response largely comes from the

outer retina (photoreceptors and bipolar cells), with little contribution from the ganglion cells, and optic nerve. (Tomita, 1982) The mass response is accrued from the entire retina, and the contribution from the central area particularly is small, as it will also be for small patchy eccentric areas of the retina. Small areas of abnormality (even in visually important areas) may be readily missed on standard ERG.

Other available electrophysiology tools include the focal ERG, and the pattern ERG or PERG (that provides a largely foveal response). The PERG, in contrast to the standard ERG, has a clear discriminant of primary ganglion cell disease in that the N95 component is reduced or absent in primary ganglion cell disease. (Manca et al., 1984)

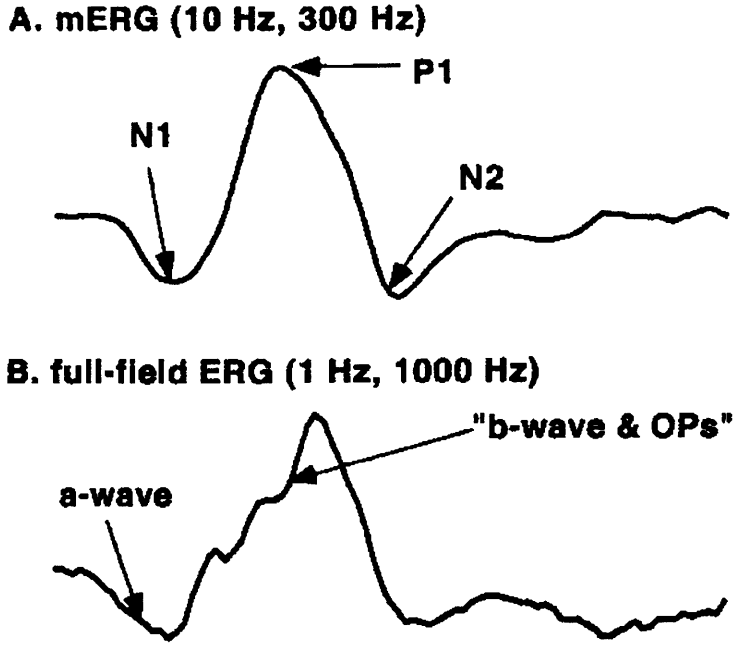
The mfERG was conceived as a topographical representation of regional responses to overcome the problem of missed abnormalities from massed responses. (Poloschek and Sutter, 2002) In combination with perimetry, localisation and monitoring of disease can be effected. (Hood et al., 2003b)

The mfERG also largely represents outer retina responses. The waveform bears a resemblance to the ERG, with a negative first wave (N1), comparing with the a-wave of the ERG, and a positive second wave (P1), similar to the b-wave of the ERG, with a trailing 'ledge'. (Figure 5.1) Whilst definitive certainties cannot be made regarding the derivation of individual mfERG waveforms (the traces are mathematically derived

rather than generated in the truest sense of the word); plausible speculation (Hood et al., 2003a) accompanied by experimental design (Hood et al., 2002) and clinical correlation allows a reasonable attribution of retinal structure and correlation.

The mfERG (a cone response) is predominantly representative of bipolar cell activity, with a smaller contribution from photoreceptor cells. Damage at or before the bipolar cells results in reduction in amplitude of the mfERG, although bipolar cell damage may not result in delayed responses; delay in timing of the mfERG is likely to reflect photoreceptor cell or outer plexiform cell damage. Contribution from inner retinal cells (amacrine and ganglion cells) is relatively small; damage to the amacrine or ganglion cells seems to affect the waveform, in particular that of the trailing ledge of P1. Ganglion cell damage seems either not to affect timing of the mfERG, or may even shorten the P1 implicit time. (Seiple et al., 2002, Fortune et al., 2004)

Figure 5.1.1 Comparison of Full-Field ERG with mfERG



In summary, the multifocal electroretinogram (mfERG) comprises numerous simultaneously recorded local ERG responses from several regions across the retina. In keeping with the standard full-field ERG, the response is largely derived from the outer retina, and has little contribution from the ganglion cells. The mfERG may therefore be of most benefit in distinguishing between diseases of the outer retina and the ganglion cells/optic nerve. Clinically, mfERG has been mooted as a useful tool in monitoring retinal disease, particularly patchy retinal disease (eg retinitis pigmentosa) where the mass response of the standard ERG may remain normal for some time into the clinical syndrome. In addition, together with mfVEP, the techniques may add useful information in suspected non-organic visual loss.

5.1.3 mfERG: Principles

The regional traces are made possible by the stimulus presented and the software required to process the response. The classic stimulus is of 61 or 103, scaled hexagons, which subtend 50° at 32 cm viewing distance. The hexagons are scaled to produce approximately equivalent response sizes across the retina based on normal retinas; typically a central sector subtends 3° whilst a peripheral sector subtends 7°. The 61 sector stimulus uses slightly larger sectors but with the consequent price of poorer resolution.

The hexagons or sectors all independently undergo a unique pattern of frame shifting, either shifting from white to black (or vice versa) or staying unchanged every 13.3 milliseconds in a unique way, different from all other sectors (Figure 2.3.2). In fact, this sequence is not pseudo-random, but a set binary change sequence called a m-sequence, typically composed of a minimum of 64 frame shifts. Each sector begins this same sequence at a different point. The recorded response is a single continuous trace, from which the individual sector traces are extracted via a software algorithm. These individual traces are determined by the serial correlation between the stimulation sequence of a particular hexagon with the continuous single trace. (Jurklies et al., 2002) This system is patented by EDI, and marketed as the VERIS system (used in this study); other production systems use alternative similar techniques to extract the individual traces.

5.1.4 mfERG: Acquisition.

The multifocal response is recorded in accordance with ISCEV guidelines. (Marmor et al., 2003) Recordings take place in the light adapted patients. The response is recorded with either contact-lens electrodes (typically the glass Burian-Allen electrode) (Burian and Allen, 1954) or with non-contact-lens electrodes, the most popular of which is the Dawson- Trick-Litzkow (DTL) electrode employed in this thesis. (Dawson et al., 1979) Corneal electrodes provide larger better quality responses, but almost invariably require local anaesthetic and are less comfortable than non contact-lens electrodes. The stimulus is presented with a luminance of

between 100 and 200 cd/m^2 .(Marmor et al., 2003)

5.1.5 mfERG: Display and Interpretation

The responses may be displayed in a variety of ways; the individualised traces are seen in the trace array, and any combination of these individual traces may be grouped for summing or averaging (Figure 2.4.2). This trace is invaluable in identifying local defects, and also demonstrates the normal nasotemporal trend typical of the healthy retina. (Seeliger et al., 2001) The software also routinely displays annular rings around the fixation point. These rings are presented as response densities (nV/deg^2) as if merely response size were displayed, then the responses would increase in amplitude from the centre to the periphery due to increasing area size. Presenting the values in a response density format enables a more useful comparison across the annuli. Also presented is the 3D plot. This is a three dimensional representation of responses across the retina. Although this plot is appealing and can help identify the blind spot and fovea and thus provide some information regarding accuracy of fixation, it must never be interpreted in isolation, as it can be misleading. The chief problem with the 3D plot is that noise may be interpreted as meaningful signal. This is because the peak response (signal per area) will always be at the fovea, due to the central area being smallest. Therefore, in an example where no recorded response is seen, and only noise is recorded, the peak noise will be seen at the fovea.

5.1.6 mfERG: Limitations

Traditionally, a LCD monitor system has been used to display the stimulus, and allows binocular recordings, and therefore halved acquisition time. However, the newer VERIS model also offers the option to display the stimulus monocularly with a built-in fundal camera for monitoring fixation stability.

5.1.7 mfERG and VGB: Rationale

In keeping with limited VGB exposed populations and a still evolving technology, there is little data regarding the use of mfERG in VGB exposed populations. Lawden et al (Lawden et al., 1999) reported reduced peripheral amplitudes in 2 people with VAVFL, a finding echoed in a further 2 adults with VAVFL from an Australian cohort (Mackenzie and Klistorner, 1998), whilst a more specific finding of reduced b-wave amplitude (now called the P1 in standard nomenclature) was found in 6 of 12 adults with VAVFL. (Ponjavic and Andreasson, 2001) These groups did not report alteration in implicit times, although study with the wide-field mfERG, which assesses out to 90° of the visual field, reported a significant difference in implicit times, with reduced peripheral P1 implicit times compared with central times. (McDonagh et al., 2003) This admittedly limited data in VGB in conjunction with current understanding regarding localisation of mfERG data encouraged me to apply the technology for the reasons outlined below (Section 5.2.1).

5.2 mfERG: Study In VGB

5.2.1 Aims

The overall aim of this study was to investigate whether the mfERG technology identified retinal dysfunction attributable to VGB and the relationship of any abnormality to the visual field. More specifically, the aims were fourfold. Firstly, to determine whether a reduction in amplitude was present for one or more of the mfERG waveforms thereby implicating bipolar dysfunction in the pathophysiology of VGB toxicity. Secondly, to determine whether abnormalities in the implicit time of one or more of the mfERG waveforms were present, thereby implicating photoreceptor dysfunction (in the case of delayed implicit time) or retinal ganglion cell dysfunction (in the case of a shortened P1 implicit time) in the pathophysiology of VGB toxicity. Thirdly, to quantify the relationship between any abnormality in amplitude and/or implicit time of one or more of the mfERG waveforms and the daily dose and duration of VGB intake. Fourthly, to quantify the relationship between any abnormality in amplitude and/or implicit time of one or more of the mfERG waveforms and the severity of the visual field loss.

5.2.2 Methods

5.2.2.1 Cohort

A normal cohort was recruited for a pilot study and is described in Table 5.2.1. A cohort comprising 24 normal individuals was recruited to derive the normative dataset and is described in Table 5.2.2. However, for reasons described below (5.2.3.1) this

dataset had to be discarded. A further set of normal individuals containing 6 of the normal cohort from 6.2.2 formed part of the final study cohort used to investigate VAVFL and this cohort is detailed in Table 6.2.3.

Group (Total)	Gender		Mean Age (yrs) (SD)
	Male	Female	
Normal Controls (20)	9	11	28.5 (3.4)

Table 5.2.1 The summary measures (Group Mean, Standard Deviation [SD]) of the demographical characteristics of the pilot data cohort

Group (Total)	Gender		Mean Age (yrs) (SD)
	Male	Female	
Normal Controls (24)	9	15	36 (9.4)

Table 5.2.2 The summary measures (Group Mean, Standard Deviation [SD]) of the demographical characteristics of the original normative dataset

Group (Total)	Gender		Mean Age (yrs) (SD)	Mean Duration of Epilepsy (yrs) (SD)	Mean Duration of Vigabatrin (yrs) (SD)	Cumulative dose of Vigabatrin (kg) (SD)
	Male	Female				
VAVFL (4)	1	4	49.0 (7.2)	25.6 (8.2)	9.49 (1.63	11.03 (2.53)
VGB-Exposed (9)	2	7	43.0 (17.4)	24.89 (10.1)	7.64 (2.48)	6.96 (2.46)
Controls (13)	4	9	41.69 (20.5)	0	0	0

Table 5.2.3 The summary measures (Group Mean, Standard Deviation [SD]) of the demographical characteristics for each of the three groups)

5.2.2.2 Examination Protocol

Direct contact and included colleagues, fellow post-graduate students, friends and family recruited the normal control individuals. The VGB-exposed adults were recruited from the Welsh Epilepsy Unit, University Hospital of Wales, Cardiff, and the adolescents from the Paediatric Neurology and Adolescent services at the University Hospital of Wales, Cardiff. All individuals conformed to rigid inclusion criteria in each eye including a distance refractive error of less than or equal to 5 dioptres mean sphere and less than 2.5 dioptres cylinder; open angles, clear ocular media; no fundal or optic nerve head abnormalities characteristic of known disease other than VGB toxicity; no previous ocular surgery or trauma; no history of diabetes mellitus and no family history of glaucoma. All individuals exhibited a visual acuity of 6/9 or better in each eye. All patients underwent standard neurological examination at visit 1.

5.2.2.3 Equipment

The mfERG was recorded using the VERIS^{TM} Multifocal System with Integrated Fundus Monitoring System, (supplied by EDI) with software version VERIS 5.1.

This multifocal system utilises a built-in fundus camera designed to allow direct observation of fixation. However, the disadvantage of the camera is that only monocular mfERGs can be recorded, thereby doubling the chair time. Pragmatically, it was felt that, in children and learning-disabled adults, the advantage of observing

fixation was outweighed by the increase in recording time and by the likely subsequent reduction in cooperation and, therefore, lower completion rates.

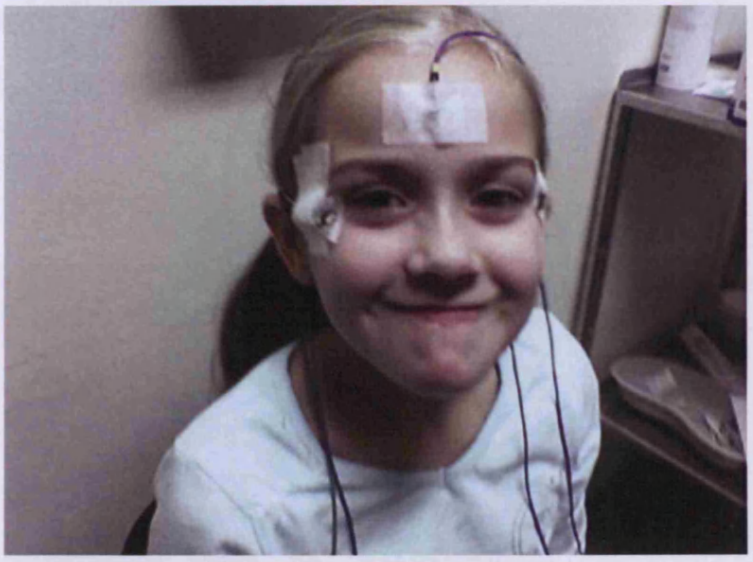
A pilot study in 20 normal adults was therefore undertaken to compare the results from the camera system to those obtained with a high-resolution CRT monitor system and binocular mfERG. (Appendix section A.2.2). The traces from each technique were treated identically in terms of smoothing and averaging. A subjective inspection of the data suggested similar trace quality across the two techniques. Both techniques yielded similar values for amplitude and implicit times although the amplitudes produced from the monitor system were slightly smaller. In addition, the adults were asked to score their experience with each technique, on a rating score from 1 to 5, with 5 being the most positive outcome. All consistently rated the monitor more highly.

5.2.2.4 Set-up

The set-up used for all participants in this study agreed with current ISCEV multifocal guidelines for mfERG. (Marmor et al., 2003) Pupils were dilated with 1% tropicamide to ensure a minimum pupil diameter of 7 mm and placed in a fully lighted room (the recording room) for 15 minutes prior to recording. The skin was prepared with standard abrasive gel used in electrophysiology (NuPrep). DTL fibre electrodes with disposable electrode heads were used for the reference and ground electrodes. (Dawson et al., 1979) The electrodes were applied at the outer canthus and forehead respectively with EEG paste and secured with cotton gauze and micropore.

(Figure 5.1.1) The preparation and placement of the electrodes were in accordance with ISCEV mfERG standards,(Marmor et al., 2003) Impedance was measured for the reference and ground electrodes, using a customised ohmmeter made by the Medical Physics Department at the Queens Medical Centre from a design pioneered by Mitchell Brigell, and was deemed acceptable if the value was <10kΩ, and if both recording channels were within 2kΩ of recorded values.

Figure 5.1.1 Subject prepared for binocular mfERG recording

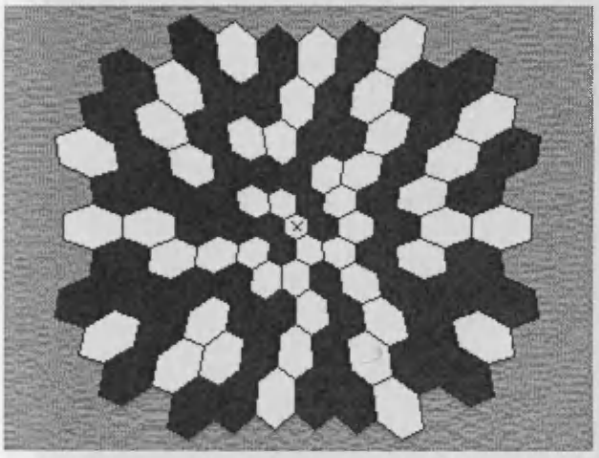


Patients were sat in front of the screen at a recording distance of 48-54 cm . Fixation was observed by direct observation of the patient by the researcher. Refraction was applied as prescription with plus 2 dioptres as a standard adjunct allowing for full dilation, in all individuals aged over 25 years.

Stimulus Properties

The frame rate of the high-resolution monitor was a CRT monitor was 75 Hz and the screen luminance was 150 cd/m^2 in full light. The stimulus pattern was a standard hexagon sized for retinal eccentricity with 61 local stimuli. The VERIS system uses an m-sequence and no extra frames or specialist inserts were applied. The stimulus pattern subtended an angle of 30° on either side of fixation. (Figure 5.2.2) The gain settings were 100,000 with bandpass filters of 3 and 100 Hz. The notch filter was not used. No averaging with neighbouring responses was applied. A red cross, 0.5°in diameter, served as the fixation target.

Figure 5.2.2 An example of the stimulus scaled for retinal eccentricity and similar to that applied in the current study



5.2.3.1 Artefactual Change in the First Normative Dataset for mfERG

The characteristics of the cohort forming the first normative database are given in

Table 5.2.2.

This first dataset revealed significant differences in P1 implicit times, as compared with all 8 VGB exposed individuals examined up to the date of the preliminary analysis. However, this latency delay was present for all waveform at all locations and affected both central and peripheral regions equally and was identical in each individual.

One possibility for the delay in implicit times could have resulted from the older ages of the 8 VGB-exposed individuals compared to the majority of the normal individuals.

The P1 implicit times of the normal individuals were re-analysed by age and also by the calendar date of the examination. In addition, some individuals were repeated to validate the magnitude of the P1 implicit times.

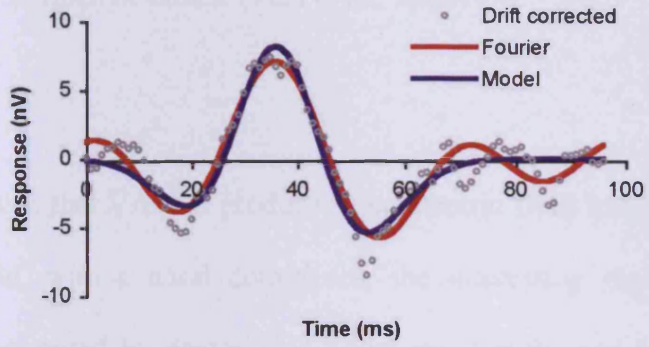
The P1 implicit times showed a tendency to increase with increasing age. As a consequence, the range in the age of the normative database was expanded to include individuals from 18 to 77 years of age. The expanded database showed a clear increase in P1 implicit times with increasing age.

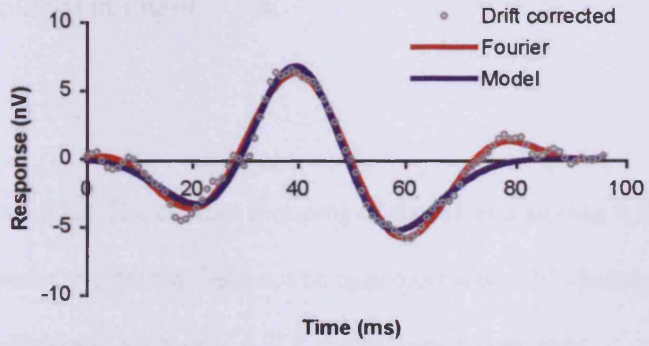
However, the calendar-based analysis showed a clear difference in P1 implicit times (and on further analysis also N1 and N2 implicit times) before and after August 2006. Further analysis of those individuals in whom traces had been recorded before and after August 2006 was undertaken. In particular, one individual had had bilateral recordings taken 5 times over this time period and her data clearly showed an increase in implicit time of approximately 5 ms in all traces performed after mid-August (Figure 5.2.3). The remaining four other individuals also exhibited a similar increase in implicit times by 5ms at all three waveforms and in a uniform manner and over the same time period.

The only identifiable change to the VERIS system during this time period was a software upgrade on the VERIS system from V.1 to V.3 uploaded from disc seemingly without incident. The software was uploaded to address a problem with malfunctioning software that had led to system failures on attempted execution of certain analysis settings. It was entirely feasible that the software upgrade could have been responsible for the increase in implicit timings as they are derived from the continuous trace by a complex application of the mathematics of the M-Sequence which was adjusted for the software upgrade.

As a consequence, the original normative dataset was discarded and a new normative dataset was collected with the new software. Fortunately, all the patients exposed to VGB had been examined with the revised software. No further upgrades to the VERIS system were permitted during the course of this study.

Figure 5.2.3 An example of a typical mfERG trace recorded from a normal individual with the original software (top) and with the software revision (bottom) showing a marked increase in all implicit times. Note, the amplitudes remain approximately the same for the two software versions.



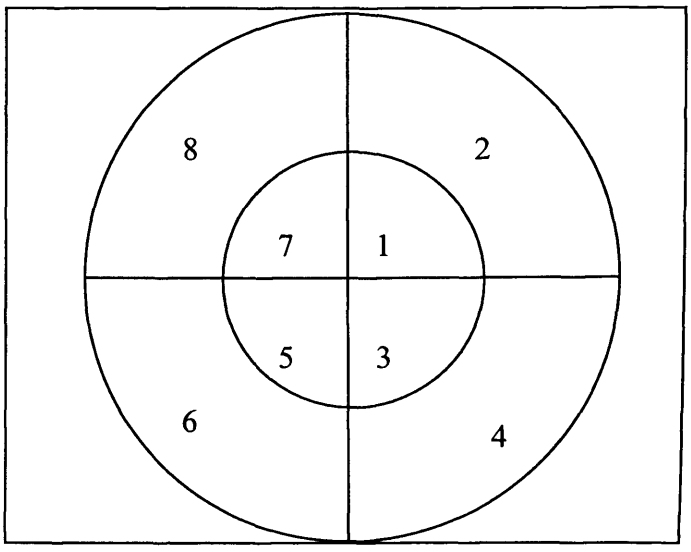


5.2.3.2 Sector Analysis Of mfERG

The traces produced from each of the 61 locations for each eye of each individual represents a huge array of data for analysis. Grouping of data, either in annuli or in customised clusters, is readily achievable using the VERIS software and produces an improvement in trace quality with the consequent narrowing of confidence intervals for normative values. (Tam et al., 2006)

Given that VAVFL produces a concentric field loss, predominantly in the peripheral field, with a nasal dominance, the interesting regions for comparison were best represented by central and peripheral annuli, and by annuli further quartered into nasal and temporal regions. The grouping of the 61 traces into 8 sectors for analysis is displayed in Figure 5.2.4.

Figure 5.2.4 The custom grouping of the 61 traces into 8 Sectors, Sectors 1, 3, 5 and 7 reresent the central field out to approximately 15°; Sectors 1,2,3,4 represent the nasal hemifield and sectors 5, 6, 7,8 the temporal hemifield.

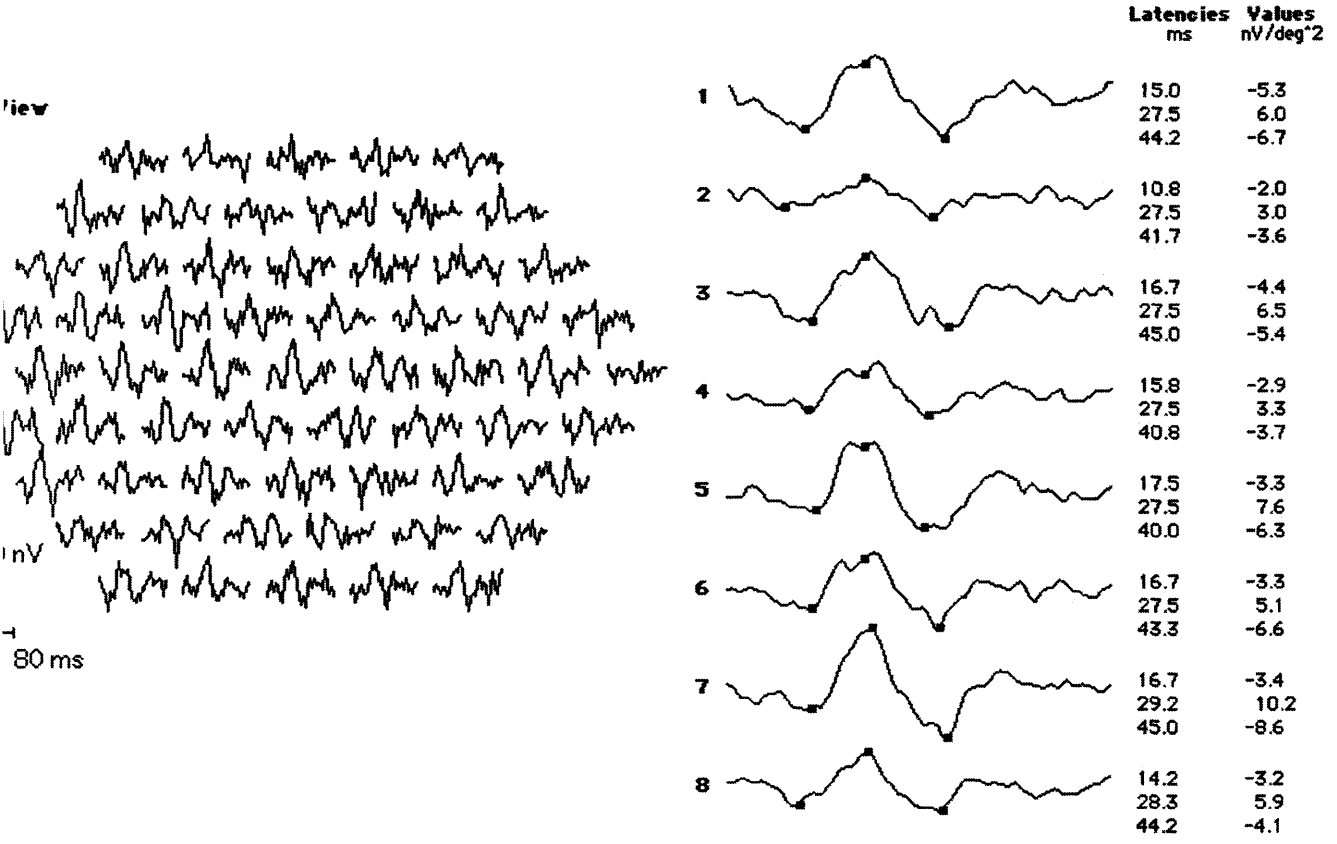


Modelling the mfERG data

One of the major technical difficulties with mfERG analysis relates to the quality of the raw data. The low signal to noise ratio inherent in such responses gives rise to difficulty in determining amplitude and implicit time for each of the three designated waveforms.

An example of an electrically noisy trace is provided in Figure 5.2.5a. An illustration of the VERIS software-assigned values from which the implicit times and amplitudes are calculated is shown in Figure 5.2.5b. It is standard practice to attempt to model raw mfERG traces in order to reduce, if not eliminate, extraneous sources of noise.

Figure 5.2.5a (left) and 5.25.b (right) depicting the raw data from a mfERG trace from the right eye of a healthy individual in 61 traces (5.25a) and grouped into 8 sectors with values determined by VERIS software (5.25b)



5.2.3.3 Fourier Analysis

One standard approach used to reduce noise is to apply a Fourier transformation, with a sharp cut-off after the third harmonic. (Hood et al., 2003a, Seiple et al., 2004) This was applied to all the sector traces before the analysis both of implicit times and amplitudes.

5.2.3.5 Analysis Of The Fourier-Transformed Data

The Fourier-transformed traces for each sector, for each eye, for each individual exposed to VGB were compared to the maxima and minima of the range of Fourier-transformed values, for the right and left eyes respectively, derived from the normal individuals for each of the N1, P1 and N2 amplitude and implicit time. All VGB exposed individuals exhibited normal amplitudes and implicit times for all waveforms in each eye for all sectors. The results are shown in Tables 5.3.1 to 5.3.12.

Sector		1	2	3	4	5	6	7	8
Status		(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)
VAVFL	Mean	47.00	47.80	45.20	44.80	44.40	44.20	44.40	44.20
	N	5	5	5	5	5	5	5	5
	SD	1.58	1.64	1.79	2.17	2.30	2.17	2.30	2.28
	Minima	45.00	46.00	43.00	42.00	42.00	41.00	42.00	42.00
	Maxima	49.00	50.00	48.00	47.00	47.00	47.00	48.00	48.00
VGB-	Mean	47.5	48.00	47.38	47.75	45.88	45.75	46.50	46.38
Exposed	N	8	8	8	8	8	8	8	8
	SD	3.07	3.82	3.46	3.85	2.80	2.92	3.46	3.66
	Minima	44.00	44.00	43.00	44.00	43.00	43.00	43.00	43.00
	Maxima	52.00	55.00	52.00	53.00	50.00	50.00	52.00	53.00
Normal	Mean	46.33	45.93	45.40	45.73	44.40	44.20	44.60	44.73
Control	N	15	15	15	15	15	15	15	15
	SD	4.27	4.92	4.32	3.69	4.22	4.38	4.14	4.30
	Minima	40.00	39.00	39.00	40.00	38.00	38.00	39.00	39.00
	Maxima	57.00	57.00	56.00	54.00	55.00	54.00	55.00	55.00

Table 5.3.1 P1 Implicit times for the right eye only

Sector		1	2	3	4	5	6	7	8
Status		(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)
VAVFL	Mean	44.60	43.40	44.20	43.80	45.80	46.40	45.68	45.00
	N	5	5	5	5	5	5	5	5
	SD	2.61	2.79	2.49	2.17	1.92	1.95	1.82	2.55
	Minima	43.00	41.00	42.00	42.00	44.00	44.00	44.00	42.00
	Maxima	49.00	48.00	48.00	48.00	49.00	49.00	48.40	49.00
VGB-	Mean	47.22	45.67	45.98	46.00	47.00	46.56	47.33	48.22
Exposed	N	9	9	9	9	9	9	9	9
	SD	2.87	2.65	2.16	2.40	3.08	2.40	2.65	3.93
	Minima	43.00	42.00	42.80	43.00	44.00	44.00	44.00	43.00
	Maxima	51.00	49.00	49.00	50.00	52.00	51.00	52.00	55.00
Normal	Mean	45.91	44.83	45.08	45.08	45.33	45.67	46.18	44.75
Control	N	12	12	12	12	12	12	12	12
	SD	4.66	4.59	4.18	3.96	4.60	4.29	4.60	5.33
	Minima	40.00	38.00	39.00	38.00	40.00	40.00	40.00	37.00
	Maxima	57.00	55.00	55.00	54.00	57.00	55.00	57.00	57.00

Table 5.3.2 P1 Implicit Times for the left eye only

Sector		1	2	3	4	5	6	7	8
Status		(nV)	(nV)	(nV)	(nV)	(nV)	(nV)	(nV)	(nV)
VAVFL	Mean	3.16	1.05	2.00	1.67	2.97	2.46	3.35	2.06
	N	5	5	5	5	5	5	5	5
	SD	0.65	0.42	0.80	0.27	0.40	0.42	0.71	0.51
	Minima	2.30	0.53	1.14	1.21	2.48	1.95	2.51	1.43
	Maxima	4.09	1.49	3.06	1.87	3.59	3.02	4.28	2.64
VGB-	Mean	3.26	1.36	3.01	2.28	3.41	2.46	3.13	2.00
Exposed	N	9	9	9	9	9	9	9	9
	SD	2.34	0.68	1.72	0.93	1.12	1.50	1.50	1.34
	Minima	1.69	0.61	1.17	1.34	1.99	1.10	1.82	1.03
	Maxima	9.34	2.70	7.17	4.36	5.38	5.56	6.80	5.44
Normal	Mean	3.94	1.84	3.65	2.56	4.42	2.93	4.58	2.49
Control	N	16	16	16	16	16	16	16	16
	SD	0.98	0.59	0.98	0.88	1.18	1.03	1.12	0.82
	Minima	2.01	0.88	1.68	0.61	0.76	0.76	2.45	1.03
	Maxima	5.77	2.87	5.15	4.13	4.76	4.76	6.29	5.44

Table 5.3.3 P1 Amplitude Values for the right eye only

Sector / Status		1 (nV)	2 (nV)	3 (nV)	4 (nV)	5 (nV)	6 (nV)	7 (nV)	8 (nV)
VAVFL	Mean	2.91	1.44	2.55	1.99	2.60	1.57	2.71	1.43
	N	5	5	5	5	5	5	5	5
	SD	0.67	1.10	0.70	0.67	0.70	0.84	0.49	0.71
	Minima	2.10	0.20	1.89	1.20	1.67	0.72	2.31	0.52
	Maxima	3.79	3.02	3.37	2.86	3.61	2.96	3.48	2.47
VGB-Exposed	Mean	3.73	2.42	3.82	2.63	3.32	2.17	2.63	1.65
	N	8	8	8	8	8	8	8	8
	SD	2.17	1.78	2.22	1.83	2.34	1.98	1.87	1.32
	Minima	1.62	0.63	1.49	1.09	1.37	0.38	0.91	0.42
	Maxima	8.50	6.22	8.47	6.63	8.70	6.48	6.52	4.48
Normal Control	Mean	4.53	2.56	4.14	3.01	4.09	2.04	3.75	2.11
	N	11	11	11	11	11	11	11	11
	SD	1.31	0.96	1.33	1.00	1.72	1.14	1.36	0.58
	Minima	1.96	1.11	2.21	1.51	2.00	1.17	2.17	1.05
	Maxima	6.38	3.61	6.13	4.26	6.97	6.79	6.97	3.17

Table 5.3.4 P1 Amplitude Values for the left eye only

Sector		1	2	3	4	5	6	7	8
Status		(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)
VAVFL	Mean	26.40	26.40	25.40	24.80	24.40	24.00	24.40	24.50
	N	5	5	5	5	5	5	5	4
	SD	1.82	2.70	2.07	1.64	2.30	1.58	1.95	1.91
	Minima	24.00	23.00	23.00	23.00	22.00	22.00	23.00	23.00
	Maxima	29.00	30.00	28.00	26.00	27.00	26.00	27.00	27.00
VGB-	Mean	26.25	26.50	26.00	26.00	25.13	26.13	25.75	25.25
Exposed	N	8	8	8	8	8	8	8	8
	SD	2.38	1.60	2.67	1.93	2.47	2.47	2.25	2.19
	Minima	24.00	25.00	22.00	24.00	23.00	23.00	23.00	22.00
	Maxima	31.00	29.00	30.00	29.00	29.00	30.00	29.00	28.00
Normal	Mean	25.00	25.00	25.25	25.44	24.31	23.94	24.00	24.56
Control	N	16	16	16	16	16	16	16	16
	SD	3.25	3.88	3.39	2.80	2.80	3.32	3.01	2.87
	Minima	20.00	20.00	20.00	20.00	20.00	20.00	19.00	20.00
	Maxima	33.00	34.00	34.00	34.00	31.00	32.00	31.00	32.00

Table 5.3.5 N1 Implicit Times for the right eye only

Table 5.3.6 N1 Implicit Times for the left eye only

Sector		1	2	3	4	5	6	7	8
Status		(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)
VAVFL	Mean	24.60	24.60	24.00	24.20	24.80	26.60	25.00	25.00
	N	5	5	5	5	5	5	5	5
	SD	1.95	1.82	1.87	1.30	1.30	1.82	1.87	1.41
	Minima	23.00	22.00	22.00	23.00	24.00	25.00	23.00	23.00
	Maxima	28.00	27.00	27.00	26.00	27.00	29.00	27.00	26.00
VGB-	Mean	25.67	24.22	25.11	25.22	26.00	25.56	25.33	26.67
Exposed	N	9	9	9	9	9	9	9	9
	SD	1.87	3.46	1.69	1.72	2.34	1.81	2.91	3.50
	Minima	23.00	19.00	23.00	23.00	24.00	23.00	20.00	23.00
	Maxima	29.00	30.00	28.00	29.00	30.00	29.00	30.00	33.00
Normal	Mean	24.72	23.72	25.09	24.90	25.09	25.45	25.54	24.81
Control	N	11	11	11	11	11	11	11	11
	SD	3.52	4.00	3.59	3.27	3.44	3.30	3.70	4.90
	Minima	20.00	16.00	20.00	20.00	20.00	20.00	20.00	18.00
	Maxima	33.00	31.00	33.00	32.00	32.00	31.00	34.00	35.00

Sector		1	2	3	4	5	6	7	8
Status		(nV)	(nV)	(nV)	(nV)	(nV)	(nV)	(nV)	(nV)
VAVFL	Mean	-1.94	-1.02	-1.62	-1.16	-1.58	-1.79	-1.46	-1.08
	N	5	5	5	5	5	5	5	5
	SD	0.92	0.26	0.49	0.64	0.20	0.54	0.86	0.92
	Minima	-3.04	-1.42	-2.28	-2.01	-1.83	-2.74	-2.38	-2.45
	Maxima	-1.01	-0.74	-1.10	-0.29	-1.32	-1.44	-0.16	-0.01
VGB-	Mean	-2.30	-0.97	-2.23	-1.76	-2.70	-2.03	-3.16	-1.62
Exposed	N	9	9	9	9	9	9	9	9
	SD	1.22	0.300	1.15	1.27	2.50	1.55	2.03	0.59
	Minima	-5.21	-1.54	-4.99	-4.88	-9.19	-5.98	-8.31	-2.82
	Maxima	-0.64	-0.62	-1.34	-0.87	-1.15	-1.15	-1.81	-0.72
Normal	Mean	-3.29	-1.49	-3.24	-2.23	-3.46	-2.33	-3.09	-1.97
Control	N	15	15	15	15	15	15	15	15
	SD	1.19	0.62	1.27	0.77	1.12	0.75	1.16	0.64
	Minima	-5.72	-2.55	-5.75	-3.29	-5.46	-3.10	-5.17	-3.05
	Maxima	-1.46	-0.57	-0.93	-1.12	-1.55	-0.87	-1.33	-1.10

Table 5.3.7 N1 Amplitude Values for the right eye only

Sector		1	2	3	4	5	6	7	8
Status		(nV)	(nV)	(nV)	(nV)	(nV)	(nV)	(nV)	(nV)
VAVFL	Mean	-2.29	-1.16	-2.09	-1.47	-1.79	-1.22	-1.81	-1.14
	N	5	5	5	5	5	5	5	5
	SD	0.60	0.17	0.61	0.56	0.59	0.59	0.64	0.19
	Minima	-3.10	-1.42	-3.03	-2.13	-1.88	-1.88	-2.30	-1.42
	Maxima	-1.54	-0.96	-1.34	-0.60	-0.29	-0.29	-0.80	-0.97
VGB-	Mean	-2.58	-1.56	-2.52	-2.24	-2.92	-1.85	-3.05	-1.60
Exposed	N	8	8	8	8	8	8	8	8
	SD	2.17	1.37	2.33	1.47	2.63	1.00	2.18	0.98
	Minima	-7.46	-4.66	-7.93	-5.63	-9.29	-3.75	-7.92	-3.69
	Maxima	-0.55	-0.48	-1.06	-0.87	-1.23	-0.59	-0.82	-0.31
Normal	Mean	-3.36	-1.51	-2.87	-2.01	-2.86	-2.18	-2.84	-1.54
Control	N	12	12	12	12	12	12	12	12
	SD	1.12	0.77	1.28	0.74	1.15	0.86	0.73	0.75
	Minima	-5.44	-3.26	-5.75	-4.17	-4.17	-3.59	-4.23	-2.43
	Maxima	-1.92	-0.36	-0.90	-0.12	-0.12	-0.75	-1.29	-0.23

Table 5.3.8 N1 Amplitude Values for the left eye only

Sector		1	2	3	4	5	6	7	8
Status		(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)
VAVFL	Mean	67.40	67.00	65.80	64.80	64.80	64.00	65.60	64.80
	N	5	5	5	5	5	5	5	5
	SD	2.07	4.06	2.17	2.17	2.39	2.74	3.36	3.42
	Minima	65.00	63.00	64.00	63.00	62.00	60.00	61.00	61.00
	Maxima	70.00	73.00	69.00	68.00	68.00	67.00	70.00	70.00
VGB-	Mean	68.22	68.00	67.25	67.78	65.89	66.89	67.22	66.44
Exposed	N	9	8	8	9	8	9	9	9
	SD	4.05	4.87	4.78	4.63	3.30	4.31	4.21	4.88
	Minima	62.00	64.00	61.00	64.00	62.00	62.00	62.00	62.00
	Maxima	75.00	79.00	74.00	78.00	71.00	74.00	74.00	78.00
Normal	Mean	67.27	65.14	65.53	65.07	64.13	65.00	65.00	64.67
Control	N	15	14	15	15	15	15	15	15
	SD	5.40	4.91	4.53	4.08	5.00	5.10	5.10	6.79
	Minima	61.00	60.00	59.00	60.00	58.00	58.00	58.00	58.00
	Maxima	82.00	80.00	76.00	74.00	78.00	77.00	77.00	82.00

Table 5.3.9 N2 Implicit Time for the right eye only

Sector		1	2	3	4	5	6	7	8
Status		(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)
VAVFL	Mean	64.40	62.60	64.40	63.60	65.60	65.40	65.20	63.80
	N	5	5	5	5	5	5	5	5
	SD	3.21	3.65	2.88	2.30	2.30	2.30	2.77	3.03
	Minima	62.00	60.00	62.00	62.00	62.00	62.00	62.00	62.00
	Maxima	70.00	69.00	69.00	67.00	67.00	67.00	69.00	69.00
VGB-	Mean	68.50	65.75	65.88	65.25	66.50	65.13	66.88	67.63
Exposed	N	8	8	8	8	8	8	8	8
	SD	4.75	3.54	3.39	2.55	3.12	3.13	3.14	4.98
	Minima	62.00	62.00	62.00	63.00	64.00	61.00	63.00	63.00
	Maxima	74.00	71.00	73.00	70.00	72.00	71.00	71.00	76.00
Normal	Mean	66.92	64.64	64.83	64.83	65.25	65.42	66.79	64.67
Control	N	12	11	12	12	12	12	12	12
	SD	7.17	5.61	5.18	4.32	6.08	5.60	7.99	6.07
	Minima	59.00	58.00	56.00	58.00	58.00	57.00	59.50	56.00
	Maxima	85.00	80.00	77.00	75.00	81.00	79.00	90.00	79.00

Table 5.3.10 N2 Implicit Time for the left eye only

Sector		1	2	3	4	5	6	7	8
Status		(nV)	(nV)	(nV)	(nV)	(nV)	(nV)	(nV)	(nV)
VAVFL	Mean	-1.89	-0.68	-1.95	-1.18	-2.68	-2.09	-2.29	-1.21
	N	5	5	5	5	5	5	5	5
	SD	0.45	0.11	0.71	0.56	0.98	0.55	0.64	0.49
	Minima	-2.46	-0.81	-3.00	-1.69	-3.98	-2.67	-3.07	-1.67
	Maxima	-1.28	-0.51	-1.03	-0.24	-1.45	-1.52	-1.44	-0.56
VGB-	Mean	-1.94	-1.21	-1.43	-1.05	-2.55	-1.81	-2.64	-1.30
Exposed	N	9	7	9	8	8	9	9	9
	SD	1.50	0.95	1.03	0.78	1.76	1.07	1.18	0.72
	Minima	-5.45	-2.98	-3.26	-2.40	-6.56	-3.67	-4.83	-2.43
	Maxima	-0.68	-0.20	-0.42	-0.17	-1.02	-0.12	-0.96	-0.05
Normal	Mean	-2.76	-1.16	-2.56	-1.87	-3.34	-2.12	-2.94	-1.68
Control	N	15	14	15	15	15	15	15	15
	SD	1.06	-.48	1.01	0.79	1.33	0.74	1.01	0.73
	Minima	-5.10	-2.30	-4.51	-3.42	-5.57	-3.34	-3.93	-2.86
	Maxima	-0.67	-0.44	-0.47	-0.98	-1.16	-0.50	-0.71	-0.28

Table 5.3.11 N2 Amplitude Values for the right eye only

Sector		1	2	3	4	5	6	7	8
Status		(nV)	(nV)	(nV)	(nV)	(nV)	(nV)	(nV)	(nV)
VAVFL	Mean	-1.89	-0.68	-1.95	-1.18	-2.68	-2.09	-2.29	-1.21
	N	5	5	5	5	5	5	5	5
	SD	0.45	0.11	0.71	0.56	0.98	0.56	0.64	0.49
	Minima	-2.46	-0.81	-3.00	-1.69	-3.98	-2.67	-3.07	-1.67
	Maxima	-1.28	-0.51	-1.03	-0.24	-1.45	-1.52	-1.44	-0.56
VGB-	Mean	-1.94	-1.21	-1.41	-1.05	-2.55	-1.81	-2.64	-1.30
Exposed	N	9	7	9	8	8	9	9	9
	SD	1.50	0.95	1.03	0.71	1.76	1.07	1.18	0.72
	Minima	-5.45	-2.98	-3.26	-6.56	-6.56	-3.67	-4.83	-2.43
	Maxima	-0.68	-0.20	-0.42	-1.02	-1.02	-0.12	-0.96	-0.05
Normal	Mean	-2.76	-1.16	-2.60	-1.87	-3.34	-2.12	-2.94	-1.68
Control	N	15	14	15	15	15	15	15	15
	SD	1.06	0.48	1.01	0.79	1.33	0.74	1.01	0.73
	Minima	-5.10	-2.30	-4.51	-3.42	-5.57	-3.34	-3.93	-2.86
	Maxima	-0.67	-0.44	-0.47	-0.98	-1.16	-0.50	-0.71	-0.28

Table 5.3.12 N2 Amplitude Values for the left eye only

The data (usually from N1 or N2) from some sectors of some individuals in the normal and VGB-exposed groups could not be modelled and therefore is automatically excluded.

In keeping with amplitude data from large normative databases, the amplitude data from this dataset is highly variable across individuals – yielding greater variability and wide confidence intervals.

The minima values for P1 amplitude data were smaller in the VAVFL groups for one individual in sectors 2 and 3 for the right eye and several sectors including 2 and 3 for the left eye. VAVFL N1 amplitudes were similarly smaller but in different sector locations, whilst all bar one sector of the N2 VAVFL amplitudes fell within the range demonstrated by the controls. However, the amplitude values are very small and even the modelled data fails to identify particularly N1 and N2 in both the control and VGB-exposed groups highlighting difficulty interpreting this data.

Analysis Adjusted For Age

It has previously been suggested that the implicit time increases and the amplitude reduces for all mfERG waveforms with increasing age. (Seiple et al., 2003, Tam et al., 2006, Langrova et al., 2008). As a consequence, age-specific confidence intervals were calculated from the normal individuals. These are reproduced in full in the Appendix A.2.1.

The original values for amplitude and implicit time are presented in Tables 5.3.13 to 5.3.24 for each of the waveforms, in each eye, of each of the VGB-exposed

individuals, subdivided into with and without field loss. Any sector values that fall outside the age-adjusted 95% confidence intervals are highlighted in bold. Missing values reflect instances where the data could not be modelled due to excessive noise.

Case	Age (years)	R1 (ms)	R2 (ms)	R3 (ms)	R4 (ms)	R5 (ms)	R6 (ms)	R7 (ms)	R8 (ms)
VAVFL	39	29	28	25	26	27	25	26	
VAVFL	44	26	26	24	26	25	24	23	25
VAVFL	52	26	25	27	23	22	23	23	23
VAVFL	54	27	30	28	26	26	26	27	27
VAVFL	56	24	23	23	23	22	22	23	23
VGB-Exposed	16	24	25	24	24	23	23	23	22
VGB-Exposed	18	**31**	26	**30**	29	**29**	27	**29**	28
VGB-Exposed	31	24	25	26	27	24	24	24	23
VGB-Exposed	43	27	28	27	26	27	25	26	26
VGB-Exposed	48	25	28	26	26	25	26	24	25
VGB-Exposed	55	25	25	22	24	23	23	25	24
VGB-Exposed	55	28	29	29	28	27	30	29	28
VGB-Exposed	59	26	26	24	24	23	23	26	26

Table 5.3.13 N1 Implicit Time values for each of the VAVFL and VGB-Exposed individuals in the right eye only

Case	Age (years)	L1 (ms)	L2 (ms)	L3 (ms)	L4 (ms)	L5 (ms)	L6 (ms)	L7 (ms)	L8 (ms)
VAVFL	39	24	25	24	24	25	29	27	26
VAVFL	44	24	25	24	25	24	26	23	24
VAVFL	52	24	24	22	23	24	25	24	26
VAVFL	54	28	27	27	26	27	28	27	26
VAVFL	56	23	22	23	23	24	25	24	23
VGB-Exposed	16	23	23	23	24	24	23	24	23
VGB-Exposed	18	28	**30**	**27**	29	**30**	27	20	**33**
VGB-Exposed	31	26	28	25	25	25	26	28	27
VGB-Exposed	43	26	19	24	25	29	29	30	31
VGB-Exposed	48	25	24	26	25	24	26	25	25
VGB-Exposed	55	24	23	23	24	24	24	23	23
VGB-Exposed	55	25	22	25	26	25	26	25	26
VGB-Exposed	59	26	22	25	23	25	25	26	28
VGB-Exposed	62	29	27	28	26	28	24	27	24

Table 5.3.14 N1 Implicit Time values for each of the VAVFL and VGB-Exposed individuals in the left eye only

Case	Age (years)	L1 (nV)	L2 (nV)	L3 (nV)	L4 (nV)	L5 (nV)	L6 (nV)	L7 (nV)	L8 (nV)
VAVFL	39	-1.01	-0.74	-1.96	-2.01	-1.68	-1.64	**-0.16**	**-0.01**
VAVFL	44	-1.66	-1.12	-1.10	-0.88	-1.83	-1.70	-1.58	-1.18
VAVFL	52	-3.04	-1.42	-2.28	**-0.29**	-1.58	-2.74	-2.38	-2.45
VAVFL	54	-1.21	-0.9	-1.29	-1.45	-1.32	-1.45	-1.19	**-0.52**
VAVFL	56	-2.77	-0.9	-1.45	-1.16	-1.47	-1.44	-2.01	-1.23
VGB-Exposed	16	-2.08	-0.97	-1.94	-1.08	-2.26	-1.85	-2.19	-1.67
VGB-Exposed	18	-2.57	-0.62	-2.89	-2.02	-3.06	-2.33	-3.69	-1.98
VGB-Exposed	31	-2.38	-0-.70	-2.16	-1.57	-1.69	-1.77	-3.22	-0.72
VGB-Exposed	43	-2.03	-1.19	-2.07	-1.17	-2.35	-1.32	-2.07	-1.65
VGB-Exposed	48	-5.21	-1.54	-4.99	-4.88	-9.19	-5.98	-8.31	-2.82
VGB-Exposed	55	-2.20	-1.16	-1.39	-1.14	-1.15	-1.71	-2.79	-1.80
VGB-Exposed	55	-0.64	-0.63	-1.98	-2.21	-1.50	-1.40	-1.81	-1.55
VGB-Exposed	59	-1.76	-0.93	-1.34	-0.88	-1.35	-0.61	-2.18	-1.19
VGB-Exposed	62	-1.84	-1.02	-1.34	-0.87	-1.76	-1.33	-2.17	-1.17

Table 5.3.16 N1 Amplitude Values for each of the VAVFL and VGB-Exposed individuals in the left eye only

Case	Age (years)	R1 (nV)	R2 (nV)	R3 (nV)	R4 (nV)	R5 (nV)	R6 (nV)	R7 (nV)	R8 (nV)
VAVFL	39	-1.54	-1.12	-3.03	-0.60	-1.00	-0.29	-2.10	-1.42
VAVFL	44	-2.57	-1.42	-2.02	-1.73	-2.17	-1.22	-0.80	-0.97
VAVFL	52	-3.10	-1.10	-2.11	-1.47	-1.88	-1.88	-2.30	-1.25
VAVFL	54	-1.91	-1.22	-1.34	-1.42	-1.93	-1.17	-1.57	-1.06
VAVFL	56	-2.33	-0.96	-1.95	-2.13	-1.98	-1.55	-2.29	-1.00
VGB-Exposed	16	-1.76	-0.79	-1.70	-1.52	-1.67	-0.59	-1.60	-0.90
VGB-Exposed	18	-1.92	-1.85	-1.06	-2.38	-2.26	-1.55	-1.51	-1.95
VGB-Exposed	31	**-0.55**	-0.80	-2.72	-1.94	-1.62	-2.34	-2.86	-1.28
VGB-Exposed	43	-2.01	-0.60	-1.95	-0.87	-2.90	-1.66	-3.22	-1.66
VGB-Exposed	48	-7.46	-4.66	-7.93	-5.63	-9.29	-3.75	-7.92	-3.69
VGB-Exposed	55	-2.26	-1.36	-1.61	-2.34	-2.18	-2.56	-3.40	-1.54
VGB-Exposed	55	-3.60	-1.93	-1.77	-2.07	-2.23	-1.31	-3.05	-1.49
VGB-Exposed	59	-1.04	-0.48	-1.42	-1.16	-1.23	-1.06	-0.82	-0.31

Table 5.3.15 N1 amplitude values for each of the VAVFL and VGB-Exposed individuals in the right eye only

Case	Age (years)	R1 (ms)	R2 (ms)	R3 (ms)	R4 (ms)	R5 (ms)	R6 (ms)	R7 (ms)	R8 (ms)
VAVFL	39	48	49	45	46	46	45	45	44
VAVFL	44	47	46	45	46	45	44	44	44
VAVFL	52	46	47	45	42	42	44	42	43
VAVFL	54	49	50	48	47	47	47	48	48
VAVFL	56	45	47	43	43	42	41	43	42
VGB-Exposed	16	45	45	44	44	43	43	43	43
VGB-Exposed	18	**52**	**52**	51	**53**	49	48	51	50
VGB-Exposed	31	46	47	46	46	44	44	45	44
VGB-Exposed	43	50	49	51	52	48	49	48	48
VGB-Exposed	48	45	44	46	45	46	46	45	44
VGB-Exposed	55	44	45	43	45	43	43	43	43
VGB-Exposed	55	51	55	52	52	50	50	52	53
VGB-Exposed	59	47	47	46	45	44	43	45	46

Table 5.3.17 P1 Implicit Time values for each of the VAVFL and VGB-Exposed individuals in the right eye only

Case	Age (years)	L1 (ms)	L2 (ms)	L3 (ms)	L4 (ms)	L5 (ms)	L6 (ms)	L7 (ms)	L8 (ms)
VAVFL	39	43	42	44	42	45	47	46	45
VAVFL	44	45	44	45	45	46	47	46	45
VAVFL	52	43	42	42	43	45	45	44	44
VAVFL	54	49	48	48	47	49	49	48	49
VAVFL	56	43	41	42	42	44	44	44	42
VGB-Exposed	16	43	42	42	43	44	44	44	43
VGB-Exposed	18	51	49	48	50	52	49	49	**55**
VGB-Exposed	31	47	47	45	45	45	45	49	49
VGB-Exposed	43	50	48	49	47	52	51	52	54
VGB-Exposed	48	46	46	46	47	45	46	45	46
VGB-Exposed	55	43	42	43	43	45	45	44	46
VGB-Exposed	55	49	47	46	47	47	48	48	48
VGB-Exposed	59	47	43	46	44	45	44	47	47
VGB-Exposed	62	49	47	48	48	48	47	48	46

Table 5.3.18 P1 Implicit Time values for each of the VAVFL and VGB-Exposed individuals in the left eye only

Case	Age (years)	L1 (nV)	L2 (nV)	L3 (nV)	L4 (nV)	L5 (nV)	L6 (nV)	L7 (nV)	L8 (nV)
VAVFL	39	2.30	0.71	1.38	1.21	2.48	2.40	3.86	2.30
VAVFL	44	2.93	0.53	2.55	1.87	2.97	2.20	3.01	1.43
VAVFL	52	4.09	1.11	1.14	1.81	2.98	3.02	2.51	2.30
VAVFL	54	3.12	1.40	1.90	1.78	3.59	2.73	4.28	2.64
VAVFL	56	3.37	1.49	3.06	1.66	2.85	1.95	3.09	1.63
VGB-Exposed	16	3.21	1.47	2.78	1.93	3.10	2.01	3.85	2.20
VGB-Exposed	18	2.30	0.61	1.17	1.43	1.99	1.34	2.48	1.30
VGB-Exposed	31	2.77	1.07	2.08	1.78	3.85	3.25	2.59	1.65
VGB-Exposed	43	1.69	0.74	1.84	1.34	2.01	1.76	2.46	1.04
VGB-Exposed	48	9.34	2.70	7.17	4.36	4.68	5.56	6.80	5.44
VGB-Exposed	55	1.94	0.85	3.32	2.03	5.38	2.98	3.07	1.84
VGB-Exposed	55	3.05	1.64	3.44	2.86	3.51	1.91	3.06	2.23
VGB-Exposed	59	2.05	1.12	2.78	2.09	3.25	2.24	1.82	1.29
VGB-Exposed	62	2.96	2.08	2.49	2.70	2.94	1.10	2.06	1.03

Table 5.3.20 P1 Amplitude values for each of the VAVFL and VGB-Exposed individuals in the left eye only

Case	Age (years)	R1 (nV)	R2 (nV)	R3 (nV)	R4 (nV)	R5 (nV)	R6 (nV)	R7 (nV)	R8 (nV)
VAVFL	39	2.10	0.20	1.89	1.97	3.61	2.96	2.31	1.63
VAVFL	44	2.44	0.63	2.21	1.20	1.67	0.72	2.42	**0.52**
VAVFL	52	3.22	1.76	3.24	2.86	2.54	1.23	2.42	1.25
VAVFL	54	3.79	3.02	3.37	2.42	2.79	1.62	3.48	2.47
VAVFL	56	3.00	1.57	2.04	1.51	2.40	1.34	2.90	1.28
VGB-Exposed	16	3.61	2.01	3.52	2.43	2.81	1.92	3.16	1.17
VGB-Exposed	18	2.82	0.63	3.38	1.67	2.54	1.25	1.14	1.08
VGB-Exposed	31	3.37	1.34	3.21	2.43	2.21	0.38	0.91	**0.42**
VGB-Exposed	43	1.62	2.35	1.49	1.20	1.77	1.16	1.47	0.85
VGB-Exposed	48	8.50	6.22	8.47	6.63	8.70	6.48	6.52	4.48
VGB-Exposed	55	3.62	3.14	3.85	1.78	2.87	2.01	3.04	2.49
VGB-Exposed	55	4.58	2.80	5.09	3.81	4.28	3.47	3.50	1.92
VGB-Exposed	59	1.74	0.83	1.56	1.09	1.37	0.68	1.35	0.78

Table 5.3.19 P1 Amplitude values for each of the VAVFL and VGB-Exposed individuals in the right eye only

Case	Age (years)	R1 (ms)	R2 (ms)	R3 (ms)	R4 (ms)	R5 (ms)	R6 (ms)	R7 (ms)	R8 (ms)
VAVFL	39	63	61	62	62	63	62	64	62
VAVFL	44	64	62	65	65	67	67	62	62
VAVFL	52	62	60	64	62	67	67	67	64
VAVFL	54	70	69	69	67	68	67	69	69
VAVFL	56	63	61	62	62	63	64	64	62
VGB-Exposed	16	69	66	66	63	65	61	66	66
VGB-Exposed	18	69	66	64	65	65	64	68	66
VGB-Exposed	31	62	63	63	63	64	64	64	64
VGB-Exposed	43	63	62	62	63	64	64	63	63
VGB-Exposed	48	74	64	67	67	70	64	68	76
VGB-Exposed	55	74	71	73	70	72	70	71	74
VGB-Exposed	55	65	63	65	64	64	65	64	63
VGB-Exposed	59	72	71	67	67	68	69	71	69

Table 5.3.21 N2 Implicit Time values for each of the VAVFL and VGB-Exposed individuals in the right eye only

Case	Age (years)	L1 (ms)	L2 (ms)	L3 (ms)	L4 (ms)	L5 (ms)	L6 (ms)	L7 (ms)	L8 (ms)
VAVFL	39	65	64	64	63	62	60	64	61
VAVFL	44	67	63	65	64	65	63	66	64
VAVFL	52	69	73	67	66	66	66	67	66
VAVFL	54	70	69	69	68	68	67	70	70
VAVFL	56	66	66	64	63	63	64	61	63
VGB-Exposed	16	68	66	67	65	64	63	65	65
VGB-Exposed	18	67	67	65	65	64	64	66	65
VGB-Exposed	31	62	64	61	64	62	62	62	62
VGB-Exposed	43	64	64	64	64	63	63	63	62
VGB-Exposed	48	72			67	66	73	72	69
VGB-Exposed	55	71	70	74	**78**	70	73	69	66
VGB-Exposed	55	66	66	64	66	64	66	64	64
VGB-Exposed	59	75	**79**	74	72	71	70	74	78
VGB-Exposed	62	69	68	69	69	69	68	70	67

Table 5.3.22 N2 Implicit Time values for each of the VAVFL and VGB-Exposed individuals in the left eye only

Table 5.3.23 N2 Amplitude values for each of the VAVFL and VGB-Exposed individuals in the right eye only

Case	Age (years)	R1 (nV)	R2 (nV)	R3 (nV)	R4 (nV)	R5 (nV)	R6 (nV)	R7 (nV)	R8 (nV)
VAVFL	39	-2.45	-1.54	-5.00	-2.13	-2.92	-0.51	-3.64	-1.42
VAVFL	44	-1.60	-1.1	-1.84	-1.42	-1.29	-1.13	**-0.06**	**-0.32**
VAVFL	52	-3.55	-2.49	-2.72	-1.25	-1.25	-1.34	-2.01	-1.27
VAVFL	54	-1.82	-1.09	-1.23	-1.47	-1.20	-0.16	0.57	
VAVFL	56	-2.53	-1.52	-2.46	-2.31	-2.23	-1.27	-1.97	-0.96
VGB-Exposed	16	-2.20	-1.16	-2.19	-1.47	-2.15	-0.98	-1.29	-0.77
VGB-Exposed	18	-0.82	-0.99	-0.33	**-0.20**	-0.38			**-0.68**
VGB-Exposed	31	-1.30	-0.39	-2.43	-2.23	-1.87	-2.45	-1.66	**-0.38**
VGB-Exposed	43	-2.18	-0.47	-1.35	-1.63	-0.94	-0.39	-1.17	
VGB-Exposed	48	-9.32	-3.60	-5.16	-3.60	-2.95	-2.07	-6.34	-3.04
VGB-Exposed	55	-2.44	-1.26	-1.55	-2.01	-1.38	-0.91	-2.24	
VGB-Exposed	55	-2.24	-1.19	-1.25	-1.26	-1.78	-0.17	-1.97	-0.98
VGB-Exposed	59	-0.95	-0.54	-1.00	-0.56	-1.02	-0.45	-0.62	-0.33

Table 5.3.24 N2 Amplitude values for each of the VAVFL and VGB-Exposed individuals in the left eye only

Case	Age (years)	L1 (nV)	L2 (nV)	L3 (nV)	L4 (nV)	L5 (nV)	L6 (nV)	L7 (nV)	L8 (nV)
VAVFL	39	-2.05	-0.69	-3.00	-1.20	-3.22	-2.66	-2.52	-0.56
VAVFL	44	-1.28	-0.75	-1.03	-0.24	-1.62	-1.62	-1.85	-1.59
VAVFL	52	-2.01	-0.81	-2.10	-1.69	-3.07	-2.67	-3.07	-1.39
VAVFL	54	-1.63	-0.51	-1.88	-1.43	-1.44	-1.52	-1.44	-0.84
VAVFL	56	-2.46	-0.65	-1.72	-1.35	-2.55	-2.00	-2.55	-1.67
VGB-Exposed	16	-1.96	-0.87	-1.74	-0.90	-2.47	-2.21	-2.47	-1.21
VGB-Exposed	18	**-0.78**		-0.77		-1.61	-1.20	-1.61	-0.98
VGB-Exposed	31	-2.66	-0.52	-2.36	-1.23	-2.97	-2.54	-2.97	-1.88
VGB-Exposed	43	-1.34	-0/82	**-0.42**	-0.55	-0.96	-0.12	-0.96	**-0.05**
VGB-Exposed	48	-5.45	-2.98	-3.26	-2.40	-4.83	-3.67	-4.83	-1.18
VGB-Exposed	55	-1.39	-1.18	-0.66	-1.39	-3.93	-1.61	-3.93	-2.43
VGB-Exposed	55	-0.76		-0.72	**-0.17**	-2.04	-2.18	-2.04	-1.31
VGB-Exposed	59	-0.68	-0.20	-0.48	-0.41	-2.08	-0.60	-2.08	-0.68
VGB-Exposed	62	-2.40	-1.89	-2.31	-1.34	-2.85	02.15	-2.85	-1.98

The age-adjusted amplitude data largely falls within the 95% confidence intervals for age. Although there are exceptions, these occur in varied individuals and varied sectors, with no clear topographical or individual patterns. These outlying values are very likely to reflect technical limitations of the mfERG with respect to signal-to-noise ratios.

5.2.4 Discussion

The results indicate that exposure to VGB, at least within the range for 12 of the 13 individuals included in the study does not influence either the implicit time or the amplitude of the three mfERG waveforms when compared to the maxima and minima of the range of values from a control group of 13 individuals and in 12 of 13 individuals when compared to the age-adjusted confidence intervals. However, the outlying individual is different in the two modes of analysis. This finding applies whether the stimuli were of central or peripheral origin or of nasal or temporal origin.

The outlier in the age- adjusted analysis was an 18-year-old male with normal fields who yielded increased implicit times at the 95% confidence intervals in certain sectors for both N1 and P1. The N2 response was within normal limits. However, even this individual had values that fell within the 90% confidence intervals for these same sectors. He was myopic and was corrected for the study according to his current refraction prescription. Uncorrected myopia increases implicit time for all three waveforms of mfERG (Chen et al., 2006a) and it is possible that his prescription represented an under-correction of the myopia.

The negative outcome for the detection of VGB toxicity of the mfERG in the current study should be placed in the context of results from other mfERG studies of VGB toxicity. Such studies have been conducted, as in the current study, with small cohorts of individuals exposed to VGB and have reported low frequencies of various types of mfERG abnormality. The low frequency of abnormality combined with the diversity of the abnormality suggests that the mfERG recorded out to 30° eccentricity is of limited value in the detection of VGB toxicity. This is unfortunate, given the apparent utility of mfERG for the investigation of visual dysfunction in children and learning-disabled adults.

The findings are seemingly at odds with those found for the wide-field mfERG, which exhibited 100% sensitivity for the detection of VAVFL and 86% specificity in 19 of 32 patients exposed to VGB for greater than 3 years. (McDonagh et al., 2003) The discrepancy between the wide-field mfERG literature and the standard mfERG literature may arise simply from the fact that VAVFL is a predominantly peripheral abnormality. However, all 4 individuals with VAVFL in this study manifested substantial field loss within the central field out to 30° eccentricity.

An alternative explanation for the negative outcome of the mfERG in the current study is that the underlying generators of the mfERG waveforms are not influenced by VGB toxicity, at least in the early stages of VGB dysfunction. It is entirely plausible that the standard mfERG does not identify VGB toxicity. Given the advances since beginning this thesis in structural quantification of VGB toxicity,

it seems more likely that there is retinal ganglion cell loss either as a primary pathology, or at least as an early stage in the pathogenesis. As the mfERG is largely derived from bipolar cells (amplitude) and photoreceptors (implicit time) and there is little contribution from the ganglion cells (occasionally producing a trailing ledge on the P1) it is perhaps not surprising that the mfERG results should be normal. This latter argument is consistent with the increased implicit time in the periphery on wide field –mfERG examination which can be explained by an advanced cellular dysfunction peripherally with likely involvement of the reduced numbers of bipolar and photoreceptor cells with increasing eccentricity.

5.2.5 Conclusions

There is no evidence from this study that standard mfERG can identify VGB toxicity. The most likely reason for this finding lies in the cellular derivation of the mfERG. It would seem reasonable to conclude that retinal toxicity attributable to VGB is not primarily mediated by bipolar or photoreceptor cells. Given that retinal ganglion cell dysfunction does not affect the mfERG, the latter cell type still remains a candidate for the primary lesion in VGB toxicity.

More directed electrophysiology aimed at determining ganglion cell abnormality including the PERG would seem a fruitful area for further research.

Chapter 6: The mfVEP In VGB Toxicity

6.1 Utility Of mfVEP In Assessing Vigabatrin Toxicity

6.1.1 Introduction to mfVEP

Standard VEP technology has been available for some 60 years. The VEP is the electroencephalographic response, recorded at the primary visual cortex (V1), to a visual stimulus. The response reflects the combined function of the visual pathway and will contain contributions from the retina, optic nerve, optic chiasm and radiation, and cortex. The VEP is, therefore, anatomically non-specific; however, it provides the only functional visual cortical measure currently available. Localisation of any abnormality is difficult without consideration of other findings from ophthalmological and neurological examinations.

Some of the general principles of standard VEP also apply to the multifocal VEP. Since both are recorded at the primary visual cortex (V1) an understanding of neuroanatomy is essential for interpretation of the VEP and mfVEP responses. The primary visual cortex is infolded to form the calcarine sulcus. Visual mapping of the visual field at the cortex is in keeping with the semi-decussation of the retinal ganglion fibres at the optic chiasm; consequently the right hemifield is represented in the left cortex and the left hemifield is represented in the right cortex. Importantly, the upper visual field is represented below the calcarine sulcus, and the lower visual field is represented above the calcarine sulcus. However, a further principle of cortical sense mapping also applies the primary visual cortex; the cortex devotes more

space and neural ability to those areas considered to be of greater importance. This is ensured by the greater representation of the projections of the retinal ganglion cells from the fovea. Thus, the central 10° radius is represented by a disproportionately large caudal part of the cortex. Consequently, the VEP and the mfVEP are dominated by the central visual field. In contrast, the peripheral visual field is less well represented for two reasons. Firstly, there are fewer projections of the retinal ganglion cells from the periphery and less cortical area devoted to the peripheral field. Secondly, this latter area of V1 lies deeper in the calarine sulcus and an electrophysiogical response is, as a consequence, difficult to obtain.

6.1.2 Description Of The mfVEP

Multifocal technology permits simultaneous recording of multiple local mfVEP responses. As with the mfERG, a single continuous trace recording is taken; from these 60 responses are derived from a mathematical model based in the pseudorandom sequence of frame shifts referable to set locations in the multifocal display. Thus, 60 responses are derived which represent an area within a radius of 25° from fixation (i.e. the central visual field).

6.1.3 Normative values and repeatability

Normative values have been published for the mfVEP but the results from any one centre are not generalisable to other centres. Each centre, therefore, is required to establish its own normative age-related dataset and corresponding local

confidence limits. Clearly, any limitations relating to a given centre, such as the presence and magnitude of ambient electrical noise, will affect the magnitude of the confidence limits. However, there are also large between-individual differences in the normal mfVEP response that mitigates against the establishment of narrow confidence intervals. The major reason for the between-individual variation in the normal mfVEP response is the between-individual variation both in surface anatomy and also in neuroanatomy. The mfVEP recordings are taken with reference to the inion, a skull notch over the occipital lobe. This anatomical surface marker varies between individuals with respect to size, projection and location and hence with respect to the relative location of the visual cortex. Whilst brain MRI with marking could improve the accuracy of mfVEP recording, it is not a pragmatic clinical option.

The brain foldings (sulci) of each individual are unique in both depth and position and this holds true for the calcarine sulcus. Consequently, mfVEP recordings may originate from across, above or below the calcarine sulcus dependent upon its positioning. The depth of the sulcus will also affect the amplitude mfVEP but should not impact upon the latency.

Most attempts to analyse the mfVEP are based upon the identification of a change in amplitude. (Zhang et al., 2002, Fortune et al., 2004, Klistorner and Graham, 2005)

The analysis of amplitude is limited by between-individual variations in the normal response, which affect the size of any confidence interval for the mfVEP response. Such sources of between-individual variability include the skull thickness and the

localisation of the visual cortical midline (with reference to the inion) that in turn defines the proportion of stimuli analysed by the respective cortical hemifields. The within-individual between-test variability is a further confounding factor in the interpretation of the mfVEP. A between-test variability of 20-30% has been reported in normal individuals based upon the sampling of 12 pre-determined locations of the mfVEP. (Klistorner and Graham, 2005) A slightly lower between-test variability, 15%, is present when all locations are considered individually and in clusters. (Fortune et al., 2004)

A widely adopted method to improve diagnostic ability in the presence of highly variable responses is that of between-eye comparison. (Hood et al., 2004b) However, such a between-eye approach is not well suited to a bilateral and symmetrical disease such as that seen in VGB toxicity.

The analysis of mfVEP implicit time is, in general, also based upon a between-eye approach (Hood et al., 2004b) although analyses of monocular latency have been reported using customised software via a method of 'template stretching' where the waveforms are stretched to a 'best fit' within pre-ordained limits. (Hood et al., 2004a, Rodarte et al., 2006) However, at the time of writing no commercially available software exists for the analysis of implicit time in this manner. Furthermore, there is no accepted method for the analysis of latency and the lack of a standardised method makes comparison of implicit times across studies difficult.

6.1.4 The Relationship Of The mfVEP To The Conventional VEP

The mfVEP response bears a superficial resemblance to the conventional VEP; there are two identifiable deflections. The first (C1) is a positive deflection at 65ms analogous to N75 and the second (C2) is a negative deflection at approximately 95ms analogous to P100. However, these responses are not 'little' VEPs, (Fortune et al., 2004) and the slight latency differences in C2, along with the smaller C2 response compared with P100, bear testimony to this. It is speculated that the mfVEP response has a larger extrastriate contribution than the conventional VEP. (Hood et al., 2003b)

6.1.5 Relationship Of The mfVEP To Static Perimetry

The results of mfVEP have been compared to the results from static threshold perimetry, i.e. the differential light threshold. One comparative approach is simply to overlay the two sets of results at each location within the visual field. This approach requires the scaling of the mfVEP responses to account for the central dominance of mfVEP. (Hood and Greenstein, 2003) A moderate correlation between mfVEP amplitude and differential light sensitivity has been demonstrated for optic neuritis (Klistorner et al., 2008) and glaucoma using this method. (Hood and Greenstein, 2003)

6.1.6 The mfVEP In Vigabatrin Toxicity

The majority of studies which have used the VEP to investigate vigabatrin toxicity in adults have reported normal results (Liegeois-Chauvel et al., 1989, Eke et al., 1997, Mauguiere et al., 1997, Wilson and Brodie, 1997, Reuther, 1998) Lawden et al., 1999). A normal VEP has also been found in children.(Uldall et al., 1995) However, three papers involving two adult cohorts have reported an abnormal VEP in 25% of four cases of VAVFL (Krauss et al., 1998), 22% in 18 cases of VAVFL (including the 4 individuals reported by Krauss and associates) (Miller et al., 1999) and 30% of 10 cases, respectively. (Daneshvar et al., 1999) However, the majority of patients in the latter cohort exhibited severe VAVFL. An abnormal VEP has also been reported in 30% of 15 children exposed to VGB; of the 5 deemed to have abnormal VEPs, 4 had VAVFL. (Gross-Tsur et al., 2000) A possible explanation for abnormal VEPs in some cohorts might be related to the central dominance of the conventional VEP and the late involvement of the central visual field in severe VAVFL. At the time of writing, no reports are available of the utility of mfVEP in VAVFL.

The pathogenesis of VGB toxicity remains uncertain; however; the retinal ganglion cell layer remains a likely location. Whilst cellular correlation is less well understood for the mfVEP as compared with the mfERG, some functional-pathological correlations are accepted. Ganglion cell loss in glaucoma is inferred by the reduction of the mfVEP signal amplitude. (Hood and Greenstein, 2003) However, alterations in mfVEP latency/ implicit time do not seem to be altered in glaucoma. (Grippo et al.,

2006, Rodarte et al., 2006) Retinal disease (autoimmune retinopathy, vascular retinal disease and non-specific retinopathies) has been reported to show increases in implicit time. (Chen et al., 2006b)

6.2 The utility of the mfVEP in VGB toxicity

6.2.1 Aims

The overall aim of the study was to investigate whether the mfVEP identified retinal dysfunction attributable to VGB and, if so, the topographical relationship between the abnormality and the visual field loss. More specifically, the aims were threefold. Firstly, to investigate whether a reduction in amplitude was present for the mfVEP waveform thereby implicating retinal ganglion cell dysfunction in the pathophysiology of VGB toxicity. Secondly, to quantify the relationship between any abnormality in amplitude of the mfVEP waveform and the severity of the visual field loss. Thirdly, to quantify the relationship between any abnormality in amplitude of the mfVEP waveform and the daily dose and duration of VGB intake.

6.2.2 Methods

6.2.2.1 Demographics of the study cohort

The cohort comprised three groups of individuals; one group of 5 individuals with

VAVFL, one group of 9 individuals exposed to VGB but with normal fields and a third group of 16 normal individuals. Normal individuals were recruited from colleagues, fellow post-graduate students, friends and family. The VGB exposed adults were recruited from the Welsh Epilepsy Unit, University Hospital of Wales, Cardiff, and the adolescents from the Paediatric Neurology and Adolescent services at the University Hospital of Wales, Cardiff.

6.2.2.2 Inclusion Criteria

All individuals conformed to rigid inclusion criteria in as described in Chapter 3.2. Briefly, each eye exhibited a distance refractive error of less than or equal to 5 dioptres mean sphere and less than 2.5 dioptres cylinder; a visual acuity of 6/9 or better; open angles, clear ocular media; no fundal or optic nerve head abnormalities characteristic of known disease other than VGB toxicity; no previous ocular surgery or trauma; no history of diabetes mellitus and no family history of glaucoma.

The demographics of the cohort are detailed in Table 6.2.1. The cohort control group was younger than either of the VGB-exposed groups by approximately 7 and 12 years respectively. However, no effect of age has been reported in mfVEP, in contrast to mfERG. (Fortune et al., 2004)

Group (Total)	Gender		Mean Age (yrs) (SD)	Mean Duration of Epilepsy (yrs) (SD)	Mean Duration of Vigabatrin (yrs) (SD)	Cumulative dose of Vigabatrin (kg) (SD)
	Male	Female				
VAVFL (5)	1	4	49.00 (7.2)	25.60 (8.2)	9.49 (1.6)	11.03 (2.5)
VGB-Exposed (9)	4	5	42.60 (16.5)	26.30 (10.53)	7.30 (2.6)	6.79 (2.37)
Controls (16)	7	9	37.62 (12.4)	0.0	0.0	0.0

Table 6.2.1 The summary measures (Group Mean, Standard Deviation [SD]) of the demographical characteristics for each of the three groups

6.2.2.3 Patient Set-up

Prior to recording the mfVEP, all patients underwent standard neurological examination. At the time of the study, and at the time of writing, no agreed ISCEV mfVEP guidelines are available. Therefore, the set-up used for all patients in this study was in accordance with that of Hood and colleagues who use an identical VERIS system. (Hood et al., 2003b)

Patients were placed in a fully lighted room (the recording room) for 15 minutes prior to recording and, therefore, were light adapted. Disposable electrodes were used

as described for the mfERG. Skin preparation and electrode fixation was achieved using the same products as that described for the mfERG in Chapter 5.2. The recording electrodes were secured with cotton gauze squares. The ground electrode was secured at the forehead with micropore. The standard two vertical and horizontal recording channels were used which were both referenced to the palpated inion. The vertical channels were applied 3 cm above and 1 cm below the inion, respectively. The horizontal channels were applied 1cm above the inion, and 2 cm either side of the midline, respectively.

Impedance was measured for all the recording electrodes, compared with the ground, using a commissioned ohmmeter made by the Department of Medical Physics at the Queens Medical Centre, University of Nottingham. Impedance was deemed acceptable if <5kΩ, and if both recording channels were within 2kΩ of each other (Figure 3.3.2). Patients sat in front of the screen at a recording distance of 50 cm wearing the refractive correction optimised for the viewing distance. Fixation was observed by direct observation of the patient. Responses were recorded monocularly for each eye.

6.2.2.4 Stimulus Properties

A high-resolution CRT monitor with a frame rate of 75 Hz was used with a screen luminance of 150 cd/m^2 in full room lighting. The stimulus pattern was a pre-set standard of 60 polygons. The size of the polygons had been scaled for cortical

magnification by the manufacturer; the central sectors subtended 1° at the eye whilst the peripheral sectors subtend 7° at the eye. The stimuli were presented in the default manner using the m-sequence, i.e., with no additional or modified stimulus frames. The stimulus field subtended a radius of 25°. A red cross, set at a diameter of 0.5°, served as the fixation target. The gain settings were 100,000 with a bandpass filter of 3 and 100 Hz. The notch filter was not used.

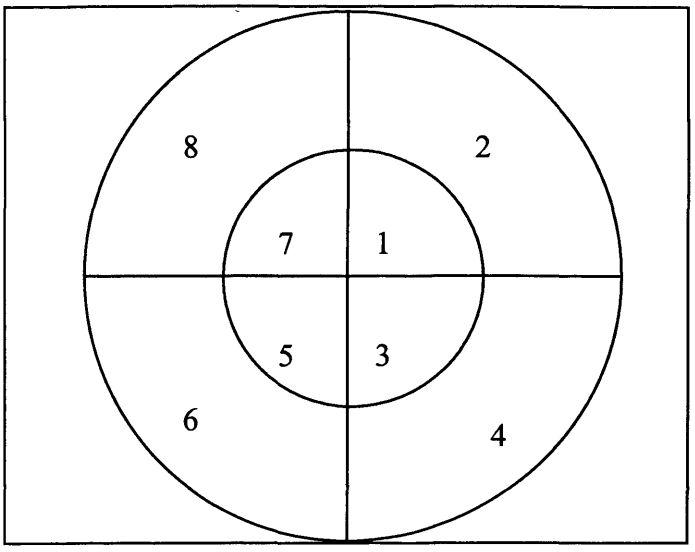
6.2.3 Analysis

All responses were analysed using the VERIS 5.1 software with the standard mfVEP Analysis 60 Protocol. The averaging function, which permits averaging of each trace with 17% of its neighbours, was not activated.

6.2.3 Sector Analysis Of mfVEP

As discussed in Chapter 5 (5.2.3.2), the customised grouping of traces is desirable as the procedure permits specific regional comparisons and produces improvements in trace quality and narrower confidence intervals for normative data. The mfVEP traces were grouped in identical sectors as those used for the analysis of the mfERG. (Figure 6.2.3.1)

Figure 6.2.3.1 The Custom Grouping Of The 61 Traces Into 8 Sectors, Sectors 1, 3, 5 and 7 represent the central field out to approximately 15°; Sectors 1,2,3,4 represent the nasal hemifield and sectors 5, 6, 7,8 the temporal hemifield.



6.2.3.3 Establishing signal-to–noise ratios and estimating amplitude

As discussed above (Section 6.1.3) there are wide between-individual variations in the normal mfVEP trace and also the inherent difficulty of recording a small signal in the presence of electrical and muscular noise of any magnitude.

The analytical approach utilised signal-to-noise ratios as a measure of amplitude. This technique calculates the root-mean-square analyses of the signal window divided by the averaged signal of the noise sector. (Zhang et al., 2002) Thus, two time periods of the complete mfVEP trace are analysed; firstly, the signal window (45- 150ms); and secondly, a noise window (325 – 430ms). The traces at each location were custom

grouped as illustrated in Figure 6.2 above and SNR measures were used as a marker of amplitude.

6.2.4 Results

The group mean mfVEP amplitude and the group maximum and the group minimum values for the group with VAVFL and for the group exposed to VGB but with normal fields are compared to the corresponding values for the normal individuals in Table 6.2.2. The vast majority of individuals exposed to VGB exhibited an amplitude in each eye which was greater than the lowest value exhibited by any individual in the normal group for any sector. As is evident from Table 6.2.1 VGB- exposed individuals manifesting normal fields fell outside the normal range for the right eye in sectors 4 and 5. These represent two solitary individuals with one low amplitude value in sector 4 and 5 respectively. The remainder of the individuals in the VGB-exposed group exhibited values greater than the second lowest value demonstrated in the normal group. Table 6.2.2 demonstrates greater abnormality for both VAVFL and VGB-exposed groups. Again, all the abnormalities represent solitary individuals falling outside the range and only one individual in the VAVFL group exhibited low values in two sectors; the remainder only exhibited one abnormal sector. As with the right eye values, all those individuals falling within the normal range, had minimum values greater than the second lowest values in the normal group, or in the case of sectors 2 and 4 for the VAVFL group, greater than the third lowest value. Thus, there was no similarity in the pattern of apparent abnormality between eyes of an individual or between individuals.

Table 6.2.1 RMS SNR as markers for amplitude values for the right eye only

Sector		1	2	3	4	5	6	7	8
Status									
VAVFL	Mean	2.73	3.91	6.38	7.48	2.04	2.14	3.13	2.95
	N	5	5	5	5	5	5	5	5
	SD	0.89	7.81	7.81	6.43	0.75	1.04	1.51	1.82
	Minima	1.81	1.49	1.49	1.71	1.41	1.35	1.41	1.08
	Maxima	3.57	15.39	15.39	14.41	2.86	3.32	4.23	4.71
VGB-	Mean	2.56	1.82	2.60	2.83	2.11	2.79	4.81	1.54
Exposed	N	9	9	9	9	9	9	9	9
	SD	2.35	0.45	1.13	2.49	1.62	2.60	5.07	0.44
	Minima	0.92	1.15	1.25	0.56	0.50	0.94	1.04	0.94
	Maxima	5.95	2.12	3.74	6.14	4.26	6.64	12.28	1.90
Normal	Mean	5.18	4.09	2.98	4.21	5.76	5.51	3.58	3.21
Control	N	16	16	16	16	16	16	16	16
	SD	2.91	2.55	1.70	2.51	5.94	4.94	1.89	2.09
	Minima	0.80	0.73	0.99	1.09	1.72	0.82	0.61	0.78
	Maxima	11.31	10.21	6.60	10.82	26.30	21.02	8.09	9.43

Table 6.2.2 RMS SNR as markers for amplitude values for the left eye only

Sector		1	2	3	4	5	6	7	8
Status									
VAVFL	Mean	2.42	2.06	4.01	5.13	2.93	3.16	2.25	2.11
	N	5	5	5	5	5	5	5	5
	SD	1.46	1.44	3.46	3.43	0.80	0.44	0.63	1.09
	Minima	0.78	0.68	1.18	2.27	2.01	3.11	1.81	1.13
	Maxima	3.58	3.55	7.87	8.93	3.41	3.19	2.97	3.28
VGB-	Mean	2.66	3.95	3.61	3.80	2.80	3.53	2.08	2.27
Exposed	N	9	9	9	9	9	9	9	9
	SD	1.12	3.23	3.15	2.82	2.11	1.94	1.16	1.65
	Minima	1.25	0.58	1.23	1.62	0.86	0.82	0.58	0.76
	Maxima	4.04	8.84	8.31	8.50	5.39	5.60	3.65	5.08
Normal	Mean	5.36	4.48	3.07	3.39	5.29	4.74	3.19	5.12
Control	N	15	15	15	15	15	15	15	15
	SD	3.50	3.01	1.48	1.74	2.63	3.14	1.56	4.77
	Minima	1.34	1.28	1.25	1.54	0.98	1.05	0.45	1.14
	Maxima	12.90	13.25	5.62	6.96	10.08	13.17	5.92	18.05

6.2.5 Discussion

The mfVEP amplitude failed to identify the effects of VGB toxicity. There are two major explanations for this finding.

The first possibility is that retinal ganglion cells do not demonstrate dysfunction in VGB toxicity. This theory cannot be discounted. However, retinal ganglion cell disruption has been observed in the description of the one post mortem eye with VAVFL (Ravindran et al., 2001) and given the structural changes to the RNFL in VGB toxicity, the retinal ganglion cell layer seems a highly plausible pathological target. (Wild et al., 2006, Durnian and Clearkin, 2007)

The second, more plausible, theory relates to the discriminatory ability of the mfVEP to identify regional retinal ganglion cell layer dysfunction. Retinal ganglion cell layer dysfunction in glaucoma is represented by a reduction in mfVEP amplitude. (Hood and Greenstein, 2003) An interocular approach has been recommended for clinical monitoring with the mfVEP in glaucoma due to the accepted difficulties with establishing satisfactory confidence intervals for normative databases, (Hood and Greenstein, 2003) and to the additional difficulties of high within-individual between-test variability. However, scrutiny of the illustrated cases within the literature suggests that the predominant field loss detected by mfVEP lies close to fixation, i.e. within a 15° radius. This is consistent with the finding that the mfVEP is useful in the

confirmation of those cases of optic neuritis manifesting abnormality in the extreme central location. (Klistorner et al., 2008) It is likely, therefore, that although the mfVEP stimuli are located out to 30° eccentricity, and that such stimuli are scaled for eccentricity, there remains a strong central dominance in each response due to the cortical representation of the visual field within the visual cortex. Consequently, the mfVEP may not identify dysfunction beyond approximately 15°. In the most severe form of VAVFL, the field loss never encroaches within 15° eccentricity.

A further factor inhibiting the identification by mfVEP of ganglion cell layer dysfunction attributable to VGB is the technical limitations of the monocular analysis with respect to wide normative confidence intervals and high variability encountered in the normal individual. (Fortune et al., 2004, Klistorner and Graham, 2005). Given the bilateral and symmetrical nature of VGB dysfunction, any interocular analysis of the mfVEP amplitude, is not feasible. It is possible that the prospective repetitive monitoring of a given individual treated with VGB may identify an amplitude reduction of the mfVEP within that expected from standard confidence intervals, thus obviating the need for comparison with a normative database.

Implicit time was not assessed in this study. Given that no abnormality was identified in amplitude arising from either VAVFL or VGB dysfunction, and given the topography of VAVFL relative to the stimulus location and central dominance of the response, it seemed unlikely that any abnormality would have arisen in the implicit time. Nevertheless, attempts were undertaken to design a custom template stretching

program using MATLAB, but the software had not been completed at the time of writing.

6.2.5 Conclusion

The mfVEP as recorded in this study identified neither VGB toxicity nor exposure to VGB. The lack of evidence is in direct conflict with the unequivocal evidence for RNFL thinning by OCT. Failure to identify any abnormality in amplitude is likely to arise from the disparity between the cortical functional topography of the mfVEP and the location of VAVFL and also from the technical limitations associated with the monocular analyses of the mfVEP necessary in individuals manifesting a bilateral, symmetrical dysfunction, such as that arising from VGB toxicity.

On the basis of the evidence from this study, the mfVEP as recorded in this study cannot be recommended as a screening tool for identifying VGB toxicity.

Chapter 7: General Summary Of Results And Proposals For Future Work

7.1 Summary Of Results And Conclusions

7.1.1 Utility of Retinal Nerve Fibre Layer Thickness as Measured by OCT in VGB-Attributed Visual Dysfunction

VGB is currently under application with the FDA for licensing in the United States for use in Infantile Spasms and adjunctive use in partial-onset epilepsy. (Ovation, 2008) This renewed interest, and the likely increase in VGB prescribing worldwide, should lead to greater emphasis on the visual monitoring of VGB-exposed patients. This thesis has shown that an attenuated nasal quadrant retinal nerve fibre layer thickness with a normal temporal quadrant thickness, as measured by optical coherence tomography (OCT) is a characteristic indicator of VGB toxicity. The ease and accessibility of OCT lends credence to the argument that OCT should now become the principal means for monitoring VGB toxicity and, indeed, should be mandatory for all individuals receiving VGB. Given the limitations of perimetry with respect to patient understanding and cooperation, particularly prevalent in those with refractory epilepsy, and the unacceptably long chair time, perimetry should no longer be relied upon in isolation for the detection of VGB toxicity.

The chair time for OCT is much shorter than for perimetry, the results are objectively acquired and the technique is applicable to children as young as 3 years old. The size

of the current equipment necessary for OCT is a practical limitation for the examination of neonates and young infants. Modification of OCT technology (including the possibility of a hand-held imaging component) would enable the imaging of neonates, which is of importance in other ocular conditions as well as in West Syndrome. The creation of OCT normative databases for infants would enable greater clinical confidence in paediatric decision-making.

The finding of nasal quadrant RNFL attenuation together with a normal temporal quadrant is unique to VGB toxicity (Chapter 3) and will prove invaluable in unequivocally identifying VGB toxicity. The pattern of attenuation is consistent with the presence of inverse optic atrophy assocated with VGB toxicity. (Frisen and Malmgren, 2003, Buncic et al., 2004) Furthermore, the identification of nasal quadrant attenuation in VGB-exposed individuals not yet manifesting visual field loss suggests that OCT might be used as an indicator for the withdrawal of VGB on the grounds of the risk of imminent visual loss. The latter represents a potential breakthrough in that the prediction of imminent visual loss has hitherto not been possible.

The prediction of imminent VAVFL might be further improved by assessing, at regular intervals, the given individual's proportionate change in nasal RNFL. The development of nasal atrophy inside the confidence intervals for normality but outside range of the between-visit normal variability could then be used as a clinical indicator to withdraw VGB. This might reasonably provide an earlier pointer to the presence of

VGB toxicity compared with that dependent upon nasal attenuation identified cross-sectionally by comparison with the generic normative database.

Clinical issues are inevitably affected by resource implications. Generic issues important in all health care costing relate to the cost/benefit as determined by primary drug outcomes. When considering the cost/benefit analysis of AEDs, the socio-economic differences between seizure reduction and seizure freedom are important. In paediatric practice, the cost/benefit analysis is weighted by the greatly improved chances of developmental gain in children free from infantile spasms compared with those who continue to experience spasms. However, a further consideration in those patients treated with VGB is the additional costs, arising from the requirement for regular visual monitoring. The difference in costing between OCT and perimetry is difficult to quantify. There is also a potential significant cost saving arising from the prevention of VAVFL, in the event that nasal RNFL attenuation permits withdrawal of VGB prior to the development of VAVFL. In our current UK system, new prescriptions of VGB are very uncommon in adult practice (I have never initiated any new prescriptions of VGB whilst working in tertiary epilepsy clinics over the last 8 years) and this situation is likely to remain unchanged in the UK given other developments in AEDs. Worldwide prescription of VGB may increase, however, with the advent of a successful application for VGB licensing in the USA.

7.1.2 HRT Measures In Vigabatrin Toxicity

Scanning laser ophthalmoscopy, as evidenced by the HRT II, also identifies the pattern of inverse nasal atrophy seen in OCT (Section 7.1.1). However, the measure of reduction in NRR area is not as sensitive a marker of VGB toxicity as is RNFL measurement by OCT and identifyies only moderate to advanced cases of VAVFL. The reasons for this lack of sensitivity include the lack of a normative database for measurement of regional RNFL thickness, which in fact, is a height difference as opposed to a RNFL defined by anatomical boundaries, The measure of NRR area employed in this thesis, for which a robust formative database exists, is a surrogate marker of RNFL thickness only. Thus, HRT, in current commercially available format, cannot be recommended for the identification of VGB toxicity.

7.1.3 Placental transfer of vigabatrin: no indication of visual field loss

The licence request for VGB usage as an AED in refractory partial-onset seizures will, if granted, allow prescription in the US to women of child-bearing age. There is no indication of visual dysfunction in 4 individuals exposed to VGB in utero, as assessed by both static perimetry and RNFL measurement by OCT (Section 4.2). This result will be of interest to epileptologists, and potentially psychiatrists with an interest in addiction (Section 7.1.4) as, if generalisable, this finding provides useful information for women planning pregnancies on VGB and could obviate the need for visual screening in individuals exposed to VGB in utero.

7.1.4 Vigabatrin-Attributed Visual Field Loss In Uniquely Low Cumulative Dosing: Implications For Potential Use In Anti-Addiction Therapy.

VGB is currently undergoing Phase II trials as an anti-addiction agent. (Ovation, 2008) The recommended dosing regimen is based upon a two-week titration dose and treatment on a 9-week basis with high peak dosing of 3g/day. Although ocular safety data has been published (Fechtner et al., 2006), OCT was not used for monitoring safety. Wide between-individual variations occur in the duration and/or in the cumulative and/or mean daily dose of VGB necessary to produce VAVFL. The development of VAVFL in an individual (described in Chapter 4.3) treated for epilepsy with VGB in the range proposed for addiction must represent cause for concern with respect to the potential widespread use of a 'safe' dose of VGB as an anti-addiction agent..

7.1.5 Utility of mfERG in VGB-Attributed Visual Dysfunction

The mfERG does not seemingly identify VGB toxicity. This is consistent with what we now believe regarding the location of pathogenesis in VGB toxicity. The mfERG largely reflects photoreceptor cells, with additional subtle changes reflecting involvement of bipolar cells. Whilst previous electrical studies have suggested that VGB toxicity might involve photoreceptors and/or bipolar cells, the structural changes seen on OCT implicates RNFL loss as an early marker of VGB toxicity and therefore implicates ganglion cells as being primarily affected.

The limitations of multi-focal technology are numerous and include technical difficulties inherent in recording small, derived responses; difficulties in dealing with electrically noisy data and the additional technology required to further analyse the modelled data. Despite the large number of multifocal electrophysiology centres, worldwide, at the time of writing no commercial package exists to deal with analysis in a uniform manner.

7.1.6 Utility of mfVEP in VGB-Attributed Visual Dysfunction

The mfVEP did not identify VGB toxicity in the cohort used in this thesis. Given that the ganglion cell is a plausible location for the retinal toxicity seen in VAVFL and this is reflected in mfVEP amplitude, this requires explanation. The large inter-individual differences recorded in mfVEP amplitudes render normative databases very difficult to interpret. Skull thickness affects the recorded signal of the mfVEP, whilst electrode placement affects the relative positive and negative waveforms according to the position of the sulcal folding in the brain, in turn affecting inter-reliability results for mfVEP. (Klistorner and Graham, 2005) Many authors prefer to use interocular analyses for this reason (Hood and Greenstein, 2003) – not a helpful strategy in an essentially symmetrical bilateral disease such as VGB toxicity. Others have devised complex analyses to address the issue of normative databases (Klistorner and Graham, 2005) though as with mfERG no commercially available approach exists, as yet. Thus, ganglion cell involvement remains a distinct possibility in VGB toxicity but the limitations inherent in mfVEP recording and analysis might

fail to reveal important differences in bilateral disease.

7.2 Proposals For Future Work

7.2.1 The Potential Of OCT In VGB Toxicity

The examination of the retinal nerve fibre layer by OCT should be mandatory for all VGB-exposed individuals including individuals participating in clinical studies of VGB as an addiction therapy.

OCT technology continues to improve and the resolution of individual retinal layers is now possible. (Drexler and Fujimoto, 2008) Imaging using High Resosolution (HR) OCT and adaptive optics enables resolution of 5-6 μm, The use of such technology might delineate the retinal site(s) of VGB toxicity, as well as providing clues as to the pathogenesis.

7.2.2 The Potential Further Investigation Of The Retinal Ganglion Cell In VGB Toxicity

If ganglion cells are of prime importance in the pathogenesis of VGB toxicity, then an appropriate direct discriminator of ganglion cell dysfunction is the PERG. (Manca et al., 1984) Electrophysiology studies utilising the pattern ERG (PERG) in VGB-exposed individuals might provide information on the pathogenesis of VAVFL. Alternatively, prospective studies utilising mfVEP amplitude in individuals currently receiving VGB might allow identification of retinal ganglion cell dysfunction with amplitude reduction outside that allowed for by inter-test variability, alone.

Electrophysiology has, thus far, proved a less sensitive indicator of VGB toxicity, and remains less clinically accessible, compared to measurement of the RNFL with OCT.

7.2.3 Pharmacogenomics In VGB

Cumulative dose of VGB and daily dose of VGB have emerged as significant factors in the development of VAVFL. Still, there remain some individuals on long-term VGB who do not develop VAVFL. Conversely, some individuals develop VAVFL on low dose VGB after short-term exposure. The reason for this is not established, though between-individual differences related to genetic susceptibilities seem a plausible reason.

There has been one published attempt to relate the development of VAVFL to allelic heterogeneity in the ornithine delta aminotransferase gene. (Hisama et al., 2001) This group did not identify any mutations but identified a common intronic polymorphism. It is possible that differences in single nucleotide polymorphisms in candidate genes relate to the pathogenesis of VGB toxicity. A multi-centre trial investigating the pharmacogenomics of VGB has recently been established in the UK. (Ovation, 2008)

Identification and stratification of epileptic patients in this way would be informative to clinicians and might enable many more individuals to be treated with VGB. In particular, this would enable children and adults to be treated according to clinical need and efficacy rather than at the behest of currently unquantifiable risks of adverse

VGB effects.

Clinical-genetic stratification and estimation of individualised visual field risk in VGB will inform clinical outcomes and thereby improve service organisation and delivery, since prescribing will be based on clearer risk/benefit analyses.

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Appendix

A.1 Key To Abbreviations In The Text

ACTH Adrenocorticotrophic Hormone

AED Anti-epileptic Drug

AI Arden Index

CBZ Carbamazepine

CI Confidence Intervals

DANA Dopamine levels in the nucleus accumbens

DTL Dawson-Trick-Lithgo

ERG Electro-Oculogram

ERG Electro-Retinogrram

HFA Humphrey Visual Field Analyzer

HRT Heidelberg Retinal Tomograph

IS Infantile Spasms

ISCEV International Society for Clinical Electrophysiology of Vision

mfERG Multifocal Electroretinography

mfVEP Multifocal Visual Evoked Potential

MRA Moorfields Regression Analysis

NRR Neuroretinal Rim

OCT Optical Coherence Tomography

PERG Pattern Electroretinography

QoL Quality of Life

RCT Randomised Control Trial

RGC Retinal Ganglion Cell

RNFL Retinal Nerve Fibre Layer

RPE Retinal Pigment Epithelium

SLD Super Luminescent Diode

SLO Scanning Laser Opthalmoscopy

TS Tuberous Sclerosis

UKISS United Kingdom Infantile Spasm Study

VAVFL Vigabatrin-Attributed Visual Field Loss

VEP Visual Evoked Potential

VERIS Trademark software of EDI

VGB Vigabatrin

A.2.1 Age-Adjusted Confidence Intervals For The mfERG

The confidence intervals for the mfERG data based on the controls from the cohort are detailed below.

95% Confidence Limits 18:35 Wednesday, November 19, 2008 1

data	var	cl25	cl30	cl35	cl40	cl45

nlamp	l1	-5.83 - -1.32	-5.77 - -1.25	-5.71 - -1.19	-5.64 - -1.13	-5.58 - -1.06
	l2	-3.20 - -0.05	-3.17 - -0.01	-3.13 - 0.02	-3.10 - 0.06	-3.06 - 0.09
	l3	-5.71 - -0.67	-5.61 - -0.58	-5.52 - -0.48	-5.43 - -0.39	-5.33 - -0.29
	l4	-3.56 - -0.46	-3.56 - -0.46	-3.56 - -0.46	-3.56 - -0.46	-3.56 - -0.46
	l5	-5.22 - -0.39	-5.24 - -0.40	-5.25 - -0.42	-5.27 - -0.43	-5.29 - -0.45
	l6	-4.09 - -0.56	-4.05 - -0.52	-4.00 - -0.47	-3.96 - -0.43	-3.92 - -0.39
	l7	-4.48 - -1.53	-4.43 - -1.49	-4.39 - -1.44	-4.34 - -1.39	-4.29 - -1.35
	l8	-3.20 - -0.25	-3.14 - -0.19	-3.09 - -0.14	-3.04 - -0.09	-2.98 - -0.03
	r1	-6.00 - -1.37	-5.89 - -1.26	-5.78 - -1.15	-5.67 - -1.04	-5.57 - -0.94
	r2	-2.85 - -0.29	-2.82 - -0.27	-2.80 - -0.25	-2.78 - -0.22	-2.75 - -0.20
	r3	-5.99 - -0.77	-5.95 - -0.73	-5.91 - -0.69	-5.88 - -0.65	-5.84 - -0.62
	r4	-3.77 - -0.58	-3.79 - -0.59	-3.81 - -0.61	-3.82 - -0.63	-3.84 - -0.64
	r5	-5.86 - -1.22	-5.84 - -1.20	-5.82 - -1.18	-5.79 - -1.15	-5.77 - -1.13
	r6	-3.96 - -0.88	-3.94 - -0.85	-3.91 - -0.83	-3.89 - -0.80	-3.86 - -

0.78

r7 -5.67 - -1.64 -5.51 - -1.48 -5.36 - -1.33 -5.20 - -1.17 -5.04 - -

1.01

r8 -3.41 - -1.10 -3.33 - -1.02 -3.25 - -0.95 -3.18 - -0.87 -3.10 - -

0.79

data cl50 cl55 cl60 cl65 cl70

nlamp -5.51 - -1.00 -5.45 - -0.93 -5.38 - -0.87 -5.32 - -0.80 -5.25 - -

0.74

-3.03 - 0.13 -2.99 - 0.16 -2.96 - 0.20 -2.92 - 0.23 -2.89 - 0.27

-5.24 - -0.20 -5.14 - -0.10 -5.05 - -0.01 -4.95 - 0.09 -4.86 - 0.18

-3.56 - -0.46 -3.56 - -0.46 -3.56 - -0.46 -3.56 - -0.46 -3.56 - -0.46

-5.30 - -0.47 -5.32 - -0.48 -5.33 - -0.50 -5.35 - -0.51 -5.37 - -0.53

-3.88 - -0.35 -3.84 - -0.31 -3.80 - -0.27 -3.75 - -0.22 -3.71 - -0.18

-4.25 - -1.30 -4.20 - -1.25 -4.16 - -1.21 -4.11 - -1.16 -4.06 - -1.11

-2.93 - 0.02 -2.87 - 0.08 -2.82 - 0.13 -2.77 - 0.18 -2.71 - 0.24

-5.46 - -0.83 -5.35 - -0.72 -5.24 - -0.61 -5.13 - -0.50 -5.03 - -0.40

-2.73 - -0.18 -2.71 - -0.15 -2.68 - -0.13 -2.66 - -0.11 -2.64 - -0.08

-5.80 - -0.58 -5.76 - -0.54 -5.73 - -0.50 -5.69 - -0.47 -5.65 - -0.43

-3.85 - -0.66 -3.87 - -0.67 -3.89 - -0.69 -3.90 - -0.71 -3.92 - -0.72

-5.74 - -1.11 -5.72 - -1.08 -5.70 - -1.06 -5.67 - -1.04 -5.65 - -1.01

-3.84 - -0.75 -3.81 - -0.73 -3.79 - -0.70 -3.76 - -0.68 -3.74 - -0.66

-4.89 - -0.86 -4.73 - -0.70 -4.57 - -0.55 -4.42 - -0.39 -4.26 - -0.23

-3.02 - -0.72 -2.95 - -0.64 -2.87 - -0.56 -2.79 - -0.49 -2.72 - -0.41

data var cl25 cl30 cl35 cl40 cl45

nlimptim 11 17.25 - 28.40 17.75 - 28.90 18.25 - 29.40 18.75 - 29.90 19.25 - 30.40

12 14.84 - 29.14 15.30 - 29.59 15.76 - 30.05 16.21 - 30.51 16.67 - 30.97

data cl50 cl55 cl60 cl65 cl70

nlimptim 19.75 - 30.90 20.25 - 31.40 20.75 - 31.90 21.25 - 32.40 21.76 - 32.90

17.13 - 31.42 17.59 - 31.88 18.04 - 32.34 18.50 - 32.79 18.96 - 33.25

95% Confidence Limits 18:35 Wednesday, November 19, 2008 2

data var cl25 cl30 cl35 cl40 cl45

nlimptim 13 17.99 - 27.70 18.58 - 28.29 19.17 - 28.88 19.76 - 29.47 20.36 - 30.06

14 18.25 - 27.66 18.77 - 28.18 19.28 - 28.69 19.79 - 29.20 20.31 - 29.72

15 17.88 - 28.43 18.39 - 28.94 18.90 - 29.45 19.41 - 29.96 19.92 - 30.47

16 18.34 - 29.23 18.78 - 29.67 19.22 - 30.11 19.66 - 30.55 20.10 - 30.99

17 17.73 - 29.31 18.27 - 29.85 18.80 - 30.38 19.33 - 30.91 19.86 - 31.44

	I8	14.16 - 30.70	14.78 - 31.33	15.41 - 31.96	16.04 - 32.59
16.67 - 33.22					
	rl	18.17 - 28.39	18.69 - 28.91	19.20 - 29.42	19.72 - 29.94
20.23 - 30.45					
	r2	16.38 - 30.92	16.79 - 31.32	17.19 - 31.73	17.59 - 32.13
18.00 - 32.54					
	r3	18.65 - 27.58	19.29 - 28.22	19.93 - 28.86	20.57 - 29.50
21.21 - 30.14					
	r4	17.98 - 29.56	18.48 - 30.06	18.98 - 30.56	19.48 - 31.06
19.98 - 31.56					
	r5	18.65 - 26.70	19.14 - 27.19	19.63 - 27.68	20.12 - 28.17
20.61 - 28.67					
	r6	16.90 - 27.56	17.41 - 28.07	17.92 - 28.58	18.44 - 29.10
18.95 - 29.61					
	r7	17.59 - 27.35	18.05 - 27.81	18.51 - 28.27	18.96 - 28.73
19.42 - 29.18					
	r8	18.73 - 27.05	19.23 - 27.55	19.73 - 28.05	20.23 - 28.56
20.73 - 29.06					

data	cl50	cl55	cl60	cl65	cl70
	20.95 - 30.66	21.54 - 31.25	22.13 - 31.84	22.72 - 32.43	23.32 -
33.02					
	20.82 - 30.23	21.33 - 30.74	21.85 - 31.26	22.36 - 31.77	22.87 -
32.28					
	20.43 - 30.98	20.94 - 31.49	21.45 - 32.00	21.96 - 32.51	22.47 -
33.02					

20.54 - 31.43 20.98 - 31.87 21.42 - 32.31 21.86 - 32.75 22.30 -
33.19

20.39 - 31.97 20.93 - 32.51 21.46 - 33.04 21.99 - 33.57 22.52 -
34.10

17.30 - 33.85 17.93 - 34.47 18.56 - 35.10 19.19 - 35.73 19.82 -
36.36

20.75 - 30.97 21.26 - 31.48 21.77 - 32.00 22.29 - 32.51 22.80 -
33.02

18.40 - 32.94 18.81 - 33.35 19.21 - 33.75 19.61 - 34.15 20.02 -
34.56

21.85 - 30.78 22.49 - 31.42 23.13 - 32.06 23.77 - 32.70 24.41 -
33.34

20.48 - 32.06 20.98 - 32.56 21.47 - 33.06 21.97 - 33.55 22.47 -
34.05

21.10 - 29.16 21.59 - 29.65 22.08 - 30.14 22.58 - 30.63 23.07 -
31.12

19.46 - 30.12 19.97 - 30.63 20.48 - 31.14 20.99 - 31.65 21.50 -
32.16

19.88 - 29.64 20.34 - 30.10 20.79 - 30.56 21.25 - 31.01 21.71 -
31.47

21.23 - 29.56 21.74 - 30.06 22.24 - 30.56 22.74 - 31.06 23.24 -
31.57

data	var	cl25	cl30	cl35	cl40	cl45
n2amp	11	-5.90 - -0.45	-5.81 - -0.35	-5.71 - -0.26	-5.62 - -0.17	-5.53 - -0.08

	l2	-3.35 - 0.38	-3.36 - 0.38	-3.36 - 0.37	-3.36 - 0.37	-3.37 - 0.37
	l3	-5.23 - 0.10	-5.27 - 0.05	-5.32 - 0.01	-5.37 - -0.04	-5.41 - -0.09

data	cl50	cl55	cl60	cl65	cl70
n2amp	-5.44 - 0.02	-5.34 - 0.11	-5.25 - 0.20	-5.16 - 0.29	-5.07 - 0.39
	-3.37 - 0.36	-3.38 - 0.36	-3.38 - 0.35	-3.38 - 0.35	-3.39 - 0.35
	-5.46 - -0.14	-5.51 - -0.18	-5.55 - -0.23	-5.60 - -0.28	-5.65 - -0.32

data	var	cl25	cl30	cl35	cl40	cl45
n2amp	l4	-3.74 - -0.35	-3.71 - -0.33	-3.69 - -0.31	-3.67 - -0.28	-3.65 - -0.26
	l5	-6.09 - 0.04	-6.09 - 0.04	-6.09 - 0.05	-6.08 - 0.05	-6.08 - 0.05
	l6	-3.42 - -0.37	-3.37 - -0.31	-3.32 - -0.26	-3.27 - -0.21	-3.21 - -0.15
	l7	-5.26 - 0.19	-5.13 - 0.32	-5.00 - 0.45	-4.87 - 0.58	-4.74 - 0.71
	l8	-2.71 - -0.72	-2.63 - -0.63	-2.54 - -0.54	-2.46 - -0.46	-2.37 - -0.37
	r1	-5.15 - -1.20	-5.04 - -1.08	-4.93 - -0.97	-4.81 - -0.86	-4.70 - -

0.74

r2 -2.12 - -0.12 -2.13 - -0.13 -2.15 - -0.14 -2.16 - -0.16 -2.18 - -

0.17

r3 -4.83 - -0.74 -4.78 - -0.69 -4.73 - -0.64 -4.67 - -0.58 -4.62 - -

0.53

r4 -3.51 - -0.22 -3.51 - -0.22 -3.51 - -0.22 -3.51 - -0.22 -3.51 - -

0.22

r5 -6.33 - -1.10 -6.23 - -1.00 -6.12 - -0.89 -6.02 - -0.79 -5.91 - -

0.69

r6 -3.75 - -0.73 -3.72 - -0.70 -3.68 - -0.66 -3.65 - -0.63 -3.61 - -

0.59

r7 -5.07 - -2.05 -4.90 - -1.88 -4.72 - -1.71 -4.55 - -1.54 -4.38 - -

1.37

r8 -3.31 - -0.73 -3.22 - -0.64 -3.12 - -0.54 -3.03 - -0.45 -2.93 - -

0.35

data	cl50	cl55	cl60	cl65	cl70
	-3.62 - -0.24	-3.60 - -0.22	-3.58 - -0.19	-3.56 - -0.17	-3.53 - -0.15
	-6.08 - 0.05	-6.08 - 0.06	-6.07 - 0.06	-6.07 - 0.06	-6.07 - 0.07
	-3.16 - -0.10	-3.11 - -0.05	-3.06 - 0.00	-3.00 - 0.06	-2.95 - 0.11
	-4.61 - 0.84	-4.48 - 0.97	-4.35 - 1.10	-4.22 - 1.23	-4.09 - 1.36
	-2.28 - -0.29	-2.20 - -0.20	-2.11 - -0.11	-2.02 - -0.03	-1.94 - 0.06
	-4.59 - -0.63	-4.47 - -0.51	-4.36 - -0.40	-4.24 - -0.29	-4.13 - -0.17
	-2.19 - -0.19	-2.20 - -0.20	-2.22 - -0.21	-2.23 - -0.23	-2.25 - -0.24
	-4.57 - -0.48	-4.51 - -0.42	-4.46 - -0.37	-4.41 - -0.32	-4.36 - -0.26
	-3.51 - -0.22	-3.51 - -0.22	-3.51 - -0.22	-3.51 - -0.22	-3.51 - -0.22

-5.81 - -0.58 -5.71 - -0.48 -5.60 - -0.37 -5.50 - -0.27 -5.40 - -0.17

-3.58 - -0.56 -3.55 - -0.53 -3.51 - -0.49 -3.48 - -0.46 -3.45 - -0.42

-4.21 - -1.20 -4.04 - -1.03 -3.87 - -0.86 -3.70 - -0.68 -3.53 - -0.51

-2.84 - -0.26 -2.74 - -0.16 -2.65 - -0.07 -2.55 - 0.03 -2.46 - 0.12

data	var	cl25	cl30	cl35	cl40	cl45
n2imptim	11	53.48 - 72.04	54.69 - 73.26	55.91 - 74.47	57.13 - 75.69	58.35 - 76.91
	12	53.11 - 71.64	53.86 - 72.39	54.62 - 73.15	55.37 - 73.90	56.13 - 74.66
	13	54.48 - 69.73	55.28 - 70.52	56.08 - 71.32	56.88 - 72.12	57.68 - 72.92
	14	55.84 - 69.71	56.45 - 70.31	57.05 - 70.91	57.65 - 71.52	58.25 - 72.12

data	cl50	cl55	cl60	cl65	cl70
n2imptim	59.56 - 78.12	60.78 - 79.34	62.00 - 80.56	63.22 - 81.78	64.43 - 82.99
	56.88 - 75.41	57.63 - 76.17	58.39 - 76.92	59.14 - 77.68	59.90 - 78.43
	58.48 - 73.72	59.28 - 74.52	60.07 - 75.32	60.87 - 76.11	61.67 - 76.91
	58.85 - 72.72	59.46 - 73.32	60.06 - 73.92	60.66 - 74.53	61.26 - 75.13

data	var	cl25	cl30	cl35	cl40	cl45
n2imptim	l5	52.98 - 71.27	53.90 - 72.18	54.81 - 73.10	55.73 - 74.01	56.64 - 74.93
	l6	53.78 - 71.72	54.56 - 72.50	55.34 - 73.28	56.12 - 74.06	56.90 - 74.84
	l7	50.58 - 74.88	51.77 - 76.07	52.96 - 77.25	54.15 - 78.44	55.34 - 79.63
	l8	52.32 - 70.89	53.22 - 71.79	54.11 - 72.68	55.01 - 73.58	55.90 - 74.47
	r1	56.60 - 70.38	57.65 - 71.42	58.69 - 72.47	59.74 - 73.51	60.78 - 74.56
	r2	54.67 - 70.39	55.50 - 71.23	56.34 - 72.06	57.17 - 72.90	58.01 - 73.73
	r3	56.33 - 68.68	57.17 - 69.51	58.01 - 70.35	58.85 - 71.19	59.69 - 72.03
	r4	56.18 - 69.48	56.80 - 70.10	57.42 - 70.72	58.04 - 71.34	58.65 - 71.96
	r5	53.48 - 68.84	54.30 - 69.66	55.13 - 70.48	55.95 - 71.31	56.77 - 72.13
	r6	53.76 - 68.05	54.58 - 68.87	55.40 - 69.69	56.23 - 70.51	57.05 - 71.33
	r7	54.49 - 68.89	55.41 - 69.80	56.33 - 70.72	57.24 - 71.63	58.16 - 72.55
	r8	50.09 - 71.36	51.18 - 72.45	52.27 - 73.54	53.36 - 74.63	

54.45 - 75.72

data	cl50	cl55	cl60	cl65	cl70	
	57.55 - 75.84	58.47 - 76.75	59.38 - 77.67	60.30 - 78.58	61.21 -	79.50
	57.68 - 75.62	58.46 - 76.40	59.24 - 77.18	60.02 - 77.97	60.80 -	78.75
	56.53 - 80.82	57.72 - 82.01	58.91 - 83.20	60.09 - 84.39	61.28 -	85.58
	56.80 - 75.37	57.70 - 76.26	58.59 - 77.16	59.49 - 78.05	60.38 -	78.95
	61.83 - 75.60	62.88 - 76.65	63.92 - 77.69	64.97 - 78.74	66.01 -	79.78
	58.84 - 74.57	59.68 - 75.40	60.51 - 76.24	61.35 - 77.07	62.19 -	77.91
	60.52 - 72.87	61.36 - 73.71	62.20 - 74.55	63.04 - 75.38	63.88 -	76.22
	59.27 - 72.58	59.89 - 73.19	60.51 - 73.81	61.13 - 74.43	61.75 -	75.05
	57.60 - 72.95	58.42 - 73.78	59.24 - 74.60	60.07 - 75.42	60.89 -	76.25
	57.87 - 72.16	58.69 - 72.98	59.51 - 73.80	60.33 - 74.62	61.15 -	75.44
	59.07 - 73.47	59.99 - 74.38	60.91 - 75.30	61.82 - 76.22	62.74 -	77.13
	55.54 - 76.82	56.64 - 77.91	57.73 - 79.00	58.82 - 80.09	59.91 -	

81.18

data var cl25 cl30 cl35 cl40 cl45

plamp I1 2.25 - 7.49 2.16 - 7.40 2.07 - 7.31 1.98 - 7.22 1.89 - 7.13

I2 0.40 - 4.09 0.48 - 4.17 0.57 - 4.26 0.65 - 4.34 0.74 - 4.43

I3 1.32 - 6.94 1.32 - 6.94 1.33 - 6.95 1.33 - 6.95 1.33 - 6.95

I4 0.75 - 4.59 0.84 - 4.68 0.93 - 4.77 1.02 - 4.86 1.11 - 4.95

I5 0.46 - 7.71 0.46 - 7.71 0.46 - 7.71 0.46 - 7.71 0.46 - 7.72

data cl50 cl55 cl60 cl65 cl70

plamp 1.80 - 7.04 1.71 - 6.95 1.62 - 6.86 1.53 - 6.77 1.44 - 6.68

0.82 - 4.51 0.91 - 4.59 0.99 - 4.68 1.07 - 4.76 1.16 - 4.85

1.33 - 6.95 1.34 - 6.95 1.34 - 6.96 1.34 - 6.96 1.34 - 6.96

1.20 - 5.04 1.29 - 5.13 1.39 - 5.22 1.48 - 5.31 1.57 - 5.40

0.46 - 7.72 0.47 - 7.72 0.47 - 7.72 0.47 - 7.72 0.47 - 7.72

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data var cl25 cl30 cl35 cl40 cl45

plamp	l6	-0.52 - 4.04	-0.45 - 4.11	-0.37 - 4.19	-0.30 - 4.26	-0.22 - 4.34
	l7	0.99 - 6.72	0.96 - 6.69	0.94 - 6.67	0.91 - 6.64	0.88 - 6.61
	l8	0.92 - 3.36	0.91 - 3.35	0.91 - 3.34	0.90 - 3.34	0.89 - 3.33
	r1	1.93 - 5.97	1.93 - 5.97	1.92 - 5.96	1.92 - 5.96	1.91 - 5.95
	r2	0.54 - 2.94	0.57 - 2.97	0.60 - 3.00	0.63 - 3.03	0.66 - 3.06
	r3	1.78 - 5.77	1.74 - 5.73	1.70 - 5.70	1.66 - 5.66	1.63 - 5.62
	r4	0.85 - 4.45	0.82 - 4.42	0.80 - 4.40	0.77 - 4.37	0.74 - 4.34
	r5	2.37 - 7.05	2.28 - 6.96	2.19 - 6.87	2.10 - 6.79	2.02 - 6.70
	r6	0.74 - 5.00	0.76 - 5.02	0.78 - 5.04	0.80 - 5.06	0.82 - 5.08
	r7	2.63 - 7.06	2.55 - 6.98	2.47 - 6.90	2.39 - 6.82	2.31 - 6.74
	r8	0.77 - 4.17	0.78 - 4.18	0.78 - 4.18	0.78 - 4.18	0.79 - 4.19

data	cl50	cl55	cl60	cl65	cl70
	-0.15 - 4.41	-0.07 - 4.49	0.00 - 4.56	0.08 - 4.64	0.15 - 4.71

0.86 - 6.59	0.83 - 6.56	0.80 - 6.53	0.78 - 6.51	0.75 - 6.48
0.88 - 3.32	0.88 - 3.32	0.87 - 3.31	0.86 - 3.30	0.86 - 3.29
1.91 - 5.95	1.90 - 5.94	1.90 - 5.94	1.89 - 5.93	1.89 - 5.93
0.69 - 3.09	0.73 - 3.13	0.76 - 3.16	0.79 - 3.19	0.82 - 3.22
1.59 - 5.58	1.55 - 5.54	1.51 - 5.51	1.47 - 5.47	1.44 - 5.43
0.71 - 4.31	0.68 - 4.29	0.66 - 4.26	0.63 - 4.23	0.60 - 4.20
1.93 - 6.61	1.84 - 6.52	1.75 - 6.43	1.66 - 6.34	1.57 - 6.26
0.84 - 5.10	0.86 - 5.12	0.88 - 5.14	0.90 - 5.16	0.92 - 5.18
2.23 - 6.66	2.15 - 6.59	2.08 - 6.51	2.00 - 6.43	1.92 - 6.35
0.79 - 4.19	0.80 - 4.20	0.80 - 4.20	0.81 - 4.21	0.81 - 4.21

data	var	cl25	cl30	cl35	cl40	cl45
plimptim	11	37.10 - 49.39	37.88 - 50.17	38.66 - 50.96	39.44 - 51.74	40.23 - 52.52
	12	35.07 - 50.58	35.66 - 51.16	36.25 - 51.75	36.84 - 52.34	37.42 - 52.93
	13	36.56 - 49.39	37.18 - 50.00	37.79 - 50.62	38.41 - 51.24	39.03 - 51.86
	14	36.89 - 49.44	37.45 - 50.00	38.01 - 50.56	38.57 - 51.12	39.14 - 51.69
	15	36.02 - 49.96	36.70 - 50.65	37.39 - 51.33	38.08 - 52.02	38.76 - 52.71
	16	36.73 - 50.53	37.33 - 51.13	37.92 - 51.72	38.52 - 52.32	39.11 - 52.91

data	cl50	cl55	cl60	cl65	cl70

plimptim 41.01 - 53.30 41.79 - 54.09 42.57 - 54.87 43.36 - 55.65 44.14 - 56.43

38.01 - 53.52 38.60 - 54.10 39.19 - 54.69 39.77 - 55.28 40.36 - 55.87

39.65 - 52.48 40.27 - 53.09 40.88 - 53.71 41.50 - 54.33 42.12 - 54.95

39.70 - 52.25 40.26 - 52.81 40.82 - 53.37 41.38 - 53.93 41.95 - 54.49

39.45 - 53.39 40.13 - 54.08 40.82 - 54.76 41.51 - 55.45 42.19 - 56.14

39.71 - 53.51 40.30 - 54.11 40.90 - 54.70 41.49 - 55.30 42.09 - 55.89

data	var	cl25	cl30	cl35	cl40	cl45
plimptim	17	36.97 - 50.58	37.67 - 51.28	38.38 - 51.99	39.09 - 52.69	39.79 - 53.40
	18	33.76 - 50.56	34.52 - 51.32	35.28 - 52.07	36.03 - 52.83	36.79 - 53.59
	r1	37.72 - 49.17	38.52 - 49.97	39.32 - 50.77	40.12 - 51.57	40.92 - 52.36
	r2	35.35 - 50.83	36.14 - 51.62	36.93 - 52.40	37.71 - 53.19	38.50 - 53.98
	r3	36.47 - 48.75	37.24 - 49.52	38.02 - 50.29	38.79 - 51.06	

39.56 - 51.84

	r4	37.96 - 48.94	38.59 - 49.57	39.23 - 50.20	39.86 - 50.84
40.49 - 51.47					
	r5	35.72 - 47.56	36.49 - 48.32	37.25 - 49.09	38.01 - 49.85
38.78 - 50.61					
	r6	34.88 - 48.29	35.60 - 49.02	36.32 - 49.74	37.05 - 50.46
37.77 - 51.19					
	r7	35.93 - 48.09	36.65 - 48.81	37.36 - 49.53	38.08 - 50.24
38.80 - 50.96					
	r8	36.00 - 47.73	36.79 - 48.52	37.59 - 49.32	38.38 - 50.11
39.18 - 50.90					

data	cl50	cl55	cl60	cl65	cl70
	40.50 - 54.10	41.20 - 54.81	41.91 - 55.52	42.61 - 56.22	43.32 -
56.93					
	37.55 - 54.35	38.31 - 55.11	39.07 - 55.87	39.83 - 56.63	40.59 -
57.39					
	41.72 - 53.16	42.52 - 53.96	43.32 - 54.76	44.12 - 55.56	44.92 -
56.36					
	39.29 - 54.76	40.07 - 55.55	40.86 - 56.34	41.65 - 57.12	42.43 -
57.91					
	40.33 - 52.61	41.11 - 53.38	41.88 - 54.15	42.65 - 54.93	43.42 -
55.70					
	41.12 - 52.10	41.75 - 52.73	42.38 - 53.36	43.01 - 53.99	43.65 -
54.62					
	39.54 - 51.38	40.30 - 52.14	41.07 - 52.90	41.83 - 53.67	42.59 -

54.43

38.50 - 51.91 39.22 - 52.64 39.94 - 53.36 40.67 - 54.08 41.39 -

54.81

39.51 - 51.67 40.23 - 52.39 40.94 - 53.10 41.66 - 53.82 42.38 -

54.54

39.97 - 51.70 40.77 - 52.49 41.56 - 53.29 42.36 - 54.08 43.15 -

54.88

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data	var	cl25	cl30	cl35	cl40	cl45
nlamp	11	-5.55 - -1.60	-5.49 - -1.54	-5.42 - -1.47	-5.36 - -1.41	-5.29 - -1.34
	12	-3.01 - -0.25	-2.97 - -0.21	-2.94 - -0.18	-2.90 - -0.14	-2.87 - -0.11
	13	-5.39 - -0.99	-5.30 - -0.89	-5.20 - -0.80	-5.11 - -0.70	-5.02 - -0.61
	14	-3.36 - -0.65	-3.36 - -0.65	-3.36 - -0.65	-3.36 - -0.65	-3.36 - -0.65
	15	-4.92 - -0.69	-4.94 - -0.71	-4.95 - -0.72	-4.97 - -0.74	-4.98 - -0.75
	16	-3.87 - -0.78	-3.83 - -0.74	-3.78 - -0.69	-3.74 - -0.65	-3.70 - -0.61
	17	-4.30 - -1.72	-4.25 - -1.67	-4.20 - -1.62	-4.16 - -1.58	-4.11 - -1.53
	18	-3.01 - -0.43	-2.96 - -0.38	-2.91 - -0.32	-2.85 - -0.27	-2.80 - -

0.22

r1 -5.71 - -1.66 -5.60 - -1.55 -5.49 - -1.44 -5.39 - -1.33 -5.28 - -

1.23

r2 -2.69 - -0.45 -2.66 - -0.43 -2.64 - -0.41 -2.62 - -0.38 -2.59 - -

0.36

r3 -5.66 - -1.09 -5.63 - -1.06 -5.59 - -1.02 -5.55 - -0.98 -5.51 - -

0.94

r4 -3.57 - -0.78 -3.59 - -0.79 -3.61 - -0.81 -3.62 - -0.83 -3.64 - -

0.84

r5 -5.57 - -1.51 -5.55 - -1.49 -5.53 - -1.47 -5.50 - -1.44 -5.48 - -

1.42

r6 -3.77 - -1.07 -3.74 - -1.04 -3.72 - -1.02 -3.69 - -1.00 -3.67 - -

0.97

r7 -5.42 - -1.89 -5.26 - -1.73 -5.10 - -1.58 -4.95 - -1.42 -4.79 - -

1.27

r8 -3.26 - -1.24 -3.19 - -1.17 -3.11 - -1.09 -3.03 - -1.01 -2.96 - -

0.94

data cl50 cl55 cl60 cl65 cl70

nlamp -5.23 - -1.28 -5.17 - -1.21 -5.10 - -1.15 -5.04 - -1.09 -4.97 - -

1.02

-2.83 - -0.07 -2.80 - -0.04 -2.76 - -0.00 -2.73 - 0.03 -2.69 - 0.07

-4.92 - -0.51 -4.83 - -0.42 -4.73 - -0.32 -4.64 - -0.23 -4.54 - -0.13

-3.36 - -0.65 -3.36 - -0.65 -3.36 - -0.65 -3.36 - -0.65 -3.36 - -0.65

-5.00 - -0.77 -5.02 - -0.78 -5.03 - -0.80 -5.05 - -0.82 -5.06 - -0.83

-3.66 - -0.57 -3.62 - -0.53 -3.57 - -0.49 -3.53 - -0.44 -3.49 - -0.40

	-4.06 - -1.49	-4.02 - -1.44	-3.97 - -1.39	-3.92 - -1.35	-3.88 - -1.30
	-2.74 - -0.16	-2.69 - -0.11	-2.64 - -0.06	-2.58 - -0.00	-2.53 - 0.05
	-5.17 - -1.12	-5.06 - -1.01	-4.95 - -0.90	-4.85 - -0.79	-4.74 - -0.69
	-2.57 - -0.34	-2.55 - -0.31	-2.52 - -0.29	-2.50 - -0.27	-2.48 - -0.24
	-5.47 - -0.90	-5.44 - -0.87	-5.40 - -0.83	-5.36 - -0.79	-5.32 - -0.75
	-3.65 - -0.86	-3.67 - -0.87	-3.69 - -0.89	-3.70 - -0.91	-3.72 - -0.92
	-5.45 - -1.40	-5.43 - -1.37	-5.41 - -1.35	-5.38 - -1.33	-5.36 - -1.30
	-3.65 - -0.95	-3.62 - -0.92	-3.60 - -0.90	-3.57 - -0.87	-3.55 - -0.85
	-4.64 - -1.11	-4.48 - -0.95	-4.32 - -0.80	-4.17 - -0.64	-4.01 - -0.48
	-2.88 - -0.86	-2.80 - -0.78	-2.73 - -0.71	-2.65 - -0.63	-2.57 - -0.55

data	var	cl25	cl30	cl35	cl40	cl45
nlimptim	11	17.95 - 27.70	18.45 - 28.20	18.95 - 28.70	19.45 - 29.20	19.95 - 29.71
	12	15.74 - 28.24	16.19 - 28.70	16.65 - 29.16	17.11 - 29.62	17.57 - 30.07

data	cl50	cl55	cl60	cl65	cl70
nlimptim	20.45 - 30.21	20.95 - 30.71	21.45 - 31.21	21.95 - 31.71	22.45 - 32.21
	18.02 - 30.53	18.48 - 30.99	18.94 - 31.44	19.39 - 31.90	19.85 - 32.36

data	var	cl25	cl30	cl35	cl40	cl45
nlimptim	l3	18.59 - 27.09	19.19 - 27.68	19.78 - 28.27	20.37 - 28.86	20.96 - 29.46
	l4	18.84 - 27.07	19.35 - 27.59	19.87 - 28.10	20.38 - 28.62	20.89 - 29.13
	l5	18.54 - 27.77	19.05 - 28.28	19.56 - 28.79	20.07 - 29.30	20.58 - 29.81
	l6	19.02 - 28.54	19.46 - 28.99	19.90 - 29.43	20.34 - 29.87	20.78 - 30.31
	l7	18.46 - 28.59	18.99 - 29.12	19.52 - 29.65	20.05 - 30.19	20.59 - 30.72
	l8	15.19 - 29.67	15.82 - 30.30	16.45 - 30.92	17.08 - 31.55	17.71 - 32.18
	rl	18.81 - 27.75	19.33 - 28.27	19.84 - 28.78	20.35 - 29.30	20.87 - 29.81
	r2	17.29 - 30.01	17.69 - 30.42	18.10 - 30.82	18.50 - 31.22	18.91 - 31.63
	r3	19.20 - 27.02	19.84 - 27.66	20.48 - 28.30	21.13 - 28.94	21.77 - 29.58
	r4	18.71 - 28.84	19.20 - 29.34	19.70 - 29.84	20.20 - 30.34	20.70 - 30.83
	r5	19.15 - 26.20	19.64 - 26.69	20.13 - 27.18	20.62 - 27.67	21.11 - 28.16
	r6	17.57 - 26.90	18.08 - 27.41	18.59 - 27.92	19.10 - 28.43	19.61 - 28.94
	r7	18.20 - 26.74	18.66 - 27.20	19.12 - 27.66	19.57 - 28.12	

20.03 - 28.57

r8 19.25 - 26.53 19.75 - 27.03 20.25 - 27.53 20.75 - 28.03

21.25 - 28.54

data cl50 cl55 cl60 cl65 cl70

21.55 - 30.05 22.15 - 30.64 22.74 - 31.23 23.33 - 31.82 23.92 -

32.42

21.41 - 29.64 21.92 - 30.16 22.44 - 30.67 22.95 - 31.18 23.46 -

31.70

21.09 - 30.32 21.60 - 30.83 22.11 - 31.34 22.62 - 31.85 23.13 -

32.36

21.22 - 30.75 21.66 - 31.19 22.10 - 31.63 22.54 - 32.07 22.98 -

32.51

21.12 - 31.25 21.65 - 31.78 22.18 - 32.31 22.71 - 32.85 23.24 -

33.38

18.33 - 32.81 18.96 - 33.44 19.59 - 34.07 20.22 - 34.70 20.85 -

35.33

21.38 - 30.33 21.90 - 30.84 22.41 - 31.36 22.93 - 31.87 23.44 -

32.39

19.31 - 32.03 19.72 - 32.44 20.12 - 32.84 20.52 - 33.24 20.93 -

33.65

22.41 - 30.22 23.05 - 30.86 23.69 - 31.50 24.33 - 32.15 24.97 -

32.79

21.20 - 31.33 21.70 - 31.83 22.20 - 32.33 22.70 - 32.83 23.20 -

33.33

21.61 - 28.65 22.10 - 29.14 22.59 - 29.64 23.08 - 30.13 23.57 -

30.62

20.12 - 29.45 20.63 - 29.96 21.14 - 30.47 21.65 - 30.98 22.16 -
31.49

20.49 - 29.03 20.95 - 29.49 21.40 - 29.95 21.86 - 30.40 22.32 -
30.86

21.75 - 29.04 22.26 - 29.54 22.76 - 30.04 23.26 - 30.54 23.76 -
31.05

data var cl25 cl30 cl35 cl40 cl45

n2amp I1 -5.56 - -0.79 -5.47 - -0.70 -5.37 - -0.60 -5.28 - -0.51 -5.19
- -0.42

I2 -3.12 - 0.15 -3.12 - 0.14 -3.13 - 0.14 -3.13 - 0.14 -3.13 -
0.13

I3 -4.89 - -0.23 -4.94 - -0.28 -4.99 - -0.33 -5.03 - -0.37 -5.08 - -
0.42

data cl50 cl55 cl60 cl65 cl70

n2amp -5.10 - -0.33 -5.00 - -0.23 -4.91 - -0.14 -4.82 - -0.05 -4.73 -
0.04

-3.14 - 0.13 -3.14 - 0.12 -3.15 - 0.12 -3.15 - 0.12 -3.15 - 0.11

-5.13 - -0.47 -5.17 - -0.52 -5.22 - -0.56 -5.27 - -0.61 -5.31 - -0.66

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data var cl25 cl30 cl35 cl40 cl45

n2amp	l4	-3.52 - -0.56	-3.50 - -0.54	-3.48 - -0.52	-3.46 - -0.49	-3.43 - -0.47
	l5	-5.71 - -0.34	-5.71 - -0.34	-5.70 - -0.34	-5.70 - -0.34	-5.70 - -0.33
	l6	-3.23 - -0.56	-3.18 - -0.50	-3.13 - -0.45	-3.08 - -0.40	-3.02 - -0.35
	l7	-4.92 - -0.15	-4.79 - -0.02	-4.66 - 0.11	-4.53 - 0.24	-4.40 - 0.37
	l8	-2.59 - -0.84	-2.50 - -0.76	-2.42 - -0.67	-2.33 - -0.58	-2.24 - -0.50
	r1	-4.91 - -1.44	-4.79 - -1.33	-4.68 - -1.22	-4.56 - -1.10	-4.45 - -0.99
	r2	-1.99 - -0.24	-2.01 - -0.26	-2.02 - -0.27	-2.04 - -0.28	-2.05 - -0.30
	r3	-4.58 - -1.00	-4.52 - -0.94	-4.47 - -0.89	-4.42 - -0.84	-4.36 - -0.78
	r4	-3.30 - -0.42	-3.30 - -0.42	-3.30 - -0.42	-3.30 - -0.42	-3.31 - -0.42
	r5	-6.00 - -1.43	-5.90 - -1.32	-5.80 - -1.22	-5.69 - -1.12	-5.59 - -1.01
	r6	-3.56 - -0.92	-3.53 - -0.88	-3.49 - -0.85	-3.46 - -0.82	-3.43 - -0.78
	r7	-4.88 - -2.24	-4.71 - -2.07	-4.54 - -1.90	-4.36 - -1.73	-4.19 - -1.56
	r8	-3.15 - -0.89	-3.06 - -0.80	-2.96 - -0.70	-2.87 - -0.61	-2.77 - -0.51

data	cl50	cl55	cl60	cl65	cl70
	-3.41 - -0.45	-3.39 - -0.43	-3.37 - -0.40	-3.34 - -0.38	-3.32 - -0.36
	-5.69 - -0.33	-5.69 - -0.33	-5.69 - -0.32	-5.69 - -0.32	-5.68 - -0.32
	-2.97 - -0.29	-2.92 - -0.24	-2.86 - -0.19	-2.81 - -0.14	-2.76 - -0.08
	-4.27 - 0.50	-4.14 - 0.63	-4.01 - 0.76	-3.88 - 0.89	-3.74 - 1.02
	-2.16 - -0.41	-2.07 - -0.32	-1.98 - -0.24	-1.90 - -0.15	-1.81 - -0.06
	-4.34 - -0.88	-4.22 - -0.76	-4.11 - -0.65	-4.00 - -0.53	-3.88 - -0.42
	-2.06 - -0.31	-2.08 - -0.33	-2.09 - -0.34	-2.11 - -0.35	-2.12 - -0.37
	-4.31 - -0.73	-4.26 - -0.68	-4.21 - -0.63	-4.15 - -0.57	-4.10 - -0.52
	-3.31 - -0.43	-3.31 - -0.43	-3.31 - -0.43	-3.31 - -0.43	-3.31 - -0.43
	-5.48 - -0.91	-5.38 - -0.80	-5.28 - -0.70	-5.17 - -0.60	-5.07 - -0.49
	-3.39 - -0.75	-3.36 - -0.71	-3.32 - -0.68	-3.29 - -0.65	-3.26 - -0.61
	-4.02 - -1.39	-3.85 - -1.21	-3.68 - -1.04	-3.51 - -0.87	-3.34 - -0.70
	-2.68 - -0.42	-2.58 - -0.32	-2.49 - -0.23	-2.39 - -0.14	-2.30 - -0.04

data	var	cl25	cl30	cl35	cl40	cl45
n2imptim	l1	54.64 - 70.88	55.85 - 72.10	57.07 - 73.31	58.29 - 74.53	59.51 - 75.75
	l2	54.26 - 70.48	55.02 - 71.23	55.77 - 71.99	56.53 - 72.74	57.28 - 73.50
	l3	55.44 - 68.77	56.24 - 69.57	57.03 - 70.37	57.83 - 71.17	58.63 - 71.97
	l4	56.71 - 68.84	57.31 - 69.45	57.91 - 70.05	58.52 - 70.65	59.12 - 71.25

data cl50 cl55 cl60 cl65 cl70

n2imptim 60.72 - 76.96 61.94 - 78.18 63.16 - 79.40 64.38 - 80.62 65.59 - 81.83

58.04 - 74.25 58.79 - 75.01 59.55 - 75.76 60.30 - 76.52 61.06 - 77.27

59.43 - 72.77 60.23 - 73.56 61.03 - 74.36 61.83 - 75.16 62.62 - 75.96

59.72 - 71.85 60.32 - 72.45 60.92 - 73.06 61.53 - 73.66 62.13 - 74.26

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data var cl25 cl30 cl35 cl40 cl45

n2imptim l5 54.13 - 70.13 55.04 - 71.04 55.95 - 71.95 56.87 - 72.87 57.78 - 73.78

l6 54.90 - 70.60 55.68 - 71.38 56.46 - 72.16 57.24 - 72.94 58.02 - 73.72

l7 52.10 - 73.36 53.29 - 74.55 54.48 - 75.74 55.67 - 76.93 56.86 - 78.12

l8 53.48 - 69.73 54.38 - 70.63 55.27 - 71.52 56.17 - 72.42 57.06 - 73.31

r1 57.46 - 69.51 58.51 - 70.56 59.55 - 71.61 60.60 - 72.65 61.65 - 73.70

r2 55.65 - 69.41 56.49 - 70.24 57.32 - 71.08 58.16 - 71.92

58.99 - 72.75

r3 57.10 - 67.90 57.94 - 68.74 58.78 - 69.58 59.62 - 70.42
60.46 - 71.26

r4 57.01 - 68.65 57.63 - 69.27 58.25 - 69.89 58.87 - 70.51
59.49 - 71.13

r5 54.44 - 67.88 55.26 - 68.70 56.09 - 69.52 56.91 - 70.35
57.73 - 71.17

r6 54.66 - 67.16 55.48 - 67.98 56.30 - 68.80 57.12 - 69.62
57.94 - 70.44

r7 55.39 - 67.99 56.31 - 68.90 57.23 - 69.82 58.14 - 70.74
59.06 - 71.65

r8 51.42 - 70.03 52.51 - 71.12 53.60 - 72.21 54.69 - 73.30
55.78 - 74.39

data cl50 cl55 cl60 cl65 cl70

58.70 - 74.70 59.61 - 75.61 60.53 - 76.53 61.44 - 77.44 62.36 -
78.36

58.80 - 74.50 59.58 - 75.28 60.36 - 76.06 61.14 - 76.84 61.92 -
77.62

58.05 - 79.30 59.23 - 80.49 60.42 - 81.68 61.61 - 82.87 62.80 -
84.06

57.96 - 74.21 58.86 - 75.10 59.75 - 76.00 60.65 - 76.89 61.54 -
77.79

62.69 - 74.74 63.74 - 75.79 64.78 - 76.83 65.83 - 77.88 66.87 -
78.92

59.83 - 73.59 60.66 - 74.42 61.50 - 75.26 62.33 - 76.09 63.17 -

76.93

61.30 - 72.10 62.13 - 72.94 62.97 - 73.77 63.81 - 74.61 64.65 -
75.45

60.10 - 71.74 60.72 - 72.36 61.34 - 72.98 61.96 - 73.60 62.58 -
74.22

58.56 - 71.99 59.38 - 72.82 60.20 - 73.64 61.03 - 74.46 61.85 -
75.29

58.76 - 71.26 59.58 - 72.08 60.40 - 72.90 61.22 - 73.73 62.04 -
74.55

59.97 - 72.57 60.89 - 73.48 61.81 - 74.40 62.72 - 75.32 63.64 -
76.23

56.87 - 75.49 57.97 - 76.58 59.06 - 77.67 60.15 - 78.76 61.24 -
79.85

data	var	cl25	cl30	cl35	cl40	cl45
plamp	11	2.57 - 7.16	2.48 - 7.07	2.39 - 6.98	2.30 - 6.90	2.22 - 6.81
	12	0.63 - 3.86	0.71 - 3.94	0.80 - 4.03	0.88 - 4.11	0.97 - 4.20
	13	1.67 - 6.59	1.67 - 6.59	1.68 - 6.59	1.68 - 6.60	1.68 - 6.60
	14	0.99 - 4.35	1.08 - 4.44	1.17 - 4.53	1.26 - 4.62	1.35 - 4.71
	15	0.91 - 7.26	0.91 - 7.26	0.91 - 7.26	0.92 - 7.26	0.92 - 7.26

data		cl50	cl55	cl60	cl65	cl70
plamp		2.13 - 6.72	2.04 - 6.63	1.95 - 6.54	1.86 - 6.45	1.77 - 6.36
		1.05 - 4.28	1.14 - 4.36	1.22 - 4.45	1.30 - 4.53	1.39 - 4.62
		1.68 - 6.60	1.69 - 6.60	1.69 - 6.61	1.69 - 6.61	1.69 - 6.61
		1.44 - 4.80	1.53 - 4.89	1.62 - 4.98	1.72 - 5.07	1.81 - 5.17
		0.92 - 7.26	0.92 - 7.26	0.92 - 7.27	0.92 - 7.27	0.92 - 7.27

data	var	cl25	cl30	cl35	cl40	cl45
plamp	l6	-0.24 - 3.75	-0.16 - 3.83	-0.09 - 3.90	-0.01 - 3.98	0.06 - 4.05
	l7	1.35 - 6.36	1.32 - 6.33	1.29 - 6.31	1.27 - 6.28	1.24 - 6.26
	l8	1.07 - 3.21	1.07 - 3.20	1.06 - 3.19	1.05 - 3.18	1.04 - 3.18
	r1	2.19 - 5.72	2.18 - 5.71	2.18 - 5.71	2.17 - 5.70	2.17 - 5.70
	r2	0.69 - 2.79	0.72 - 2.82	0.75 - 2.85	0.78 - 2.88	0.81 - 2.91
	r3	2.03 - 5.52	1.99 - 5.48	1.95 - 5.45	1.91 - 5.41	1.87 - 5.37
	r4	1.08 - 4.23	1.05 - 4.20	1.02 - 4.17	0.99 - 4.14	0.97 - 4.12

	r5	2.66 - 6.76	2.57 - 6.67	2.48 - 6.58	2.40 - 6.49	2.31 - 6.41
	r6	1.00 - 4.73	1.02 - 4.75	1.04 - 4.77	1.06 - 4.79	1.08 - 4.81
	r7	2.90 - 6.78	2.82 - 6.70	2.75 - 6.62	2.67 - 6.54	2.59 - 6.47
	r8	0.98 - 3.96	0.99 - 3.96	0.99 - 3.97	1.00 - 3.97	1.00 - 3.98

data	cl50	cl55	cl60	cl65	cl70
	0.14 - 4.13	0.21 - 4.20	0.29 - 4.28	0.36 - 4.35	0.44 - 4.43
	1.21 - 6.23	1.19 - 6.20	1.16 - 6.18	1.14 - 6.15	1.11 - 6.12
	1.04 - 3.17	1.03 - 3.16	1.02 - 3.16	1.02 - 3.15	1.01 - 3.14
	2.16 - 5.69	2.16 - 5.69	2.15 - 5.68	2.15 - 5.68	2.14 - 5.67
	0.84 - 2.94	0.88 - 2.98	0.91 - 3.01	0.94 - 3.04	0.97 - 3.07
	1.84 - 5.33	1.80 - 5.29	1.76 - 5.26	1.72 - 5.22	1.69 - 5.18
	0.94 - 4.09	0.91 - 4.06	0.88 - 4.03	0.85 - 4.01	0.83 - 3.98
	2.22 - 6.32	2.13 - 6.23	2.04 - 6.14	1.95 - 6.05	1.87 - 5.96
	1.10 - 4.83	1.12 - 4.85	1.14 - 4.87	1.16 - 4.89	1.18 - 4.91
	2.51 - 6.39	2.43 - 6.31	2.35 - 6.23	2.27 - 6.15	2.20 - 6.07
	1.01 - 3.98	1.01 - 3.98	1.01 - 3.99	1.02 - 3.99	1.02 - 4.00

data	var	cl25	cl30	cl35	cl40	cl45
plimptim	11	37.86 - 48.62	38.65 - 49.40	39.43 - 50.19	40.21 - 50.97	40.99 - 51.75

	12	36.04 - 49.61	36.63 - 50.20	37.22 - 50.78	37.81 - 51.37
38.39 - 51.96					
	13	37.36 - 48.58	37.98 - 49.20	38.60 - 49.82	39.21 - 50.44
39.83 - 51.06					
	14	37.67 - 48.65	38.24 - 49.22	38.80 - 49.78	39.36 - 50.34
39.92 - 50.90					
	15	36.89 - 49.09	37.57 - 49.78	38.26 - 50.46	38.95 - 51.15
39.63 - 51.83					
	16	37.59 - 49.67	38.19 - 50.27	38.79 - 50.86	39.38 - 51.46
39.98 - 52.05					

data	cl50	cl55	cl60	cl65	cl70
plimptim	41.78 - 52.53	42.56 - 53.32	43.34 - 54.10	44.12 - 54.88	44.91
- 55.66					
	38.98 - 52.55	39.57 - 53.13	40.16 - 53.72	40.74 - 54.31	41.33 -
54.90					
	40.45 - 51.67	41.07 - 52.29	41.69 - 52.91	42.30 - 53.53	42.92 -
54.15					
	40.48 - 51.46	41.04 - 52.02	41.61 - 52.59	42.17 - 53.15	42.73 -
53.71					
	40.32 - 52.52	41.01 - 53.21	41.69 - 53.89	42.38 - 54.58	43.06 -
55.27					
	40.57 - 52.65	41.17 - 53.24	41.76 - 53.84	42.36 - 54.43	42.95 -
55.03					

data	var	cl25	cl30	cl35	cl40	cl45
plimptim	l7	37.82 - 49.73	38.52 - 50.43	39.23 - 51.14	39.94 - 51.84	40.64 - 52.55
	l8	34.81 - 49.51	35.57 - 50.27	36.33 - 51.02	37.08 - 51.78	37.84 - 52.54
	r1	38.44 - 48.45	39.24 - 49.25	40.04 - 50.05	40.84 - 50.85	41.64 - 51.65
	r2	36.32 - 49.86	37.11 - 50.65	37.89 - 51.44	38.68 - 52.22	39.47 - 53.01
	r3	37.24 - 47.98	38.01 - 48.75	38.78 - 49.52	39.56 - 50.30	40.33 - 51.07
	r4	38.65 - 48.25	39.28 - 48.89	39.91 - 49.52	40.54 - 50.15	41.17 - 50.78
	r5	36.46 - 46.82	37.23 - 47.58	37.99 - 48.35	38.75 - 49.11	39.52 - 49.87
	r6	35.71 - 47.45	36.44 - 48.18	37.16 - 48.90	37.89 - 49.63	38.61 - 50.35
	r7	36.69 - 47.33	37.41 - 48.05	38.12 - 48.77	38.84 - 49.48	39.56 - 50.20
	r8	36.73 - 46.99	37.53 - 47.79	38.32 - 48.58	39.12 - 49.38	39.91 - 50.17

data	cl50	cl55	cl60	cl65	cl70
	41.35 - 53.25	42.05 - 53.96	42.76 - 54.67	43.47 - 55.37	44.17 -

56.08

38.60 - 53.30 39.36 - 54.06 40.12 - 54.82 40.88 - 55.58 41.64 -
56.34

42.43 - 52.45 43.23 - 53.25 44.03 - 54.05 44.83 - 54.85 45.63 -
55.65

40.25 - 53.80 41.04 - 54.58 41.83 - 55.37 42.61 - 56.16 43.40 -
56.94

41.10 - 51.84 41.87 - 52.61 42.65 - 53.39 43.42 - 54.16 44.19 -
54.93

41.81 - 51.41 42.44 - 52.04 43.07 - 52.68 43.70 - 53.31 44.33 -
53.94

40.28 - 50.64 41.04 - 51.40 41.81 - 52.16 42.57 - 52.93 43.33 -
53.69

39.33 - 51.07 40.06 - 51.80 40.78 - 52.52 41.51 - 53.24 42.23 -
53.97

40.27 - 50.91 40.99 - 51.63 41.70 - 52.34 42.42 - 53.06 43.14 -
53.78

40.70 - 50.97 41.50 - 51.76 42.29 - 52.55 43.09 - 53.35 43.88 -
54.14

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data var cl25 cl30 cl35 cl40 cl45

nlamp 11 -5.29 - -1.86 -5.23 - -1.80 -5.16 - -1.73 -5.10 - -1.67 -5.03
- -1.60

12 -2.83 - -0.43 -2.79 - -0.39 -2.76 - -0.36 -2.72 - -0.32 -2.69 - -

0.29

l3	-5.10 - -1.28	-5.01 - -1.18	-4.92 - -1.09	-4.82 - -0.99	-4.73 - -

0.90

l4	-3.19 - -0.83	-3.19 - -0.83	-3.19 - -0.83	-3.19 - -0.83	-3.19 - -

0.83

l5	-4.64 - -0.97	-4.66 - -0.98	-4.67 - -1.00	-4.69 - -1.02	-4.71 - -

1.03

l6	-3.66 - -0.98	-3.62 - -0.94	-3.58 - -0.90	-3.54 - -0.86	-3.50 - -

0.81

l7	-4.13 - -1.89	-4.08 - -1.84	-4.03 - -1.79	-3.99 - -1.75	-3.94 - -

1.70

l8	-2.84 - -0.60	-2.79 - -0.55	-2.74 - -0.49	-2.68 - -0.44	-2.63 - -

0.39

r1	-5.44 - -1.92	-5.33 - -1.82	-5.23 - -1.71	-5.12 - -1.60	-5.01 - -

1.49

r2	-2.54 - -0.60	-2.52 - -0.58	-2.49 - -0.55	-2.47 - -0.53	-2.45 - -

0.51

r3	-5.36 - -1.39	-5.32 - -1.36	-5.29 - -1.32	-5.25 - -1.28	-5.21 - -

1.24

r4	-3.39 - -0.96	-3.41 - -0.98	-3.42 - -0.99	-3.44 - -1.01	-3.45 - -

1.03

r5	-5.31 - -1.78	-5.28 - -1.76	-5.26 - -1.73	-5.24 - -1.71	-5.21 - -

1.69

r6	-3.59 - -1.25	-3.57 - -1.22	-3.54 - -1.20	-3.52 - -1.17	-3.49 - -

1.15

r7	-5.18 - -2.12	-5.03 - -1.97	-4.87 - -1.81	-4.72 - -1.65	-4.56 - -

1.50

r8 -3.13 - -1.38 -3.05 - -1.30 -2.98 - -1.22 -2.90 - -1.15 -2.82 - -
1.07

data cl50 cl55 cl60 cl65 cl70

nlamp -4.97 - -1.54 -4.91 - -1.47 -4.84 - -1.41 -4.78 - -0.80 -4.71 - -
0.74

-2.65 - -0.25 -2.62 - -0.22 -2.58 - -0.18 -2.55 - 0.23 -2.51 - 0.27

-4.63 - -0.80 -4.54 - -0.71 -4.44 - -0.61 -4.35 - 0.09 -4.25 - 0.18

-3.19 - -0.83 -3.19 - -0.83 -3.19 - -0.83 -3.19 - -0.46 -3.19 - -0.46

-4.72 - -1.05 -4.74 - -1.06 -4.75 - -1.08 -4.77 - -0.51 -4.79 - -0.53

-3.46 - -0.77 -3.41 - -0.73 -3.37 - -0.69 -3.33 - -0.22 -3.29 - -0.18

-3.89 - -1.65 -3.85 - -1.61 -3.80 - -1.56 -3.75 - -1.16 -3.71 - -1.11

-2.57 - -0.33 -2.52 - -0.28 -2.47 - -0.22 -2.41 - 0.18 -2.36 - 0.24

-4.90 - -1.38 -4.79 - -1.28 -4.69 - -1.17 -4.58 - -0.50 -4.47 - -0.40

-2.42 - -0.48 -2.40 - -0.46 -2.38 - -0.44 -2.35 - -0.11 -2.33 - -0.08

-5.17 - -1.21 -5.14 - -1.17 -5.10 - -1.13 -5.06 - -0.47 -5.02 - -0.43

-3.47 - -1.04 -3.49 - -1.06 -3.50 - -1.07 -3.52 - -0.71 -3.53 - -0.72

-5.19 - -1.66 -5.16 - -1.64 -5.14 - -1.62 -5.12 - -1.04 -5.09 - -1.01

-3.47 - -1.12 -3.44 - -1.10 -3.42 - -1.07 -3.39 - -0.68 -3.37 - -0.66

-4.40 - -1.34 -4.25 - -1.18 -4.09 - -1.03 -3.94 - -0.39 -3.78 - -0.23

-2.75 - -0.99 -2.67 - -0.92 -2.59 - -0.84 -2.52 - -0.49 -2.44 - -0.41

data var cl25 cl30 cl35 cl40 cl45

nlimptim 11 18.59 - 27.06 19.09 - 27.56 19.59 - 28.06 20.09 - 28.56
20.59 - 29.06

	I2	16.56 - 27.42	17.02 - 27.88	17.47 - 28.34	17.93 - 28.79
18.39 - 29.25					

data	cl50	cl55	cl60	cl65	cl70
nlimptim	21.09 - 29.56	21.59 - 30.07	22.09 - 30.57	22.59 - 32.40	23.09
- 32.90					
	18.84 - 29.71	19.30 - 30.16	19.76 - 30.62	20.22 - 32.79	20.67 -
33.25					

data	var	cl25	cl30	cl35	cl40	cl45
nlimptim	I3	19.15 - 26.53	19.74 - 27.12	20.34 - 27.71	20.93 - 28.31	
21.52 - 28.90						
	I4	19.38 - 26.53	19.90 - 27.05	20.41 - 27.56	20.92 - 28.07	
21.44 - 28.59						
	I5	19.14 - 27.16	19.65 - 27.67	20.16 - 28.18	20.67 - 28.69	
21.18 - 29.20						
	I6	19.64 - 27.92	20.08 - 28.36	20.52 - 28.80	20.96 - 29.24	
21.40 - 29.68						
	I7	19.12 - 27.92	19.66 - 28.46	20.19 - 28.99	20.72 - 29.52	
21.25 - 30.05						
	I8	16.14 - 28.72	16.77 - 29.34	17.40 - 29.97	18.03 - 30.60	
18.66 - 31.23						
	rl	19.40 - 27.17	19.91 - 27.68	20.43 - 28.20	20.94 - 28.71	

21.46 - 29.22

	r2	18.13 - 29.18	18.53 - 29.58	18.93 - 29.98	19.34 - 30.39
19.74 - 30.79					
	r3	19.72 - 26.51	20.36 - 27.15	21.00 - 27.79	21.64 - 28.43
22.28 - 29.07					
	r4	19.37 - 28.17	19.87 - 28.67	20.37 - 29.17	20.87 - 29.67
21.37 - 30.17					
	r5	19.61 - 25.73	20.10 - 26.22	20.59 - 26.72	21.09 - 27.21
21.58 - 27.70					
	r6	18.18 - 26.28	18.69 - 26.80	19.20 - 27.31	19.71 - 27.82
20.22 - 28.33					
	r7	18.76 - 26.18	19.22 - 26.64	19.68 - 27.10	20.14 - 27.56
20.59 - 28.01					
	r8	19.72 - 26.05	20.23 - 26.55	20.73 - 27.05	21.23 - 27.56
21.73 - 28.06					

data	cl50	cl55	cl60	cl65	cl70
	22.11 - 29.49	22.70 - 30.08	23.30 - 30.67	23.89 - 32.43	24.48 -
33.02					
	21.95 - 29.10	22.46 - 29.61	22.98 - 30.13	23.49 - 31.77	24.00 -
32.28					
	21.69 - 29.71	22.20 - 30.22	22.71 - 30.73	23.22 - 32.51	23.73 -
33.02					
	21.85 - 30.12	22.29 - 30.56	22.73 - 31.00	23.17 - 32.75	23.61 -
33.19					
	21.78 - 30.58	22.31 - 31.12	22.85 - 31.65	23.38 - 33.57	23.91 -

34.10

19.29 - 31.86 19.91 - 32.49 20.54 - 33.12 21.17 - 35.73 21.80 -
36.36

21.97 - 29.74 22.49 - 30.25 23.00 - 30.77 23.52 - 32.51 24.03 -
33.02

20.15 - 31.20 20.55 - 31.60 20.96 - 32.00 21.36 - 34.15 21.76 -
34.56

22.92 - 29.71 23.56 - 30.35 24.20 - 30.99 24.84 - 32.70 25.48 -
33.34

21.87 - 30.67 22.37 - 31.17 22.86 - 31.67 23.36 - 33.55 23.86 -
34.05

22.07 - 28.19 22.56 - 28.68 23.05 - 29.17 23.54 - 30.63 24.03 -
31.12

20.74 - 28.84 21.25 - 29.35 21.76 - 29.86 22.27 - 31.65 22.78 -
32.16

21.05 - 28.47 21.51 - 28.93 21.97 - 29.39 22.42 - 31.01 22.88 -
31.47

22.23 - 28.56 22.74 - 29.06 23.24 - 29.56 23.74 - 31.06 24.24 -
31.57

data var cl25 cl30 cl35 cl40 cl45

n2amp I1 -5.25 - -1.10 -5.15 - -1.01 -5.06 - -0.92 -4.97 - -0.82 -4.88
- -0.73

I2 -2.90 - -0.07 -2.91 - -0.07 -2.91 - -0.07 -2.92 - -0.08 -2.92 - -
0.08

I3 -4.59 - -0.54 -4.63 - -0.59 -4.68 - -0.63 -4.73 - -0.68 -4.77 - -

0.73

data cl50 cl55 cl60 cl65 cl70

n2amp -4.78 - -0.64 -4.69 - -0.55 -4.60 - -0.45 -4.51 - 0.29 -4.41 -
0.39

-2.92 - -0.09 -2.93 - -0.09 -2.93 - -0.09 -2.94 - 0.35 -2.94 - 0.35

-4.82 - -0.77 -4.87 - -0.82 -4.91 - -0.87 -4.96 - -0.28 -5.01 - -0.32

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data var cl25 cl30 cl35 cl40 cl45

n2amp l4 -3.33 - -0.76 -3.31 - -0.73 -3.29 - -0.71 -3.26 - -0.69 -3.24
- -0.67

l5 -5.36 - -0.70 -5.35 - -0.69 -5.35 - -0.69 -5.35 - -0.69 -5.35 - -
0.68

l6 -3.06 - -0.73 -3.00 - -0.68 -2.95 - -0.63 -2.90 - -0.57 -2.85 - -
0.52

l7 -4.61 - -0.47 -4.48 - -0.34 -4.35 - -0.20 -4.21 - -0.07 -4.08 -
0.06

l8 -2.48 - -0.96 -2.39 - -0.87 -2.30 - -0.78 -2.22 - -0.70 -2.13 - -
0.61

r1 -4.68 - -1.67 -4.56 - -1.56 -4.45 - -1.44 -4.34 - -1.33 -4.22 - -
1.22

r2 -1.88 - -0.36 -1.89 - -0.37 -1.91 - -0.38 -1.92 - -0.40 -1.93 - -
0.41

r3 -4.34 - -1.23 -4.29 - -1.18 -4.24 - -1.13 -4.18 - -1.07 -4.13 - -1.02

r4 -3.11 - -0.61 -3.11 - -0.61 -3.11 - -0.61 -3.12 - -0.61 -3.12 - -0.61

r5 -5.70 - -1.73 -5.60 - -1.62 -5.49 - -1.52 -5.39 - -1.42 -5.29 - -1.31

r6 -3.39 - -1.09 -3.35 - -1.06 -3.32 - -1.02 -3.29 - -0.99 -3.25 - -0.96

r7 -4.70 - -2.41 -4.53 - -2.24 -4.36 - -2.07 -4.19 - -1.90 -4.02 - -1.73

r8 -3.00 - -1.04 -2.91 - -0.95 -2.81 - -0.85 -2.72 - -0.76 -2.62 - -0.66

data	cl50	cl55	cl60	cl65	cl70
	-3.22 - -0.64	-3.20 - -0.62	-3.17 - -0.60	-3.15 - -0.17	-3.13 - -0.15
	-5.34 - -0.68	-5.34 - -0.68	-5.34 - -0.68	-5.33 - 0.06	-5.33 - 0.07
	-2.79 - -0.47	-2.74 - -0.42	-2.69 - -0.36	-2.64 - 0.06	-2.58 - 0.11
	-3.95 - 0.19	-3.82 - 0.32	-3.69 - 0.45	-3.56 - 1.23	-3.43 - 1.36
	-2.04 - -0.53	-1.96 - -0.44	-1.87 - -0.35	-1.78 - -0.03	-1.70 - 0.06
	-4.11 - -1.10	-4.00 - -0.99	-3.88 - -0.88	-3.77 - -0.29	-3.66 - -0.17
	-1.95 - -0.43	-1.96 - -0.44	-1.98 - -0.45	-1.99 - -0.23	-2.00 - -0.24
	-4.08 - -0.97	-4.02 - -0.91	-3.97 - -0.86	-3.92 - -0.32	-3.86 - -0.26
	-3.12 - -0.61	-3.12 - -0.61	-3.12 - -0.62	-3.12 - -0.22	-3.12 - -0.22
	-5.18 - -1.21	-5.08 - -1.11	-4.98 - -1.00	-4.87 - -0.27	-4.77 - -0.17
	-3.22 - -0.92	-3.18 - -0.89	-3.15 - -0.85	-3.12 - -0.46	-3.08 - -0.42
	-3.85 - -1.56	-3.68 - -1.39	-3.51 - -1.22	-3.34 - -0.68	-3.16 - -0.51

-2.53 - -0.57 -2.43 - -0.47 -2.34 - -0.38 -2.24 - 0.03 -2.15 - 0.12

data var cl25 cl30 cl35 cl40 cl45

n2imptim 11 55.70 - 69.81 56.92 - 71.03 58.14 - 72.25 59.36 - 73.46
60.57 - 74.68

12 55.33 - 69.41 56.08 - 70.17 56.84 - 70.92 57.59 - 71.68
58.35 - 72.43

13 56.31 - 67.90 57.11 - 68.70 57.91 - 69.49 58.71 - 70.29
59.51 - 71.09

14 57.51 - 68.05 58.11 - 68.65 58.71 - 69.25 59.31 - 69.85
59.92 - 70.45

data cl50 cl55 cl60 cl65 cl70

n2imptim 61.79 - 75.90 63.01 - 77.11 64.23 - 78.33 65.44 - 81.78 66.66
- 82.99

59.10 - 73.19 59.86 - 73.94 60.61 - 74.70 61.37 - 77.68 62.12 -
78.43

60.31 - 71.89 61.10 - 72.69 61.90 - 73.49 62.70 - 76.11 63.50 -
76.91

60.52 - 71.06 61.12 - 71.66 61.72 - 72.26 62.32 - 74.53 62.93 -
75.13

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data var cl25 cl30 cl35 cl40 cl45

n2imptim	l5	55.18 - 69.07	56.09 - 69.99	57.01 - 70.90	57.92 - 71.82	58.83 - 72.73
	l6	55.93 - 69.57	56.71 - 70.35	57.49 - 71.13	58.27 - 71.91	59.05 - 72.69
	l7	53.50 - 71.96	54.68 - 73.15	55.87 - 74.34	57.06 - 75.53	58.25 - 76.72
	l8	54.55 - 68.66	55.45 - 69.56	56.34 - 70.45	57.24 - 71.35	58.13 - 72.25
	r1	58.26 - 68.72	59.30 - 69.77	60.35 - 70.81	61.39 - 71.86	62.44 - 72.90
	r2	56.55 - 68.50	57.39 - 69.34	58.22 - 70.18	59.06 - 71.01	59.90 - 71.85
	r3	57.81 - 67.19	58.65 - 68.03	59.49 - 68.87	60.33 - 69.71	61.17 - 70.55
	r4	57.78 - 67.89	58.40 - 68.51	59.01 - 69.12	59.63 - 69.74	60.25 - 70.36
	r5	55.32 - 66.99	56.15 - 67.82	56.97 - 68.64	57.79 - 69.46	58.62 - 70.29
	r6	55.48 - 66.34	56.30 - 67.16	57.12 - 67.98	57.94 - 68.80	58.76 - 69.62
	r7	56.22 - 67.16	57.14 - 68.08	58.05 - 68.99	58.97 - 69.91	59.88 - 70.82
	r8	52.64 - 68.81	53.73 - 69.90	54.82 - 70.99	55.91 - 72.08	57.01 - 73.17

data	cl50	cl55	cl60	cl65	cl70

		59.75 - 73.65	60.66 - 74.56	61.58 - 75.47	62.49 - 78.58	63.41 - 79.50
		59.83 - 73.47	60.61 - 74.25	61.39 - 75.03	62.17 - 77.97	62.95 - 78.75
		59.44 - 77.91	60.63 - 79.10	61.82 - 80.29	63.01 - 84.39	64.20 - 85.58
		59.03 - 73.14	59.92 - 74.04	60.82 - 74.93	61.71 - 78.05	62.61 - 78.95
		63.48 - 73.95	64.53 - 75.00	65.57 - 76.04	66.62 - 78.74	67.66 - 79.78
		60.73 - 72.68	61.57 - 73.52	62.40 - 74.35	63.24 - 77.07	64.07 - 77.91
		62.00 - 71.39	62.84 - 72.23	63.68 - 73.06	64.52 - 75.38	65.36 - 76.22
		60.87 - 70.98	61.49 - 71.60	62.11 - 72.22	62.72 - 74.43	63.34 - 75.05
		59.44 - 71.11	60.26 - 71.93	61.09 - 72.76	61.91 - 75.42	62.73 - 76.25
		59.58 - 70.44	60.40 - 71.26	61.22 - 72.08	62.04 - 74.62	62.87 - 75.44
		60.80 - 71.74	61.72 - 72.66	62.63 - 73.57	63.55 - 76.22	64.47 - 77.13
		58.10 - 74.26	59.19 - 75.35	60.28 - 76.45	61.37 - 80.09	62.46 - 81.18

data	var	cl25	cl30	cl35	cl40	cl45

plamp I1 2.88 - 6.86 2.79 - 6.77 2.70 - 6.68 2.61 - 6.59 2.52 - 6.50

I2 0.84 - 3.65 0.93 - 3.73 1.01 - 3.81 1.10 - 3.90 1.18 - 3.98

I3 2.00 - 6.27 2.00 - 6.27 2.00 - 6.27 2.00 - 6.27 2.01 - 6.28

I4 1.21 - 4.13 1.30 - 4.22 1.39 - 4.31 1.48 - 4.40 1.57 - 4.49

I5 1.33 - 6.84 1.33 - 6.84 1.33 - 6.84 1.33 - 6.84 1.33 - 6.85

data cl50 cl55 cl60 cl65 cl70

plamp 2.43 - 6.41 2.34 - 6.32 2.25 - 6.23 2.16 - 6.77 2.07 - 6.68

1.26 - 4.07 1.35 - 4.15 1.43 - 4.24 1.52 - 4.76 1.60 - 4.85

2.01 - 6.28 2.01 - 6.28 2.01 - 6.28 2.01 - 6.96 2.02 - 6.96

1.66 - 4.58 1.75 - 4.67 1.85 - 4.76 1.94 - 5.31 2.03 - 5.40

1.33 - 6.85 1.34 - 6.85 1.34 - 6.85 1.34 - 7.72 1.34 - 7.72

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data var cl25 cl30 cl35 cl40 cl45

plamp I6 0.02 - 3.49 0.10 - 3.57 0.17 - 3.64 0.25 - 3.72 0.33 - 3.79

I7	1.68 - 6.03	1.65 - 6.01	1.62 - 5.98	1.60 - 5.95	1.57 - 5.93
I8	1.21 - 3.07	1.21 - 3.06	1.20 - 3.05	1.19 - 3.04	1.18 - 3.04
r1	2.42 - 5.49	2.41 - 5.48	2.41 - 5.48	2.40 - 5.47	2.40 - 5.47
r2	0.83 - 2.65	0.86 - 2.68	0.89 - 2.71	0.92 - 2.74	0.95 - 2.78
r3	2.26 - 5.29	2.22 - 5.25	2.18 - 5.22	2.14 - 5.18	2.10 - 5.14
r4	1.28 - 4.02	1.26 - 3.99	1.23 - 3.97	1.20 - 3.94	1.17 - 3.91
r5	2.93 - 6.49	2.84 - 6.40	2.75 - 6.31	2.67 - 6.22	2.58 - 6.14
r6	1.25 - 4.49	1.27 - 4.51	1.29 - 4.53	1.31 - 4.55	1.33 - 4.57
r7	3.16 - 6.52	3.08 - 6.45	3.00 - 6.37	2.92 - 6.29	2.84 - 6.21
r8	1.18 - 3.76	1.18 - 3.77	1.19 - 3.77	1.19 - 3.78	1.20 - 3.78

data	cl50	cl55	cl60	cl65	cl70
	0.40 - 3.87	0.48 - 3.94	0.55 - 4.02	0.63 - 4.64	0.70 - 4.71
	1.54 - 5.90	1.52 - 5.87	1.49 - 5.85	1.47 - 6.51	1.44 - 6.48
	1.18 - 3.03	1.17 - 3.02	1.16 - 3.02	1.16 - 3.30	1.15 - 3.29
	2.39 - 5.46	2.39 - 5.46	2.38 - 5.45	2.38 - 5.93	2.37 - 5.93

0.98 - 2.81	1.01 - 2.84	1.04 - 2.87	1.08 - 3.19	1.11 - 3.22
2.07 - 5.10	2.03 - 5.06	1.99 - 5.03	1.95 - 5.47	1.92 - 5.43
1.14 - 3.88	1.12 - 3.85	1.09 - 3.83	1.06 - 4.23	1.03 - 4.20
2.49 - 6.05	2.40 - 5.96	2.31 - 5.87	2.22 - 6.34	2.13 - 6.26
1.35 - 4.59	1.37 - 4.61	1.39 - 4.62	1.41 - 5.16	1.43 - 5.18
2.76 - 6.13	2.69 - 6.05	2.61 - 5.97	2.53 - 6.43	2.45 - 6.35
1.20 - 3.78	1.21 - 3.79	1.21 - 3.79	1.21 - 4.21	1.22 - 4.21

data	var	cl25	cl30	cl35	cl40	cl45
plimptim	l1	38.57 - 47.92	39.35 - 48.70	40.14 - 49.48	40.92 - 50.26	41.70 - 51.04
	l2	36.93 - 48.72	37.52 - 49.30	38.11 - 49.89	38.70 - 50.48	39.28 - 51.07
	l3	38.10 - 47.85	38.72 - 48.46	39.33 - 49.08	39.95 - 49.70	40.57 - 50.32
	l4	38.40 - 47.93	38.96 - 48.49	39.52 - 49.06	40.08 - 49.62	40.64 - 50.18
	l5	37.69 - 48.29	38.38 - 48.97	39.06 - 49.66	39.75 - 50.35	40.43 - 51.03
	l6	38.39 - 48.88	38.98 - 49.47	39.58 - 50.07	40.17 - 50.66	40.77 - 51.26

data	cl50	cl55	cl60	cl65	cl70
plimptim	42.48 - 51.83	43.27 - 52.61	44.05 - 53.39	44.83 - 55.65	45.61 - 56.43

39.87 - 51.65 40.46 - 52.24 41.05 - 52.83 41.64 - 55.28 42.22 -
55.87

41.19 - 50.94 41.80 - 51.55 42.42 - 52.17 43.04 - 54.33 43.66 -
54.95

41.20 - 50.74 41.77 - 51.30 42.33 - 51.87 42.89 - 53.93 43.45 -
54.49

41.12 - 51.72 41.81 - 52.41 42.49 - 53.09 43.18 - 55.45 43.87 -
56.14

41.36 - 51.85 41.96 - 52.45 42.56 - 53.04 43.15 - 55.30 43.75 -
55.89

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data	var	cl25	cl30	cl35	cl40	cl45
plimptim	l7	38.60 - 48.94	39.31 - 49.65	40.01 - 50.35	40.72 - 51.06	41.42 - 51.77
	l8	35.77 - 48.54	36.53 - 49.30	37.29 - 50.06	38.05 - 50.82	38.81 - 51.58
	rl	39.10 - 47.79	39.90 - 48.59	40.70 - 49.39	41.49 - 50.19	42.29 - 50.99
	r2	37.21 - 48.97	38.00 - 49.76	38.78 - 50.55	39.57 - 51.33	40.36 - 52.12
	r3	37.94 - 47.27	38.72 - 48.05	39.49 - 48.82	40.26 - 49.59	41.03 - 50.36
	r4	39.28 - 47.62	39.91 - 48.25	40.54 - 48.89	41.17 - 49.52	41.81 - 50.15

r5	37.14 - 46.14	37.91 - 46.90	38.67 - 47.67	39.43 - 48.43	40.20 - 49.19
r6	36.49 - 46.68	37.21 - 47.41	37.93 - 48.13	38.66 - 48.85	39.38 - 49.58
r7	37.39 - 46.63	38.11 - 47.35	38.82 - 48.07	39.54 - 48.78	40.26 - 49.50
r8	37.41 - 46.32	38.20 - 47.11	39.00 - 47.91	39.79 - 48.70	40.58 - 49.50

data	cl50	cl55	cl60	cl65	cl70
	42.13 - 52.47	42.84 - 53.18	43.54 - 53.88	44.25 - 56.22	44.95 - 56.93
	39.57 - 52.34	40.33 - 53.09	41.09 - 53.85	41.84 - 56.63	42.60 - 57.39
	43.09 - 51.79	43.89 - 52.59	44.69 - 53.39	45.49 - 55.56	46.29 - 56.36
	41.14 - 52.91	41.93 - 53.69	42.72 - 54.48	43.50 - 57.12	44.29 - 57.91
	41.81 - 51.14	42.58 - 51.91	43.35 - 52.68	44.12 - 54.93	44.90 - 55.70
	42.44 - 50.78	43.07 - 51.41	43.70 - 52.04	44.33 - 53.99	44.96 - 54.62
	40.96 - 49.96	41.72 - 50.72	42.49 - 51.48	43.25 - 53.67	44.01 - 54.43
	40.11 - 50.30	40.83 - 51.03	41.55 - 51.75	42.28 - 54.08	43.00 - 54.81

40.97 - 50.21 41.69 - 50.93 42.40 - 51.65 43.12 - 53.82 43.83 - 54.54

41.38 - 50.29 42.17 - 51.09 42.97 - 51.88 43.76 - 54.08 44.56 - 54.88

80% Confidence Limits 18:35 Wednesday, November 19, 2008 1

data	var	cl25	cl30	cl35	cl40	cl45
nlamp	l1	-5.09 - -2.06	-5.02 - -2.00	-4.96 - -1.94	-4.90 - -1.87	-4.83 - -1.81
	l2	-2.68 - -0.57	-2.65 - -0.53	-2.61 - -0.50	-2.58 - -0.46	-2.54 - -0.43
	l3	-4.88 - -1.50	-4.78 - -1.41	-4.69 - -1.31	-4.59 - -1.22	-4.50 - -1.12
	l4	-3.05 - -0.97	-3.05 - -0.97	-3.05 - -0.97	-3.05 - -0.97	-3.05 - -0.97
	l5	-4.42 - -1.19	-4.44 - -1.20	-4.46 - -1.22	-4.47 - -1.23	-4.49 - -1.25
	l6	-3.51 - -1.14	-3.46 - -1.10	-3.42 - -1.06	-3.38 - -1.01	-3.34 - -0.97
	l7	-3.99 - -2.02	-3.95 - -1.97	-3.90 - -1.93	-3.86 - -1.88	-3.81 - -1.83
	l8	-2.71 - -0.74	-2.66 - -0.68	-2.60 - -0.63	-2.55 - -0.57	-2.50 - -0.52
	rl	-5.23 - -2.13	-5.13 - -2.02	-5.02 - -1.92	-4.91 - -1.81	-4.80 - -1.70

r2 -2.43 - -0.71 -2.40 - -0.69 -2.38 - -0.67 -2.36 - -0.64 -2.33 - -
0.62

r3 -5.13 - -1.63 -5.09 - -1.59 -5.05 - -1.55 -5.01 - -1.52 -4.98 - -
1.48

r4 -3.25 - -1.10 -3.26 - -1.12 -3.28 - -1.14 -3.29 - -1.15 -3.31 - -
1.17

r5 -5.10 - -1.99 -5.07 - -1.97 -5.05 - -1.94 -5.03 - -1.92 -5.00 - -
1.90

r6 -3.45 - -1.39 -3.43 - -1.36 -3.40 - -1.34 -3.38 - -1.31 -3.35 - -
1.29

r7 -5.00 - -2.30 -4.85 - -2.15 -4.69 - -1.99 -4.53 - -1.83 -4.38 - -
1.68

r8 -3.03 - -1.48 -2.95 - -1.40 -2.87 - -1.33 -2.80 - -1.25 -2.72 - -
1.17

data cl50 cl55 cl60 cl65 cl70

nlamp -4.77 - -1.74 -4.70 - -1.68 -4.64 - -1.61 -4.57 - -1.55 -4.51 - -
1.48

-2.51 - -0.39 -2.47 - -0.36 -2.44 - -0.32 -2.40 - -0.29 -2.37 - -0.25

-4.40 - -1.03 -4.31 - -0.93 -4.21 - -0.84 -4.12 - -0.74 -4.02 - -0.65

-3.05 - -0.97 -3.05 - -0.97 -3.05 - -0.97 -3.05 - -0.97 -3.05 - -0.97

-4.50 - -1.26 -4.52 - -1.28 -4.54 - -1.30 -4.55 - -1.31 -4.57 - -1.33

-3.30 - -0.93 -3.25 - -0.89 -3.21 - -0.85 -3.17 - -0.81 -3.13 - -0.76

-3.76 - -1.79 -3.72 - -1.74 -3.67 - -1.69 -3.62 - -1.65 -3.58 - -1.60

-2.44 - -0.47 -2.39 - -0.41 -2.33 - -0.36 -2.28 - -0.30 -2.23 - -0.25

-4.69 - -1.59 -4.59 - -1.48 -4.48 - -1.38 -4.37 - -1.27 -4.26 - -1.16

-2.31 - -0.60 -2.29 - -0.58 -2.26 - -0.55 -2.24 - -0.53 -2.22 - -0.51

-4.94 - -1.44 -4.90 - -1.40 -4.86 - -1.36 -4.83 - -1.33 -4.79 - -1.29

-3.33 - -1.19 -3.34 - -1.20 -3.36 - -1.22 -3.37 - -1.23 -3.39 - -1.25

-4.98 - -1.87 -4.96 - -1.85 -4.93 - -1.83 -4.91 - -1.80 -4.89 - -1.78

-3.33 - -1.26 -3.30 - -1.24 -3.28 - -1.21 -3.26 - -1.19 -3.23 - -1.16

-4.22 - -1.52 -4.07 - -1.37 -3.91 - -1.21 -3.75 - -1.05 -3.60 - -0.90

-2.64 - -1.10 -2.57 - -1.02 -2.49 - -0.94 -2.41 - -0.87 -2.34 - -0.79

data var cl25 cl30 cl35 cl40 cl45

nlimptim l1 19.09 - 26.56 19.59 - 27.06 20.09 - 27.56 20.59 - 28.06 21.09 - 28.56

l2 17.20 - 26.78 17.66 - 27.24 18.12 - 27.69 18.57 - 28.15 19.03 - 28.61

data cl50 cl55 cl60 cl65 cl70

nlimptim 21.59 - 29.06 22.09 - 29.56 22.59 - 30.06 23.09 - 30.56 23.60 - 31.07

19.49 - 29.06 19.94 - 29.52 20.40 - 29.98 20.86 - 30.44 21.32 - 30.89

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data var cl25 cl30 cl35 cl40 cl45

nlimptim l3 19.59 - 26.09 20.18 - 26.69 20.77 - 27.28 21.37 - 27.87

21.96 - 28.46

	I4	19.81 - 26.11	20.32 - 26.62	20.83 - 27.14	21.35 - 27.65

21.86 - 28.16

	I5	19.62 - 26.69	20.13 - 27.20	20.64 - 27.71	21.15 - 28.22

21.66 - 28.73

	I6	20.13 - 27.43	20.57 - 27.87	21.01 - 28.31	21.45 - 28.75

21.89 - 29.19

	I7	19.64 - 27.40	20.18 - 27.94	20.71 - 28.47	21.24 - 29.00

21.77 - 29.53

	I8	16.89 - 27.97	17.51 - 28.60	18.14 - 29.23	18.77 - 29.86

19.40 - 30.49

	r1	19.86 - 26.71	20.37 - 27.22	20.89 - 27.74	21.40 - 28.25

21.92 - 28.76

	r2	18.78 - 28.52	19.19 - 28.93	19.59 - 29.33	19.99 - 29.73

20.40 - 30.14

	r3	20.12 - 26.10	20.76 - 26.75	21.40 - 27.39	22.04 - 28.03

22.68 - 28.67

	r4	19.89 - 27.65	20.39 - 28.15	20.89 - 28.65	21.39 - 29.15

21.89 - 29.65

	r5	19.97 - 25.37	20.47 - 25.86	20.96 - 26.35	21.45 - 26.84

21.94 - 27.34

	r6	18.66 - 25.80	19.17 - 26.32	19.68 - 26.83	20.19 - 27.34

20.70 - 27.85

	r7	19.20 - 25.74	19.66 - 26.20	20.12 - 26.66	20.58 - 27.12

21.03 - 27.57

	r8	20.10 - 25.68	20.60 - 26.18	21.10 - 26.68	21.60 - 27.18

22.11 - 27.68

data	cl50	cl55	cl60	cl65	cl70	
	22.55 - 29.05	23.14 - 29.65	23.73 - 30.24	24.32 - 30.83	24.92 - 31.42	
	22.37 - 28.68	22.89 - 29.19	23.40 - 29.70	23.91 - 30.22	24.43 - 30.73	
	22.17 - 29.24	22.68 - 29.75	23.19 - 30.26	23.70 - 30.77	24.21 - 31.28	
	22.34 - 29.63	22.78 - 30.07	23.22 - 30.51	23.66 - 30.95	24.10 - 31.39	
	22.30 - 30.06	22.84 - 30.60	23.37 - 31.13	23.90 - 31.66	24.43 - 32.19	
	20.03 - 31.12	20.66 - 31.74	21.29 - 32.37	21.92 - 33.00	22.55 - 33.63	
	22.43 - 29.28	22.95 - 29.79	23.46 - 30.31	23.98 - 30.82	24.49 - 31.34	
	20.80 - 30.54	21.21 - 30.95	21.61 - 31.35	22.01 - 31.75	22.42 - 32.16	
	23.32 - 29.31	23.96 - 29.95	24.60 - 30.59	25.24 - 31.23	25.88 - 31.87	
	22.39 - 30.15	22.89 - 30.65	23.39 - 31.14	23.88 - 31.64	24.38 - 32.14	
	22.43 - 27.83	22.92 - 28.32	23.41 - 28.81	23.90 - 29.30	24.40 - 29.79	
	21.22 - 28.36	21.73 - 28.87	22.24 - 29.38	22.75 - 29.89	23.26 - 30.40	

21.49 - 28.03 21.95 - 28.49 22.40 - 28.95 22.86 - 29.40 23.32 - 29.86

22.61 - 28.19 23.11 - 28.69 23.61 - 29.19 24.11 - 29.69 24.62 - 30.19

data	var	cl25	cl30	cl35	cl40	cl45
n2amp	11	-5.00 - -1.35	-4.91 - -1.25	-4.82 - -1.16	-4.72 - -1.07	-4.63 - -0.98
	12	-2.74 - -0.23	-2.74 - -0.24	-2.74 - -0.24	-2.75 - -0.25	-2.75 - -0.25
	13	-4.35 - -0.78	-4.39 - -0.83	-4.44 - -0.87	-4.49 - -0.92	-4.53 - -0.97

data	cl50	cl55	cl60	cl65	cl70
n2amp	-4.54 - -0.88	-4.45 - -0.79	-4.35 - -0.70	-4.26 - -0.61	-4.17 - -0.51
	-2.76 - -0.25	-2.76 - -0.26	-2.76 - -0.26	-2.77 - -0.27	-2.77 - -0.27
	-4.58 - -1.01	-4.63 - -1.06	-4.68 - -1.11	-4.72 - -1.15	-4.77 - -1.20

data	var	cl25	cl30	cl35	cl40	cl45
n2amp	14	-3.18 - -0.91	-3.16 - -0.89	-3.13 - -0.86	-3.11 - -0.84	-3.09 - -0.82

	l5	-5.08 - -0.97	-5.08 - -0.97	-5.07 - -0.97	-5.07 - -0.96	-5.07 - -
0.96						
	l6	-2.92 - -0.87	-2.87 - -0.82	-2.81 - -0.76	-2.76 - -0.71	-2.71 - -
0.66						
	l7	-4.36 - -0.71	-4.23 - -0.58	-4.10 - -0.45	-3.97 - -0.32	-3.84 - -
0.19						
	l8	-2.39 - -1.05	-2.30 - -0.96	-2.21 - -0.87	-2.13 - -0.79	-2.04 - -
0.70						
	r1	-4.50 - -1.85	-4.39 - -1.74	-4.27 - -1.62	-4.16 - -1.51	-4.05 - -
1.39						
	r2	-1.79 - -0.45	-1.80 - -0.46	-1.82 - -0.47	-1.83 - -0.49	-1.84 - -
0.50						
	r3	-4.16 - -1.42	-4.10 - -1.36	-4.05 - -1.31	-4.00 - -1.26	-3.95 - -
1.20						
	r4	-2.97 - -0.76	-2.97 - -0.76	-2.97 - -0.76	-2.97 - -0.76	-2.97 - -
0.76						
	r5	-5.47 - -1.96	-5.36 - -1.86	-5.26 - -1.76	-5.16 - -1.65	-5.05 - -
1.55						
	r6	-3.25 - -1.23	-3.22 - -1.19	-3.18 - -1.16	-3.15 - -1.13	-3.12 - -
1.09						
	r7	-4.57 - -2.55	-4.40 - -2.38	-4.23 - -2.21	-4.06 - -2.04	-3.88 - -
1.87						
	r8	-2.89 - -1.16	-2.79 - -1.06	-2.70 - -0.97	-2.60 - -0.87	-2.51 - -
0.78						

data	cl50	cl55	cl60	cl65	cl70

-3.07 - -0.80	-3.04 - -0.77	-3.02 - -0.75	-3.00 - -0.73	-2.98 - -0.71
-5.07 - -0.96	-5.06 - -0.95	-5.06 - -0.95	-5.06 - -0.95	-5.05 - -0.95
-2.66 - -0.61	-2.60 - -0.55	-2.55 - -0.50	-2.50 - -0.45	-2.45 - -0.40
-3.71 - -0.06	-3.58 - 0.07	-3.45 - 0.20	-3.32 - 0.33	-3.19 - 0.46
-1.95 - -0.61	-1.87 - -0.53	-1.78 - -0.44	-1.69 - -0.36	-1.61 - -0.27
-3.93 - -1.28	-3.82 - -1.17	-3.71 - -1.05	-3.59 - -0.94	-3.48 - -0.83
-1.86 - -0.52	-1.87 - -0.53	-1.89 - -0.54	-1.90 - -0.56	-1.91 - -0.57
-3.89 - -1.15	-3.84 - -1.10	-3.79 - -1.05	-3.73 - -0.99	-3.68 - -0.94
-2.97 - -0.76	-2.97 - -0.76	-2.97 - -0.76	-2.97 - -0.76	-2.97 - -0.77
-4.95 - -1.44	-4.84 - -1.34	-4.74 - -1.24	-4.64 - -1.13	-4.53 - -1.03
-3.08 - -1.06	-3.05 - -1.02	-3.01 - -0.99	-2.98 - -0.96	-2.95 - -0.92
-3.71 - -1.69	-3.54 - -1.52	-3.37 - -1.35	-3.20 - -1.18	-3.03 - -1.01
-2.41 - -0.68	-2.32 - -0.59	-2.22 - -0.49	-2.13 - -0.40	-2.03 - -0.30

data	var	cl25	cl30	cl35	cl40	cl45
n2imptim	I1	56.54 - 68.98	57.76 - 70.19	58.97 - 71.41	60.19 - 72.63	61.41 - 73.84
	I2	56.16 - 68.58	56.92 - 69.33	57.67 - 70.09	58.43 - 70.84	59.18 - 71.60
	I3	57.00 - 67.21	57.80 - 68.01	58.60 - 68.81	59.39 - 69.61	60.19 - 70.41
	I4	58.13 - 67.42	58.73 - 68.02	59.34 - 68.63	59.94 - 69.23	60.54 - 69.83

data	cl50	cl55	cl60	cl65	cl70

n2imptim 62.63 - 75.06 63.84 - 76.28 65.06 - 77.50 66.28 - 78.71 67.50 - 79.93

59.94 - 72.35 60.69 - 73.11 61.45 - 73.86 62.20 - 74.62 62.96 - 75.37

60.99 - 71.20 61.79 - 72.00 62.59 - 72.80 63.39 - 73.60 64.19 - 74.40

61.14 - 70.43 61.74 - 71.03 62.35 - 71.64 62.95 - 72.24 63.55 - 72.84

data	var	cl25	cl30	cl35	cl40	cl45
n2imptim	l5	56.00 - 68.25	56.91 - 69.17	57.83 - 70.08	58.74 - 70.99	59.66 - 71.91
	l6	56.74 - 68.76	57.52 - 69.54	58.30 - 70.32	59.08 - 71.10	59.86 - 71.88
	l7	54.59 - 70.87	55.78 - 72.06	56.97 - 73.25	58.16 - 74.44	59.35 - 75.62
	l8	55.39 - 67.83	56.28 - 68.72	57.18 - 69.62	58.07 - 70.51	58.97 - 71.41
	r1	58.88 - 68.10	59.92 - 69.15	60.97 - 70.19	62.01 - 71.24	63.06 - 72.28
	r2	57.26 - 67.80	58.10 - 68.63	58.93 - 69.47	59.77 - 70.30	60.60 - 71.14
	r3	58.37 - 66.64	59.21 - 67.48	60.05 - 68.32	60.88 - 69.15	61.72 - 69.99

r4	58.38 - 67.29	58.99 - 67.91	59.61 - 68.53	60.23 - 69.14	60.85 - 69.76
r5	56.01 - 66.30	56.84 - 67.13	57.66 - 67.95	58.48 - 68.77	59.31 - 69.60
r6	56.12 - 65.69	56.94 - 66.51	57.76 - 67.34	58.58 - 68.16	59.40 - 68.98
r7	56.87 - 66.51	57.78 - 67.43	58.70 - 68.34	59.62 - 69.26	60.53 - 70.18
r8	53.60 - 67.85	54.69 - 68.94	55.78 - 70.03	56.87 - 71.12	57.96 - 72.21

data	cl50	cl55	cl60	cl65	cl70
	60.57 - 72.82	61.49 - 73.74	62.40 - 74.65	63.32 - 75.57	64.23 - 76.48
	60.64 - 72.66	61.42 - 73.44	62.20 - 74.22	62.98 - 75.00	63.76 - 75.78
	60.54 - 76.81	61.73 - 78.00	62.91 - 79.19	64.10 - 80.38	65.29 - 81.57
	59.86 - 72.30	60.76 - 73.20	61.65 - 74.10	62.55 - 74.99	63.45 - 75.89
	64.10 - 73.33	65.15 - 74.38	66.19 - 75.42	67.24 - 76.47	68.28 - 77.51
	61.44 - 71.97	62.27 - 72.81	63.11 - 73.64	63.94 - 74.48	64.78 - 75.32
	62.56 - 70.83	63.40 - 71.67	64.24 - 72.51	65.08 - 73.35	65.91 - 74.19

61.47 - 70.38 62.09 - 71.00 62.70 - 71.62 63.32 - 72.24 63.94 - 72.85

60.13 - 70.42 60.95 - 71.24 61.78 - 72.07 62.60 - 72.89 63.42 - 73.71

60.23 - 69.80 61.05 - 70.62 61.87 - 71.44 62.69 - 72.26 63.51 - 73.08

61.45 - 71.09 62.36 - 72.01 63.28 - 72.92 64.20 - 73.84 65.11 - 74.76

59.05 - 73.31 60.15 - 74.40 61.24 - 75.49 62.33 - 76.58 63.42 - 77.67

data	var	cl25	cl30	cl35	cl40	cl45
plamp	l1	3.11 - 6.63	3.02 - 6.54	2.93 - 6.45	2.84 - 6.36	2.75 - 6.27
	l2	1.01 - 3.48	1.09 - 3.56	1.18 - 3.65	1.26 - 3.73	1.35 - 3.82
	l3	2.25 - 6.01	2.25 - 6.02	2.25 - 6.02	2.26 - 6.02	2.26 - 6.02
	l4	1.38 - 3.95	1.47 - 4.05	1.56 - 4.14	1.66 - 4.23	1.75 - 4.32
	l5	1.66 - 6.51	1.66 - 6.52	1.66 - 6.52	1.66 - 6.52	1.66 - 6.52

data	cl50	cl55	cl60	cl65	cl70
plamp	2.66 - 6.18	2.57 - 6.09	2.48 - 6.00	2.39 - 5.91	2.30 -

5.82

1.43 - 3.90	1.51 - 3.99	1.60 - 4.07	1.68 - 4.15	1.77 - 4.24
2.26 - 6.03	2.26 - 6.03	2.27 - 6.03	2.27 - 6.03	2.27 - 6.03
1.84 - 4.41	1.93 - 4.50	2.02 - 4.59	2.11 - 4.68	2.20 - 4.77
1.66 - 6.52	1.66 - 6.52	1.66 - 6.52	1.66 - 6.52	1.67 - 6.52

80% Confidence Limits 18:35 Wednesday, November 19, 2008 5

data	var	cl25	cl30	cl35	cl40	cl45
plamp	l6	0.23 - 3.28	0.31 - 3.36	0.38 - 3.44	0.46 - 3.51	0.53 - 3.59
	l7	1.93 - 5.77	1.91 - 5.75	1.88 - 5.72	1.85 - 5.69	1.83 - 5.67
	l8	1.32 - 2.96	1.31 - 2.95	1.31 - 2.94	1.30 - 2.93	1.29 - 2.93
	r1	2.60 - 5.30	2.59 - 5.30	2.59 - 5.29	2.58 - 5.29	2.58 - 5.28
	r2	0.94 - 2.54	0.97 - 2.57	1.00 - 2.61	1.03 - 2.64	1.06 - 2.67
	r3	2.44 - 5.11	2.40 - 5.07	2.36 - 5.04	2.32 - 5.00	2.28 - 4.96
	r4	1.44 - 3.86	1.42 - 3.83	1.39 - 3.80	1.36 - 3.78	1.33 - 3.75
	r5	3.14 - 6.28	3.05 - 6.19	2.96 - 6.10	2.88 - 6.01	2.79 - 5.93
	r6	1.44 - 4.30	1.46 - 4.31	1.48 - 4.33	1.50 - 4.35	1.52 -

4.37

	r7	3.36 - 6.32	3.28 - 6.25	3.20 - 6.17	3.12 - 6.09	3.04 - 6.01
	r8	1.33 - 3.61	1.34 - 3.61	1.34 - 3.62	1.35 - 3.62	1.35 - 3.63

data	cl50	cl55	cl60	cl65	cl70
	0.61 - 3.66	0.68 - 3.74	0.76 - 3.81	0.83 - 3.89	0.91 - 3.96
	1.80 - 5.64	1.78 - 5.62	1.75 - 5.59	1.72 - 5.56	1.70 - 5.54
	1.29 - 2.92	1.28 - 2.91	1.27 - 2.91	1.27 - 2.90	1.26 - 2.89
	2.57 - 5.28	2.57 - 5.27	2.56 - 5.27	2.56 - 5.26	2.55 - 5.26
	1.09 - 2.70	1.12 - 2.73	1.15 - 2.76	1.18 - 2.79	1.21 - 2.82
	2.25 - 4.92	2.21 - 4.88	2.17 - 4.85	2.13 - 4.81	2.09 - 4.77
	1.31 - 3.72	1.28 - 3.69	1.25 - 3.66	1.22 - 3.64	1.20 - 3.61
	2.70 - 5.84	2.61 - 5.75	2.52 - 5.66	2.43 - 5.57	2.35 - 5.48
	1.54 - 4.39	1.56 - 4.41	1.58 - 4.43	1.60 - 4.45	1.62 - 4.47
	2.96 - 5.93	2.89 - 5.85	2.81 - 5.78	2.73 - 5.70	2.65 - 5.62
	1.35 - 3.63	1.36 - 3.64	1.36 - 3.64	1.37 - 3.65	1.37 - 3.65

data	var	cl25	cl30	cl35	cl40	cl45
plimptim	11	39.12 - 47.36	39.91 - 48.14	40.69 - 48.93	41.47 - 49.71	42.25 - 50.49
	12	37.63 - 48.02	38.22 - 48.61	38.81 - 49.19	39.40 - 49.78	39.98 - 50.37
	13	38.68 - 47.27	39.29 - 47.89	39.91 - 48.51	40.53 - 49.12	

41.15 - 49.74

l4 38.96 - 47.37 39.52 - 47.93 40.08 - 48.49 40.65 - 49.05
41.21 - 49.61

l5 38.32 - 47.66 39.00 - 48.35 39.69 - 49.03 40.38 - 49.72
41.06 - 50.41

l6 39.01 - 48.26 39.60 - 48.85 40.20 - 49.45 40.80 - 50.04
41.39 - 50.64

data	cl50	cl55	cl60	cl65	cl70
plimptim	43.04 - 51.27	43.82 - 52.06	44.60 - 52.84	45.38 - 53.62	46.17 - 54.40
	40.57 - 50.96	41.16 - 51.54	41.75 - 52.13	42.33 - 52.72	42.92 - 53.31
	41.76 - 50.36	42.38 - 50.98	43.00 - 51.59	43.62 - 52.21	44.24 - 52.83
	41.77 - 50.18	42.33 - 50.74	42.89 - 51.30	43.45 - 51.86	44.02 - 52.42
	41.75 - 51.09	42.43 - 51.78	43.12 - 52.46	43.81 - 53.15	44.49 - 53.84
	41.99 - 51.23	42.58 - 51.83	43.18 - 52.42	43.77 - 53.02	44.37 - 53.61

data	var	cl25	cl30	cl35	cl40	cl45

plimptim	l7	39.21 - 48.33	39.92 - 49.04	40.63 - 49.74	41.33 - 50.45	42.04 - 51.15
	l8	36.53 - 47.79	37.29 - 48.54	38.05 - 49.30	38.81 - 50.06	39.56 - 50.82
	rl	39.61 - 47.28	40.41 - 48.08	41.21 - 48.88	42.01 - 49.68	42.81 - 50.48
	r2	37.91 - 48.28	38.69 - 49.06	39.48 - 49.85	40.27 - 50.64	41.05 - 51.42
	r3	38.50 - 46.72	39.27 - 47.49	40.04 - 48.27	40.81 - 49.04	41.59 - 49.81
	r4	39.77 - 47.13	40.40 - 47.76	41.04 - 48.39	41.67 - 49.02	42.30 - 49.66
	r5	37.68 - 45.61	38.44 - 46.37	39.20 - 47.13	39.97 - 47.90	40.73 - 48.66
	r6	37.09 - 46.08	37.81 - 46.80	38.54 - 47.53	39.26 - 48.25	39.99 - 48.97
	r7	37.94 - 46.09	38.66 - 46.80	39.37 - 47.52	40.09 - 48.23	40.80 - 48.95
	r8	37.93 - 45.79	38.73 - 46.59	39.52 - 47.38	40.32 - 48.17	41.11 - 48.97

data	cl50	cl55	cl60	cl65	cl70
	42.74 - 51.86	43.45 - 52.56	44.15 - 53.27	44.86 - 53.98	45.57 - 54.68
	40.32 - 51.58	41.08 - 52.34	41.84 - 53.10	42.60 - 53.86	43.36 - 54.61

	43.61 - 51.28	44.41 - 52.07	45.21 - 52.87	46.01 - 53.67	46.80 - 54.47
	41.84 - 52.21	42.63 - 53.00	43.41 - 53.78	44.20 - 54.57	44.99 - 55.36
	42.36 - 50.58	43.13 - 51.36	43.90 - 52.13	44.68 - 52.90	45.45 - 53.67
	42.93 - 50.29	43.56 - 50.92	44.19 - 51.55	44.83 - 52.18	45.46 - 52.81
	41.49 - 49.42	42.26 - 50.19	43.02 - 50.95	43.78 - 51.71	44.55 - 52:48
	40.71 - 49.70	41.43 - 50.42	42.16 - 51.15	42.88 - 51.87	43.60 - 52.59
	41.52 - 49.67	42.23 - 50.38	42.95 - 51.10	43.67 - 51.81	44.38 - 52.53
	41.91 - 49.76	42.70 - 50.56	43.50 - 51.35	44.29 - 52.15	45.08 - 52.94

A.2.2 Pilot Data For The mfVEP

The pilot data from the mfVEP is as detailed in Table A.2.2 below. This data was not divided into custom sectors but analysed in the conventional annuli. The mean experience score (and SD) as rated by the normal controls were 2.8 (1.5) and 3.6 (0.9) for the camera and monitor system respectively. All individuals rated the monitor system more highly.

Table A2.2 mfVEP Pilot Data For 20 Normal Controls Detailing Mean Raw Amplitude Values and Standard Deviation (SD)

Annulus Location / Group (n)	1 nV (SD)	2 nV (SD)	3 nV (SD)	4 nV (SD)	5 nV (SD)
Camera (20)	54.5 (20.3)	40.3 (17.2)	37.4 (15.7)	35.2 (18.2)	31.5 (14.9)
Monitor (20)	38.3 (15.2)	27.3 (15.3)	28.2 (13.2)	26.7 (12.4)	22.5 (11.4)

A.2.3 Publications And Presentations

Papers

Lawthom C, Smith PEM, Wild JM. In Utero exposure to vigabatrin: no indication of visual field loss. Epilepsia 2008 Jun 26 (Epub ahead of print)

Lawthom C, Smith PEM, Wild JM. Nasal Retinal Nerve Fibre Layer Attenuation: A Bio-marker For Vigabatrin Toxicity. Ophthalmology (In Press)

Abstracts From Conference Presentations

Lawthom C, Wild JM, Smith PEM Vigabatrin in utero: visual assessment using standard and novel techniques. Journal of Neurology, Neurosurgery & Psychiatry 2006; 77 (S): 1395

Lawthom C, Smith PEM, Wild JM Optical Coherence Tomography identifies Vigabatrin-Attributed Visual Field Loss in children and learning-disabled adults. Investigative Ophthalmology & Visual Science 2007,48: E-Abstract 955

Epilepsia, **(*):1–4, 2008
doi: 10.1111/j.1528-1167.2008.01700.x

BRIEF COMMUNICATION

In utero exposure to vigabatrin: No indication of visual field loss

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SUMMARY

The purpose of the study was to determine whether in utero exposure to vigabatrin caused visual field loss.

Three mothers with four children who had been exposed to vigabatrin in utero and who were subsequently formula fed were identified. All seven individuals underwent perimetry and imaging of the retinal nerve fiber layer (RNFL).

All individuals yielded reliable outcomes to perimetry and RNFL images of acceptable quality. Two of the three mothers exhibited vigabatrin-attributed visual field loss and an abnormally attenuated RNFL. The third exhibited an upper left quadrantanopia, consistent with previous temporal lobe surgery, and a normal RNFL. All four children yielded normal visual fields and RNFL thicknesses.

The presence of the normal findings for the children is reassuring and, if representative, suggests a lack of vigabatrin visual toxicity and therefore obviates the need for ophthalmological examination of those exposed to vigabatrin prenatally.

KEY WORDS: Vigabatrin, Visual field loss, Retinal nerve fiber layer thinning, Prenatal exposure.

The antiepileptic drug vigabatrin is associated with irreversible visual field loss (Eke et al., 1997). The prevalence of vigabatrin-attributed visual field loss is at least 30% (Kalviainen & Nousiainen, 2001) and increases with duration and extent of exposure to the drug (Wild et al., 2007). Vigabatrin-attributed visual field loss manifests as a bilateral concentric constriction. In the mild to moderate stages, the field loss for static perimetry within the central visual field (i.e., within a radius of 30° from fixation) manifests as an annular defect at the extremities of the nasal field, extending to varying amounts above and below the horizontal midline, and also centripetally, with relative sparing of the temporal field. In the advanced stages, the defect is concentric within the central field (Wild et al., 1999). Vigabatrin-attributed visual field loss is initially asymptomatic: the visual acuity remains normal or near-normal, and the nasal field loss is compensated by the relatively well-preserved temporal field in the contralateral eye.

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Perimetry is the "gold standard" for detecting vigabatrin toxicity. However, a developmental age of 9 years is usually necessary to understand the requirements of the examination, thereby excluding many children. In addition, 20%–25% of adults with epilepsy are unable to produce a reliable outcome. Fortunately, a structural measure, namely, attenuation of the retinal nerve fiber layer (RNFL) thickness as estimated by optical coherence tomography (OCT), identifies vigabatrin toxicity with seemingly excellent sensitivity and specificity (Wild et al., 2006). OCT is a noninvasive imaging technique that provides high-resolution cross-sectional images of the retina and is already used in the management of glaucoma and, increasingly, of multiple sclerosis. Vigabatrin toxicity has a characteristic pattern of RNFL attenuation: the nasal quadrant is seemingly universally affected, with or without superior and/or inferior quadrant involvement, while the temporal quadrant is spared. This appearance thus differs from the temporal quadrant atrophy seen in optic neuritis and in optic nerve head hypoplasia.

It can be surmised that, worldwide, a substantial number of women of childbearing age will have received vigabatrin. Uncertainty persists regarding the potential for visual dysfunction in individuals exposed in utero to vigabatrin. To our knowledge, only one report has described the outcome of systematic visual field examination of prenatally

Table 1. The demographics and results for the three families

	Family 1			Family 2		Family 3	
	Mother 1	Daughter 1.1	Daughter 1.2	Mother 2	Daughter 2.1	Mother 3	Son 3.1
Age at assessment (years)	44.3	6.8	10.3	39.8	8.6	39.2	15.8
Vigabatrin gestational dose (kg)	0.560	0.560	0.560	0.662	0.662	0.287	0.287
Vigabatrin dose mg/kg/day equivalent		1200	1200		1410		600
Visual Field	**VAVFL**	Normal	Normal	**VAVFL**	Normal	Normal	Normal
Mean RNFLT (μm)	**55** **Abnormal**	109	106	**70** **Abnormal**	110	104	115
Nasal quadrant RNFLT (μm)	**31** **Abnormal**	102	81	**34** **Abnormal**	71	74	76

Bold type indicates an abnormal finding. VAVFL, vigabatrin-attributed visual field loss.

exposed individuals; two children, unrelated to each other, each yielded inconclusive visual field examinations (Sorri et al., 2005). However, many children exposed to vigabatrin prenatally are now reaching the age where complete ophthalmological examination is possible. The aim of this case series was to assess whether vigabatrin-attributed visual field loss was present in children with in utero exposure to vigabatrin.

METHODS

Three families (four children) were identified in which each mother completed one or more successful pregnancies while receiving vigabatrin for refractory partial epilepsy. All four children were born at term and were exclusively formula fed. Vigabatrin placental transfer, which may reach 100% (Tran et al., 1998; Abdulrazzaq et al., 2001), represented the only mechanism of vigabatrin exposure.

The three mothers were aged 44.3, 39.8, and 39.2 years at the time of the study. The duration of the refractory partial epilepsy was 26, 17, and 24 years, and the duration of vigabatrin exposure was 8.5, 9.8, and 6.7 years, respectively. The cumulative vigabatrin doses were 8.75, 10.50, and 7.32 kg, respectively, and the mean daily dose was 2.82, 2.74, and 2.99 g, respectively. Two mothers (M2 and M3) had taken one other antiepileptic drug (carbamazepine in both cases) within the conception period which was continued throughout the pregnancy in the case of mother M3.

Three of the four children were female. The ages of the children at the time of the study were 6.8, 10.3, 8.6, and 15.8 years, respectively. Cumulative vigabatrin gestational dosage was recorded, and all three mothers reported compliance with medication before and during pregnancy. The estimated in utero exposure to vigabatrin using area under the curve estimates for fetal growth is shown in Table 1.

Perimetry

Each of the mothers underwent two-level (three zone) suprathreshold perimetry of the full field and threshold

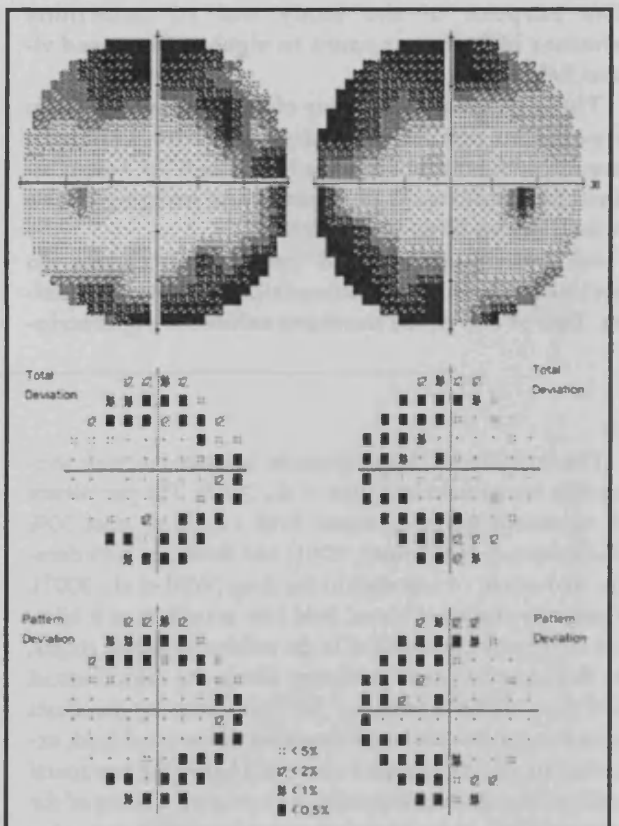

Figure 1.
The appearance of the central visual field of the left eye (left) and of the right eye (right) by threshold static automated perimetry for mother M1, exhibiting the typical binasal annular defect of vigabatrin-attributed field loss, illustrated in terms of the gray scale (top) and of the height (middle) and shape (bottom) deviation probability maps. Abnormality is indicated by increasing levels of gray and by the increasing darkness of the symbols indicating the level of probability associated with the measured value lying within the normal range. *Epilepsia* © ILAE

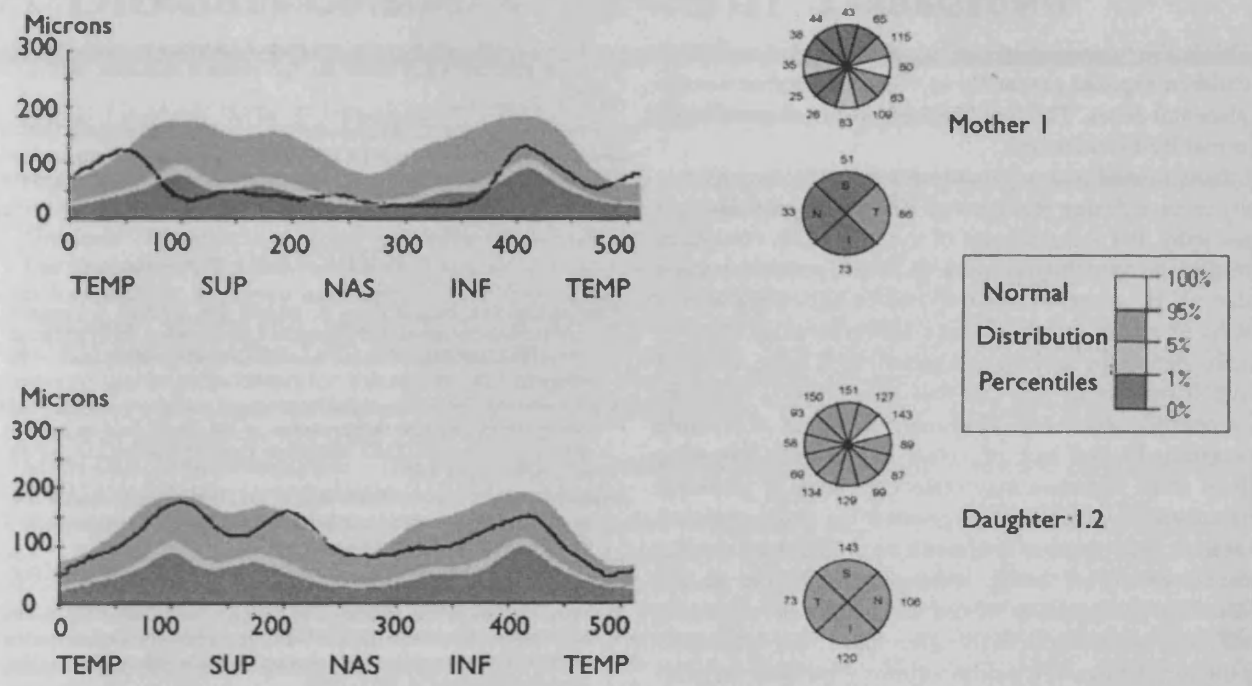

Figure 2.

The printout of the abnormal peripapillary RNFL thickness (solid black line) displayed in terms of Cartesian coordinates (left) for the right eye of mother M1 (top left) and for the normal thickness of the right eye of her daughter (D1.2) (bottom left) and in terms of the various segmental displays (right). The associated percentiles for the normal range are indicated in color (yellow and red represent abnormality).

perimetry of the central field, as recommended by the marketing authorization holder (Aventis Pharma, 2001), using the Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, CA, U.S.A.). Reliable outcomes to the visual field examination, in terms of incorrect responses to the false-negative, false-positive, and fixation-loss catch-trials, were obtained in all three mothers. Each of the children underwent perimetry of the full field in an identical manner to that of their mother. Reliable results were obtained in all four children.

Imaging with OCT

The RNFL thickness was determined for each mother and child, using the RNFL Thickness 3.4 Protocol of the StratusOCT (Carl Zeiss Meditec, Dublin, CA, U.S.A.). All scans exhibited the requirements of a signal-to-noise ratio greater than 25 dB and at least 90% good quality A-scans.

The study had approval from the South East Wales Ethics Committee, and informed consent was obtained from each adult and, in the case of the children, from their legal guardians.

Results

The three mothers and the four children were visually asymptomatic. The visual acuity and the fundus, examined through dilated pupils, were normal in each individual.

Two of the three mothers each exhibited bilateral vigabatrin-attributed visual field loss and an abnormally attenuated RNFL thickness. The third mother exhibited a left upper temporal partial quadrantanopia, secondary to an anterior temporal lobectomy, and a RNFL thickness within the normal range.

All four children manifested normal visual fields. The mean and nasal quadrant RNFL thickness for each child were well within the normal range for young adults (the StratusOCT software does not contain a database for children, although children are considered to exhibit comparable RNFL thicknesses to adults).

The appearance of the central visual field for mother M1, exhibiting typical vigabatrin-attributed field loss, is given in Fig. 1. The printout of the abnormal RNFL thickness for mother M1 and of the normal thickness of her daughter D1.2 is given in Fig. 2, top and bottom, respectively.

DISCUSSION

This is the first report of definitively normal visual fields in children exposed prenatally to vigabatrin across a range of placental doses. The four children also each manifested a normal RNFL thickness.

Infants treated with vigabatrin for infantile spasms normally receive dosing regimens of 100–150 mg/kg/day. It is noteworthy that the estimates of the maximum fetal daily dosing of the four children were up to 10 times this amount (Table 1). However, infants exposed to vigabatrin after 6 months of age are approximately 2.5 times more likely to exhibit vigabatrin toxicity compared with those exposed before 6 months of age (Westall et al., 2007), suggesting a possible physiological immaturity effect. A possible explanation for the lack of visual dysfunction following high in utero exposure may reflect the lack of placental metabolism of vigabatrin, suggested by equal amounts of active and inactive enantiomers, in contrast to the maternal excess of active enantiomer (Challier et al., 1992). The pathogenesis of retinal toxicity in vigabatrin is still not understood. It is speculative, but plausible, that the mechanism of toxicity requires vigabatrin metabolites that may be absent in utero and relatively underproduced in neonates. All children were exposed via placental transfer alone. No assertion can therefore be made regarding the safety of maternal breastfeeding while on vigabatrin.

In terms of the possible visual toxicity of vigabatrin, the findings are clinically reassuring and, if representative, obviate the need to identify and then ophthalmologically examine the children exposed to vigabatrin in utero. Furthermore, for women of childbearing age receiving vigabatrin, the findings can aid informed discussion about potential visual aspects of vigabatrin toxicity. The latter should also be placed in the context of possible unplanned pregnancy in women treated with vigabatrin as an antiaddiction drug (Fechtner et al., 2006).

ACKNOWLEDGMENTS

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflict of interest: C.L. is supported by unrestricted grants from Cardiff and Vale NHS Trust and from UCB Pharma. The authors have no conflicts of interest; however, J.M.W. has formerly provided expert opinion to the marketing authorization holder of vigabatrin.

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Nasal Retinal Nerve Fiber Layer Attenuation: A Biomarker for Vigabatrin Toxicity

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Purpose: To investigate whether nasal peripapillary retinal nerve fiber layer (RNFL) attenuation is associated with visual field loss attributed to the anti-epileptic drug vigabatrin.

Design: Prospective cross-sectional observational study.

Participants: Twenty-seven individuals with focal-onset epilepsy exposed to vigabatrin and 13 individuals with focal-onset epilepsy exposed to non-GABAergic anti-epileptic drug monotherapy.

Methods: At one visit, suprathreshold perimetry of the central and peripheral field (3-zone, age-corrected Full Field 135 Screening Test) and threshold perimetry of the central field (Program 30-2 and the FASTPAC strategy) were undertaken for the right eye using the Humphrey Field Analyzer (Carl Zeiss Meditech, Dublin, CA). At a second visit, ocular coherence tomography was undertaken for the same eye using the 3.4 RNFL thickness protocol of the StratusOCT (Carl Zeiss Meditech).

Main Outcome Measures: The magnitude, for each individual, of the RNFL thickness, averaged across the 4 oblique quadrants, and for each separate quadrant.

Results: Of the 27 individuals exposed to vigabatrin, 11 (group I) exhibited vigabatrin-attributed visual field loss, 15 exhibited a normal field, and 1 exhibited a homonymous quadrantanopia (group II). All 13 individuals exposed to non-GABAergic therapy had normal fields (group III). All individuals in group I exhibited abnormal average and nasal quadrant RNFL thicknesses in the presence of a normal temporal quadrant thickness. Most also exhibited additional RNFL attenuation in either the superior or inferior quadrant, or both. Four individuals in group II exhibited an identical pattern of RNFL attenuation suggesting that nasal RNFL thinning is a more sensitive marker for vigabatrin toxicity than visual field loss. None of the 13 individuals in group III exhibited nasal quadrant RNFL attenuation.

Conclusions: Vigabatrin-attributed visual field loss is associated with a characteristic pattern of RNFL attenuation: nasal quadrant thinning and normal temporal quadrant thickness with or without superior or inferior quadrant involvement. Nasal attenuation may precede visual field loss. Ocular coherence tomography of the peripapillary RNFL should be considered in patients previously exposed to vigabatrin and at baseline and follow-up in those commencing vigabatrin for treatment of epilepsy or in trials for anti-addiction therapy. The pattern of RNFL thinning seems to be a useful biomarker to identify vigabatrin toxicity.

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Ophthalmology 2008;xx:xxx

Vigabatrin is a highly effective antiepileptic drug[1,2] licensed outside of the United States for the adjunctive therapy of focal-onset epilepsy. The first of the new generation of antiepileptic drugs, vigabatrin is a selective and irreversible inhibitor of the enzyme γ-aminobutyric acid (GABA)-transaminase, which catalyzes the inactivation of GABA. The increase in concentration of presynaptic GABA is thought to produce the anticonvulsant effect. Vigabatrin has been widely used beyond the initial licensing to include idiopathic generalized epilepsies, monotherapy prescription, and pediatric use. It is also of considerable benefit in West syndrome[3] and seizures secondary to tuberous sclerosis.[4] Vigabatrin, administered over a short duration and at a low cumulative dose, has also been studied as an anti-addiction therapy for misuse of stimulant drugs;[5] the elevated levels of GABA in the cortex reduce the extracellular dopamine increase, which is responsible for the heightened stimulatory effect in substance misuse.[6]

Eight years after its introduction, vigabatrin was found to be associated with visual field loss.[7] The prevalence of the field loss is at least 30%[8] and increases with the duration and extent of exposure to the drug.[9] Vigabatrin-attributed visual field loss presents as a severe, bilateral/symmetric, "concentric" constriction of sudden/rapid, but variable time to, onset that affects the peripheral field (i.e., beyond a radius of 30 degrees from fixation) more nasally than temporally. The field loss encroaches on the central field where it manifests, in varying extent of severity, as a characteristic deep and steeply bordered bilateral nasal annulus with a relative sparing of the temporal field.[10] In the severest manifestation, the defect is completely concentric to within approximately 15 degrees from fixation. The field loss is usually asymptomatic: The predominantly nasal loss in the ipsilateral eye is compensated by the comparatively well-preserved temporal field in the contralateral eye, and the visual acuity always remains normal or near-normal.[10] Once manifest, the field loss remains stable on with-

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drawal of the drug[11] and seemingly does not progress[12] or progresses very slowly[13] with continued vigabatrin therapy. The visual electrophysiology[14,15] and histopathology[16] of vigabatrin-attributed visual field loss indicates a retinal origin. The nasal predominance of the field loss is less obvious by kinetic perimetry.

As a consequence of the emerging reports of visual field loss attributable to vigabatrin and the variable time to onset of the defect, many patients were withdrawn from the drug; however, a substantial number are still receiving treatment. Vigabatrin is now only prescribed, de novo, for children, learning-disabled adults, and the small number of adults whose epilepsy is refractory to all other antiepileptic drugs. At least 25% of adults with epilepsy and particularly those exposed to vigabatrin are unable to perform perimetry because of their associated cognitive limitations. In addition, noncompliance to perimetry is a problem in those children and learning-disabled adults for whom treatment with vigabatrin is particularly useful, thereby creating an ethical dilemma. In other patients exposed to vigabatrin, the outcome of the visual field examination is frequently inconclusive and often requires 1 or more confirmatory examinations, even after which the results often remain equivocal. Thus, there are substantial numbers of patients in whom the presence or absence of vigabatrin toxicity cannot be established by perimetry.

The fundal abnormalities associated with vigabatrin-attributed visual field loss are subtle when viewed by fundoscopy. The field loss can occur in the presence of a seemingly normal retina or normal optic nerve head.[17,18] However, field loss can also occur with optic nerve atrophy[10,19–21] with or without 1 or more of a variety of retinal abnormalities, including surface wrinkling retinopathy,[21,22] peripheral retinal arterial narrowing,[10,22] peripheral retinal hypopigmentation,[23] irregular sheen at the macula,[22] and thinning of the retinal nerve fiber layer (RNFL).[20,21,24] The attenuation of the RNFL, by both fundoscopy[21] and image enhancement of fundus photographs,[20] can show a nasal predilection that can frequently be associated with corresponding secondary nasal optic atrophy.

The subtlety and variation of the associated optic nerve head and retinal abnormalities preclude the use of fundal examination by ophthalmoscopy as a biomarker of vigabatrin-attributed field loss. Visual electrophysiology has identified biomarkers of vigabatrin-attributed field loss, particularly the 30 Hz flicker electroretinogram;[14,15] however, no one standalone electroretinogram criterion possesses a clinically acceptable sensitivity and specificity.

Measurement of the RNFL thickness using scanning laser ophthalmoscopy,[25,26] optical coherence tomography,[26–28] or nerve fiber layer polarimetry[29] shows considerable potential as a biomarker for vigabatrin toxicity. However, such potential is based on case histories,[25,27] retrospective case analysis,[28] or uncontrolled studies of small numbers of individuals with field loss.[29] Only 1 case-controlled prospective study has been undertaken.[26]

Our case-controlled prospective study[26] measured RNFL thickness using ocular coherence tomography (OCT) with the StratusOCT (Carl Zeiss Meditech, Dublin, CA) and the proportional circle scan set at a scan radius corresponding to the vertical diameter of the individual optic nerve head. At 100% specificity, based on the 90% confidence interval from 20 age-matched normal individuals, the mean of the RNFL thickness over the circular scan yielded 100% sensitivity for 13 individuals with vigabatrin-attributed visual field loss. However, 3 of 8 individuals exposed to vigabatrin but with normal fields and 2 of 14 individuals receiving carbamazepine monotherapy (a non-GABAergic antiepileptic drug) and exhibiting normal fields also manifested mean RNFL thicknesses outside the apparent normal range. The former raises the possibility of an earlier manifestation of vigabatrin toxicity than the predominantly nasal field loss, whereas the latter questions the validity of the confidence intervals.

The Proportional Circle Scan is used with a scan diameter based on a function of the vertical diameter of the individual optic nerve head. This is in contrast with the more commonly used alternative: a fixed scan radius that does not account for variation in the optic nerve head size. The former has the advantage of overcoming the between-individual differences in the topographic variation of the normal nerve fiber layer thickness, inherent with the use of a fixed scan radius, and arising from between-individual variations in the size of the optic nerve head. However, only the fixed scan radius protocol benefits from the manufacturer's standardized and substantive generic database of normal values contained within the instrument software and available to all users. Thus, there is a pressing need to validate the previous findings of RNFL attenuation, obtained with the Proportionate Circle Scan and the small proprietary database,[26] against the fixed scan radius and the corresponding generic database of normal values as a biomarker of vigabatrin-attributed visual field loss, particularly for the nasal quadrant.

The purpose of the study was 2-fold: (1) to investigate the validity of retinal nerve fiber attenuation measured by OCR as a biomarker of vigabatrin-attributed visual field loss, with particular reference to a standardized set of generic normal values (i.e., those of the 3.4 RNFL thickness protocol of the StratusOCT); and (2) to determine whether retinal nerve fiber attenuation within the nasal quadrant is a more sensitive marker of vigabatrin-attributed visual field loss than that for the remaining quadrants.

Materials and Methods

The study used a cross-sectional prospective observational design.

Cohort

The cohort comprised 27 consecutively presenting individuals with focal-onset epilepsy (7 male and 20 female) and exposure to vigabatrin who had volunteered to take part in the study and 13 consecutively presenting volunteers with focal-onset epilepsy (4 male and 9 female), no exposure to vigabatrin or other GABAergic antiepileptic drugs, and current treatment with non-GABAergic monotherapy, primarily carbamazepine.

The mean age of the individuals exposed to vigabatrin was 39.6 years (standard deviation, 14.1). This latter group included 3 learning-disabled adults and 3 adolescents aged 13 years, 13 years,

and 15 years. The individuals with no exposure to vigabatrin (designated as belonging to group III) served as a control. The mean of this group was 47.8 years (standard deviation, 14.2).

Adults were recruited from the Welsh Epilepsy Unit, University Hospital of Wales, Cardiff, and adolescents were recruited from the Pediatric Neurology and Adolescent Services at the University Hospital of Wales, Cardiff. All individuals conformed to rigid inclusion criteria in each eye, including a distance refractive error of ≤5 diopters mean sphere and <2.5 diopters cylinder; open angles, clear ocular media; no fundal or optic nerve head abnormalities characteristic of known disease other than possible vigabatrin toxicity; no previous ocular surgery or trauma; and no history of diabetes mellitus and no family history of glaucoma. All individuals exhibited a visual acuity of 20/30 or better in each eye.

The individuals attended for 2 visits. Visual field examination of the right eye was undertaken at 1 visit, and RNFL imaging of the same eye was undertaken at a second visit. The order of the perimetry and imaging visits was randomized between individuals.

Perimetry

Perimetry comprised 2 examinations: 3-zone, age-corrected suprathreshold perimetry undertaken with the Full Field 135 Screening Test followed by threshold perimetry undertaken with Program 30–2 and the FASTPAC strategy of the Humphrey Field Analyzer 750 (Carl Zeiss Meditec). Distance refraction corrected, where appropriate, for the viewing distance of the perimeter bowl was used for examination of the central field. No correction was used for examination of the peripheral field (i.e., that beyond 30 degrees eccentricity). The left eye was covered with an opaque occluder. Individuals were given frequent rest periods, both throughout and between perimetric examinations, and occasionally required more than 1 visit to provide a conclusive visual field outcome.

Imaging

Measurement of the RNFL thickness was undertaken using OCR with the Stratus OCT (Carl Zeiss Meditec) and the 3.4 RNFL thickness protocol. This approach undertakes 512 sequentially obtained A-scans in 1.3 seconds along a circle 3.4 mm in diameter positioned at the center of the optic nerve head. The contralateral eye was occluded, and individuals viewed the internal fixation target. The z-offset and polarization were obtained before each scan. Three scans were obtained, and the mean of the 3 scans was calculated by the instrument software. All scans exhibited a signal to noise ratio >25 dB and at least 90% good quality A-scans. The mean image was analyzed by Stratus OCT software Version 3.0. In addition, a single representative image was analyzed by Stratus OCT software Version 3.0 to produce an RNFL Thickness Average Analysis Report. The pupil of the right eye was dilated, using tropicamide 1%, for all 10 individuals aged 55 years or more.

Analysis

The visual fields for each individual were evaluated by one of the authors (JMW), who was masked to the antiepileptic drug status and the results for the RNFL thickness and has 25 years of experience of interpreting the results of automated perimetry and 10 years of experience in interpreting vigabatrin-attributed visual field loss. In all cases, but particularly for those in whom the loss was concentric within the central field, the evaluation was confirmed by exclusion of all other potential or confounding ophthalmologic or neurologic causes determined at examination by the corresponding lead clinicians and based on standard investigative techniques, as appropriate, including ocular electrophysiology and brain imaging.

Vigabatrin-attributed visual field loss was defined before the onset of the study as a bilateral, symmetric, "concentric," steeply sided absolute defect in the peripheral field, recorded by 2-level 3-zone suprathreshold perimetry, consistent with the field loss in the central field recorded by threshold perimetry and with an appearance characteristic of that attributable to vigabatrin. In mild to moderate cases, the field loss characteristic of vigabatrin in the central field extends, to varying amounts, in an annulus above and below the horizontal midline at the nasal extremities of the central field and centripetally with varying amounts of sparing of the temporal field. In the most severe cases, the defect is completely concentric within the central field.

The RNFL thickness for each individual was analyzed in terms of the absolute values of thickness displayed in the RNFL Thickness Average Analysis Report (i.e., the average thickness of all 4 oblique quadrants and the thickness for each individual oblique quadrant) and in terms of the corresponding percentile (≤1st, ≤5th, ≤100th percentile) of the result within the manufacturer's generic database of age-corrected normal values. The adolescents were compared with the percentiles for an 18-year-old because the StratusOCT software does not contain a database for individuals aged less than 18 years. Descriptive statistics of the magnitudes of the RNFL were used as appropriate.

Written informed consent was obtained from each individual after an explanation had been given of the nature and possible consequences of the study. In the case of the adolescents and learning-disabled adults, written informed consent was obtained from the parent or legal guardian, as appropriate. The study had approval from the local institutional review board. The study adhered to the tenets of the Declaration of Helsinki.

Results

Of the 27 individuals exposed to vigabatrin, 11 exhibited vigabatrin-attributed visual field loss (group I), 15 exhibited normal fields, and 1 exhibited a superior homonymous quadrantanopsia secondary to temporal lobectomy (group II). Four individuals were receiving vigabatrin at the time of the study (1 in group I and 3 in group II). Four

Table 1. Summary Measures of the Demographic Characteristics for Each of the 3 Groups

Group (No. of Individuals)	Gender		Mean Age (y) (SD; Range)	Mean Duration of Epilepsy (y) (SD)	Mean Duration of Vigabatrin (y) (SD)	Cumulative Dose of Vigabatrin (kg) (SD)
	Male	*Female*				
I (11)	4	7	41.5 (11.1; 20.1–56.4)	25.0 (6.0)	8.8 (2.3)	9.8 (2.7)
II (16)	3	13	38.3 (16.1; 13.4–62.3)	20.1 (8.4)	7.6 (2.8)	6.8 (2.6)
III (13)	4	9	47.7 (14.2; 22.8–67.4)	22.7 (11.4)	—	0

SD = standard deviation.
Group I comprises individuals exhibiting vigabatrin-attributed visual field loss. Group II comprises individuals exposed to vigabatrin but with no vigabatrin-attributed visual field loss. Group III comprises individuals receiving non-GABAergic anti-epileptic drug therapy and with normal fields.

Table 2. Summary Measures of the Retinal Nerve Fiber Layer Thickness Averaged Across the 4 Quadrants and for Each Individual Quadrant

	Retinal Nerve Fiber Layer Thickness (microns) (Group Mean, SD)				
Group (No. of Individuals)	*Average*	*Nasal*	*Superior*	*Temporal*	*Inferior*
I (11)	64.0 (7.6)	37.1 (4.8)	73.9 (15.4)	68.5 (9.7)	76.2 (19.4)
II (16)	91.1 (11.9)	62.0 (14.5)	98.6 (21.6)	79.9 (16.1)	103.6 (17.0)
III (13)	93.2 (11.0)	74.8 (15.1)	107.0 (20.4)	71.4 (13.9)	117.9 (17.3)

SD = standard deviation.
Group I comprises individuals exhibiting vigabatrin-attributed visual field loss. Group II comprises individuals exposed to vigabatrin but with no vigabatrin-attributed visual field loss. Group III comprises individuals receiving non-GABAergic anti-epileptic drug therapy and with normal fields.

individuals in group I and 4 individuals in group II had taken part in the previous study of RNFL thickness using the Proportionate Circle scan.[26] All 13 individuals in group III exhibited normal fields. The summary measures of the demographic characteristics T1 for each of the 3 groups are given in Table 1.

All 27 individuals exposed to vigabatrin were able to undertake suprathreshold perimetry, and all exhibited incorrect responses within the normal range to each of the 3 types of catch trials. Two adolescents and 1 adult could not perform threshold perimetry. For threshold perimetry, 1 individual, exposed to vigabatrin but with normal fields, exhibited incorrect responses to the fixation loss catch trials that were outside of the normal range, although fixation mediated by the gaze tracker was considered stable. Two individuals, both with vigabatrin-attributed visual field loss, exhibited incorrect responses to the false-negative catch trials that were outside of the normal range. Of these latter 2 individuals, 1 also exhibited incorrect responses to the fixation loss catch trials that were outside of the normal range, although fixation mediated by the gaze tracker was considered stable.

The summary measures (group mean, standard deviation, and range) of the RNFL thickness, as a function of quadrant, for each T2 of the 3 groups are given in Table 2.

The frequency, across individuals, of the magnitude of the percentile (≤1st, ≤5th, ≤100th) associated with the measured value of the RNFL thickness for each quadrant in each of the 3 T3 groups is given in Table 3.

All 11 individuals with vigabatrin-attributed visual field loss (group I) exhibited an abnormally attenuated RNFL (i.e., ≤1st or ≤5th percentile) when averaged across the 4 quadrants. All 11 individuals also manifested an abnormal nerve fiber layer thickness in the nasal quadrant; 7 of the 11 individuals exhibited additional abnormal thinning in the superior or inferior quadrant. All 11 individuals exhibited a normal RNFL thickness for the temporal quadrant. An example, from an individual in group I, of the nasal attenuation and temporal sparing of the RNFL together with the appearance of the central visual field is given in Figure 1. F1

Twelve of the 16 individuals in group II (i.e., those exposed to vigabatrin but with normal visual fields and the individual with the temporal lobectomy but otherwise normal fields) exhibited a normal RNFL thickness when averaged across the 4 quadrants and for each individual quadrant. The remaining 4 individuals all exhibited abnormally attenuated average and nasal RNFL thicknesses in the presence of a normal temporal nerve fiber layer. Two of these 4 individuals were 2 of the 3 individuals who had exhibited an abnormal nerve fiber layer in the previous study from our group using the variable scan protocol.

Ten of the 13 individuals in group III (i.e., those exposed to non-GABAergic anti-epileptic drugs) exhibited a normal RNFL thickness when averaged across the 4 quadrants and for each individual quadrant. However, 3 of the 13 individuals each exhibited a normal average thickness but an abnormal thickness in only one of the superior, inferior, or temporal quadrants, respectively. The RNFL thickness in the nasal quadrant was normal for all 13 individuals.

A moderate correlation ($r = -0.47$, $P \leq 0.01$) was present between the RNFL thickness in the nasal quadrant and the cumulative dose of vigabatrin (Fig 2). The magnitude of the correlation F2 is limited by the floor effect of the retinal nerve fiber thickness measurement at approximately 35 to 40 μm, which presumably arises from glial cell hypertrophy replacing the nerve fiber layer[30] and from the finite thickness of the internal limiting membrane.

Discussion

This study confirms that attenuation of the RNFL thickness, relative to the manufacturer's generic database of normal

Table 3. Frequency, Across Individuals, of Magnitude of the Percentile (≤1st, ≤5th) of Measured Value of Retinal Nerve Fiber Layer Thickness, Averaged Across the 4 Quadrants and for Each Individual Quadrant

	Percentile														
	Average RNFL			*Nasal RNFL*			*Superior RNFL*			*Temporal RNFL*			*Inferior RNFL*		
Group (No. of Individuals)	*N*	≤5%	≤1%	*N*	≤5%	≤1%	*N*	≤5%	≤1%	*N*	≤5%	≤1%	*N*	≤5%	≤1%
I (11)	0	3	8	0	3	8	4	5	2	11	0	0	3	5	3
II (16)	12	4	0	12	4	0	15	1	0	16	0	0	15	1	0
III (13)	13	0	0	13	0	0	11	2	0	12	1	0	12	1	0

RNFL = retinal nerve fiber layer thickness; N = a normal valueyy (i.e., >5th percentile).
Group I comprises individuals exhibiting vigabatrin-attributed visual field loss. Group II comprises individuals exposed to vigabatrin but with no vigabatrin-attributed visual field loss. Group III comprises individuals receiving non-GABAergic anti-epileptic drug therapy and with normal fields.

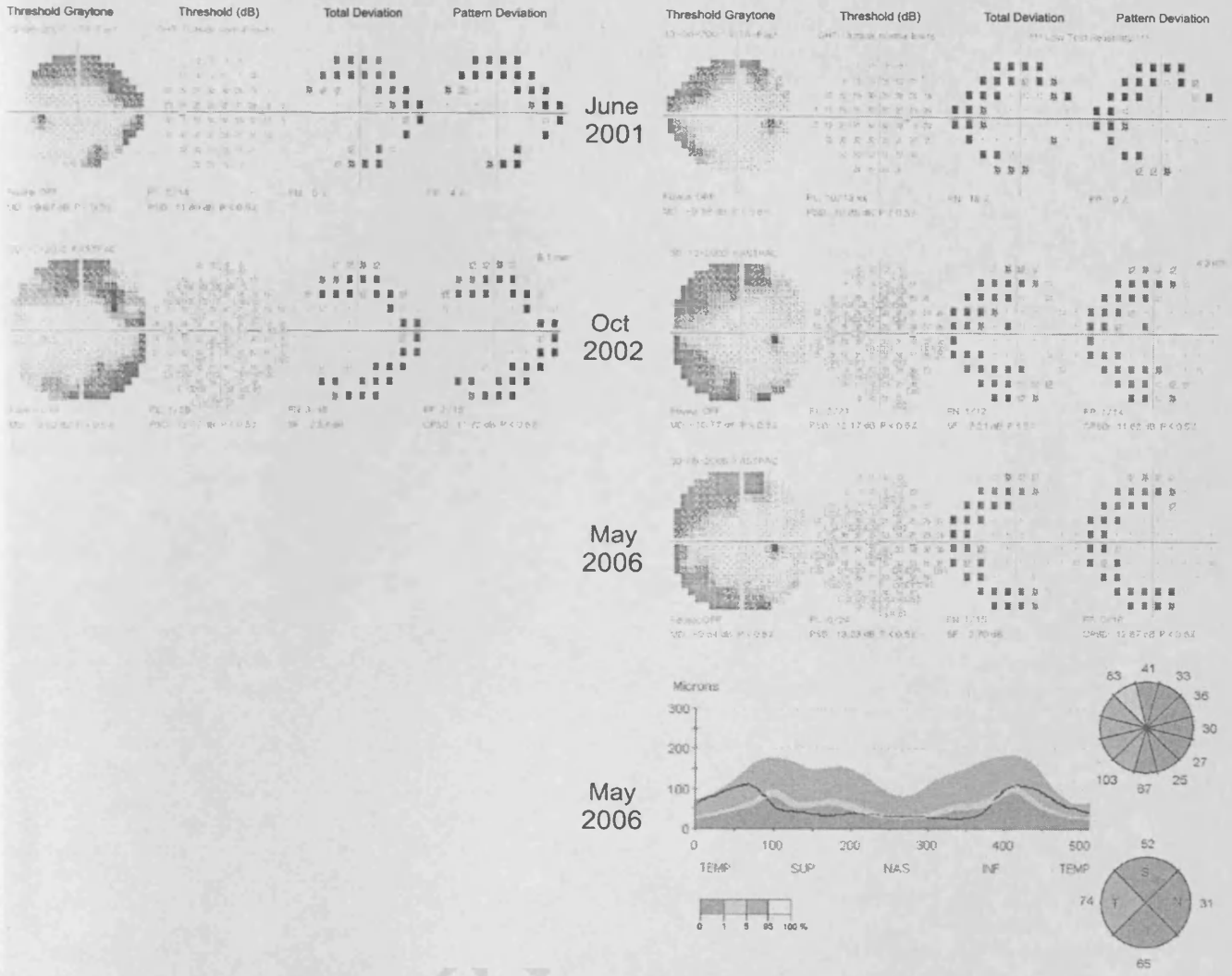

Figure 1. Vigabatrin-attributed visual field loss recorded before the study with Program 24-2 and the SITA Fast algorithm of the Humphrey Field Analyzer (Carl Zeiss Meditech, Dublin, CA) (top), with Program 30-2 and the FASTPAC algorithm (middle top), and as part of the study for only the right eye with Program 30-2 and the FASTPAC algorithm (middle bottom). The corresponding RNFL attenuation for only the right eye recorded with StratusOCT (Carl Zeiss Meditech) (bottom) and illustrated in terms of the height profile (left) and quadrant and sector distributions (right).

values, is associated with vigabatrin-attributed visual field loss. Moreover, it shows that the toxicity is particularly associated with RNFL thinning in the nasal quadrant and preservation in the temporal quadrant.

The predilection for attenuation of the RNFL in the nasal quadrant with temporal quadrant sparing is in agreement with a characteristic pattern of peripheral RNFL atrophy, sparing of the central region, and corresponding secondary optic atrophy that has been found in some individuals with vigabatrin toxicity and labeled, variously, as "C-shaped" or "temporal sparing atrophy"[20] or "inverse atrophy."[21] Such a pattern of optic atrophy is distinct from that of acquired and congenital optic neuropathies. However, the inverse retinal nerve fiber atrophy of vigabatrin toxicity is seemingly difficult to recognize using fundoscopy alone, and, when visible, probably indicates an advanced stage of atrophy. Recognition can also be further confounded by the presence of coexisting optic nerve hypoplasia in some patients treated with vigabatrin. Temporal quadrant nerve fiber layer attenuation is likely to be present only when the field loss is concentric within the central field.

The attenuation of the nasal quadrant RNFL and the preservation of temporal quadrant nerve fiber layer are compatible with the characteristics of the field loss attributable to vigabatrin, that is, a concentric constriction that, in the mild to moderate levels of severity, exhibits a nasal predominance and relative sparing of the temporal field. The attenuated nerve fiber layer in the nasal quadrant of the retina accounts for the temporal field loss. The retinotopic correspondence at the optic nerve head with the visual field is such that the retinal nerve fibers in the unaffected temporal quadrant largely originate from the papillomacular bundle and the fovea, whereas the temporal fibers corresponding to those locations that exhibit nasal vigabatrin-attributed visual field loss enter the optic nerve head immediately on either side of the superior pole (but with a slightly greater preponderance superior nasally) and nasally to the inferior pole.[31]

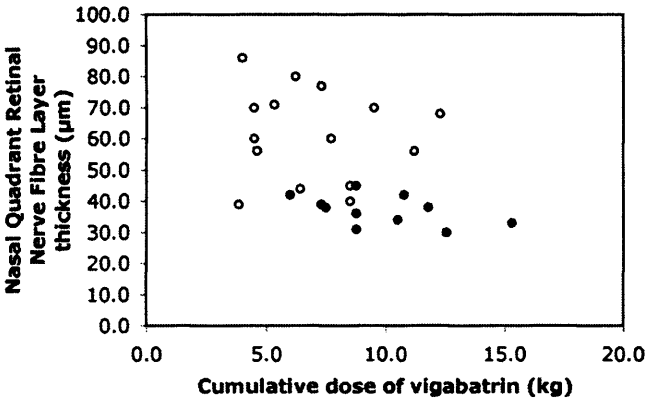

Figure 2. Nasal RNFL thickness (micrometer) as a function of cumulative dose of vigabatrin (kilograms) for the 16 patients exposed to vigabatrin but with no vigabatrin-attributed visual field loss (*open circles*) and the 11 patients manifesting vigabatrin-attributed visual field loss ($r = -0.47$, $P<0.01$).

A similar temporal quadrant nerve fiber layer preservation/sparing is also present in retinitis pigmentosa.[32]

The predilection for nasal quadrant nerve fiber layer thinning in vigabatrin toxicity, measured here by optical coherence tomography, is also compatible with that found by nerve fiber layer polarimetry.[29]

The mechanism of vigabatrin toxicity in the retina is unknown, and the etiologic agent may be vigabatrin itself, the resulting elevated level of GABA within the retina, or a combination thereof. The presence and pattern of nerve fiber layer atrophy may represent primary or secondary, ganglion cell body or nerve fiber, damage.

All 13 patients treated with non-GABAergic drugs manifested a normal nerve fiber layer averaged across the quadrants. Such a finding is in agreement with the presence of a normal RNFL thickness in patients treated with the non-GABAergic anti-epileptic drug carbamazepine[26,33] and the mildly GABAergic drug sodium valproate.[26,33] However, 3 of the 13 individuals manifested an abnormally thin nerve fiber layer (≤5th percentile) in the superior or inferior quadrant. No clinical reason was found for this mild attenuation. It is possible that the attenuation in these 3 cases may have arisen from transsynaptic degeneration because of a longstanding preexisting cerebral lesion. It is also conceivable that transsynaptic degeneration may also be a confounding factor in the vigabatrin-exposed groups. However, as part of their ongoing care for epilepsy, all 40 individuals had undergone epilepsy protocol brain magnetic resonance imaging to identify intracerebral lesions. Only one individual, with the homonymous superior quadrantanopia secondary to a temporal lobectomy, had visual field loss attributable to an intracerebral lesion. She had received vigabatrin, but there was no visual field loss attributable to vigabatrin and her RNFL thickness was normal. We have also recorded a normal RNFL thickness in all 9 cases of a consecutive series of 9 individuals manifesting visual field loss from a cortical lesion. This suggests that transsynaptic degeneration of the retinal ganglion cell axons is not present at all or is not identifiable with the resolution of the StratusOCT. A more likely explanation for the 3 cases of abnormally thin superior or inferior quadrant nerve fiber layer in the non-GABAergic group is inadvertent vertical misalignment of the patient or scan circle leading to an apparent reduction in the nerve fiber layer thickness in the region of the corresponding vertical pole.

On the basis of the various findings, it would seem that an attenuated RNFL, measured by OCT, in at least the nasal quadrant combined with a normal nerve fiber layer in the temporal quadrant is a highly sensitive and specific indicator of vigabatrin-attributed visual field loss. Because of the small numbers of individuals exposed to vigabatrin managed at any one center, including our own, it will be difficult to estimate accurately the magnitude of the sensitivity and specificity. More precise estimates of the magnitudes will only become available as a consequence of a pooling of experiences between centers.

Four of the 16 individuals exposed to vigabatrin but exhibiting normal visual fields exhibited abnormally attenuated average and nasal quadrant RNFL thicknesses in the presence of a normal temporal quadrant thickness. This pattern of nerve fiber layer thinning is identical to that encountered in the individuals with vigabatrin-attributed visual field loss and suggests that measurement of RNFL thickness, at least by OCT, is a more sensitive measure of vigabatrin toxicity than perimetry. The finding is not surprising given that structural abnormality manifests before functional abnormality in, for example, open angle glaucoma[34] and multiple sclerosis.[35]

The proposed use of vigabatrin as an anti-addiction therapy is based on short-term exposure (9 weeks and a total dose of 0.137 kg). Nevertheless, it would be advisable for such patients to undergo regular ocular examination, including RNFL assessment.

The presence of nasal quadrant RNFL attenuation determined by OCT with the 3.4 RNFL thickness protocol can be used as biomarker of vigabatrin toxicity. The technique should be considered as a baseline and follow-up measure to augment perimetry in all patients commencing treatment with vigabatrin for epilepsy or substance abuse.[5] It should also be considered to supplement the examination routine of those patients already exposed to vigabatrin. It is suitable for the assessment of vigabatrin toxicity in learning-disabled adults. For optimum interpretation of the findings in children, normal values will be required for the OCT generic database. In the absence of a definitive visual field result, the development of nasal RNFL attenuation should be considered as a clinical indicator for the evaluation of withdrawal of vigabatrin.

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Footnotes and Financial Disclosures

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