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INVESTIGATING THE EFFECTS OF ANTIEPILEPTIC DRUGS ON THE ELECTROPHYSIOLOGY OF VISION

EMMA LOUISE SPENCER
Doctor of Philosophy

THE UNIVERSITY OF ASTON IN BIRMINGHAM
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Investigating the effects of antiepileptic drugs on the electrophysiology of vision

Emma Louise Spencer

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The principal aim of this work was to examine the effects of antiepileptic drugs (AEDs) on vision. Vigabatrin acts by increasing GABA at brain inhibitory synapses by irreversibly binding to GABA-transaminase. Remacemide is a novel non-competitive NMDA receptor antagonist and fast sodium channel inhibitor that results in the inhibition of the NMDA receptors located in the neuronal membrane calcium channels increasing glutamate in the brain. Vigabatrin has been shown to cause a specific pattern of visual field loss, as one in three adults taking vigabatrin have shown a bilateral concentric constriction. Remacemide has unknown effects on vision. The majority of studies of the effects of AEDs on vision have not included the paediatric population due to difficulties assessing visual field function using standard perimetry testing. Evidently an alternative test is required to establish and monitor visual field problems associated with AEDs both in children and in adults who cannot comply with perimetry.

In order to test paediatric patients exposed to vigabatrin, a field-specific visual evoked potential was developed. Other tests performed on patients taking either vigabatrin or remacemide were electroretinograms, electro-oculograms multifocal VEPs and perimetry. Comparing these tests to perimetry results from vigabatrin patients the field specific VEP was found to have a high sensitivity and specificity, as did the 30Hz flicker amplitude. The multifocal VEP was also found to provide useful results in vigabatrin patients. Remacemide did not produce a similar visual field loss to vigabatrin although macular vision was affected.

The field specific VEP is a useful method for detecting vigabatrin associated visual field loss that is well tolerated by young children. This technique combined with the ERG under light adapted (30Hz flicker) condition is presently the superior method for detecting vigabatrin-attributed peripheral field defects present in children below the developmental age of 9. The effects of AEDs on vision should be monitored carefully and the use of multifocal stimulation allows for specific areas of the retina and visual pathway to be monitored.

Key words: vigabatrin, remacemide, VEPs, ERGs, multifocal

This thesis is dedicated to my parents, George and Phyl Spencer

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CHAPTER 1
A REVIEW OF EPILEPSY

1.1 Classification of epilepsy

Epilepsy is a common neurological condition affecting around 500,000 people in the UK (Brodie, 1990). This condition arises due to abnormal electrical activity in the brain, which gives rise to seizures, which can occur in a number of ways from a short blank daze to falling to the ground and convulsing. Epilepsy is diagnosed when an individual suffers two or more unprovoked seizures. The incidence and prevalence of epilepsy is difficult to assess. The disease has a higher incidence at the extremes of life, both in young children and the older generation over 70 years old, although in developing countries a higher incidence of epilepsy is seen in young adults. Hauser (1997) stated that in general the incidence of epilepsy was highest in males who had a greater risk of unprovoked seizures.

The Commision on Classification and Terminology of the International League Against Epilepsy developed a classification of epileptic seizures (1981), based on a number of factors. Partial and generalised seizures were grouped separately with aetiology and electroencephalography (EEG) results as distinguishing factors. In partial epilepsies, the nature of the seizures is dependent on where in the brain the lesion is located. In complex partial seizures the location is generally in the frontal or temporal lobes. A severe neurological disorder of development, such as abnormal neural migrations or biochemical disorders is associated with generalised epilepsy. Epilepsy can also be classified by the underlying condition of the seizures. Idiopathic epilepsies have an unknown cause but are often age related. Symptomatic epilepsies usually occur as a result of a lesion in the brain, which may be caused by a trauma, tumour or a vascular focal brain involvement. Cryptogenic epilepsy denotes seizures in which an underlying symptomatic cause is almost certain by association with an abnormality but the cause remains elusive.

With the growth of knowledge on brain disorders and the central nervous system, the complexity of the epilepsy classification system has increased. Additionally, the

classification system implies a stereotype of epileptic disorders whereas in real life there are no clear borders separating major classifications and subdivisions. Blume et al (1997) stated that a number of attributes were essential in order to create an ideal classification system. This included the use of clear terms and definitions, with a clinical and laboratory evaluation that allowed for flexibility for future findings.

1.2 Seizure types

Partial seizures are those that begin locally in one hemisphere of the brain, and if the seizure remains confined in one area then the seizure takes on its specific characteristics. A simple partial seizure is identified if consciousness is not impaired and a complex partial seizure is defined if consciousness becomes impaired. Depending on the site of origin of the attack motor, autonomic, somatosensory and visual symptoms may be seen. In complex partial seizures the inhibition of the limbic system is likely as this is required for the maintenance of consciousness. In addition the seizures are likely to involve complex interrelations between sensory information and memory. The ictal EEG results would show localised spike and wave activity. Partial seizures may show secondary generalisation in which the disturbance spreads to both hemispheres after beginning in one localised area.

Generalised epilepsy involves both cerebral hemispheres being simultaneously activated, in which consciousness is usually impaired. Motor manifestations are bilateral. The ictal EEG would show bilateral disturbance of brain activity. An absence seizure, one such generalised seizure, is characterised by a sudden interruption of ongoing activities including a blank stare. During this time the patient will be unresponsive and the attack may last from a few seconds to half a minute. Tonic-clonic seizures are the most frequently encountered of the generalised seizures. Some patients may have some sort of warning, or aura if there is a cortical focus preceding the generalisation, whilst others lose consciousness without any symptoms. A sudden tonic contraction of muscles is followed by a tonic state in which the patient has fallen to the ground and lies rigid. A clonic phase invariably follows during which convulsive movements last for a variable period of time. Patients may also suffer from one of these seizure types without the other. Atonic

seizures are characterised by a sudden attenuation in muscle tone, which may lead to a head drop or to a slumping to the ground. Myoclonic jerks are sudden, brief shock-like contractions that may affect the whole body or may be restricted to the face or trunk.

1.3 Specific seizure types in children

Certain seizure types exist which occur exclusively in children. Infantile spasms constitute such an age-specific epilepsy syndrome. Also known as West's syndrome, the disorder has unique features including hypsarrhythmia, a characteristic EEG pattern consisting of high voltage, multifocal spikes, chaotic slowing and asynchrony (Appleton, 1993). Individual spasms are characterised by symmetric, salaam-like contractions of the trunk, with extension and elevation of the arms and tonic extension of the legs. The aetiology of infantile spasms is either symptomatic or cryptogenic epilepsy. The overall prognosis of this syndrome is poor, with mental retardation occurring in 90% of cases, and 65% likely to develop chronic epilepsy in later life (Appleton, 1993). Consequently, treatment is usually initiated quickly and aggressively after diagnosis in the hope that the course of the disease can be changed. It is clear that an effective treatment of infantile spasms is essential in this battle.

1.4 Neuronal activity during seizures

Nerve cell behaviour is dependent on receptor- and voltage-gated ionic channels and open leak channels. Receptor-gated channels mediate information between cells, whereas voltage-gated channels determine how a neuron integrates information and propagates it to other neurones or effectors. The channels also regulate membrane potential, influence dendrites, generate action potentials and influence calcium loading. Ions are able to cross membranes via energy-driven pumps using adenosine triphosphate (ATP), which set up the ionic gradient underlying current flow and affect the membrane potential to be either positive or negative. Ion pumps are proteins, which generate and maintain the concentration gradient of sodium, potassium, calcium, hydrogen and chloride ions across the plasma membrane, hydrogen across the vesicular membrane and calcium across mitochondrial membrane and endoplasmic reticulum. The release of neurotransmitters are dependent on the behaviour of nerve cells determined by ionic channels which are

large proteins that form ion-permeant pores which may be located in the lipid membrane, dendrites, cell bodies and axons. An action potential, a regenerative process that allows nerve cells to carry electrical impulses along axons, is usually fired at the axon hillock and relies on voltage-dependent sodium channels.

Voltage-gated ion channels control the flow of cations across the cell membranes. One such channel is the sodium ion channel, which plays an important role in the neuronal action potential. The channel consists of a multi-subunit structure that forms a sodium selective, voltage-gated pore through the plasma membrane. This protein structure undergoes conformational changes depending on changes in membrane potential and so regulates conductance through the intrinsic pore. The majority of sodium channels are in a closed, resting state at normal membrane potentials. During depolarisation the channel is activated and facilitates an ion influx. The sodium channel then enters an inactivated state from which it cannot be readily activated. Repolarisation converts the channel back to a resting state from which it can respond to subsequent depolarisations. Such a cycle occurs within a few milliseconds, which is an essential characteristic for maintaining normal brain functions. However, it is this type of activity that has been implicated in the production of epileptic seizures and so this site has been deemed one of the most important targets for seizure control. Such drugs may act by blocking the sustained high-frequency repetitive firing of action potentials by blocking voltage-dependent sodium channels.

Sodium accumulates within neurones and potassium accumulates in the extracellular space causing the activation of the electrogenic sodium pump. The hyperpolarsing drive following this results in the cessation of the seizure, although if the sodium pump loses efficiency the after hyperpolarisation becomes smaller causing the continuing of the seizure and this may account for continuing seizures as seen in status epilepticus. The accumulation of intracellular calcium could also impair function, possibly due to either the depolarisation of mitochondrial membranes interfering with ATP generation as ATP is required to pump calcium out of cells or by the direct modulation of sodium-potassium ATPase. The gradient could also be affected by secondary active transporters, which

transport a second molecule against the concentration gradient such as glucose or amino acids, which may influence membrane potential. Neurotransmitters may affect the membrane potential either by the activation of ligand-gated channels or the activation of the electrogenic transport system.

Voltage-gated potassium currents are thought to be the most diverse ion channel known. The currents are sensitive to voltage, kinetics of activation and inactivation, single-channel behaviour and pharmacological modulation. Three main types of current have been found. The outward delaying rectifying potassium currents have a high activation threshold, the fast transient potassium currents display a fast activation following depolarisation and inactivate fast and inward rectifying potassium currents, which activate negative to the potassium equilibrium potential. Voltage-gated currents have been shown to contribute to seizures by various lines of evidence, such as toxins that prolong sodium channel opening causes seizures and drugs that prevent the activation of potassium currents induce seizures. Burster cells are neurons that when excited produce an all-or-nothing response which generates seizures. Such bursting behaviour consists of depolarising, which triggers a burst of action potentials and have been found in the neocortex, hippocampal areas and especially in the subiculum, which comprises part of the hippocampal formation. In the subiculum, in which 50% of pyramidal cells have bursting properties, seizures are caused by slowly inactivating sodium currents. Voltage-gated currents are also involved in the synchronisation of neurones, which are necessary for the development of seizures.

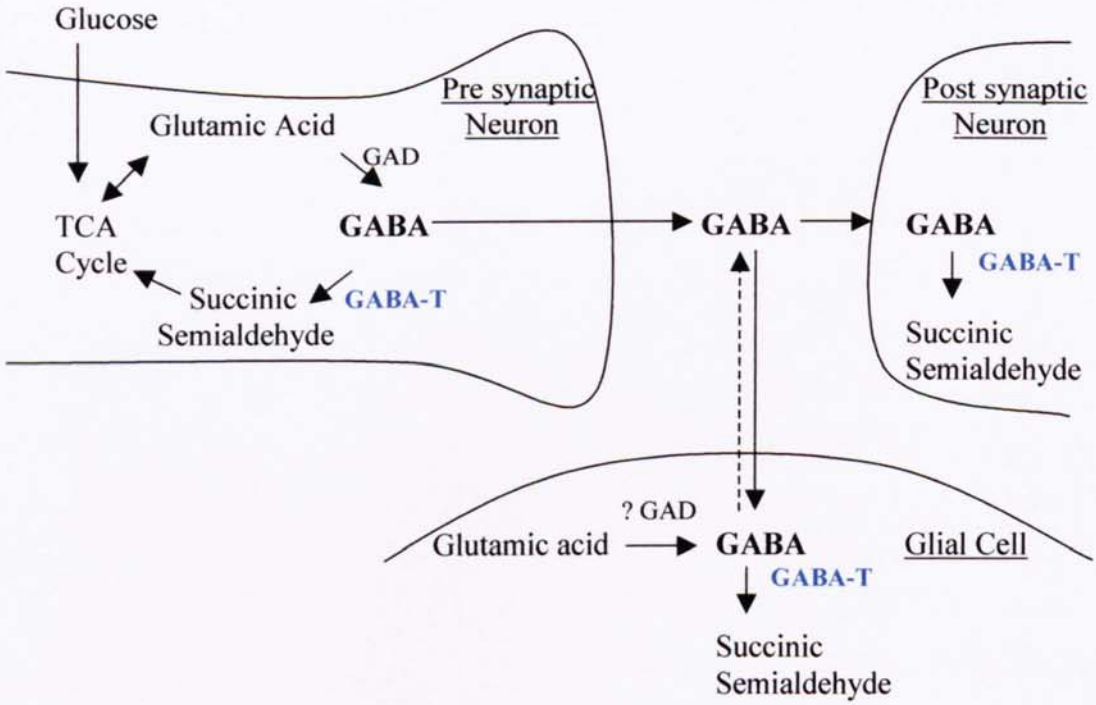
1.5 Inhibitory synaptic transmission

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain and the loss of GABA inhibition has been associated with epileptogenesis. GABA was first localised in the mammalian brain in 1950 (Awapara et al, 1950) and was shown to have inhibitory effects soon after (Bazemore et al, 1957). GABA is mainly found in short interneurons although long GABA-ergic tracts exist running to the cerebellum and striatum. GABA mediates the synaptic events through two types of receptors, namely ionotropic and metabotropic. GABAergic inhibition is the primary form of fast inhibition

in the forebrain and can be presynaptic or postsynaptic. Presynaptic inhibition mediated by GABA occurs when GABAergic nerve terminals release GABA onto presynaptic nerve terminals resulting in a reduction in neurotransmitter release. Postsynaptic inhibition is mediated by the interaction of the neurotransmitter with specific postsynaptic receptors.

The synthesis of GABA occurs in GABAergic presynaptic terminals by the removal of a carboxyl group from glutamate by the enzyme glutamic acid decarboxylase (GAD). In the central nervous system, GAD is the most reliable marker for GABAergic neurones locating GABA inhibitory activity in the mammalian brain and the visual cortex (Iverson et al, 1971). The main supplier of glutamate is the tricarboxylic acid (TCA) cycle and so GABA is ultimately derived from glucose. At least 50% of GAD in the brain is inactive which allows for a controlling mechanism for the rate of GABA synthesis (Martin & Rimvall, 1993). Once synthesised, GABA is kept in synaptic vesicles and is only released into the extracellular space when the GABAergic terminal is depolarised by an action potential. Synaptic release involves calcium dependent exocytosis of vesicles from the synaptic terminal of the presynaptic cell onto the postsynaptic target neurone. GABA then interacts with all GABA receptors located either on the adjacent neurons or on the GABAergic terminal. The high affinity uptake of GABA into nerve terminals or glial cells results in GABA being metabolised into succinic semialdehyde by GABA-transaminase (GABA-T). GABA-T, a mitochondrial aminotransferase that also binds to pyridoxal phosphate, therefore terminates the inhibitory effect of GABA. This synthetic and catabolic pathway is called the GABA shunt (figure 1.1).

Figure 1.1
The GABA shunt.



The effects of GABA are multiple, as actions are mediated by at least three receptor classes. The $GABA_A$ and $GABA_C$ receptors are ionotropic receptors associated with a chloride channel. The $GABA_A$ receptor is a ligand-gated ion channel, which is selectively permeable to chloride ions (Bormann et al, 1987). GABA may activate $GABA_A$ channels presynaptically to produce an increase in chloride conductance thus decreasing the size of the action potential, or activate potassium conductance via $GABA_B$ receptors mediated via G-protein or it may act presynaptically via $GABA_B$ receptors to decrease calcium influx into cells by inhibiting voltage sensitive calcium channels. $GABA_A$ receptors are stimulated by GABA inhibited by convulsants and are directly associated with a chloride ion channel. $GABA_B$ receptors, part of the metabotropic family of G-protein coupled receptors, are stimulated by GABA and appear to be coupled to calcium and potassium channels via second messenger systems. $GABA_C$ receptors appear to have a higher affinity for GABA than the $GABA_A$ receptor (Feigenspan & Bormann, 1994).

$GABA_A$ agonists can cause a decrease in GABA-induced current due to a change in the transmembrane chloride gradient and a decrease in conductance resulting in receptor desensitisation. However the properties of $GABA_A$ receptors may differ in different brain regions so GABA may have a greater inhibitory effect in certain areas. Following activation of GABA receptors with agonists, the receptors may become desensitised and removed from the cell surface membrane entering an internal membrane pool. This process of internalisation, or sequestration, can be followed either by recycling or degradation of the receptors (Sieghart, 1995).

1.6 Excitatory synaptic inhibition

The processing of information in the central nervous system is controlled by the chemical synapse transmission between neurones coupled via excitatory synapses. The excitability of these circuits is controlled by both the amount of excitatory neurotransmitter release and by the way the postsynaptic neuron responds to the released transmitter. Mechanisms are in place that prevent the over excitation within the central nervous system. A number of endogenous compounds have been identified as excitatory neurotransmitters, including glutamate and aspartate which act as agonists at ionotropic glutamate receptors.

Glutamate is the main excitatory neurotransmitter in the mammalian brain. The neurotransmitter has been associated with the propagation of seizures in animal and human models. Similar to GABA, glutamate exerts its effects by receptors from both the ionotropic and metabotropic families. The ionotropic receptors are comprised of various subunits and comprise three specific subtypes of receptors; α-amino-3-hydroxy-5-methylisooxazole-4propionic acid (AMPA), kainite or N-methyl-D-aspartate (NMDA) receptors. All three receptors types form ligand-gated ion channels, permeable to Na^+ and in some cases permeable to Ca^{2+} (Trist, 2000). The AMPA and kainate receptors are implicated in fast excitatory neurotransmission, and the NMDA receptor is quiet at resting membrane potential and is recruited during prolonged depolarisation. The NMDA receptor also differs by having glycine as a co-agonist. The metabotropic group of glutamate receptors are also split into subtypes, termed groups I, II and III. These receptors are G-protein linked and predominantly presynaptic and so possibly control neurotransmitter release (Meldrum, 2000).

1.7 Therapeutic targets of epilepsy

Evidently, many sites exist in which it is possible to alter neuronal function in order to reduce the propensity to have seizures. For example, in order to block generalised tonic-clonic seizures and some partial seizures it was thought that a drug would be required to block sustained repetitive firing, whereas drugs that prevented a broader range of seizures blocked both sustained repetitive firing and enhanced GABAergic inhibition (Macdonald, 1999).

The direct activation of voltage-dependent potassium channels hyperpolarises the neuronal membrane and limits action potential firing. The potentiation of voltage-sensitive potassium channel currents may prove to be an important target for antiepileptic drugs (AEDs). Voltage-dependent calcium channels can be classified into low or high threshold depending on the membrane potential that they are activated. The low-threshold T-type calcium channel is expressed particularly in thalamocortical relay neurones where it is thought to be instrumental in the generation of the rhythmic 3Hz spike and wave discharge, which is characteristic of generalised absence seizures. The high-threshold

calcium channels are distributed throughout the nervous system on dendrites, cell bodies and nerve terminals and have been implicated in controlling the release of neurotransmitters at the synapse. Several AEDs may block the slow pacemaker-driven repetitive firing of the calcium current.

The pharmacological properties of GABA make it a target for epilepsy control, as it is the principal inhibitory neurotransmitter in the brain, with a widespread distribution in the cerebellum, cerebral cortex, hippocampus and striatum. The involvement of the GABA system in the generation of seizures has been the subject of many investigations. As one of the main inhibitory neurotransmitters in the mammalian central synapses, GABA (or the lack of it) is thought to be a key factor in the production of seizures. To support this idea, studies have focused on severe dietary deficiencies of vitamin B_6, which results in seizures in which the supplement of B_6 causes the cessation. Tower (1976) reported on a vitamin B6 dependency although noted that this was a rare syndrome. This can be explained by the fact that vitamin B_6 in the form of pyridoxal 5′-phosphate acts as a coenzyme for GAD, hence resulting in an increase in the synthesis of GABA from glutamic acid. It would be expected that epileptic patients would respond well to such GABA treatment, but this is a rare outcome due to the blood-brain barrier preventing the passing of exogenous GABA.

As GABA synthesis, uptake and degradation occurs in GABA-containing nerve terminals and in cells (glial and neuronal) that do not use GABA, it is difficult to determine exactly how much influence GABA is having on synaptic transmission. Ideally, the question that needs to be answered is how much of an increase in GABA is required to prevent a seizure. However this is difficult to answer by the fact that AEDs have different chemical and pharmacological actions, for example being ineffective in several animal seizure tests but anticonvulsant in others. By dispelling some GABA myths, Gale (1989) attempted to at least partly answer this question. Firstly, an increase in GABA in most brain regions does not give seizure protection, as it is the increase in GABA in the substantia nigra that is necessary and sufficient. Secondly, an increase in GABA does not necessarily result in an increase in GABA synaptic transmission as different cellular components of GABA

exists. Thirdly, blocking is not always a convulsant, for example a GABA antagonist in the substantia nigra does not cause seizures. Changes in GABA-T occur in all areas of the brain although, due to the uneven distribution of GABAergic neurons in the brain, this occurs to differing extents.

The $GABA_A$ receptor has been the main target for AED development as they are the main source of fast inhibitory neurotransmission. The $GABA_A$ receptor complex consists of many binding sites including sites for GABA, benzodiazepines and barbiturates. When GABA binds to the receptor the chloride channel opens causing an influx of chloride anions into a neuron resulting in its hyperpolarisation. The effect of GABA on the $GABA_B$ receptor results in the activation of phospholipase A_2 which catalyses the synthesis of arachidonic acid from phospholipids. This is important as arachidonic acid is involved in modulating cyclic adenosine monophosphate (cAMP) levels. $GABA_C$ receptors are primarily localised in the retina (chapter 3) and their physiological significance is uncertain. GABA-T is a useful target in order to increase synaptic concentrations of GABA and is found in cells other than GABAergic neurons such as glial cells. Highly specific irreversible enzyme inhibitors of GABA-T can be designed by incorporating latent reactive groups into substrates for the target enzymes.

Despite the evidence to suggest GABA is a useful target for epilepsy control, it is clear that GABA-mediated inhibition is not always beneficial. Both experimental and clinical studies have suggested that GABAergic neurotransmission exacerbates absence seizures. Such a fact has been shown in rats (Marescaux et al, 1992) and in children (Parker et al, 1998). From such evidence it is thought that GABA-mediated inhibition may be closely related to absence seizures although other AEDs such as valproic acid (chapter 2), are useful for controlling absences. Such a difference in effectiveness may be due to alternative mechanisms of action at different sites, which may account for the anti-absence effects.

AEDs may focus on the glutamate pathway to exert their effects, as the blockade of ionotropic glutamate receptors may contribute to the reduction of seizures (Meldrum,

1996). Meldrum (1995) showed that a focal injection of glutamate induces seizures in animals and an over-activation of glutamatergic transmission or abnormal glutamate receptors can be seen in seizure models and in human epilepsy models. Consequently the focus of some of the newer AEDs has been on inhibiting the neuronal release of glutamate and the blockade of its receptors (Meldrum, 2000).

CHAPTER 2

THE PAST, PRESENT AND FUTURE OF ANTIEPILEPTIC DRUGS

2.1 Antiepileptic drugs

Around forty distinct epileptic syndromes have been identified, ensuring that epilepsy is a diverse neurological condition that is difficult to control. The accurate definition of the individual epileptic seizure is essential, as it is one of the principle factors on which the choice of AEDs is predicated. By understanding the pathogenesis resulting in biochemical imbalances, which are responsible for seizure generation and propagation, it is possible to alter such changes with medication. As well as those seizure types with an established cause, such as symptomatic epilepsy, there are many other types which have no discernable cause and so very little is known about the pathophysiological basis on which to base medication. Hence medication for epilepsy focuses on the individual patient and the need to control the symptoms of epilepsy by suppressing seizures rather than treating the epilepsy.

Various strategies exist which are used to develop AEDs (Löscher, 1998). These include a random screening of a newly synthesised chemical compound of diverse structural categories for anticonvulsant activity in animal models, the structural variation of existing AEDs and the mechanism based rationale drug development based on the pathophysiology of seizures. Due to this fact many of the drugs used today were found by serendipity, in that a fortunate discovery was made by accident.

Preclinical models used in the identification of AEDs are primarily the animal model. The maximal electroshock seizure test and the pentylenetetrazole seizure test are the most commonly used animal models (Löscher & Schmidt, 1988). The maximal electroshock seizure test is thought to be predictive of generalised tonic-clonic seizures as tonic hindlimb seizures are induced by bilateral corneal or transauricular electrical stimulation. Although this model may also be predictive of partial epilepsies, this has been disputed as many AEDs have been ineffective in treating this type of epilepsy, which may be attributed to using the incorrect model (Engel, 1992). A more suitable partial epilepsy

model has been found in amygdala-kindling. The pentylenetetrazole seizure test is thought to be predictive of generalised absences and/or myoclonic seizures in humans induced by the systemic administration of the drug. Both old and new drugs can be examined to determine if the animal models can predict the clinical effect of the drug by comparing results on animal models and results on human seizures (Löscher, 1998). Numerous examples show that the animal models can be both predictive and misleading when testing different drugs. Consequently, the optimum use of AEDs is often determined once they have been used in a clinical situation.

2.2 First generation AEDs

The first AED manufactured, aimed at influencing excitatory and inhibitory neurotransmitter mechanisms, was valproic acid, which was licensed for epilepsy treatment in the early 1960's. Valproic acid is thought to act via a combination of inhibition of GABA degradation and an enhancement of GABA synthesis (Chapman et al, 1982). Despite such evidence Bernasconi et al (1985) found the drug to show no relationship between chemically induced seizures in animals and the elevation of GABA levels. Other drugs were developed and introduced between 1910-1970 and are often referred to as 'first generation' drugs (see table 2.1).

As can be seen from table 2.1, many drugs act by closing sodium channels. Three drugs mentioned in the table act via mediating an interaction with the GABA receptor system. Postsynaptic $GABA_A$ receptor currents are enhanced by barbiturates and benzodiazepines. Valproic acid has also been implicated in enhancing the release of GABA or to enhance postsynaptic GABA responses. Ethosuximide reduces T-type calcium currents in thalamic neurones and dorsal root ganglion cells.

Table 2.1
Actions of established antiepileptic drugs (adapted from Macdonald, 1999).

Drug	Sodium current	GABA current	Calcium current
Carbamazepine	↓++	-	-
Phenytoin	↓++	-	-
Valproic Acid	↓++	↑?/+	↓?/-
Barbiturates	↓+	↑+	-
Benzodiazepines	↓+	↑++	-
Ethosuximide	-	-	↓++

Key: ↓ close, ↑ open, + effect, - no effect, ? unknown effect

Table 2.2
Actions of new antiepileptic drugs (adapted from Macdonald, 1999).

Drug/Effect	Sodium Current	$GABA_A$ receptor current	Calcium current	Glutamate synaptic function
Vigabatrin	?	+/?	?	-
Lamotrigine	+	?	?	+
Gabapentin	+/?	+/?	+/?	+/?
Tiagabine	?	+/?	?	-
Topiramate	+/?	+/?	?	-
Felbamate	+/?	+/?	-	+/?

Key: + effect, - no effect, ? unknown effect

GABA mimetic drugs have an important use in therapeutic practice, especially in the treatment of epilepsy. Four main modes of action have been established. Firstly, the enhancements of affinity of $GABA_A$ receptor sites have been utilised. Benzodiazepines can enhance GABAergic inhibition by binding to benzodiazepine sites on the $GABA_A$ receptor channel (Tallmann, 1978). This action enhances the binding of GABA to its receptor and enhances the current by increasing the frequency of chloride channel opening. Alternatively, the modulation of $GABA_A$ receptor chloride channels can act to depress the physiological excitations and so enhance the synaptic inhibition of the receptor by increasing the open duration time of the chloride channel (Macdonald et al, 1989). Barbiturates, such as phenobarbital, are known to work like this. It is also possible to increase GABA-T inhibition which is a mechanism specifically targeting a brain mechanism as well as decreasing neuronal and glial uptake of GABA (Nielson et al, 1991).

Over 50 chemically distinct benzodiazepines are currently used worldwide, such as diazepam, clobazam and clonazepam. Such drugs are useful for treating partial or generalised seizures as well as treating status epilepticus. One major drawback of using such drugs is the potential of developing a tolerance to the pharmacological effects. Barbiturates have been in use since the 1900s for their anticonvulsant effects. One such drug, phenobarbital, is still commonly prescribed worldwide for epilepsy. The drug acts by allosteric activation of the $GABA_A$ receptor increasing the duration of chloride channel opening without affecting the frequency of opening or channel conductance. Other benzodiazepines, such as diazepam, act by increasing the frequency of the channel openings. Barbiturates, such as phenobarbital, act by prolonging the opening time of the channel. Additionally both benzodiazepines and barbiturates enhance the affinity of GABA to the $GABA_A$ receptor.

Carbamazepine is related to tricyclic antidepressants and was first introduced onto the epilepsy market in 1963. Both carbamazepine and phenytoin cause voltage-, frequency- and use-dependent block of sodium channels. Both agents suppress the sustained high frequency repetitive firing of neurons. This type of induced reduction is increased after

membrane depolarisation therefore sodium channels are more susceptible to blocking during a seizure activity when the channels are depolarised.

Phenytoin was discovered following a search to identify a nonsedative analogue of phenobarbital. It is thought to act primarily on voltage-dependent Na^+ channels by binding to the fast-inactivated state of the channel and reducing the frequency of sustained repetitive firing of action potentials (McLean & Macdonald, 1983). Phenytoin may have other actions on neurotransmitters such as decreasing presynaptic glutamate release.

Valproic acid is effective in a wide range of seizures although the exact mode of action is still unknown. The drug has been shown to diminish sustained repetitive firing in animal cultures, inhibit the degradative enzyme GABA-T and activates the biosynthesis of GAD although this is not a definite finding as GABA levels do not correlate with antiepileptic effects and it also modestly blocks low-threshold T-type calcium currents.

Ethosuximide has been used to target generalised absence seizures since the 1970s. Interestingly this drug has no consistently useful effect on any other seizure type. This is due to ethosuximide acting by reducing the T-type Ca^{2+} currents in thalamocortical neurones thus preventing the synchronised firing of these channels (Coulter et al, 1989). Such low threshold Ca^{2+} channels predominate in these neurones and are thought to play an important role in the generation of the 3Hz spike-and-wave activity seen during an EEG recording of an absence seizure.

Major studies undertaken globally showed that the classic AEDs had a similar overall efficacy when used to treat epilepsy (Pellock, 2000). However it should be noted that whilst an epileptic patient may not respond to one drug, but responds favourably to another drug of similar mechanism, such as carbamazepine and phenytoin, this indicates that the drugs may work via numerous mechanisms and so table 2.1 often oversimplifies the complex effects of the drugs.

2.3 Second generation AEDs

New AEDs or 'second generation' drugs have been developed by screening or structural variation from existing medication, as well as being based on a rational strategy. After a break of over 20 years, a burst of new AEDs were introduced into clinical practice. Surprisingly the majority of anticonvulsants were still found through serendipity despite the advent of modern research techniques including lamotrigine and topiramate, although the exception to this was the GABA-mimetic drugs such as vigabatrin and tiagabine, which were based on a scientific rationale. Indeed it can be seen from table 2.2, that the mechanisms of action of the newer AEDs have not yet been fully established. However, it is thought that the newer drugs act by different mechanisms to the older drugs, such as inhibiting the glutamatergic excitation, mediated by NMDA and non-NMDA types of glutamate receptors.

The ideal AED would be any drug that was as effective as existing therapy, less toxic than other drugs, easy to use, with a half-life of 12-24 hours with simple kinetics and should not cause tolerance (Richens, 1988). Indeed the new generation of AEDs exhibit fewer drug interactions, better tolerability as well as having no enzyme-inducing properties (Sabers & Gram, 2000), which makes for the newer drugs being a more useful option than the older drugs.

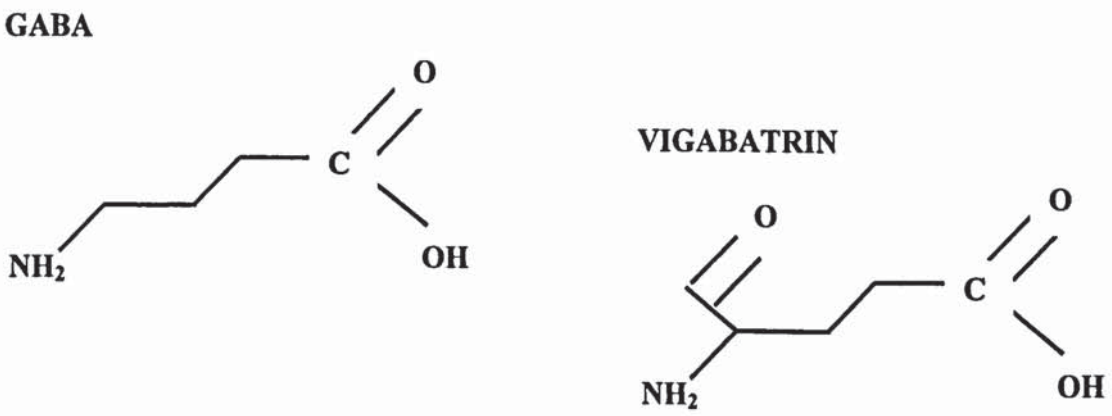
When determining the effectiveness of the new generation AEDs, studies predominantly examine the drugs as adjunctive therapy. This limits the usefulness of such studies as it becomes less clear as to whether the drug will be useful if used as monotherapy. Indeed Brodie (2001) suggested that in order to assess new AEDs a monotherapy trial should be undertaken with patients with newly diagnosed epilepsy. Such studies would establish which AEDs were useful as monotherapy and which are useful as add-on therapy due to a specific pharmacokinetic or a pharmacodynamic interaction.

2.4 Vigabatrin

Vigabatrin (Sabril) is one of the newer AEDs developed by the Merrell Dow Research Institute (Hoechst Marion Roussel, now Aventis). The drug was marketed in Great

Britain in 1989, along with 64 other countries and since 1989 170,000 patients have been treated with this AED (Hinkle, 1998). Also known as γ-vinyl GABA or d1-4-aminohex-5-enoic acid, vigabatrin is a GABA analogue, and is a rationally designed drug that bears a striking resemblance to GABA itself (figure 2.1). Vigabatrin is able to bind irreversibly to the active sites of GABA-T, and so unlike other AEDs the target is located intracellularly. This results in the inhibition of GABA breakdown as it substitutes for the natural substrate and so interferes with GABA metabolism and synthesis, receptor binding and the uptake of GABA (Löscher, 1980). Vigabatrin is able to do this, as it is part of a group of enzyme inhibitors called Kcat inhibitors. Such inhibitors are constructed to ensure that they require chemical activation by the target enzyme, after which a chemical reaction occurs which results in the target enzyme being inactivated (Rando, 1974). In this way it is the enzyme itself, in this case GABA-T, which by its own specific mode of action catalyses its own inactivation.

Figure 2.1
Chemical structure of GABA and vigabatrin.



With this mode of action, vigabatrin is a novel AED as it has a specific effect on the brains chemistry by increasing GABA levels. In theory the inhibition of GABA-T would increase the amount of GABA in the brain, and so this inhibiting neurotransmitter would reduce the brains propensity to having seizures. An alternative mechanism of action has been suggested by Engelborghs et al (1998) which states that brain excitatory amino acid levels and/or elevation of glycine levels may be responsible for the effectiveness of the drug.

Vigabatrin has a favourable pharmacokinetic profile suggesting an uncomplicated use in clinical practice. An oral administration of vigabatrin is rapidly absorbed showing a wide distribution in the body, as the drug is water-soluble. Vigabatrin is not bound to plasma protein and is not influenced by cytochrome P-450 dependent enzymes therefore is not expected to interact with other AEDs (Richens, 1991). Despite this, an interaction between vigabatrin and phenytoin has been found in which plasma phenytoin concentration was reduced (Rimmer & Richens, 1989). However vigabatrin is unlikely to significantly alter the clinical effect of other AEDs as such reductions are minimal. There is no effect of food on the absorption of vigabatrin (Frisk-Holmberg et al, 1989). The transfer of vigabatrin from maternal to foetal blood across the placenta is low (Challier et al, 1992). Vigabatrin is not metabolised in humans and so the amount absorbed is excreted unchanged in the urine. With a plasma elimination half-life range of 5-7 hours being eliminated primarily via the kidneys (Schechter, 1989). Despite the renal clearance being the same in children and adults, the bioavailability is lower in children and so higher doses are needed in children to achieve the same effect.

Vigabatrin was created as an AED on the basis of decreasing GABA in order to create an increase in GABA in the brain, and this fact has been demonstrated in animal models. Indeed vigabatrin has been shown to protect against induced seizures in animals, such as picrotoxin and bicuculline-induced seizures (Kendall et al, 1981). Studies have confirmed that vigabatrin causes an increase in GABA in the brain by examining whole rat and mice brains (Perry et al, 1979, Löscher, 1980) and by examining cat and rat cerebrospinal fluid for free and conjugated GABA (Böhlen et al, 1979). However vigabatrin was found to have differing effects depending on where it was injected in the brain of animal models subjected to maximal electroshock seizures (Gale, 1986). Vigabatrin in the thalamus, hippocampus and cortex failed to protect whereas protection was maximal in the midbrain tegmentum, including the substantia nigra and the midbrain reticular formation. Such evidence indicates that the antiepileptic effect of vigabatrin is a result of a local increase in GABA levels as opposed to a direct effect of vigabatrin.

Results also indicate that vigabatrin does not exclusively increase the amount of GABA available in the brain but also causes an increase in other cerebral enzymes and interacts with other neurotransmitter systems (Löscher & Horstermann (1993). Additionally vigabatrin has also been shown to result in a decrease in GAD activity, which is the enzyme that converts GABA from glutamate (Perry et al, 1979, Löscher, 1980). As a consequence of this action it is possible that vigabatrin may indirectly decrease GABA levels. This fact is difficult to determine as the degree to which endogenous GABA can influence GABA-mediated transmission can depend on whether it effects GABA found in either GABA-containing nerve terminals or in cells, glial and neural that do not use GABA as a neurotransmitter. The former is functionally relevant but vigabatrin can increase GABA in compartments not associated with these nerve terminals (Gale & Iadarola, 1980).

Jung et al (1977) showed that an intraperitioneal (IP) injection of vigabatrin into mice caused a rapid decrease in GABA-T, to about 20% of control activity in 3-4 hours. After several days, as the enzyme is synthesised, recovery occurs. Böhlen et al (1979) found that the small quantity of free GABA found in rat and cat brain increased 30-fold with the administration of vigabatrin. More specifically vigabatrin causes changes in the metabolism of GABA in the rat brain including GABA-T and GAD activity reduced, producing a 150% increase in brain GABA as well as β-alanine, homocarnosine and hypotaurine (Perry et al, 1979). Such increases in GABA had an anticonvulsant effect as an IP injection of vigabatrin was found to protect against bicuculline induced seizures in mice as well as other elicited seizures (Kendall et al, 1981).

Studies continued by examining vigabatrin effects on GABA-T in epileptic patients. Schechter et al (1984) gave vigabatrin orally (1-2 g daily for two weeks) and found a dose-related increase in free and total GABA and homocarnosine. No change was seen in 5-hydroxyindoleacetic acid or homovanillic acid indicating that GABA-T inhibition is both effective and selective as 70% of patients exhibited a reduction in seizures. Riekkinen et al (1989a) expanded this study by looking at excitatory amino acids, cholinergic, dopaminergic, serotonergic, peptidergic and GABAergic systems. GABA

markers were increased more in responders possibly due to the degree of destruction of GABAergic neurons. The only other marker to be increased was glycine, which showed a slight but consistent change.

By examining the CSF of humans a sensitive test was developed to detect if vigabatrin could successfully increase GABA levels in the human brain. The inhibition of GABA-T does successfully increase GABA levels in the brain (Schechter, 1989) showing that vigabatrin could be clinically useful in preventing seizures. More specifically, at a dose of 50 mg/kg/day vigabatrin causes a 200-300% increase in GABA in the CSF and brain tissue (Ben-Menachem et al, 1991). A linear relationship appears to exist between vigabatrin dose and percentage increase of GABA in the CSF, although this does not extend to vigabatrin dose and efficacy, as this appears to be dependent on other factors, such as type of epilepsy. Vigabatrin does show a very poor penetration across the blood-brain barrier due to it being a highly hydrophilic molecule. Consequently, high doses (2-3 mg) are often necessary in man in order to result in effective anticonvulsant treatment (Mattson et al, 1995). It has been suggested that the use of brain absorption enhancers with vigabatrin may both facilitate antiepileptic treatment and possibly reduce side effects (Dimitrijevic et al, 2001).

Animal studies have also focused on the neuropathology of vigabatrin in order to determine its effects on brain function. Neuropathological studies have shown microvacuolation prominent in white matter to differing extents, as in rats a clear effect was seen, reversible isolated microvacuolation was found in dogs and monkeys showed a mild vacuolation in the white matter (Hauw et al, 1988). Interestingly the study also revealed that microvacuolation favoured different brain sites in different species, being the cerebellum in rats, the columns of fornix in dogs and the optic tracts in monkeys, although on average the latter change was seen in all animals. Toxicologic reactions to vigabatrin in rats, dogs and monkeys showed myelin vacuolation of the brain which was limited to the myelinated tracts but was reversible following the discontinuation of the treatment (Butler, 1989). By examining somatosensory evoked potentials in dogs, Arezzo et al (1989) showed vigabatrin to cause slowing of conduction in somatosensory

pathways indicating a vigabatrin induced microvacuolation. All such studies indicate a toxic effect of vigabatrin may occur in humans. Using magnetic resonance imaging (MRI) vigabatrin was found to have caused delayed myelination, axonal degeneration, glial cell death, white matter edema and microvacuolation in immature rat brains (Qiao et al, 2000). This study in particular highlights the potential problems of using vigabatrin in infants and children as the developing brain may be more at risk from such harmful effects.

The fact that long-term administration of high doses of vigabatrin in various animal species caused central nervous system changes resulted in many human clinical studies. Hauw et al (1988) examined one autopsy and one biopsy to reveal no spongiosis in vigabatrin treated patients. In fact neither myelin microvacuolation (Cannon et al, 1991) nor intramyelinic edema (Cohen et al, 2000) is induced by vigabatrin in humans. Evoked potential monitoring examining nerve conduction velocity, supports these findings as many studies failed to show any significant delay in nerve conduction in epileptic patients taking vigabatrin (Hammond et al, 1988) and somatosensory evoked potentials in epileptic patients remained within normal limits (Liegeois-Chauvel et al, 1989). Even after prolonged doses of vigabatrin ranging from one to three and a half years, epileptic patients showed no changes in evoked potential responses (Cosi et al, 1989). All such evidence were against a possibility of microvacuolation changes developing and indicated a lack of neurotoxicity occurring in the brain as a result of vigabatrin treatment. Indeed, despite the concern from animal studies, vigabatrin appeared to be both a useful AED with fewer side effects than most other AEDs.

2.5 Vigabatrin and partial epilepsy

The efficacy of vigabatrin appears to be crucially dependent on the type of epilepsy seizures (Michelucci & Tassinari, 1989). Vigabatrin has been found to be more successful in treating complex partial epilepsy especially those patients exhibiting one seizure type, low seizure frequency and unifocal EEG abnormalities, as opposed to patients with generalised epilepsy who are poor responders (Michelucci & Tassinari, 1989). Perucca (1988) reported on an early double-blind placebo-controlled study in

which severe drug resistant, mostly partial, epilepsy was treated. Vigabatrin caused a greater than 50% decrease in seizure frequency in 60% of the patients. Dam (1991) produced a review of studies and found in refractory epilepsy vigabatrin to be potent and well tolerated, showing no evidence of loss of efficacy associated with long-term therapy. The concomitant use of vigabatrin and lamotrigine revealed a complementary effect, as vigabatrin increases an inhibitory neurotransmitter and lamotrigine decreases an excitatory neurotransmitter, which proved to be useful in treating complex partial refractory epilepsy (Stolarek et al, 1994). As an add-on therapy, vigabatrin has been shown to significantly reduce seizure frequency in uncontrolled complex partial epilepsy (Browne et al, 1989, Dean et al, 1999). In a retrospective study the long-term use of the newer AEDs, including vigabatrin, was assessed for chronic epilepsy, which showed the drugs to have a modest impact on seizures (Wong et al, 1999). Although a reduction in seizures has been noted following vigabatrin treatment, an improvement in the EEG recording does not appear to reflect this. Epileptiform activity may show a modest improvement in some patients, but no abolition of such activity is seen (Hammond & Wilder, 1985). Spectral quantitative EEG recordings also failed to show a significant alteration following vigabatrin monotherapy treatment (Mervaala et al, 1989).

Comparisons between older AEDs and vigabatrin have provided more information on the effectiveness of vigabatrin. A randomised double-blind study directly compared vigabatrin with carbamazepine, and found that although vigabatrin was better tolerated with fewer withdrawals, it was less effective in reducing seizures than carbamazepine (Chadwick, 1999). However in carbamazepine-resistant epilepsy a comparison of add-on therapy between vigabatrin and sodium valproate revealed both drugs to caused a positive response in half of the patients with vigabatrin being more effective in decreasing seizures with fewer dropouts, although this finding was not significant (Brodie et al, 1999). Long-term studies have also shown vigabatrin to be well tolerated with efficacy maintained over many years (Remy & Beaumont, 1989).

A number of CSF studies have been performed on epileptic patients in order to establish how and why vigabatrin is effective in partial epilepsy. A dose-related increase in the

concentration of free and total GABA and homocarnosine has been found in the CSF of patients with refractory epilepsy treated with vigabatrin, indicating a selective GABA-T inhibition (Schechter et al, 1984). Homocarnosine is synthesised by GABA and histidine although the physiological significance of this marker has not been established. Glycine has also been found to increase in vigabatrin patients with complex partial epilepsy, although a three-fold increase of GABA markers also occurred ensuring the drugs specificity (Riekkinen et al, 1989a). GABA markers have been compared in responders and non-responders to vigabatrin in epileptic patients, showing that total GABA and homocarnosine were lower in non-responders (Riekkinen et al, 1989b). This difference may be accounted to a defect in the epileptic brain or due to GABA receptors being more sensitive in responders. Additionally Kälviäinen et al (1993) has suggested that responders to vigabatrin monotherapy have higher glutamate levels in CSF before receiving the drug than non-responders.

Children studies indicate a similar efficacy of vigabatrin to the adult studies, showing partial seizures to respond more favourably (Livingston et al, 1989) and myoclonic epilepsy being aggravated (Luna et al, 1989). In particular infantile spasm, a seizure syndrome that is extremely difficult to treat, appeared to be controlled using vigabatrin (Chiron et al, 1991). Appleton et al (1995) showed how vigabatrin resulted in 13 of 20 patients becoming seizure-free, with a further 3 achieving a greater than 75% decrease in seizures. No adverse side effects were noted and a response was seen within 72 hours of taking vigabatrin.

2.6 Vigabatrin side effects

The introduction of vigabatrin for use as an antiepileptic treatment was associated with the investigation into possible side effects of the medication. The most common side effect noted in a study including 1147 patients was drowsiness and fatigue, with irritability, dizziness, headache and confusion also occurring, although such symptoms were not common for all patients and the majority found the drug to be well tolerated (Mumford, 1988). A multicentre study including 254 patients found 75% of these not to suffer any side effects at all (Reny & Beaumont, 1989). Additionally, a rare side effect

reported by Rogers et al (1993) in which the starting of vigabatrin treatment has been attributed to the development of complex partial seizures. When comparing side effects of vigabatrin with carbamazepine, vigabatrin has been found to be associated more with psychiatric problems and weight gain but less with a rash although overall vigabatrin was found to be better tolerated by epileptic patients (Chadwick, 1999).

In 1997, Eke et al reported peripheral visual field loss associated with vigabatrin and so, eight years after the introduction of vigabatrin onto the market, neurotoxicity was noted in humans. The paper reported on three case studies, which consisted of patients with complex partial epilepsy treated with vigabatrin for a range of 28-38 months who all complained of tunnel vision. In response to this revelation similar cases began to be published (Wilson & Brodie, 1997) with some postulating a dose-dependent, reversible effect of vigabatrin on visual fields (Wong et al, 1997). Conflicting evidence however, suggested that the cessation of vigabatrin treatment did not affect electrophysiological and perimetric functions (Arndt et al, 1999a). Hoechst Marion Roussel replied to these suggestions by stating a frequency of less than 0.1% of 140 000 patients taking vigabatrin showing a visual field defect since 1989 (Backstrom et al, 1997). Initial explanations of the cause of the visual field defect suggested by Harding (1997) included the history of complex partial epilepsy, the increase of GABA by vigabatrin or other drugs, carbamazepine or other drugs or vigabatrin in association with other AEDs causing a change in visual fields.

As vigabatrin had been so successful at treating complex partial epilepsy it was not taken off the market in the UK, especially as a hurried withdrawal may have adverse consequences. In particular the benefit:risk ratio (Harding, 1998) had to be appreciated as although a risk had been linked to vigabatrin, a risk is also attached to uncontrolled epilepsy such as sudden death in epilepsy (SUDEP). As Hoechst Marion Roussel began to identify more visual field defects associated with vigabatrin it became apparent that this defect could be asymptomatic rather than symptomatic (Mackenzie & Klistorner, 1998) hence making the detection of this defect more difficult.

The occurrence of visual field defects, which was predominantly binasal with relative temporal sparing in vigabatrin patients revealed by perimetry (Wild et al, 1999) were expanded to include the investigation of the electrophysiology of the retina in order to establish if the defect was retinal or cortical in origin. This marked the beginning of a plethora of investigations carried out all over the world on vigabatrin patients. As standard visual evoked potentials (chapter 4) remained within normal limits in vigabatrin patients (chapter 5), a retinal effect seemed likely. Multiple studies on the retina, including the electroretinogram (ERG, chapter 4), which examines the electrical activity of the eye and electro-oculography (EOG, chapter 4), which examines the standing potential of the eye, were performed worldwide.

Results from different studies have been examined and many theories were developed as to how and why vigabatrin caused such an unusual visual field defect. It was argued that a toxic effect of vigabatrin on the retina was not surprising as vigabatrin increases brain GABA, and so may also increase GABA in the retina where it is an established inhibitory neurotransmitter (Krauss et al, 1998). As glial cell densities are the lowest in the peripheral and temporal retina, it is possible that these would be more susceptible to a toxic influence hence affecting the peripheral and nasal visual field (Mackenzie & Klistorner, 1998). The transmission between bipolar cells and amacrine and/or ganglion cells may be affected or a loss of ganglion cell function may account for the defect (Ruether et al, 1998). Miller et al (1999) postulated that the proportion of $GABA_C$ to $GABA_A$ receptors is higher in rod bipolar cells, where $GABA_A$ receptors are involved in decreasing the b-wave amplitude in the ERG and this may be the reason why cone cells are affected more than the rod cells. Indeed, when testing patients for vigabatrin associated visual field loss, the cone-specific ERG 30Hz flicker amplitude provides a high sensitivity and specificity for identifying defects determined by perimetry (Harding et al, 2000b, Wild et al, 1999).

All evidence suggests vigabatrin is affecting vision at a retinal level. The ERG results suggest a retinal cone dysfunction, the pattern ERG (PERG) suggests a defect from the ganglion cell layer, the multifocal ERG (chapter 4) suggest the inner plexiform layer and

the EOG suggests an abnormal retinal pigment epithelium. It is possible that areas of the retina with a low density of cell types may be preferentially affected in adverse conditions. Therefore amacrine cells, Müller cells, bipolar and ganglion cells, which are all found at a lower density in the periphery, may be particularly susceptible to the toxic effects of vigabatrin thus causing a predominantly peripheral visual field defect.

It is also possible that vigabatrin may affect cellular mechanisms other than causing the increase of GABA in the retina. As the maintenance of synaptic glutamate pools are dependent on the breakdown of GABA (Pow & Rogers, 1996), it is possible that the fast synaptic transmission through photoreceptors, bipolar and ganglion cells, which is facilitated by glutamate, may be disrupted. GAD, which converts glutamate to GABA, decreases in the brain following vigabatrin administration and this down regulation may affect synaptic transmission. At a molecular level, $GABA_A$ receptors may be downregulated thereby affecting synaptic transmission. It is known that vigabatrin affects other transaminases such as alanine aminotransferase (ALAT), which is significantly reduced in plasma following vigabatrin treatment (Foletti et al, 1995). Indeed vigabatrin appears to cause metabolic disturbances in children (Vallat et al, 1996; Lahat et al, 1999). Such evidence indicates that vigabatrin may result in cross-enzyme inhibition, thus affecting more than one enzyme. Ornithine aminotransferase, when inactivated causes atrophy of the choroid and the retina, and this enzyme may be a target for vigabatrin hence causing a loss of vision (Roubertie, 1998). This constitutes another explanation as to how vigabatrin could cause a toxic effect on the retina. Overall, it is likely that the toxic effects of vigabatrin on the retina is manifold, in that a number of retinal systems are affected by the increase of GABA in this area.

More recently, the central visual function of vigabatrin patients has been tested using short-wavelength automated perimetry (SWAP). SWAP resembles a basic threshold perimetry technique that works by isolating and measuring blue-yellow ganglion cell function. More specifically, blue cones and ganglion cell connections can be tested. The SWAP test is particularly useful if these areas are specifically damaged or if a specific part of the visual pathway is damaged, as the test is designed to be more sensitive and so

a specific loss would be discovered earlier. Hilton et al (2001) performed this test on vigabatrin patients and found a defect to extend into the central retina. The test therefore highlights the possibility that vigabatrin retinal toxicity extends to the central retina by affecting the blue cones and ganglion connections. However it should be noted that standard VEP testing (chapter 5) and standard perimetry depicts central vision to be unaffected by the drug. Additionally patients exposed to vigabatrin have been found to have a reduced ocular blood flow which may have implications in the visual field loss seen in these patients (Hosking et al, 2001).

Similar retinal effects have been seen with other AEDs, which may have a toxic effect on the retina. Studies indicate a possible cause of field defects to be caused by the combination of sodium valproate and vigabatrin (Arndt et al, 1999b). The combination of vigabatrin and gabapentin results in more frequent individual abnormalities in the ERG and lamotrigine was also shown to produce ERG abnormalities in some patients (Koehler et al, 1999). Bayer et al (1991) found carbamazepine to be linked to ERG abnormalities and this has been attributed to the involvement of calcium channels in the retina (chapter 3). However in a study in which 40% of patients taking vigabatrin showed concentric visual fields, none of the patients taking carbamazepine monotherapy showed a visual field defect (Kälviäinen et al, 1999).

An important question to be answered is whether the discontinuation of vigabatrin causes any positive change on the visual field defect. Krakow et al (2000) examined a number of case studies and found that some patients with symptomatic visual field defects benefited significantly from a withdrawal of the drug and it has been shown that even a reduced dose may slow down the progression of visual field loss (Wong et al, 1997). It has also been suggested that reversibility following the discontinuation of vigabatrin treatment is seen particularly in children indicating that repair mechanisms in the young are superior to that seen in adults (Versino & Veggiotti, 1999). However the authors failed to appreciate that the learning effect of perimetry testing and Harding (1997) has suggested that this result may be more related to the practice effect of visual field testing in children rather than any cellular mechanism. Other studies have indicated that no significant

change occurs following the discontinuation of vigabatrin (Johnson et al, 2000) and as the patients that showed any improvement were those with non or minimal visual field loss, the authors suggested that the progression to a visual field defect may be permanent. Nevertheless, if such a reversal were proven to be correct this would have major implications on the use of vigabatrin in infantile spasms.

At this present time it is unclear as to what is the minimum exposure time required to cause a vigabatrin associated visual field defect. In three cases, symptoms have started after 28, 37 and 38 months of treatment (Eke et al, 1997) but the precise moment at which the defect occurs is difficult to establish. Indeed Krauss et al (1998) reported visual symptoms starting between 2-38 months of treatment. In a large cohort, Wild et al (1999) found a prevalence of visual field defects at 32% and 28% for patients taking vigabatrin for less than and more than four years respectively, indicating that after four years it is unlikely for a defect to develop or increase in severity.

At present, the most useful test to use in order to gain reliable visual field results is perimetry, which examines the visual field. Two main methods exist to examine the visual fields, namely the Humphrey automated static and the Goldmann manual kinetic tests (chapter 4). The majority of clinical studies focus on using the Humphrey test although Comaish et al (2002) found a higher rate of vigabatrin associated visual field loss with the kinetic perimetry method. Additionally, when using the Goldmann kinetic perimetry test, the classification of severity of visual field loss (mild, moderate and severe) has been determined (Wild et al, 1999). Such classification allows for a more direct comparison of perimetry results with other test results. Guidelines for clinical practice from Hoechst Marion Roussel (1998) deemed that regular perimetry is necessary to determine whether an individual exhibits visual field defects. However, as reliable perimetry results can be difficult to obtain from paediatric patients, the Vigabatrin Paediatric Advisory Group (2000) acknowledged that an alternative test was necessary for children and so vigabatrin should be prescribed to this patient population with caution.

2.7 Third generation AEDs

Although epilepsy can be controlled by various AEDs, over 30% of sufferers continue to have seizures (Brodie, 1990). This is a highly volatile situation for epileptics who often have a poor quality of life and are at constant risk of sudden death in epilepsy. Additionally even if seizures are controlled by medication, side effects often from combinations of anticonvulsants, can themselves be disabling. Consequently there has been a market for new AEDs of which the mode of action can be determined. Indeed for many epileptics, the development of a new drug is their only hope of achieving control (Loiseau, 1988). Although surgery for epilepsy may be an option in certain cases, such as those with a specifically located cause in the brain, it would be more cost effective to develop a more effective and less toxic AED by which many more could benefit. Newer AEDs have been in preclinical or clinical development, and such drugs can be referred to as 'third generation'.

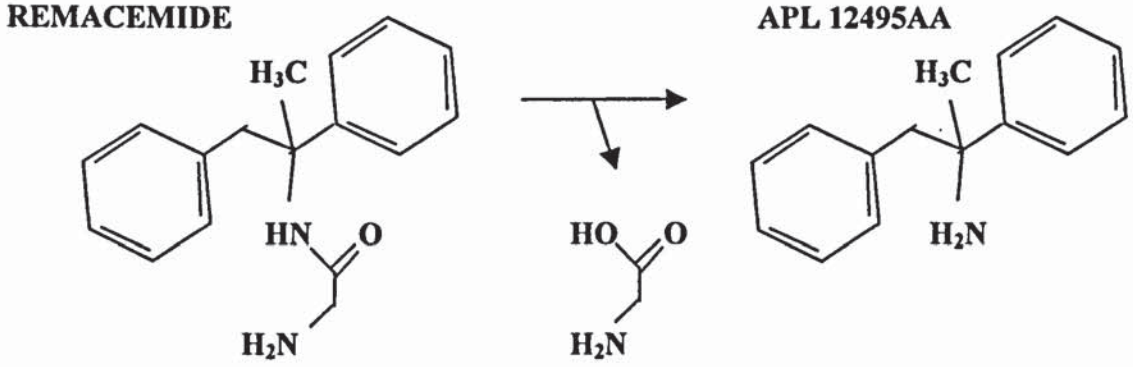
2.8 Remacemide

Remacemide hydrochloride (APL 12924, (± 2-amino-N-(1-methyl-1, 2-diphenylethyl)-acetamide monohydrochloride) is a new AED with a novel mode of action which acts by decreasing excitatory neurotransmission. The drug has been shown to exhibit anticonvulsant properties in a number of animal models, as well as promising results in phase II clinical trials. Remacemide is pharmacologically active but the desglycinyl metabolite (APL 12495AA) is more potent, in vivo and in vitro, and so remacemide hydrochloride can be classified as a prodrug. Remacemide is a non-competitive low affinity NMDA receptor antagonist, which also interacts with voltage-dependent Na+ channels (Schachter & Tarsy, 2000).

It is probable that remacemide and APL 12495AA share a common mechanism of action. They both have the ability to inhibit NMDA-induced depolarisations in a non-competitive manner in a number of preparations suggesting that the compounds are low-affinity blockers of NMDA-operated ion channel. A further possible mechanism has been suggested to inhibit sustained-repetitive firing, which implies an antagonistic action on fast sodium channels (Wamil et al, 1996). This theory has been backed up by the fact that

the inhibitory action of APL 12495AA occurs on veratridine-stimulated glutamate release, as veratridine elicits transmitter release through the prevention of sodium channel inactivation (Srinivasan et al, 1995). However it is believed that the main mechanism by which remacemide and APL 12495AA act is through the blockade of the NMDA-operated channel. By blocking NMDA receptors in the neuronal membrane, calcium channels are thought to block the Ca^{2+} influx mediated by major excitatory neurotransmitters mainly glutamate and glycine. This reduces cortical neuronal activity and so potentially enhances the suppression of seizures.

Figure 2.2
Chemical structure of remacemide transformed to its active desglycinated metabolite, APL 12495AA.



Receptor-binding studies have shown that remacemide and APL 12495AA displace [^{3}H]MK801 binding from the synaptic membrane fractions of the rat cerebral cortex and hippocampus (Ray et al, 1992). Some evidence does suggest that remacemide and APL 12495AA may act at distinct sites. When examining NMDA receptor currents in cultured rat hippocampal cells and rat forebrain membranes. Subramaniam et al (1995) found differences in blocking properties of the two drugs.

Remacemide and its desglycinated metabolite are lipid soluble and absorption into the brain and the gastrointestinal tract is rapid (Heyn et al, 1994). Remacemide hydrochloride is not extensively plasma protein bound and has a half-life of three to four hours in humans, whereas the desglycinate has a half-life of 12-19 hours (Clark et al, 1995).

Remacemide hydrochloride is completely metabolised and no intact drug or active metabolite is excreted. Interactions of remacemide have been investigated . Leach et al (1996) found carbamazepine induced the metabolism of remacemide which itself inhibited carbamazepine oxidation. Lamotrigine also had no pharmacokinetic interaction with the drug (Blakey et al, 1999).

Remacemide and APL 12495AA have been shown to have anticonvulsant action in a number of animal models, although the latter has shown to be more potent (about 2-fold) than the parent compound in all animal models. The duration of protection of remacemide against maximal electroshock convulsions following PO administration was two hours, whereas APL 12495AA showed a 50% protection after four hours (Garske et al, 1991). The potency of the two depend on the route of administration and the species used although such differences were thought not to be biologically significant. The two compounds have been found to be ineffective in seizures elicited by bicuculline, picrotoxin, strychnine or pentylenetetrazol as well as corneal-kindled seizures in rats (Palmer et al, 1992). Examining the effects of remacemide on spike-wave discharges, EEG and behaviour in rats with absence epilepsy indicated that remacemide may be effective against absence epilepsy (Van Luijtelaar & Coenen, 1995).

Remacemide hydrochloride has been investigated in phase II clinical trials in patients with partial seizures with or without secondary generalisation (Clark et al, 1995). In a double-blind, placebo-controlled crossover study Crawford et al (1992) presented 28 patients with refractory epilepsy with 33% showing a reduction in seizure frequency. The frequency of secondary generalised seizures was reduced by 50% or more in a third of all patients and four were seizure-free during treatment. Additionally, Leach et al (1997) found remacemide to be both a potent and well tolerated new AED, showing an improved responder rate at a higher dose of 800-1200mg/day. Significant EEG improvements have been reported with remacemide (Owen et al, 1992).

A further use of remacemide has been developed for the treatment of Parkinson's disease. Remacemide was administered to patients who had Parkinson's disease with dyskinesias

and was found to be effective for improving symptoms without exacerbating levodopa-induced dyskinesias (Clarke et al, 2001). Side effects associated with this drug included vomiting, dizziness and abnormal vision.

2.9 Remacemide side effects

Generally, remacemide has been shown to be well tolerated (Chadwick et al, 1994). Nausea has been associated with remacemide hydrochloride use in one patient during the washout period (Leach et al, 1997). Blakey et al (1999) found during a Phase III study that the pharmacokinetics of remacemide but not APL12495AA were affected by renal impairment although concluded that the drug could be used safely in mild hepatic impairment and with caution in more severe hepatic impairment. Besag et al (2001) showed that remacemide used as an adjunctive therapy for children with persistent refractory epilepsy was well tolerated with no serious adverse events.

CHAPTER 3
THE RETINA, VISUAL PATHWAY AND THE EFFECTS OF NEUROTRANSMITTERS

3.1 The retina

Lining the posterior three-quarter of the eyeball is the retina, which constitutes the beginning of the visual pathway. The retina is part of the central nervous system as it is derived from the neural ectoderm, which also gives rise to the brain, during development. The synaptic organisation of the retina contains five major classes of neurones; photoreceptors (rods and cones), bipolar cells, horizontal cells, amacrine cells and ganglion cells all of which are linked together via numerous connections and arranged into nuclear layers. The photoreceptors, bipolar cells and horizontal cells make synaptic connections with each other in the outer plexiform layer. The bipolar, amacrine and ganglion cells make contact in the inner plexiform layer. The bipolar cells also bridge the two layers. This arrangement of cells results in an inverted retina as light must traverse through some of these neurones before impinging upon the photoreceptors. Within the retina, two distinct regions can be found comprising the fovea, a small depression in the centre of the macula lutea and the optic disc, a site where the optic nerve exits the eyeball, which is also termed the blind spot.

Cone photoreceptors respond to bright light so are used for day vision and are most numerous in the fovea. There are three types of cones each with a different pigment that is sensitive to a different part of the visible spectrum and so they are used for colour vision. Rod photoreceptors are highly sensitive and are specialised for night vision, containing more photopigment than cones although only one type of rod photopigment is used for achromatic vision. A cone-dominated retina is responsible for visual function at high levels of light adaptation known as photopic vision, whereas at low levels of light adaptation a rod dominated retina is responsible for scotopic vision. When rods and cones contribute to vision in intermediate levels of light, this is termed mesopic vision. The fovea contains a high density of cone photoreceptors and is the area of highest visual acuity and resolution in the absence of rods. In the peripheral retina both rod and cone

photoreceptors can be found although at increasing eccentricities cone density decreases rapidly. However cone density has been found to be 40-45% higher in the nasal retina as compared to the temporal retina at increasing eccentricities (Curcio et al, 1990).

As opposed to most neurones, rods and cones do not fire action potentials but instead respond to light in graded changes in membrane potential. During darkness, sodium ions flow into the outer segment of the photoreceptors through cyclic guanosine monophosphate (cGMP) gated channels. Potassium selective, non-gated channels are found across the inner segment membrane. In darkness, current enters the photoreceptor through cGMP-gated channels, where it is carried by sodium ions and flows back out through the non-gated potassium ions channels ensuring a membrane potential of -40mV. Potassium ions are able to flow out of these channels as the photoreceptors are depolarised in respect to the potassium ions equilibrium potential. In order to maintain a steady intracellular concentration of sodium ions and potassium ions, a high density of sodium-potassium pumps can be found in the inner segment, which pump sodium ions out and potassium ions in. Light serves to hyperpolarise photoreceptor as absorption of light lowers the cytoplasmic concentration of cGMP in the outer segment causing the closure of cGMP-gated channels. The large conductance produced by the non-gated potassium channels drives the photoreceptor membrane potential to -70mV. Rods respond slowly so that photons of light absorbed can be summated, ensuring small amounts of light can be detected. Contrary to this, cones respond much faster ensuring a greater temporal resolution of the visual image.

Bipolar cells represent the most direct pathway to relay signals from the photoreceptors to the ganglion cells. Bipolar cells lack voltage-gated sodium channels and so do not generate action potentials. The cells display spatial antagonism having a centre-surround receptive field organisation, with either an on- or off-centre. Light falling on the centre of the receptive field causes excitation (depolarisation) and light falling in the surround causes inhibition (hyperpolarisation) in the on-centre bipolar cells. These cells form a synapse, called a triad, where a bipolar cell and two horizontal cells form an invagination within the photoreceptor. Those bipolar cells with an off-centre exhibit a more

conventional flat synapse with two horizontal cells onto the photoreceptor. Glutamate is believed to have different effects on the two classes of bipolar cells. In dark conditions, photoreceptors release glutamate and so light results in hyperpolarisation and a decrease in neurotransmitter. For on-centre bipolar cells it is thought that glutamate is inhibitory so that a reduction of this would cause a depolarisation of the bipolar cells. In off-centre bipolar cells the opposite occurs in which glutamate is excitatory and the hyperpolarisation of the photoreceptor occurring as a result of the neurotransmitter would cause inhibition of the bipolar cell.

Two major pathways exist in the mammalian retina, the cone bipolar pathway and the rod bipolar pathway. The pathways begin in the outer plexiform layer where the rods and cones contact separate bipolar cells. The rod and cone bipolar cells lead to the inner plexiform layer and converge on the same ganglion cell. The rod pathway is less direct as it includes amacrine cells and functions best under conditions of low illumination, whereas the cone pathway functions best under high illumination (review Freed, 1992). Similarly to bipolar cells, amacrine cells show a centre-surround organisation. Amacrine cells respond transiently to a stimulus and so play a role in detecting movement. As opposed to the other retinal cells, amacrine cells display action potentials.

Ganglion cells are the output neurones of the retina as their axons form the optic nerve, which projects to the visual cortex. The fovea contains the greatest density of ganglion cells and so has a larger neural representation than the periphery of the retina, which contains fewer ganglion cells. Such a difference in innervation is due to the shape of the eye, which is designed to rotate in its socket. As a result of this the retina cannot have more area in the centre than in the periphery and so this is compensated for by packing in retinal ganglion cells in and around the fovea. This physical limitation does not occur in the brain and so neurones are evenly distributed over a wide area. The ratio of the area in the primary visual cortex (V1) and the area in the retina is termed the magnification factor.

Ganglion cells transmit information using action potentials via bipolar, horizontal and amacrine cells. This is an important attribute for ganglion cells as the axons must traverse a long distance and action potentials do not decay over distance and myelin increases the speed of transmission. Each ganglion cell, which shows spatial antagonism, responds to light directed to a specific area of the retina, termed the receptive field. Similar to bipolar cells, the majority of ganglion cells have circular receptive fields divided into a centre and an antagonistic surround. Two major classes of retinal ganglion cells are on-centre and off-centre. On-centre ganglion cells increase action potential firing when light is directed onto the centre of their receptive field and reduces firing when light stimulates the surround. Conversely off-centre ganglion cells produce their highest firing rate when light is applied to the surround or during the period immediately after light is removed. The fact that ganglion cells respond weakly to diffuse light and best when light intensity in the centre and surround are different indicates that the cells respond best to contrast in visual input as opposed to intensity of light. The size of the receptive field increases with retinal eccentricity, so that foveal cells have small receptive fields whilst large receptive fields are found in the periphery. The receptive field of ganglion cells is governed by the proximity of central photoreceptors that are antagonistic to surrounding photoreceptors. Foveal ganglion cells may have as few as one cone contributing to the receptive field resulting in a high degree of spatial resolution, whereas the large receptive field of peripheral ganglion cells results from the summation of many cones. In this way the receptive fields of ganglion cells reflect the collective properties of neurones that precede them.

Cones in the centre of a ganglion receptive field make direct contact with bipolar cells, which then make contact with ganglion cells, termed the central pathway. Signals from cones in the surround are conveyed to ganglion cells via horizontal or amacrine cells, termed the lateral pathway. The central and lateral pathways are responsible for mediating the centre-surround antagonistic receptive fields of retinal bipolar cells and ganglion cells. Neurotransmitters involved in such processes include glutamate used in the excitatory central pathway and GABA used in the inhibitory lateral pathway. The

main function of GABA is to provide antagonistic surround inputs to bipolar and ganglion cells (Wu, 1992).

On- and off-centre ganglion cells are present in almost equal numbers and provide two parallel pathways for processing visual information. Most ganglion cells are in two classes either the M or P cells. M cells have larger cell bodies with large dendritic fields and so have large receptive fields, which respond to large objects and gross features. P cells have smaller cell bodies and dendritic fields so hence have smaller receptive fields so are used for examining small features as well as colour vision. Retinal ganglion cells can be classified tonic or phasic, which denotes their transient response to a stimulus. Tonic cells give a sustained increase in firing to a sustained stimulus, whereas phasic cells only respond to a stimulus change. In the cone system of the Rhesus monkey a greater number of tonic cells can be found throughout the retina particularly towards the fovea, whereas less phasic cell can be found in the fovea being more common in the periphery (Gouras, 1968). The difference in temporal properties may be attributed to the M cells receiving input from transient amacrine cells, whereas P cells receive input from sustained amacrine cells (Werblin & Dowling, 1969). The distribution of phasic cells may explain why the cone flicker fusion frequencies increase away from the fovea. Many differences are apparent when M and P cells are compared and the main ones are shown in table 3.1.

Table 3.1
The differences between properties of M and P cells in the monkey retina (adapted from Kaplan, 1991).

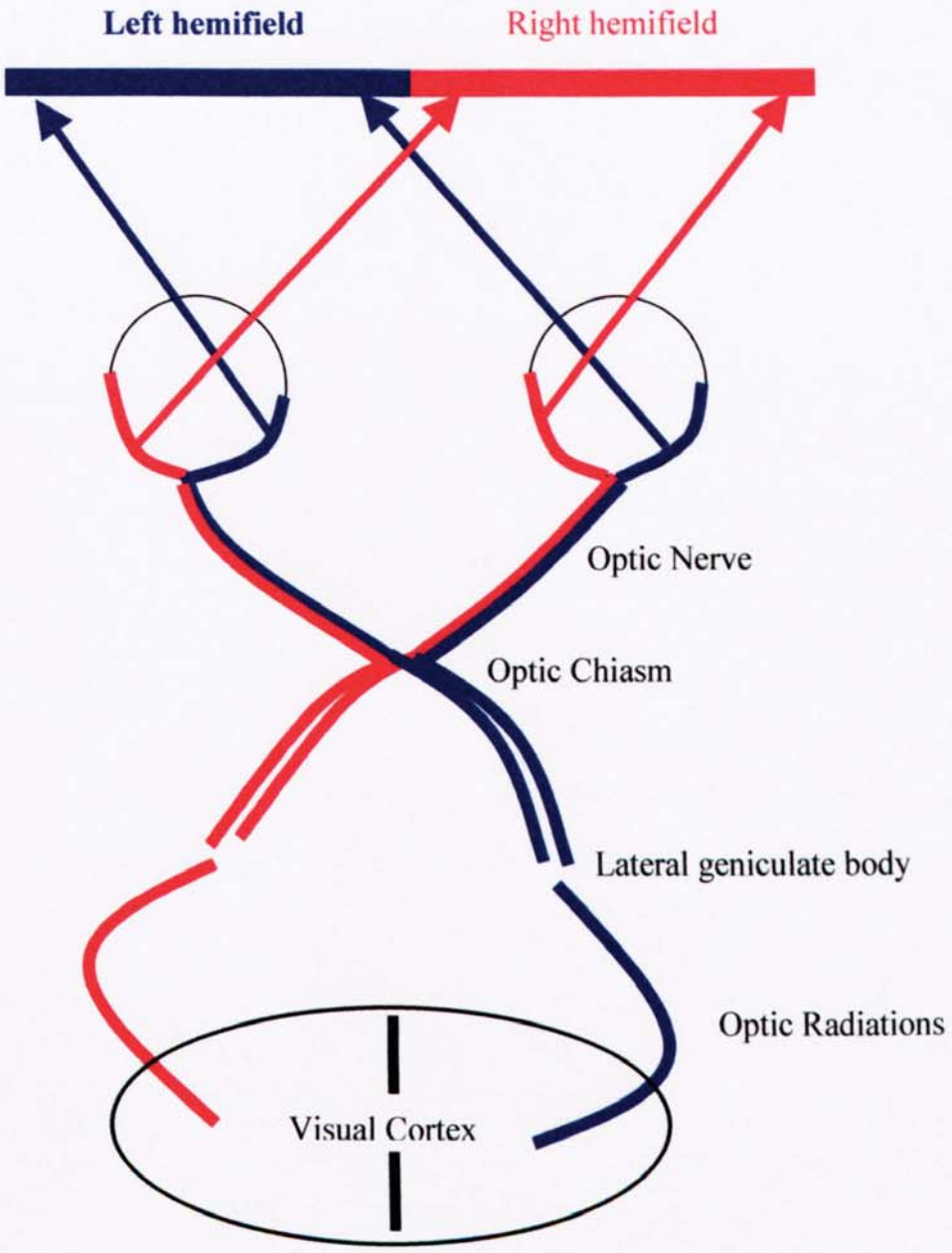
Property	M cells	P cells
Receptive field size	Larger	Smaller
LGN target	Magnocellular	Parvocellular
Conduction velocity	Higher	Lower
Cell size	Larger	Smaller
Response to light steps	Phasic	Tonic
Number of cells in millions	0.15	1.2

3.2 The visual pathway

The amount of area that is visible to each eye is termed the field of vision with its outer half being the temporal field and its inner half being the nasal field. Light from the temporal field falls on the nasal half of the retina and light from the nasal field falls on the temporal retina. The nerve fibres arising from these two halves remain separate in the optic nerve until they come together to form the optic chiasma, which is situated below the third ventricle and above the pituitary gland. The chiasma is about 12 mm wide by 8 mm deep, and the crossing of half of the nerve fibres results in stimulation of corresponding points in the visual field of the two eyes sending signals to the same half of the visual cortex in the occipital poles. Fibres in the temporal retina relating to the nasal visual field do not decussate and fibres in the nasal retina relating to the temporal visual field do decussate. As the number of decussating fibres is larger than non-decussating fibres, this results in a larger peripheral temporal field.

The nerve fibres in the chiasma are arranged in a format that is based on the area of the retina that they originate from. Decussating fibres from the macula cross more centrally and fibres from the inferior nasal part of the retina decussate more anterior in the chiasma (review Roberson, 1996). Due to this arrangement impulses from the right hand side of the field of vision, which stimulate the left hand side of each retina, run in the left optic tract to the left cerebral hemisphere into the left occipital cortex and vice versa (figure 3.1). In the optic chiasma fibres from each nasal retina cross over to join the fibres from the temporal retina of the other eye. The two sets of fibres then form the optic tracts, which project to three subcortical regions. The pretectal area of the midbrain controls pupillary reflexes, the superior colliculus controls saccadic eye movements and the lateral geniculate nucleus (LGN) processes visual information.

Figure 3.1
The visual pathway.



Despite the LGN being the main thalamic structure conveying information from the retina to V1, its role in vision is still unclear. It acts as a neuronal relay station with 80% of the visual fibres entering the LGN after partial decussation at the optic chiasma. The remaining fibres pass to the superior colliculus. Although it can be described as the group of cells that receives input from the retina and sends connections to the cortex, the LGN also consists of subdivisions that do not receive retinal input. Additionally 20-25% of the neurones within the LGN are interneurones and so do not send out axons from the nucleus.

LGN cells, similar to ganglion cells, have concentric receptive fields being either on- or off-centre and respond best to small spots of light within the receptive field centre. Similar to the retina, the on- and off-centre pathways are independent and are divided into the M and P pathways, which consist of six layers of cell bodies. Layers 1 and 2 comprise the magnocellular layer, which receives its main retinal input from M cells that are involved in the initial analysis of movement of the visual image. The four remaining dorsal layers comprise of the parvocelluar pathway, which receives its main retinal input from P cells that are involved in the analysis of fine structure and colour vision. Fibres from the contralateral nasal retina contact layers 1,4 and 6 and fibres from the ipsilateral temporal retina contact layers 2,3 and 5.

The LGN axons spread out to form optic radiations, which reach V1. Each point in the retina is represented by a wedge shaped area in the LGN, with the central 15^0 of the visual field being represented in over 50% of the LGN. Wiesel (1960) noted that the receptive field centre size was an important determinant of visual acuity as smaller receptive field centres were found in the macular region and the antagonistic effect of the surround was weaker as the centre became larger. Using a grating pattern, Enroth-Cugell & Robson (1966) found the contrast sensitivity for individual ganglion cells. Lesions introduced in the magnocellular region of the LGN cause a reduction in the ability to resolve high frequency flicker and a reduction in contrast sensitivity at low spatial frequencies (Schiller & Logothetis, 1990). Such evidence indicates that the parvocellular system analyses high spatial frequency information and is useful for detecting spatial

details and colour contrast. The magnocellular system is thought to be more primitive being sensitive to large stimuli.

Found on the dorsal surface of the midbrain, the mammalian superior colliculus is homogenous to the optic tectum in the lower vertebrate. In mammals, this area has been somewhat 'downsized' having reduced in size and significance as the visual cortex became more highly developed, hence only 20% of the optic nerve fibres leaving the optic chiasm pass to the superior colliculus. As the superior colliculus projects to the regions of the brain stem that controls eye movements, it was thought that it was primarily the centre for reflex eye movements. The superior colliculus is divided into seven layers alternating between fibrous and cellular layers. Such layers are further subdivided into superficial (I – III) and deep (IV – VIII) sections. The superficial layers receive input from the retina so cells are primarily concerned with vision, while deeper layers are concerned with eye movements. The activity of the two layers is not always coordinated as the two separate pathways can be considered separately.

The visual receptive fields of the LGN and superior colliculus differ. The LGN is divided into two magnocellular and four parvocellular layers. The parvocellular layers are sensitive to high contrast achromatic gratings presented as drifting gratings or pattern reversal modes as well as chromatic gratings of low spatial frequencies. The superior colliculus responds to flashes of lights and movement and becomes unresponsive to sustained stimulation.

3.3 The visual cortex

The lateral surface of the occipital lobe is marked by several lateral occipital gyri and the medial surface contains the cuneus between the parieto-occipital sulcus and the calcarine sulcus. V1 occupies the walls of the calcarine sulcus along its entire length, with the sulcus being 10 mm deep. It emerges onto the medial surface of the hemisphere for 5 mm above and below the sulcus and onto the occipital pole of the brain for 10 mm. V1 has gained an alternative name, the striate cortex, as in a freshly cut brain the visual cortex is easily identifiable by a thin band of white matter within gray matter. This band is called

the line of Gennari and this area is also referred to as V1, visual area 1, primary visual cortex or Brodmann's area 17.

Information from the LGN enters V1 and flows systematically from one cortical layer to another, beginning with spiny stellate cells in layer 4, which receives direct input from the LGN and projects up to layers 4B, 2 and 3. Projections from layers 2 and 3 project down to pyramidal cells in layer 5, which in turn project to pyramidal cells in layer 6 via axon collaterals. The pyramidal cells of layer 6 send axon collaterals to layer 4 to excite the inhibitory smooth stellate cells, which also modulate the firing of excitatory spiny stellate cells and so completing an inhibitory feedback loop. The striate cortex projects to the extrastriate cortex and in temporal and parietal pathways, which are not independent. Reciprocal projections from V1 also exist going back to the LGN, usually originating from the deeper layers 5 and 6. Superficial layers generally project to higher levels of extrastriate cortex (Lund et al, 1979).

The majority of neurones in V1 do not have circular receptive fields which respond to small spots of light but instead respond only to stimuli that have linear properties, for example a bar or a line (Hubel and Wiesel, 1962). These cells are have been termed either simple or complex. Simple cells do resemble cells of the LGN, having on and off zones that are more rectangular in shape. Such cells are most sensitive to a light or dark edge, and are sensitive to different orientations of a bar with a specific width that must be properly positioned within the receptive field. Antagonistic excitatory and inhibitory regions still exist. The shape of the receptive field is formed by the summation of LGN receptive fields arranged in a line together. Such creating of more complicated receptive fields is referred to as serial or hierarchical processing (Hubel & Wiesel, 1962) and resembles the process occurring in the retina as photoreceptors, horizontal, bipolar and amacrine cells combine to from the receptive fields of ganglion cells.

Complex cells are larger than simple cells with less clearly defined on and off zones, although an elongated stimulus of a specific orientation is still required. It is thought that simple cells summate to form these complex cell receptive fields, although in a nonlinear

fashion, as separate excitatory and inhibitory areas are not found. However in complex cells, the stimulus can be positioned anywhere within the receptive field. In particular complex cells are sensitive to moving stimulus. Both simple and complex cells appear to be important in analysing the form of the visual image by examining its contours and boundaries. Additionally the interaction of simple and complex cells may be essential in the perception of form.

The M and P pathways have different destinations in the visual cortex, as they project to different parts of V1 and V2. By staining the metabolic enzyme cytochrome oxidase, the distinctive modular organisation of V1 and V2 has been revealed. A further subdivision of cells, which do have circular receptive fields, can be found in layers 2 and 3 as well as layers 5 and 6, and due to their 3-dimensional shape are termed blobs. Interblobs refer to the superficial region of the striate cortex between blobs. As layers 2 and 3 receive most of their input from the parvocellular layer 4Cβ, blobs and interblobs are thought to be part of the parvocellular pathway. However, blobs also receive magnocellular input and have different response properties from interblobs indicating that they could be considered as a separate subdivision. Blobs are thought to be wavelength selective and lacking orientation, whereas interblobs are orientation selective being responsive to differences in wavelength or luminance (review Zeki & Shipp, 1988).

The two sets of layers have different projections as the parvocellular cells project to cortical layers 4a and 4cβ, whilst magnocellular cells (which comprise only 8-10% of the whole LGN) projects to the superficial half of the 4cα. In general the parvocellular cells have colour-opponent receptive fields, small receptive fields with linear spatial summation properties and conduct at medium velocities to V1. In contrast magnocellular cells are not colour selective, have large receptive fields, are less linear in their spatial summation and send high velocity signals to V1.

V1 sends projections to neighbouring cortical areas that in turn send projections to higher visual areas. Many visual areas exist which contain a map of the visual field, termed a retinotopic map. The main target of V1 is visual area 2 (V2 or Brodmann's area 18).

Higher visual areas appear to maintain, if not enhance, the segregation of properties. V5 (the middle temporal lobe) is specialised for movement a fact established by evidence from brain-injured subjects with specific cerebral lesions. V4 contains an abundance of cells with chromatic sensitivities. The inferotemporal cortex responds to complex forms such as faces. Such facts support a theory of functional specialisation in the visual cortex. However such distinct organisation is not independent as pathways communicate to allow for the effective analysis of visual information.

In general within the fovea LGN cells, as well as retinal cells, have smaller receptive fields centres than those found in the periphery of the visual field. In this way larger fields are found with increasing eccentricity from the central visual field (Perry and Cowey, 1985). This asymmetry appears to increase from the retina to the cortex as in the retina the number of ganglion cells per degree of field is 100 times higher in the fovea than the periphery, whereas in V1 the area devoted to the fovea is almost 1000 times higher than in the periphery (review Casagrande & Norton, 1991). The decline in ganglion cell distribution from fovea to the peripheral retina is not radially symmetric as the decline is less steep along the nasal horizontal meridian. The density of cones mirrors this difference but not to the same extent as ganglion cells, as the density of nasal cones is never greater than one and a half times that of temporal cones at matched eccentricity, except in the extreme periphery (Perry & Cowey, 1985).

Conflicting evidence has been found as to whether the parvocellular and magnocellular pathway are equally magnified. As the parvocellular layers have more cells than the magnocellular layers it is easy to assume they have an unequal representation. It has been suggested that the parvocellular layers are more devoted to central vision and the magnocellular layers are more devoted to the periphery. However Livingstone & Hubel (1988) indicate that neither pathway is different in the central and periphery and this was based on the anatomical ratio of magnocellular and parvocellular LGN cells which did not change with eccentricity.

Spatial vision is required for the perception of borders, lines and edges. If the visual system is thought of as a Fourier analysis, then it breaks vision into its separate spatial frequency components and reassembles them to produce the percept. In order for this to work, independent spatial frequency channels are needed within the visual system rather than one single channel. Indeed DeValois et al (1982) found that cortical neurones respond to sine wave gratings and are selective for a particular spatial frequency. Temporal vision is concerned with the analysis of changes in luminance over time, such as the detection of a flicker produced by a flashing light. Studies suggest that temporal vision is analysed by specialised visual pathways that feed to motion analysis areas within the cerebral cortex.

As colour is a property of an object, colour vision is essential in detecting patterns and objects and so enhances the visual experience for an individual. Within the retina, the trichromatic theory states that three different cone pigment types exist which allow for the perception of colour. Each pigment type is sensitive to light of a particular wavelength approximately 420, 530 and 560 nanometers respectively for short-wavelength sensitive (S cones), medium-wavelength sensitive (M cones) and long-wavelength sensitive (L cones). As a cone absorbs a photon an electrical response is generated which is always the same regardless of the wavelength of the photon. This attribute is termed univariance, and occurs due to all-or-none conformational change in the retinal molecule (Baylor et al, 1987). Although individual cones respond preferentially to a particular colour it is not possible for the nervous system to determine what colour is stimulating the cone. This is because each cone will respond equally to light of any wavelength as long as the intensity of the light compensates for the cell's rate of absorbance at that wavelength.

The distribution of the three types of cones is uneven, as S cones are scarce in the fovea increasing in the parafoveal area and declining with eccentricity. M and L cones are found mainly in the fovea and decline with eccentricity. The distribution of S cones is also different to M and L cones, being sparse and creating large gaps between

neighbouring S cones. Consequently S cones give poor acuity and can distort boundaries (Boynton, 1982).

3.4 GABA in the retina

The retina is very rich in neurochemical substances including GABA, dopamine and 5-hydroxytryptophan. Kojima et al (1958) first established the presence of GABA in the retina, although traces of the amino acid could not be found in the optic nerve and so it was established that GABA was located in cells within the eye. The highest concentration of glutamic acid decarboxlyase (GAD) was found in the inner plexiform-ganglion cell region, with GABA-T being found more diffusely in ganglion cells, inner plexiform layer and the photoreceptor layer (Hyde and Robinson, 1974). Additionally Macaione (1972) found GABA-T within Müller cells. Multiple GABA plasma membrane transporters are expressed in the vertebrate retina (Brecha et al, 1995) which play an important role in regulating synaptic activity by high affinity uptake of GABA from the extracellular space.

Problems investigating GABA in the retina have arisen due to the fact that the GABAergic neurones appear to be found in a population of amacrine cells that are small and scattered. 55% of all amacrine cells are thought to be GABAergic in the human retina (Crooks & Kolb, 1992) and with 30-40 different morphological subtypes of amacrine cells, it is believed that at least five types are GABAergic (Kolb, 1997). Additionally interplexiform cells have been shown to contain GABA (Crooks & Kolb, 1992).

Despite the finding of GABA in horizontal cells of the lower vertebrate (Marc, 1992) it remains unclear as to whether it is found in the mammalian horizontal cells. Immunoreactivity for GABA and GAD has been determined in horizontal cells (Wassle & Chum, 1989) although this has not been found in all studies (Hendrickson, 1985). It is hypothesised that the mammalian horizontal cells, whilst lacking the ability to uptake GABA, can regulate their content of GABA by altering synthesis and release (Pow & Rogers, 1996). Within horizontal cells, GABA plays an important role in retinal development including maturation and differentiation (Nag & Wadhwa, 1997). The

majority of input to the horizontal cells is from cones that act in a feedforward manner. In light, the cone is hyperpolarized and so transmitter release is suppressed causing the membrane hyperpolarisation of horizontal cells and the suppression of GABA release.

GABA is used in the lateral pathway by subpopulations of horizontal and amacrine cells, and exerts postsynaptic effects on cones, bipolar cells and ganglion cells. Major GABAergic synapses include feedback and feedforward synapses from horizontal cells to bipolar cells, feedback synapses from amacrine cells to bipolar cells, feedforward synapses from amacrine cells to ganglion cells and amacrine-amacrine cell synapses (Wu, 1992). More specifically, GABA is thought to inhibit cholinergic amacrine activity as the light-evoked release of acetylcholine is reduced by the introduction of GABA (Cunningham & Neal, 1983). Additionally Pourcho (1980) found that five classes of amacrine cells accumulate GABA and as they provide an input to the inner plexiform layer, GABA mediates the inhibitory actions on ganglion cells. Ganglion cells within the central retina appear to be more sensitive to transmitters than in the periphery (Priest et al, 1985).

The major supporting cells (marcoglia) found in the retina are the Müller cells that contact all layers of the retina. By removing transmitters from the extracellular space, Müller cells are able to control the environment within the retina. Nag & Wadhwa (1997) have located GABA in the axonal processes of Müller cells and $GABA_A$ receptors and uptake transporters have been observed (Reichenbach et al, 1997). The GABA transporter (GAT-1) is an abundant transporter of GABA found in the brain, as well as in the retina particularly in the inner plexiform layer and postsynaptically on Müller cells (Brecha & Weigmann, 1994). Another GABA transporter, GAT-3, has also been found in the retina (Durkin et al, 1995) and so transporters are in use both in neurones and glial cells within the retina.

Animal models have been widely used to investigate the activity of GABA in the retina, as they exhibit many similarities to the human visual system. Within the fish retina, GABA has been found to be present in horizontal cells acting via cone photoreceptors

(Wu & Dowling, 1980). GABA has been shown to exert a tonic hyperpolarisation in red- and green-sensitive cones in the turtle retina, so if a lack of light causes a tonic GABA release then the cones are depolarised by disinhibition (Kaneko & Tachibana, 1986). A substantial amount of work has focused on the cat retina, showing cone horizontal cells to be GABAergic (Vardi et al, 1992) and $GABA_A$ agonists to modify the time course of photopic responses in the inner and outer plexiform layer making responses slower and less phasic (Frumkes & Nelson, 1995). It has been highlighted that in the cat retina, GABA can have contrasting effects on morphologically distinct neurones, such as the many amacrine cell types and the interplexiform layer (Bolz et al, 1985). GABA-mediated inhibition plays a role in defining response properties in visual cortex cells, for example GABA effects on the discharge properties of cat visual cortex cells have been demonstrated (Duffy et al, 1976).

Investigations into GABA activity in the retina also extends to human studies, which have indicated that $GABA_A$ receptors may mediate the receptive field surround of both on and off bipolar cells as well as the inhibition between amacrine and bipolar cells being GABA mediated (Vardi & Sterling, 1994). Evidence also suggests that GABA may play a role in metabolism during retinal development, as well as in horizontal cell differentiation and maturation (Nag & Wadhwa, 1997). With such evidence from animal and human studies, it suggests that GABA plays a vital role in the activity of the retina and so any changes in GABA levels may alter retina function. GABA appears to play a fundamental role in feature extraction in the retina, which is mainly mediated by the lateral inhibitory elements (horizontal and amacrine cells), which modulate vertical flow of information from photoreceptors to retinal ganglion cells (Freed, 1992).

As GABA functions as a neurotransmitter in the retina, receptor sites for GABA must exist in this tissue. Binding sites synonymous with GABA receptors have been found in the retina (Enna & Snyder, 1976) shown by the agonist of GABA competing with [^{3}H]GABA for the binding sites. High affinity binding sites of GABA in the retina exhibit characteristics similar to those found in the brain. As well as GABAergic connections, GABA receptors also exist in synapses between photoreceptors and

horizontal cells (Yang & Wu, 1989). All the cell types within the retina express the $GABA_A$ receptor (Hughes et al, 1989) whereas $GABA_C$ receptor is mainly located on rod and cone bipolar cells (Enz et al, 1996) and some amacrine cells (Koulen et al, 1998). Within rod and cone bipolar cells $GABA_A$ receptors are thought to mediate fast transient inhibition whilst $GABA_C$ receptors mediate slower prolonged inhibition (Wassle et al, 1998). In this way, GABA has an effect on the temporal characteristics of rod and cone bipolar cells output. $GABA_A$ receptors have been shown to exist on the synaptic terminal of cones (Hughes et al, 1989) and on the dendrites of bipolar cells with the triad of cones to which horizontal cell processes contribute (Vardi et al, 1992).

3.5 GABA in the visual pathway

Based on work by Hubel & Weisel (1962) visual cortical neurons exhibit four response types to patterned light stimuli, that of simple, complex and hypercomplex as well as nonorientated. All four types have receptive fields with excitatory and inhibitory areas. As all visual afferent fibres passing from the LGN to the visual cortex are excitatory, the inhibitory area is derived from intracortical inhibitory neurons. Two types of inhibitory neurons exist, one activated by lateral geniculate fibres inhibiting cortical neurons in layers III and V and the other inhibitory cells in layers II and VI. Such inhibitory neurons may use GABA as their neurotransmitter (review Ito, 1976). GABA-mediated inhibition has a role in defining response properties of visual cortex cells. Daniels & Pettigrew (1975) observed a depression of firing rate in simple cells and changes in receptive field properties of complex and hypercomplex cells in the cat visual cortex following the administration of a GABA antagonist.

3.6 Effects of GABA and pharmacological agents on retinal function

Many studies have focused on the effects of GABA agonists and antagonists on retinal function. GABA has been shown to have a depressant effect on the spontaneous and light-induced discharge of ganglion cells (Straschill, 1968) with ON and OFF activations being depressed by GABA and the ON and OFF inhibitions being augmented and prolonged by GABA. Further studies confirmed that high doses of GABA (100-1000μm) depresses the activity of retinal ganglion cells (Ames & Pollen, 1969).

The effects of GABA antagonists have been examined on X and Y ganglion cells in the cat showing a preferential reduction in the contribution of the surround rather than the centre, to the discharge of Y ganglion cells. Indeed Y cells no longer show a response to a flashing annulus whereas X cells are unaffected by a GABA antagonist. All such results (review Morgan, 1984) indicate that X and Y ganglion cells are pharmacologically different and GABA may be important in mediating the surround influence on Y cell but not X cell discharge. This fact is further supported as X cells may be mediated via horizontal cells, whereas Y cells are mediated via the (GABAergic) amacrine cells.

GABA-T inhibitors have been shown to increase GABA levels in the retina of animal models. Cubells et al (1986) compared GAD and GABA-T activities in the retina with activity in the brain including areas of the frontal lobe, striatum and substantia nigra, following treatment with GABA-T inhibitors. GABA-T was also found in the optic nerve. Vigabatrin was found to inactivate GABA-T to a greater extent in the retina than the brain and this may be due to the blood-brain barrier or a higher retinal GABA turnover rate. The authors of this paper stated that if vigabatrin was to be used clinically, an ophthalmological examination may be warranted to monitor possible visual dysfunction that may develop. In the neurones and glial cells of the rabbit retina an increase in GABA occurs following the administration of GABA-T inhibitor, but interestingly it also results in a reduction in neuronal pools of glutamate especially in the inner plexiform layer (Pow & Rogers, 1996). It is possible that the glutamate pool is derived from the GABA shunt and so vigabatrin may work by both increasing the inhibitory GABA whilst also decreasing the excitatory glutamate.

Evidence suggests that colour vision defects in patients with vigabatrin associated visual field defects may be correlated although it is presently unclear as to whether the two mechanisms are related (Nousiainen et al, 2000). Bayer et al (1990) noted colour vision deficiencies induced by phenytoin and carbamazepine, and postulating an s-cone functional disturbance and a rod-cone interaction caused by the AED. In this way it is possible that the mode of action of AEDs significantly affect cone function. Vigabatrin appears to be the most potent of all AEDs and it is possible that s-cones are selectively

abolished during vigabatrin treatment. The s-cones are predominately located in the parafoveal area rather than in the fovea, and so this may account for a predominantly peripheral visual field defect seen in vigabatrin patients.

CHAPTER 4
THE ELECTROPHYSIOLOGY OF VISION

4.1 Visual evoked potentials

A visual evoked potential (VEP), first described by Adrian & Matthews (1934), measured the cortical activity found to be generated by a sudden change in a visual input. The assessment of the visual system was greatly advanced by the principal of averaging (Dawson, 1951). Initially VEPs to a flash stimulus were studied generating a response consisting of a series of negative and positive waves occurring from 30 msec to 300 msec. Electrodes record the electrical activity of the brain, and if situated around the visual cortex allow for the recording of VEPs. As the retina projections from the fovea are sent to the occipital lobes on the lips of the calcarine fissure, electrodes O2 and O1 are optimally placed to record the activity.

Ciganek (1961) initially used a bipolar montage to record seven waves consisting of a primary and secondary response to a flash stimulus. Common responses elicited from a diffuse flash stimulus included a negative peak occurring at 90 msec and a positive peak occurring at 120 msec although such latencies can be variable with no clear consistency between subjects. In this way it was extremely difficult to make comparisons between results, although the flash stimulus is useful when testing uncooperative subjects and so remains valuable in the clinic (Harding, 1984). A grid superimposed over the flash stimulus was found to produce consistent results (Spehlmann, 1965) and so the use of checkerboards was expanded in VEP work.

Based on anatomical studies it became apparent that a relationship existed between the number of edges (or check size) in a checkerboard pattern and consistent VEP responses. Rather than stimulate the retina using a flash stimulus, a checkerboard pattern was used to produce a triphasic negative-positive-negative complex. The use of checks as a VEP stimulus is effective as checks approximate to the alleged circular shape of retinal receptive fields. The ganglion cell discharge is determined by algebraically summing the reaction of light and dark patches falling in receptive fields. Photoreceptor signals from a

portion of the illuminated retina converge into the centre and surround of a single neurone. The ganglion cell response consists of a change in the firing rate. If both centre and surround are illuminated the modulated response decreases compared to the illumination of the centre, which is termed the antagonistic effect. Consequently the size of the centre relative to the surround establishes the spatial selectivity of the neurones. A small spot of light can stimulate the neurone but as the spot enlarges into the surround the response is reduced. The separate centre and surround areas of the bipolar neurones and ganglion cells means that the retinal output is determined by the difference of illumination between neighbouring retinal areas, rather than by the summation of separate illuminations, hence the combination of edges and corners and the areas of black and white in the squares stimulate a large number of neurones in the visual cortex. In addition, checkerboards produce no overall change in luminance, only local changes and so it is possible to investigate discrete areas of the visual field. Responses to the changes in spatial contrast are of mean luminance therefore the pattern reversal VEP may contain a luminance contribution due to the luminance modulation of separate spatial elements (Reimslag et al, 1982).

Hence the use of reversing black and white checks is a powerful method for stimulating retinal areas. Such a checkerboard pattern can be altered in many different ways to elicit varying responses and so became more useful than the flash VEP, especially as responses were more consistent between individuals. Indeed the size of the check is relevant to the response obtained. A check size smaller than 25' arc is useful to study foveal pathology and indeed a VEP could be recorded from the use of 10 min arc as this corresponds to the largest receptive field centres of retinal ganglion cells. In this way, a maculopathy may not be apparent when using 50' checks (Papakostopoulos et al, 1984).

As well as using a checkerboard pattern a sinusoidal grating pattern can also be utilised. In this stimuli the pattern element size is specified as spatial frequency, rather than check size. Spatial frequency can be denoted as the number of sinusoidally modulated dark and light bars subtended in an angle of 1° at the eye (formula 2) and is described as cycles per degree (cpd). A spatial frequency of 5 cpd elicits a large VEP. Measurements of the

visual angle in minutes or degrees can be converted into cycles per degree (cpd) by the formula 1, where w is the diagonal measure of the check in minutes of arc. The measurement of cpd defines the spatial frequency of the stimulus.

Formula 1:

$$cpd = \frac{30}{w}$$

As well as using pattern-reversal, pattern onset/offset can elicit VEP responses as the pattern is exchanged for a diffuse background. The response to the onset of the pattern consists of three components. A positive CI occurs around 70 msec, a negative CII occurs around 95 msec and a positive CIII occurs around 140 msec (Jeffreys, 1977). The responses are followed by an offset response occurring around 250 msec. CII and possibly CIII responses are thought to represent the contour response as they respond to the changes in edges and angles. The CI and offset responses are thought to respond to transient changes in contrast. As the onset and offset of neighbouring black and white pattern elements are not separated but occur simultaneously, it is probable that the P100 is a combination of the CI and offset components of the pattern onset/offset VEP. Indeed, if the pattern of progressively increasing contrast is introduced into the blank period until pattern reversal is achieved, the negative CII component reduces and the CI and offset responses become two P100 components responding to two pattern reversals. The pattern onset/offset response produces variable responses between individuals and so the more consistent pattern reversal VEP is favoured for clinical use.

4.2 Half field VEP stimulation

The use of full field pattern stimulation is not always required when examining VEP responses. The full-field VEP response provides a useful test for examining lesions affecting the anterior visual pathway (Halliday, 1978). However as nasal fibres from the retina cross to permit homonymous portions of the visual field to be represented at a cortical level, the full field VEP cannot provide useful information on lesions affecting the chiasm or retrochiasmatic visual pathways. In this way, full-field stimulation is less reliable for indicating dysfunction within the posterior visual pathway. VEP responses to

left and right half fields can be examined as well as upper and lower and central and peripheral fields in order to examine the visual pathway in more depth.

The peripheral field is mapped within the calcarine sulcus. The upper peripheral field is represented in the lower bank of the calcarine, whereas the lower peripheral field is represented in the upper bank. Additionally, the left peripheral field is represented in the right side of the calcarine and the right peripheral field is represented in the left side of the calcarine.

Cobb & Morton (1970) first examined the use of half field stimulation in VEP recordings and found, using an occipital bipolar montage, an asymmetry over each hemisphere with the major positive peak being lateralised over the contralateral hemisphere being stimulated. As a consequence of the decussation in the chiasm, a reversing checkerboard pattern in the left visual field would stimulate the right hemisphere, and similarly the left hemisphere would receive signals from the right half of visual field stimulation. An expected VEP response from such half field stimulation would include a maximal response over the contralateral hemisphere. However a maximal response is recorded from electrodes over the hemisphere ipsilateral to the field being stimulated when an Fz reference is used. A bipolar recording shows a flat response ipsilaterally but not because there is no activity rather it is due to equally high amplitude activity cancelling out. This paradoxical lateralisation (Barrett et al, 1976) is thought to be due to the cortical generator area situated on the medial and posteromedial surface of the visual cortex where neurones are transversely orientated. In this way electrodes ipsilateral are optimally placed as they are positioned perpendicular to current vectors generated by mesially located neurones of the contralateral occipital pole. Blumhardt et al (1978) showed an ipsilateral NPN is generated to a half field stimulus whereas a smaller contralateral PNP is seen.

Beauchamp et al (1976) postulated that part of the half field response was derived from the ipsilateral lobe but this was disproved by Barrett et al (1976) who examined the half field responses in four patients who had hemispherectomys. Both the NPN and PNP

responses were generated when the intact hemisphere was stimulated but no response was elicited from the blind half-field. This indicated that no significant response results from the transmission from one hemisphere to other across the corpus callosum.

Kriss & Halliday (1978) examined upper and lower half fields using a stimulus with a 32° diameter 50' arc check size and recorded from a saggital row of electrodes. When the lower half was presented the biggest NPN response was seen around the inion, with a positivity at 100 ms. However when the upper stimulus was presented, a much smaller response was recorded with a prominent negative peak (N80) followed by a broader positive peak (P120) again being largest above the inion. Jeffreys (1977) showed similar results with the upper half field showing a smaller amplitude as well as showing an opposite polarity to the lower half field. Wilderberger (1984) supported this by showing patients with a predominantly upper field loss produced a fairly unaffected VEP, whereas patients with a lower field loss produced a reduced amplitude VEP. The topographical distribution of upper and lower half fields was examined using a stimulus field of 16° diameter and 50' checks (Michael & Halliday, 1971). Peripheral stimulus (4-8^0) was compared with 0-2° and 0-8° stimulation in both upper and lower fields. The upper field results showed no comparable shift in the peripheral stimulus, although the lower field results showed bigger responses overall with the biggest being the 0-8°. The generators of upper and lower field response are thought to be spatially distinct and widely separated.

The fovea makes a major contribution to the amplitude of the P100, particularly in the pattern reversal VEP which favours a foveal response due to the higher concentration of cones in this area being more sustained in a cellular response, rather than the transient movement detectors in the periphery (Yiannikas & Walsh, 1983). Indeed the central 2° contributes 25% of the P100 amplitude and the central 4° contributes 35%. As the foveal cortex is exposed toward the occipital skull, enlarging the field size may not cause a substantial increase in VEP amplitude (Yiannikas & Walsh, 1983). Halliday et al (1979) stimulated central and peripheral areas separately in left and right half fields. A 0-16° half field produced a large ipsilateral NPN complex with a small PNP contralaterally. With the central 2.5° masked the ipsilateral complex was reduced and a larger contralateral

PNP complex appeared. This change was accentuated when 5° was masked, with the contralateral PNP increasing in amplitude. When 10° was masked a significant decrease in the size of the contralateral PNP response was recorded. It was deduced from this evidence that the ipsilateral NPN complex was evoked by macular parts of the field, and the contralateral PNP is hidden in the full field stimulation which thus becomes apparent when the central stimulus is occluded.

The same group also progressively occluded the peripheral field. A 0-16° half field was compared with a 0-10° half field which elicited an ipsilateral NPN with a reduced contralateral PNP complex. A 0-5° half field resulted in the complex being attenuated markedly. Using half field stimulation Blumhardt et al (1978) presented peripheral rings. A 1.5°-16° stimulus resulted in a contralateral PNP complex which increased in definition and amplitude when a 2.5°-16° stimulus was used. However a 10°-16° stimulus reduced the contralateral component somewhat. Harter (1970) used a 4.5°-7.5° stimulus with a larger check size of 30-60' to produce a large peripheral amplitude response. Such a response is expected as the optimal check size is related to the size of the antagonistic centre-surround receptive field, which increases with eccentricity. However it has been reported that the peripheral retina beyond 7° was not responsive to a patterned stimuli (Van Lith, 1977). Additionally a response from a large check stimulus is predominately from the central retina as a large area of the cortex is devoted to processing signals from the fovea and this area is located on the outer surface of the brain.

When considering central and peripheral half fields Blumhardt et al (1989) concluded that the early ipsilateral response (N75 and P100) has a macular origin, whereas the contralateral early response (P75 and N105) has a more peripheral predominance. Separable central and peripheral responses have been elicited to use foveal stimulation to detect multiple sclerosis (Rossini et al, 1979). Peripheral stimulation (8°-20°) decreased the latency of the P100 and this was attributed to the axons from the foveal ganglion cells being larger so increasing conduction velocity. Central (0-4°) and peripheral (4°-16°) half fields were also used to distinguish between delays in the macular and paramacular areas associated with optic neuritis (Brecelj & Kriss, 1985). The use of a small central stimulus

may however cause problems with fixation and so results need to be interpretated carefully.

The peripheral retina is mapped on the medial surface and in the calcarine sulcus. The recording of peripheral responses is complicated by the dipole positioning of the generator of the response. The location of the peripheral retina causes a difference in amplitude of responses of ipsilateral and contralateral components. The difference is attributed to the change in field radius which cause a difference in the orientation of the hypothetical dipoles for central and peripheral visual fields (Blumhardt et al, 1989). Such differences reflect the known variability of cortical representation in V1 although the potentials may also have an extrastriate origin.

A limitation of using pattern stimulation for VEP recordings is that when stimulating extramacular areas, a decrease in amplitude of responses is seen. Therefore such a stimulus is thought not to be useful when testing peripheral vision. Focus therefore turned to the use of motion stimulation in VEP recordings due to a higher sensitivity of motion in the peripheral retina. This fact is attributed to the different properties of peripheral retinal M-cells. Various types of motion stimulation has been utilised such as horizontally moving grate, checkerboard, random dot structure and dot matrices. A velocity of motion can range from 0.2-100 degrees/second. Kuba & Kubová (1992) recorded motion-onset VEPs and found a dominate negative peak occurring at 135-180msec. They deduced that this peak was a motion specific response caused only by motion stimulation.

4.3 Origin of VEP responses

The origin of the pattern VEP is difficult to establish as the response elicited is a composite waveform containing contributions from many cortical regions and generators. Indeed, Jeffreys (1977) stated that the electrical activity detected by scalp electrodes reflects contributions of several cortical regions and processes overlapping in time and space. Therefore the amplitude of a VEP response is dependent on the magnitude and orientation of the resultant dipole vector with respect to the montage used. Additionally,

varying stimulus parameters in any number of ways can selectively activate different cortical generators.

Examining the cortical generators of a VEP is a complex issue as the anatomical relation of the cortical projection areas to the recording electrodes has to be established. This issue is further complicated by the infoldings of cortical sulci which is subject to massive individual variability (Brindley, 1972). Measuring the topographical distribution of the peaks of the pattern VEPs over the scalp allows for the identification of cortical areas generating the response. The cortical generators of a VEP can be thought of as dipole layers of simultaneously active populations of cortical cells. The dipole concept is used to give an indication of the position and orientation of the hypothetical cortical sources. Studies of VEP cortical responses suggest they are generated in the primary receiving area of the visual radiation from the lateral geniculate nucleus although this can be complicated by the fact that two different cortical areas may generate a peak or a single cortical area may generate two VEP responses. Both pre- and post-synaptic components contribute to the VEP. As VEPs share some properties visual cortical cells, such as contrast adaptation, it can be assumed that such cells generate the VEP response.

Responses are labelled based on polarity (negative or positive) and latency (Harding et al, 1995a). The N75 (negative peak occurring at 75 ms) is the first negative potential which shows a variable response amplitude between individuals. It has been suggested that the component arises predominantly from the 4-15° annulus of the visual field and is generated by the transient movement detectors located in the peripheral part of the retina which contains larger, faster conducting axons (Yiannikas & Walsh, 1983).

The P100 (positive peak occurring at 100ms) is the largest and most reproducible wave thought to originate near the calcarine fissure and is therefore used to establish normal values using its latency and amplitude. It is thought to occur as a result of postsynaptic changes in the soma-dendritic polarisation of primary visual cortical neurones. The exact cortical origin of the P100 component remains unclear. Haimovic & Pedley (1982) suggest a striate origin whereas Štrucl et al (1982) suggest an extrastriate origin. Halliday

& Michael (1970) found that the P100 and CI (elicited from pattern onset) are positive peaks when the stimulus is restricted to the lower hemifield but reverse in polarity over certain parts of the scalp when the stimulus is in the upper hemifield. This indicated that the cortical neurones that generate the P100 and CI responses are retinotopically organised. However, the two peaks appear to have different cortical generators as the P100 response has been found to be paradoxically ipsilateral to the stimulus, whereas CI is not. The N145 (negative peak occurring at 145 ms) has a mid-line topography which is independent of left or right-field stimulation which supports the hypothesis that the wave reflects the activity of area 18. VEP responses recorded from the stimulation of the peripheral retina show waveforms with a variable polarity and broader duration which is thought to be related to the peripheral retina being located deeper in the striate cortex (Meredith & Celesia, 1982) and hence being more difficult to record using recording electrodes.

4.4 Recording a VEP response

The evoked potentials generated using different recording parameters can have different properties, such as different latencies due to change in luminance or field size. Distribution of potentials differs over the head and can be affected by cortical lesions. As changes to the VEP response can occur due to changes in stimuli parameters, standardisation of protocols is essential (Celesia et al, 1993, Harding et al, 1995a). Such changes are due to multiple parallel channels which process different information, as spatial, spectral and temporal codes at the retina are transmitted to higher neural centres.

When recording any evoked potential the difference in potential between two electrodes is measured so it is necessary to use a reference electrode. The site of the reference electrode is important as it results in a certain morphology of the waveform being recorded as the reference site enables all the potentials within the field to be measured relative to the potential at the reference electrode (Lehmann & Skrandies, 1980). Various sites for the reference electrode have been suggested and utilised. A common reference uses a supposed inactive site to which all recording electrodes are referred to, such as linked ears (Borda, 1977) or paracentral electrode (Harding, 1977). One problem

concerned with the use of a common reference is finding an inactive site that does not affect the recording of the evoked potential. A favoured electrode position is Fz located centrally at the front of the head (Halliday et al, 1972) as this is a large distance away from the occipital cortex but it may incorporate eye movements. A non-cephalic reference site has been favoured but electrocardiogram artefact can affect the response (Hobley and Harding, 1989). The ear reference has been shown to have a significant effect upon upper field stimulation but less effect on lower field stimulation (Halliday et al, 1977). The use of three electrodes in the form of a triangle around a centre electrode has also been used as a local referential instead of a bipolar montage in an attempt to obtain a reference-free montage (McKay, 1984). Bipolar recording has been adopted for routine EEG recordings in which a chain of electrodes is used where each electrode is referred to the neighbouring electrode. Such a montage has been used in VEP work and has proved useful in locating which hemisphere was generating a response (Harding et al, 1969). Alternatively an average reference utilises all electrodes together although this montage is not suited to evoked potential work as if a large potential is recorded it would be seen on all other channels with reversed polarity and a reduced amplitude. Despite extensive VEP studies over many years the location of the reference electrode remains controversial, as no site is truly independent.

Monocular recordings (dichoptic presentation) are preferable with a natural pupil as each eyes contribution to the response can be quantified. Binocular recordings, where both eyes view a single stimulus, are often necessary when recording from young children, but have the disadvantage that one eye could be providing all the visual response recorded. A standard VEP should consist of a full-field size greater than 8^0 with a check size ranging from 14'-64' (Harding et al, 1995a). Check size can be calculated using formula 2:

Formula 2:

$$Tan\theta = \frac{Checksize}{ViewingLength}$$

The smaller check size is optimal to stimulate the fovea but may be affected by visual acuity changes. A larger size pattern can be used to stimulate the parafoveal region.

Additionally the degree of focus can influence the VEP response, as the check-size which elicits the largest amplitude response depends on refractive error. If a pattern is sharply focused small checks (10-20') elicit a large response, but if the pattern is defocused then larger checks are needed for a large amplitude response (Harter & White, 1970). Check size is also important in peripheral stimulation where checks need to reflect the increase in receptive field with increasing eccentricity (Harter, 1970). Optical blur produces an increase in the latency of the pattern reversal VEP, which is more pronounced with both the increase in optical blur and the smaller pattern detail used. For example, Harding & Wright (1986) produced a P100 delay of 4.5 ms with a +3 dioptre blur on 56' checks. The use of 13' checks produced a P100 delay of 20.4 ms. Using the pattern onset/offset VEP, the C11 component shows a more marked increase in latency, which may be due to the fact that the response reflects the decrease in edge sharpness and a decrease in the contrast between the black and white checks caused by the blurring. In the case of the pattern reversal VEP, only the latter factor has a significant effect. As blurring does not affect the appearance of a flash, the VEP recorded is not affects. Overall, when using pattern VEPs it is essential to determine whether the patient has a refractive error and to correct for the distance of the pattern stimulus. Older patients not able to focus on a pattern stimulus at near distance may need to use convex lens.

In order to generate a suitable response it is also suggested to use a mean luminance of at least 50 cd/m^2. If the pattern stimulus is of high contrast then the amplitude of the response has a larger amplitude than if the stimulus was of low contrast (Campbell & Maffei, 1970). A contrast of 50-80% based on the Michelson contrast equation is suggested and this can be calculated using formula 3. It is thought that a low contrast stimuli triggers responses from the magnocellular layer of the LGN whereas high contrast responses originate from the parvocellular layers.

Formula 3:

$$MichelsonContrast(\%) = \frac{L\max - L\min}{L\max + L\min} \times 100$$

The rate of the pattern stimulation can alter the type of response generated. A low rate of 1 Hz (producing a reversal every 500 msec) should be used for transient VEPs (standard

VEP) which generates a response consisting of a series of wave of alternating polarity. Steady-state VEPs (sVEP) can be elicited using a high reversal rate of 4 or 8 Hz (producing a reversal rate of 8 or 16 Hz). Responses from such a high rate of reversals overlap one another and so merge into quasi-sinusoidal oscillations. Such responses are best analysed by frequency rather than time and can be assessed by Fourier analysis with the 2nd harmonic responses, occurring at twice the frequency of the stimulus, being measured. An analysis time of 250 msec is suitable for a VEP, whereas a longer time of 2 sec is more suitable for sVEPs.

4.5 Factors affecting the VEP

Pupil size has been found to affect the latency of the VEP recorded. Penne & Fonda (1981) found a decrease in pupil size to cause an increase in the latency of the P100. A high correlation between latency and amplitude is seen between both eyes (Blumhardt & Halliday, 1979) although Halliday (1982) reported a longer latency by 0.5-0.9 msec in the right eye compared to the left eye in all subgroups of age. It would be expected that no difference is seen between two eyes stimulated in a normal VEP response, and indeed Halliday could not account for this anomaly found. A significant difference between genders has been found in the mean latency of the pattern reversal VEP, in which a longer latency is found in men (Stockard et al, 1979). The reasoning behind such a difference is thought to be due to a greater head size and skull thickness in men which would cause an impulse to travel at a slower speed, and also may be due to the higher deep body temperature in women which may facilitate conduction.

The use of flash versus pattern stimulation has been the source of much discussion in the use of clinical VEPs. The flash VEP is useful in un-cooperative subjects or even comatose patients (Bergstrom & Nystrom, 1970) but produces a variable response between subjects. Halliday (1980) suggested the necessity of generating a normal database in each department in order to appreciate the nature and variability of the normal response. The pattern VEP produces a more robust response although the effect of blur can be a problem. VEP recordings have been widely used in clinical practice as the test is sensitive to abnormalities of the optic nerves, which can occur in multiple sclerosis or

optic neuritis. Waveform changes that can occur include increased latencies and decreased amplitudes.

The effect of a retinal lesion on a VEP response depends on their site and particularly the amount of intact central retina functioning. In this way, a peripheral lesion may not affect the VEP although a very localised foveal lesion may produce a normal response unless very small checks are used and an annular parafoveal lesion may produce a normal response with small checks and an abnormal response with larger checks. A lesion that predominantly affects one eye only suggests a lesion with a prechiasmal origin, as monocular fibres are still separate before this point. A postchiasmal origin is suggested if a pathology is seen in one hemisphere only. If the chiasma itself is involved in a pathology, such as in a pituitary tumour, then the nasal fibres from both eyes would be affected resulting in an abnormality of the VEP from the left hemisphere on right eye stimulation and an abnormality of the VEP from the right hemisphere on left eye stimulation.

A delay of 30 msec can be attributed to slowed conduction due to axonal demyelination. A larger delay can be caused by scotoma or reduced contrast sensitivity. Using the P100 latency as a marker for a demyelinating disease of the optic nerve is very useful as affected patients are significantly delayed when compared to the normal population. For example, in the case of multiple sclerosis, a VEP can detect a visual lesion in a patient with no history of visual problems with a normal visual acuity. An abnormal VEP can also be caused by pathology of the intraretinal optic nerve seen in glaucoma, maculopathy and synaptic malfunction due to neurotransmitter deficiency, such as in Parkinson's disease. The effect of differing check size in VEPs in patients with multiple sclerosis showed that the use of multiple checks (25′ and 100′) yielded more abnormalities than using one single check size of 50′ (Oishi et al, 1985). In order to examine other neurological conditions in which latency may not be as affected it is often necessary to resort to half field stimulation to help in the interpretation of full field responses (Halliday et al, 1982), as by separating the full field response more information may be gained about the generation of the response. It is important to note that a standard

VEP largely ignores noncentral visual function and so peripheral defects may go unnoticed using standard parameters.

With the knowledge of the functional properties of different classes of neurones in the visual pathway, it is possible to design an evoked potential experiment that selectively tests functional subunits. VEP responses can be measured to chromatic stimuli with comparable contrasts (Regan, 1973). A difference between achromatic and chromatic VEP responses can only occur if the chromatic grating bars are of equal brightness or isoluminant although an increase in eccentricity can cause isoluminacy to vary and so the chromatic stimuli may lose its selectivity. Colour and spatial properties appear to be related as many neurones with colour properties depend on the spatial nature of the stimulus. In this way the effects of colour may differ depending on whether a grating or unpatterned stimulus is used. Psychophysical experiments have shown that the sensitivities of the visual system to colour and luminance are spatial frequency dependent and so spatial variables are a critical factor when determining colour and luminance contrast sensitivities (Mullen, 1985).

When using a chromatic stimuli the parvocelluar system is stimulated as this system mediates colour vision. In this way colour VEPs can selectively examine one visual channel. For example, in glaucoma a VEP abnormality would be seen in response to an achromatic pattern but not when using an isoluminant chromatic pattern. This would be expected as the P neurones are spared in glaucoma and so mediates the response to colour. However if an abnormal VEP were seen to chromatic but not achromatic stimuli, this would suggest a loss in P channel functioning. VEP responses have been recorded to red or blue checkerboard stimuli from electrodes chronically implanted in the brain of patients with refractory epilepsy (Allison et al, 1993). Results indicated that a region of the inferior occipital cortex, primarily the posterior portion of the fusiform gyrus, is involved in colour perception as well as a region of the dorsolateral surface cortex.

4.6 Paediatric VEP testing

Inherent difficulties are associated with testing the vision of babies and young children such as crying, sleeping and not paying attention as well as the fact that babies cannot understand requests. However studies on infants have shown that, even at the young age of 6 months, a child will look at a visual stimulus and so one of the advantages of VEP recordings is that the technique is suitable to use in children and infants. The objective nature of the test allows for the interpretation of the functioning of the visual system without the child needing to participate in anything too difficult. Different methodologies have been used on infants ranging from flash stimuli to pattern stimuli, in which latencies and amplitude markers have been used to establish visual maturation. Broad-band flash VEPs showed developmental differences between the high frequency wavelets and the slow wave potentials indicating different activity occurs at the neural substrates as the later onset wavelet reflects the maturation of the primary visual cortex (Schanel-Klitsch & Siegfried, 1987). Acuity development has been examined by extrapolating data from pattern VEPs at varying spatial frequencies and contrasts, and has shown that visual acuity increases linearly during the first six months. VEP acuity reaches adult levels at 6 months, although preferential looking acuity does not reach adult levels until 3 years of age. The differing neural sites could account for this difference responsible for the responses or the different initial retinal locations used, being mainly central for VEPs and peripheral in preferential looking.

Pattern VEPs have been utilised to examine spatial vision in infants by examining the changes in VEP amplitude to check size function. Using a stationary pattern younger infants (1-4 weeks) show an initial peak at 16-24 min/arc, which disappears at 6 weeks, to be replaced by a second peak at 50 min/arc which by 6 months is shifted to adult values of 20 min/arc (Harter et al, 1977). Using a pattern reversal VEP stimulus, the check size peaked at 30-40 min/arc for 2-3 month old infants, and again reaching the adult peak of 10-20 min/arc at 6 months of age (Sokol, 1978). Spinelli et al (1980) examined the effect of increasing field size and found that at low spatial frequencies the VEP amplitude increases with increasing field size whereas at high spatial frequency the

amplitude remains constant at increasing field size. Such results indicate that in an infant's retina there is a non-uniform acuity.

The effects of aging having been investigated in flash and pattern VEP responses. An increase in age results in an increase in the latency of the flash P2 component, and the high amplitude responses recorded in childhood tends to decrease with age with an abrupt increase between the ages of 13 and 16 years. (Harding & Wright, 1986). The P100 component of the pattern VEP also showed an increase in latency with an increase in age, and this latency shift was increased further with the use of smaller check size (Harding & Wright, 1986). The increase in latency of the flash P2 with age was noted to be substantially larger than the increase in latency seen with the pattern P100 response with age. Additionally the amplitude of the P100 response was found to be 2-3 times higher in the teenage group, although no significant change was seen between the other age groups. The increase in latency of responses with age has been attributed to a decrease in pupillary distance with age, which reduces retinal illumination (Wright et al, 1985). Other studies have hypothesised a neurally based cause in a decrease in nerve conduction velocity, which could be attributed to neural demyelination, or the deterioration in the retina, LGN and cortex causing poor synaptic transmission (Sokol et al, 1981).

4.7 Electroretinogram

The retina is a complex neuronal network consisting of photoreceptors linked to bipolar cells, ganglion cells and two types of interneurones called horizontal and amacrine cells Surrounding these neurones are the structurally unique glial cells, called Müller cells. The ERG is a flash-evoked potential of the retina recorded from the cornea, which produces several prominent electrical potentials relating to these structures. Using appropriate manipulation of flash intensities, wavelength, rate of stimulation and state of light and dark adaptation it is possible to assess quantitatively the function of the preganglionic retinal neurones. The transient current generated by a flash of light reflects the complexity of the retinal wiring as each cell in the retina produces a sequence of electrical responses.

Ideally a Ganzfeld stimulator should be used to ensure the whole of the retina is stimulated and to abolish stray light during an ERG recording (Marmor & Zrenner, 1995). Light output must in the order of $2x10^6$ cd/m^2 in order to achieve maximum amplitude of responses. With an increase in stimulus intensity, an increase in amplitudes and decrease in latencies will occur as the level of visual excitation increases proportionally. Depending on whether the eye is light or dark adapted, different responses can be gained. If the eye is light adapted then a cone dominant ERG response is produced, being small and fast and is termed the photopic ERG. Introducing a smaller bandwidth by manipulating high and low frequency filters, oscillatory potentials can be accentuated in a further test and so labelled more clearly. A further method to ensure a cone dominated signal is to use a rapidly flickering stimulus as cone responses are rapid and so can follow rapid stimulation. The 30Hz Flicker test using a flashing strobe can reveal the high temporal resolution of cones as rods are unable to respond at this rate. Similarly ensuring that the eye is dark adapted can produce a rod dominant ERG, termed the scotopic ERG. The waveform recorded under such conditions becomes wider and increases in amplitude. The size of the pupil also effects ERG results as if the pupil is fixed by mydriasis, the a and b wave increase in size, the a and b wave duration is affected as is the flicker frequency (Galloway, 1981). Maximal papillary dilation is recommended for all ERG recordings in the ISCEV standards and the pupil size should be noted if not maximal (Marmor & Zrenner, 1995).

When recording an ERG response, certain factors need to be taken into account in order to deduce an accurate interpretation of the results. The age of the subject has been shown to affect the b-wave amplitude of the response. Zeidler (1959) found a linear decrease in b-wave amplitude with increasing age, although Birch & Anderson (1992) found an exponential decrease in b-wave amplitude declining gradually up to 55 years after which the decline decreases more rapidly. The b-wave amplitude has also been found to differ between genders, being smaller in men than women (Vainio-Mattila, 1951; Martin & Heckenlively, 1982).

According to ISCEV guidelines corneal contact electrodes are the best types of electrodes to use in order to record stable and reproducible ERG results (Marmor & Zrenner, 1995). Although it has been recognised that such electrodes give large amplitude results with a high signal to noise ratio, with the advent of advanced recording techniques such as filtering and averaging other electrode types are now viable for use. DTL fibres developed by Dawson, Trick and Litzkow (1979) are one such electrode type. This low mass conductive thread consisting of spun nylon fibre impregnated with metallic silver can make contact deep into the inferior conjunctival pouch. Using this fibre removes the inherent problems associated with contact lenses, which include corneal abrasions and difficulty in fitting the lens in small palpebral fissures, particularly seen in paediatrics. In comparison, DTL fibres do not require anaesthesia of the eye, can be tolerated for long periods of time, and are inexpensive and disposable.

In a study by Lachapelle et al (1993) oscillatory potentials recorded from DTL fibres were found to have similar structure and identical latency to corneal electrodes. The only significant difference found between the two types of recordings was the amplitude of the oscillatory potentials from DTL fibres, which was 50% smaller than that of the corneal electrodes. However such a difference did not result in the proper identification of waveforms becoming difficult, as there was no significant increase in noise level. Similarly Hébert et al (1996) noted that DTL fibres were stable and gave highly reproducible ERG results, although it was stressed that the fibre electrode must be positioned at the same place in all subjects as the position relative to the centre of the cornea has an effect on the amplitude of the ERG. Indeed the ideal electrode should be both comfortable and well tolerated. Electrodes are connected to a differential amplifier.

An early receptor potential (ERP) may be recorded from bright flashes and the response is from the outer segments of the photoreceptors from light-induced changes in the visual pigment molecules. The photopic response consists of an initial negative wave called the a-wave, followed by a positive wave called the b-wave on which oscillatory potentials are frequently superimposed. A second positive wave called the c-wave and the d-wave or off response can also be recorded but are not usually seen using conventional routine

recording techniques. Using animal studies to examine ERG responses associated with different pathologies the origin of the waveforms labelled could be determined. The first negative deflection, the a-wave, reflects the hyperpolarisation of retinal photoreceptors. This occurs as light reduces cell membrane conductance therefore making the cell more negative. In the dark-adapted state the retina is very sensitive to light and therefore the response would be larger. The hyperpolarised photoreceptors then cause horizontal cells to hyperpolarise although this response is not revealed in the ERG.

The origin of the b-wave is more complex due to the nature of the bipolar cells that evoke the response. A light spot in the centre of cells receptive field causes hyperpolarisation whereas a light spot in the surround region causes a depolaristion response. However in other classes of bipolar cells the opposite action occurs. The positive going b-wave of the ERG is thought to reflect the depolarising element of bipolar cells as the response disappears if the blood supply to bipolar cells is clamped (Brown & Watanabe, 1962). Many authors believe that the non-neural glial Müller cells contribute to ERG production, particularly b wave production (Miller & Dowling, 1970). Following the light evoked increase in potassium ion concentration, the Müller cells are depolarised. The Müller cells response is caused by the balance of neuronal activity involving both outer and inner synaptic layers and so the b-wave reflects postsynaptic neuronal activity. Production of the c wave is thought to be from the pigment epithelium.

Oscillatory potentials, best recorded from bright repetitive flashes, are identified as small rhythmic waves superimposed on the ascending portions of the b-wave. The response may reflect a possible feedback mechanism with an interaction between different retinal layers. Amacrine cells are believed to produce oscillatory potentials but there are many different morphological types, which result in the cells having a complex role in the ERG. A feedback mechanism is thought to be involved and a lesion of retinal circulation causes the disappearance of oscillatory potential with the b-wave amplitude maintained.

The repeating of a stimulus flash can alter the photopic response. If a stimulus is repeated every few seconds, using a weak stimulus, then the second response resembles a normal

ERG response. If the stimulus increased to 2Hz then the second response and successive responses have a photopic character but are reduced in amplitude. As the frequency of the stimulus is increased further the amplitude of the a and b waves become similar, and eventually become sinusoidal. If a flickering light is used with a frequency of 30Hz, then a pure cone response results as the rod system is unable to respond at this rate. Additionally a rod response can be elicited by stimulating the dark-adapted retina with a bright flash.

4.8 Maturation of the ERG

Recording of ERG responses in babies and children are fraught with problems due to the difficult nature of the test procedure. However, with a specially constructed stimulus flash, Zetterström (1970) was able to show no ERG responses could be recorded a few hours after birth, after a few days a small positive potential could be detected and at three months the b-wave was visible. The latency and amplitude of the ERG has been shown to reach adult levels by the age of three (Westall et al, 1999). In adult life a slow decline of the amplitude of the b-wave potential with age has been documented (Karpe et al, 1950).

4.9 Electro-oculogram

The EOG provides an electrical method of measuring eye movement and positions. It is a recording of the standing electrical potential between the cornea and the posterior pole of the eye, as the cornea is positive with respect to the negative retina at the back of the eye. Various ocular structures contribute to this corneo-retinal potential but it is mainly generated at the junction between the photoreceptors and the pigment epithelium. The transepithelial potential stands at 60mV and is permanently present, both in the presence and absence of light. Kriss (1958) documented that the magnitude of this potential difference was influenced and increased by light.

Arden and Barrada (1962) realised the clinical utility of this potential difference, and so developed a test of retinal function based on changes in light and dark and this became known as the Arden Index. A potential can be recorded using surface electrodes placed at the inner and outer canthi of the eye. To record the EOG it is necessary for the eye to be

moved from the left to the right. The size of the potential recorded is proportional to the degree of rotation. It is therefore necessary to control the horizontal rotation, which can be achieved by asking the subject to fixate alternatively on two points. A horizontal movement of 30° on both sides of the median line is used. A screen with a background illumination is used with red fixation points so as not to disadapt the retina. The large size of the response allows single trials to be analysed.

The beginning of the test requires a preliminary light adaptation period in which a resting potential is recorded for 15 seconds every minute for two minutes. A period of dark then follows in which a recording is made for 15 seconds each minute. Following this, photopic stimulation is returned for 12 minutes in which a 15 second recording is performed each minute. The Arden Index is calculated by formula 4.

Formula 4:

$$\text{Arden Index} = \frac{LightPeak}{DarkTrough} \times 100$$

The light peak is the maximum amplitude achieved in the bright photopic phase and the dark trough is the smallest signal achieved in the scotopic phase. In a healthy eye the photopic peak should be about twice the size of the smallest scotopic trough.

The recording can provide an objective and quantitative test of the functioning of the outer layers of the retina. A number of factors are responsible for the normal light rise in the EOG. These are the pigment epithelium, retinal receptors, the metabolic continuity between receptors and the pigment epithelium and the choroids. The light/dark ratio is dependent on changes in the RPE membrane potentials providing a means of assessing the clinical integrity of the RPE. The ratio is also dependent on photoreceptor activity and on RPE-photoreceptor attachment. The ratio may be reduced with the occlusion of the central retinal artery which supplies the middle and inner layers of the retina not including the RPE. In this way the Arden Index does not solely examine RPE disorders. The Arden Index measured during the EOG is therefore abnormal when any of these

layers are affected, such as in retinal pigmentosa, vitamin A deficiency, achromatopsia and retinal detachment. In the clinical environment the EOG has a major use in the diagnosis of Best's disease in which a reduced Arden's index and a normal ERG are diagnostic features.

4.10 Perimetry

A visual field can be defined as 'all the space that one eye can see at any given instant' (Tate & Lynn, 1977). The extent of a normal monocular visual field is 60° up, 75° down, 100° temporal and 60° nasal. The extent of the visual field is determined by each individual's facial anatomy as the nose, cheek bones, eyebrows and forehead all limit the size of the field of view. The nasal field of view is reduced due to the bridge of the nose and so any subject with a prominent bridge would have a reduced nasal field. Additionally, facial contours can restrict the superior field.

Sensitivity of the eye is not uniform across the visual field but instead differs with eccentricity, adaptation level and the nature of the test stimulus. The visual field has been likened to an "island of vision surrounded by a sea of blindness". The height of the island corresponds to the sensitivity of the eye. Stimuli within the island can be seen, whereas stimuli that falls outside of the island (in the sea) are outside the field of view. A visual field defect can be defined as one that does not conform to the normal limits of the hill of vision such as if a generalised reduction in the height of the hill of vision is seen. However it should be noted that the hill of vision can change with the state of adaptation, for example if the eye is dark adapted then the island will be taller with a crater in the middle due to the distribution of rods and cones. The light adapted eye is a low-lying hill with a peak at the fovea.

Many methods of subjectively investigating vision exist, such as measuring visual acuity, dark adaptation and psychophysics. Perimetry is one such test, which can be defined as the measurement of the visual field. The test is non-invasive with no adverse reactions and causes no discomfort. Perimetry also has the advantage that it is a direct measure of visual function and so can aid in a differential diagnosis. However one major

disadvantage of perimetry is that the test procedure can be lengthy and rather tedious to perform. It also requires a great deal of concentration throughout the test procedure which is necessary to give reliable results and so patients with a developmental age of less than nine are thought to be unable to provide reliable results (Wild et al, 1999).

In order to measure the visual field a number of factors need to be controlled. The position of the stimulus within the visual field is determined by its eccentricity, that is how far it is from the fixation point. The eccentricity of the target can be described in general terms as to where in the visual field it falls, such as the superior or inferior nasal or the superior or inferior temporal quadrants. The size of the stimulus, generally measured in terms of the angle subtended at the eye, is also specified.

If using a bowl type perimeter the test can be performed by placing the subject in a fixed position on a chin rest. Whilst monocularly focusing on a fixation point the subject must press a button every time a light is seen anywhere in the bowl. An internal camera is used to observe and ensure the subjects constant fixation, and it is this fixation that is often difficult to maintain in children and uncooperative patients.

Different visual field strategies are available to cater for the different questions that may need to be answered regarding visual field loss. Static perimetry describes the testing procedure in which the stimulus positions stay constant, but the luminance varies so with each luminancy step the luminancy may go closer or further from the threshold. The threshold is defined as the minimum light energy required to evoke a subjective response. In the majority of static examinations the operator need not control the test procedure which can be determined by the computer. The Humphrey perimetry test is an example of an automated static perimetry test. Responses are independent of reaction time. Such a test is sensitive to shallow focal loss and small scotomas. The nature of a static examination can vary, in that some strategies derive an estimate of the eye's threshold at a whole series of different test locations, namely the threshold test. Another test presents the stimuli at an intensity that is calculated to be slightly above the patients threshold and

so records whether or not the stimuli was seen, namely the suprathreshold test. The latter test is particularly suited for rapid screening.

Kinetic perimetry utilises a stimulus in which the luminancy remains constant but the position changes spatially coming closer to the subjects line of view. This strategy relies upon the fact that the centre of the visual field is normally more sensitive than the periphery, as a weak stimulus in the edge of the visual field becomes visible as it moves into the centre. Points of equal sensitivity form together to make an isopter and different isopters can be measured using different stimulus sizes or light intensities. Such stimulus sizes and intensities have been specified using the Goldmann system. An advantage of this type of stimulus is that the perimetrist has total control over the examination, deciding the order, direction and speed of the stimulus. In this way the test can be tailored for the individual circumstance. A disadvantage of this approach to visual field testing is that, as well as being time consuming, a moving stimulus is detected more easily in the periphery and so results may not detect a shallow focal loss of vision. Additionally these responses are dependent on patient reaction time, which may cause poor results. In recent years kinetic examinations have been replaced by static techniques, although a role does still remain for kinetic examinations for flexible testing and may particularly suit younger children or those with attentional problems.

Once results are gained from a perimetry test, difficulties still arise from questioning the accuracy of results. Short-term fluctuation may occur in which a range of values is associated with the measurement of sensitivity at one stimulus location. Long-term fluctuation may also be recorded in which a variation in the sensitivity is seen between one examination and another. A variety of reliability parameters are used to ensure the quality of the results from the patient. Firstly, an increase in the number of questions asked indicates inconsistency or difficulty as if the patients misses a stimuli then it will be presented a number of times to ensure the patient definitely cannot see it. Secondly, if the patient is not looking directly ahead then when a stimulus is presented in what should be a blind spot, the patient will respond and this is deemed a fixation loss. Thirdly, a false-negative response indicates the degree of attention, as the patient fails to respond to

a stimulus at a brighter luminancy to which they had already responded to at a dimmer luminancy. Finally, a false positive response can be recorded when the patient responds to stimuli that was not actually presented which may indicate a lack of understanding or co-operation of the test. When trying to determine if a field is abnormal a variety of methods can be used. A numerical scale of decibels (dB) gives a logarithmic value of sensitivity in which a high value indicates a high sensitivity. Similarly, a grey scale indicates high sensitivity with a light colour or a low sensitivity with a dark colour. Additionally data reduction statistics such as mean deviation, pattern deviation or probability maps can indicate sensitivity.

A detailed knowledge of the visual pathway allows for the localization of visual field defects. The amount of area that is visible to each eye is termed the field of vision with its outer half being the temporal field and its inner half being the nasal field. Light from the temporal field falls on the nasal half of the retina and the nasal field falls on the temporal retina. The nasal step is a defect associated with a difference in sensitivity above and below the horizontal midline in the nasal field. Small steps may be seen in normal subjects, but become significant when they exceed a certain value.

4.11 Ophthalmological Examination

An ophthalmological examination is required to determine if any systemic diseases, vascular abnormalities such as glaucoma may be present in a patient, which may cause a field defect. The fundus examination involves examining the retina, in particular the optic disc. The actual size of the optic disc is about 1.5 mm, subtending approximately 5°. A larger optic disc may indicate myopic discs whereas hyperopic discs appear small. The optic disc should be a round shape, and if it is vertically oval it may indicate astigmatism although a large oblique astigmatism may distort the shape. The neuroretinal margins should be distinct particularly temporally although they should not be very sharply visible. The colour of the optic disc should be a warm white with tinges of pink, and the nasal side is more likely to be darker. Within the disc the cup should have a normal extension which may slope gradually to the temporal side. The cup/disc ratio should be 0.3 and is usually measured vertically.

The blood vessels, apart from the branches of the central retinal artery and vein should be very fine although visible. Veins should be thicker than arteries, with a ratio of 3/2 in relative thickness. The course of the vessels should be traced as veins and arteries cross and a displacement or disappearance (nipping) can indicate hypertension or show signs of arterio-sclerosis. Normal crossings are oblique rather than right angled. Veins are characteristically a darker red than arteries. Any variations of width of along the course of a vessel is suspect indicating that the circulation may be compromised. The macular region is also examined to check the fovea for holes, haemorrhage or any degenerative changes.

Tonometry is a clinical technique which provides a measurement of the tension in the eye, which includes the combined resistance to deformity of its coats and the intraocular pressure. A force is required to deform the cornea and is done so by indirectly applying gas pressure to a contact probe. The extent of the distortion is then measured. Clinical convention describes tonometric measurements as intraocular pressure (IOP) in millimetres of mercury (mmHg). An abnormally high IOP can diagnose and manage glaucoma. Prior to the administration of drops to dilate the pupil (necessary when recording a dilated ERG), each patient undergoes tonometry in order to exclude open-angle glaucoma as well as ruling out any other possible cause of visual loss.

4.12 Multifocal stimulation

The visual system is considered as a nonlinear system. Such a conclusion has been based on the way in which information is processed as well as specific visual performances. Shapley (1990) quoted a numbers of examples to support the nonlinearity of the visual system including evidence that suggested a nonlinear stage in contour perception and a nonlinear image analysis to account for immediate recognition of an odd element in an array. As every receptor within the retina can be thought of as an input channel, a multi-input system needs to be analysed. In order to investigate such a complex system requires both complicated and long recording strategies. Originally stochastic techniques were adopted, in which kernel extraction was performed. Kernels, or response components, are conceptually similar to those derived from traditional white noise analysis. A Gaussian

white noise signal is an abstract entity whose value is drawn from a normal distribution. As each value is independently chosen and can vary this type of stimulus can be thought of as containing all possible stimuli. However such a random stimulation can result in poor quality kernels and it became clear that a better and faster testing strategy and extraction of kernels was required. Therefore interest turned to a deterministic technique, which utilises an exact input signal rather than stimulation by a random process.

The use of a pseudo-random stimulus, or m-sequence, is one such deterministic technique. The m-sequence is particularly suited to examine the nonlinear visual system as it is possible to obtain impulse responses from hundreds of inputs. Described simply, this multifocal technique is a much faster stimulus than that used in averaging (as described for VEPs) and acts as a more sophisticated noise reduction system. The pseudorandom stimulus is presented as a pulse stimuli and the rate of presentation is slow enough to ensure that the stimuli are resistered by the visual system as discrete events. However, as the response waveforms to consecutive pulse stimuli can overlap, kernel extraction is reduced to adding and subtracting response segments and so facilitates their interpretation. Indeed these 'shifted'versions of responses are orthogonal to another and so different kernels can be explored.

The m-sequence is a simple binary stimulation assuming the states 0 and 1, which means it can be generated quickly without requiring a memory allocation of the sequence. A binary m sequence of order n is a cyclic sequence of 2^n-1. If the stimulation of different locations is uncorrelated then it is possible to extract individual response contributions from the record. A set of pseudorandom stimulus trains, or m-sequences, that are by definition uncorrelated, ensure that the specific retinal areas can be stimulated. The m-sequence ensures that the stimulus appears random to the observer. The pseudo-random flash is present at constant intervals referred to as the base period (bp).

The duration of the flash is dependent on the persistence of the phosphor on the CRT screen. The image on the CRT screen is formed through the impact of an electron beam on a phosphorescent material. In order for an image to be built on the screen, the electron

beam is scanned from the top left of the display to the bottom right. The refresh rate of the screen is the time taken to scan a full display frame with an additional blanking interval for the beam to return to the starting point. If the frame rate is 75Hz, then the bp will be a multiple of 13.3 msec (1000/75). CRTs are therefore capable of a high refresh rate which is useful for studying non-linear systems. The fast stimulation rate also enables a large number of averages to be acquired in a short space of time, which increases the signal-to-noise ratio.

The responses are extracted from the raw data by computation of cross-correlation between the m-sequence and the response cycle, and so the response of all inputs is extracted from the same record by a single cross-correlation. An analysis technique based on the stimulation with binary m-sequences is important in that the stimulation encompasses an entire m-sequence cycle otherwise the signal-to-noise ratio and the extraction of responses can be severely degraded. The main advantage of this process is that it is extremely quick, obtaining responses for hundreds of inputs from records with up to a million data points in less than a minute. This is made possible by using the Fast Walsh Transform (FWT) (Sutter, 1991), which essentially replaces three Fast Fourier Transforms (FFT) and an array multiplication to save a considerable amount of time. The FWT requires no multiplications and is able to reduce the data analysis to a single cross-correlation between the response and the m-sequence input.

The kernels generated by the m-sequence correspond and provide information on the nonlinear dynamics of all the contributing sources. The kernels are derived from the cross-correlation technique and as the signal is sparse (slow enough to ensure the stimuli are registered as discrete events) the signal is sampled several times during each bp to capture the waveform. The response is cross-correlated with a sequence of +1s and –1s. A first order kernel response is derived from sequences which have a +1 (flash) or a –1 (no flash) at the beginning of a bp. A second order, first slice response represents an interaction between responses in consecutive bps. The response is therefore derived from bps which contain a flash or when a bp contains +1s. A second slice of a second order response represents the interaction between flashes which are separated by 2 bps.

In order to interpret such kernel derivations, three assumptions have been made. The duration of the flash response is less than the bp, the duration of the effect of one flash lasts for more that 2bp but less than 3 bp and the effect of one flash on subsequent responses causes an amplitude reduction. Based on these three asumptions, the flashes in the two preceding bps affects the response and so four possible occurrences exist. Either a flash occurs in both preceding bps, occurs in neither bps or occurs in one or the other bp. The m-sequence ensures that all four occurrences are tested an equal number of times.

According to such assumptions, a first order response is all epochs added together following a bp with a flash minus all those epochs without a flash. A first slice second order response is the effect of a flash response from a response occurring in the immediate preceding bp. A response epoch is added when either or both of the preceding bps contain flashes and subtraction if a flash occurs in either one but not both of the bps. It is therefore clear that a binary kernel can be derived by the simple adding or subtracting of response epochs, and due to the m-sequence this process can be performed in one single cross-correlation.

4.13 Multifocal ERG

A standard ERG examines the mass retinal response potential summed from different groups of cells and from different retinal regions. This fact and the problems of poor signal-to-noise ratio in ERG recordings result in the impossibility of recording responses from a number of retinal locations to allow for the derivation of response topography maps. In order to overcome this problem, small retinal areas need to be stimulated so that each area contributes to the overall response. This is important as local variability occurs within the retina, such as scotopic vision relies on rods in the periphery and central and peripheral acuity is related to the density of foveal and peripheral cone density. The retinas susceptibility to diseases may be related to these specific anatomical differences and so knowledge of the functional topography of the retina may be vital in the understanding of some disease processes. More specifically, ERG responses do not reveal glaucomatous nerve fibre loss or visual field defects. Attempts have been made to

examine small retinal areas such as utilizing a flickering spot with a constantly illuminated annulus or using a hand held stimulator ophthalmoscope, but such focal techniques only allows for a small number of areas to be tested in one session. Indeed a more sensitive and efficient technique was required to allow for a clinically more useful test to explore retinal areas.

The multifocal ERG (mERG) developed by Sutter & Tran (1992) allows for small retinal areas to be independently stimulated. Whereas in a standard ERG, responses are gained from brief full-field flashes presented as increments of intensity, mERG responses are gained from black and white hexagons each with a probability of 0.5 of being black or white on each frame. The topography of the responses can be plotted according to the properties of the system being analysed. A first-order response (kernel) is a linear approximation and the second-order kernel is a nonlinear approximation. The first-order response analysis is the response to a mean focal flash, which is the mean difference between the response to a bright and a dark stimulus event. Sutter & Vaegan (1990) gave evidence that suggested the ERG response is not linear by examining the human pattern ERG but in the mERG the first-order response dominates and so this response can be considered to reflect retinal activity. When recording from natural pupils, with a high mean luminance (375 cd/m^2), the first-order response has been shown to be dominated by the retinal component which is approximately linearly related to contrast, with the second-order component being small and unaffected by contrast changes (Bearse & Sutter, 1996). Such results can be significantly altered when the pupils are dilated and 100% contrast is used at a mean luminance of 100 cd/m^2 and both first- and second-order responses are revealed to be similar in amplitude and waveform.

The first-order response generated at the site where focal stimulation occurred can be decomposed into a retinal component whereas the second-order response is thought to be generated by the ganglion cell axons near the optic disc (Bearse & Sutter, 1996). The individual mERG waveforms, consisting of a negative (N1) and positive (P1) waveform, can be examined for latency and amplitude and these can be grouped together or examined separately. The fovea can be compared to outer peripheral rings, the lower half

can be compared to the upper half and the nasal and temporal hemifields can be compared. The array of corresponding responses to the hexagons shows the blind spot to be identifiable. 3-D densities of the response can be compared to show the luminance response topography, which shows a sharpness of the central peak. Responses from the mERG have been shown to be reproducible in normal subjects (Yoshii et al, 2000). Age appears to have a similar effect on mERG results than on standard ERG results, causing a decline in the mERG amplitude in the central retina in older subjects (Mohidin et al, 1999).

Multifocal responses consist of an initial negative trough followed by a positive peak, and such a waveform resembles the a-b wave complex seen in standard ERG recordings. The first-order response in the mERG is considered to be the mean response from a local flash but whether this has the same retinal origin as the full-field ERG is debatable. Kondo et al (1995) showed a patient with branch retinal occlusion with a preserved a-wave in the ERG but no negative configuration in the mERG indicating that the two responses did not correspond. However Hood et al (1997) found a good correspondence between mERG and full-field ERG responses in normal subjects although this was attributed to using a slow m-sequence, in which every element lasts 8 frames with an average time between successive increments of any hexagon being twice this. The negative wave was therefore thought to arise from the same components as the a-wave of the full-field ERG. Results from a mERG can be summed so when comparing full-field ERG with mERG the negative components appear similar, although the positive components do not, indicating that the positive wave of the mERG is some combination of the positive components of the full field (Hood et al, 1997).

In normal subjects, the latency of N1 and P1 were longer in the fovea than the parafovea, the amplitude was larger in upper than lower retina and the nasal and temporal retina showed no statistical difference (Nagatomo et al, 1997). The latter fact disagrees with the findings of Sutter & Tran (1992) who found a nasal-temporal asymmetry with a higher response density in the nasal retina, which is in proportion to the cone density of the human retina (Curcio et al, 1990). Examining the changes in the second-order response

from the nasal to temporal retina reveal an additive component whose latency increases with the distance of the stimulus from the optic nerve head (Sutter & Bearse, 1999). This fact can be attributed to the action potential from ganglion cells in the temporal retina travelling further to the optic disc than action potentials from the nasal retina.

The mERG has been proved to be useful in a clinical environment. A variety of known retinal diseases can cause changes in the mERG responses. For example, retinitis pigmentosa results in markedly reduced peripheral responses and a scotoma revealed by perimetry has a corresponding reduced response (Kondo et al, 1995). In glaucoma patients first- and second-order kernel responses are reduced (Chan & Brown, 1999). Seeliger et al (1998) analysed mERG responses in order to determine the implicit time topography by examining the properties of each single element across all subjects of the same group. Retinitis pigmentosa patients showed deviations when compared to the normal subjects and so this analysis could be useful in clinical work.

4.14 Multifocal VEP

The multifocal VEP (mVEP) was introduced in 1994 to allow for the objective measurement of the human visual field. The use of the mVEP was initially discouraged due to a large intersubject variability resulting from the calcarine fissure position in relation to the inion being different and the different way the cortex is folded between subjects. However the mVEP has advantages over standard perimetry as it is an objective test, requires minimum co-operation and is often the preferred test for patients (Klistorner et al, 1998). The mVEP may also have the potential to improve on the information gained from standard VEPs, in which a response is generated collectively from affected and unaffected regions thereby ignoring local VEP changes. The test has been developed primarily from the multifocal ERG test procedure. The exploration of the field topography of ERG responses was originally developed into a technique using the VERIS scientific system designed by Sutter & Tran (1992). Using this it is possible to independently stimulate small retinal areas and the individual responses can be extracted. Similarly it is possible to perform the retinotopic analysis of the VEP by examining the topography of the visual field using the mVEP.

As with mERG, the m-sequence method described by Sutter & Tran (1992) is used in the recording of the mVEP responses. Baseler et al (1994) used a pseudorandomly presented multifocal stimulation to stimulate numerous locations of the visual field simultaneously. A cortically scaled dartboard (Horton & Hoyt, 1991) is used in order to keep the area of primary visual cortex stimulated by each patch approximately constant. The stimulus consists of 60 segments which each contained 16 black-and-white checks, as opposed to the black and white hexagons used in the mERG, although some groups have used this stimulus (Klistorner et al, 1997) to no obvious advantage. Using a binary m-sequence, there is a 50% probability for the checkerboard pattern to reverse its polarity with every frame of the stimulating display. The m-sequence allows the computation of the signal by cross-correlation of the response evoked thereby allowing the collection of responses from hundreds of inputs in a fraction of a minute. Despite such short recording times maintaining fixation on the stimulus array can be difficult and so recording sessions are split into short segments in order to assist concentration. A camera can be used to monitor the pupil to ensure it remains in the same position throughout recording but this was not available during the recordings included in this study. Consequently a red cross was used to maintain concentration for short recording segments.

First-order responses (or kernels) reflect the corresponding conventional impulse response. The mVEP first-order response is flat, as a result of pattern stimulation where both pattern polarities are equal, confirming that there is no luminance change between the alternating pattern frames of the screen (Graham et al, 1999). However a first-order response can be recorded if the mVEP stimulus is flashed on and off in the same pseudo-random sequence. The response represents the sum of all responses to a white stimulus minus the sum of all responses to a black stimulus. A second order response (or second-order kernel, first slice) shows the difference between the response expected from a linear system and the response actually recorded. In this way it is the measure of the temporal nonlinearity of the visual system. The second-order kernel is considered to be analogous with a conventional pattern VEP, as the response represents the VEP to a reversal between two successive intervals, summed over all reversals in the m-sequence minus all

instances where no reversal occurred. Further 'slices' of the second-order kernel can be analysed and these represent nonlinearities when the stimulus is presented at different time intervals of 15 msec, 30 msec, 45 msec and so on. Using various contrast settings it has been shown that the second-order response first slice mimics that of the M cells and the second slice mimics P cell functions as revealed by the temporal analysis of the mVEP (Klistorner et al, 1997). Indeed Baseler & Sutter (1997) revealed M and P contributions to the VEP, in which the amplitude and latency of the response differed with eccentricity. Amplitude variations indicated that the P pathway was more active in the fovea and decreased with eccentricity. Latency variations also indicated that the P input to the cortex decreased with eccentricity.

60 traces are derived from the 60 cortically scaled individual segments and the peak-to-trough amplitude for each wave within a specified interval, around 50-150 ms, can be determined and compared for every stimulated segment of the visual field. Highly reproducible results can be gained using this technique (Klistorner & Graham, 1999). However the placement of electrodes remains vital in the recording of mVEPs and this issue, as well as using the test with vigabatrin patients, is investigated in chapter 7.

CHAPTER 5

FIELD-SPECIFIC VEPs: EXAMINING RESPONSES FROM NORMAL PARTICIPANTS AND A RANGE OF VIGABATRIN PATIENTS

5.1 Introduction

Standard VEP responses remain within normal limits in patients affected by vigabatrin associated visual field loss (Harding et al, 1995b, Mauguire et al, 1997). This is due to the fact that vigabatrin predominantly affects peripheral vision and central vision appears to be relatively well maintained. Figure 5.1 and 5.2 shows an example of vigabatrin associated visual field loss as shown by perimetry testing. As standard VEP responses originate mainly from the macular region of the retina (Blumhardt et al, 1978) such a peripheral defect caused by vigabatrin would not affect such responses. However VEP responses can give valuable information on lesions of the optic nerve and tracts when separate eyes are stimulated, as a larger VEP in the right eye would indicate a dysfunction of the fibres in the left optic tract (Borda, 1977). Furthermore evoked potential recordings have the advantage of being objective and so act as a simple test for a child to perform whereas perimetry, a test used to successfully identify visual field loss, cannot be reliably obtained on patients with a developmental age of less than 9 years. Below this age it is very often difficult for the child to concentrate on the task and so it is not possible to monitor visual field loss in paediatric patients exposed to vigabatrin.

A field-specific VEP has been developed to alleviate the problem associated with testing visual fields of young children (Harding et al, 1999b). This novel field-specific VEP, the H-Stimulus, consists of a central circle with a 5° radius containing alternating checks, which get smaller towards the centre of the circle. A blank annulus with a 5°-30° radius surrounds the centre circle. A larger checkerboard ring surrounds the annulus with a 30°-60° radius, which stimulates the surrounding regions (figure 5.3). The centre circle and the peripheral circle reverse at different rates that allows for two different responses to be recorded separately. For example, if the trigger is time locked to the central reversal rate then the response to the peripheral stimulus will be averaged out as the central response is averaged.

Figure 5.1
The visual field for a vigabatrin patient (Humphrey Field Analyzer Program 30-2 full threshold strategy) showing a concentric constriction with relative temporal sparing in the left eye. The concentric defect is evident in the top right interpolated gray scale (dark area indicates area of no sensitivity to the stimulus).

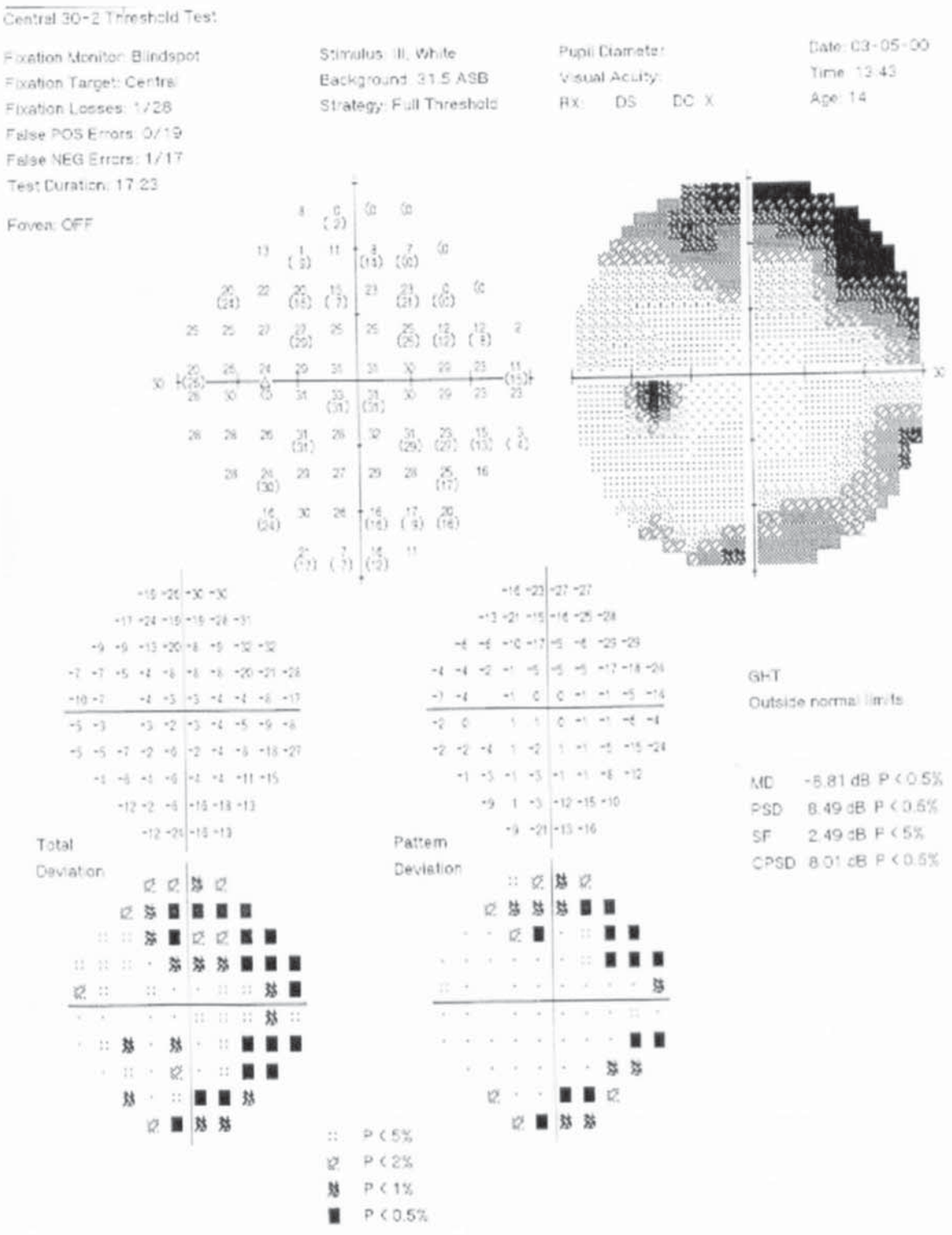


Figure 5.2
The visual field for a vigabatrin patient (Humphrey Field Analyzer Program 30-2 full threshold strategy) showing a concentric constriction with relative temporal sparing in the right eye. The concentric defect is evident in the top right interpolated gray scale (dark area indicates area of no sensitivity to the stimulus).

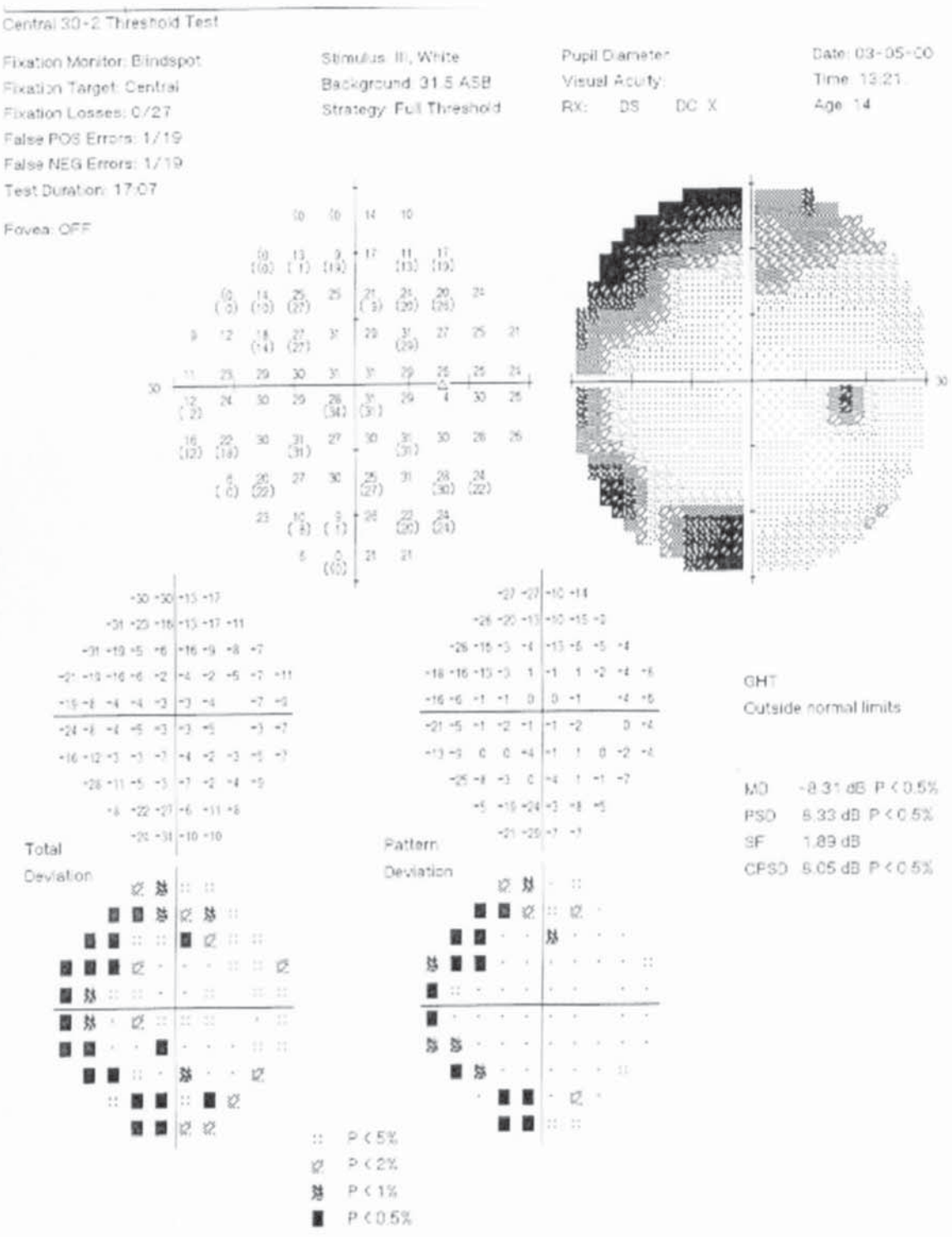
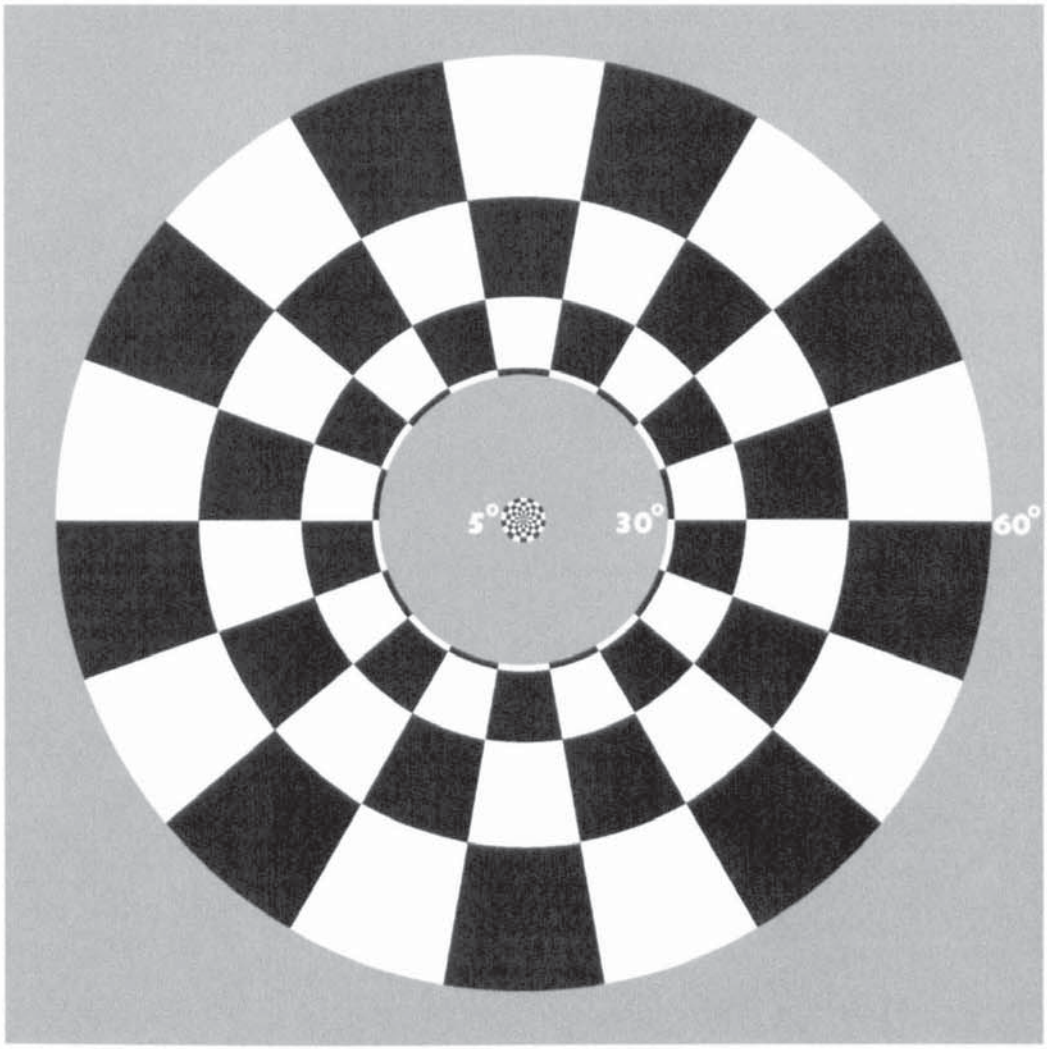


Figure 5.3
A computer generated picture of the H-Stimulus, showing the central stimulation (5° radius), blank annulus and the peripheral stimulation (30-60° radius).



The radial checks of the H-Stimulus are related to receptive field size, in that they get bigger as the radius enlarges. This theory is based on the cortical magnification factor, which indicates the amount of cortex, in millimetres associated with each degree of visual field (Daniel & Witteridge, 1961, Horton & Hoyt, 1991). The mapping of the visual field onto V1 is non-uniform, in that the fovea occupies a larger area than the peripheral field. Wässle et al (1990) examined retinal ganglion cell density in the primate and found 3-4 ganglion cells for every foveal cone compared to one ganglion cell per cone at 15-20° eccentricity. With more cones than ganglion cells in the peripheral retina, it was concluded that ganglion cell density could account for the cortical magnification factor. Horton & Hoyt (1991) calculated that 25% of the surface area of the striate cortex was attributed to the central 15° of vision. However a study combining MRI and perimetry results of patients with striate cortical diseases, indicated that the central 10° of visual field may be represented by 50-60% of the posterior striate cortex (McFadzean et al, 1994).

As it is necessary to use a large stimulus with a radius subtending to an angle of 60°, a standard television presentation is no longer suitable, as an average size screen would be too small to obtain the visual angle. A solution to this stimulus presentation problem is to use a projector, as the stimulus can be projected onto a large screen located near the subject. The stimulus is generated by programming the co-ordinates of the stimulus into the visual stimulator generator (VSG) 2/3 F 4MB VRAM. A trigger lead runs from the VSG computer to the averager and another output lead is attached to the liquid crystal projector, which projects the picture onto the screen. In this way the picture projected onto the screen can be looked at by a subject whilst, as in a conventional VEP recording, the averager machine records each response, of which an average can be displayed.

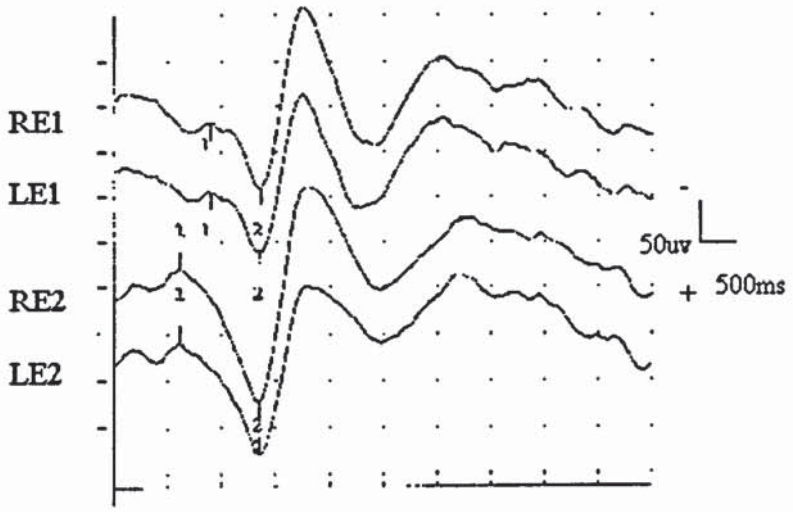
However, the internal electronics of the projector digitises the analogue signal, which it then compresses and interpolates the data spatially and temporally to produce a data stream that it can display. For example, if the frame rate (pictures per second) of the input signal is too high then the projector will drop frames to provide the correct average rate. Consequently the intervention of the signal processing circuits causes a delay between the

input and the appearance on the screen. This means that the trigger to the averager is not always time-locked to the output on the screen. It is useful to know when the stimulus onset occurs and this has been established using an oscilloscope (RS Component Ltd, LBO522 20MHz Leader) and an external trigger box. The external trigger box consists of a photodiode in which a circuit can detect an alternating black and white square pattern on the screen using a sensor. The circuit is operated from a nine-volt battery with an on/off switch. Triggering occurs on the white square to give a positive pulse of approximately five volts and the black square gives zero volts. A light emitting diode is used on the box as a visual indication of the pattern alternating. Using this technique a delay of approximately 30 ms was found. This delay degrades the response by broadening responses since the trigger is not completely time-locked to the potential thus affecting the amplitude and latency of the responses as shown in figure 5.4.

Regardless of the fact that the use of the projector incorporates a time delay, the capacity of the H-Stimulus is not compromised, as the result required from the test is whether a peripheral response can be gained. The inter-trial variation should not increase with this technique as 50 responses are averaged and all control subjects were tested using this technique, so are comparable. The presence or absence of a response could still successfully indicate preservation or loss of peripheral vision. Additionally, a similar predicament is faced when using a TV stimulator, where the pattern reversal has to be built up over the time taken to completely scan one frame. This set up also introduces a latency 'jitter' (Halliday, 1982). Consequently it was originally decide not to use a photodiode but instead rely on the 50 averaged responses that could indicate if a peripheral response was present. Additionally, the normative data was originally collected without using the photodiode and so in order to make a fair comparison of responses the original protocol was maintained. This decision was re-assessed following the initial vigabatrin study.

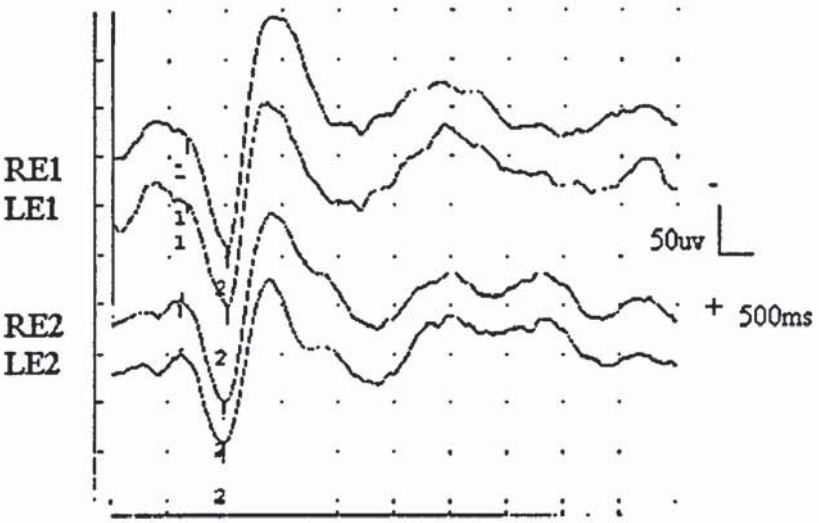
Figure 5.4
Comparison of H-Stimulus central results (a) without and (b) with an external trigger box. msec/ms denotes milliseconds, uv denotes microvolts.

(a) No external trigger box



	Latency1	Latency2	Amplitude
RE1	89ms	136ms	7.14uv
LE1	89ms	136ms	6.61uv
RE2	61ms	135ms	14.2uv
LE2	61ms	135ms	11.8uv

(b) External trigger box



	Latency1	Latency2	Amplitude
RE1	65.5ms	103ms	11.2uv
LE1	65.5ms	103ms	10.4uv
RE2	61.0ms	99.5ms	10.4uv
LE2	61.0ms	99.5ms	9.04uv

There is a steady decline with age in the maximum change in focus by accommodation. Reading (1988) presented results on Donders work from 1864 showing an almost linear trend up to the age of 60 with a decrease in dioptres. According to this data, over the age of 25 the eyes are unable to focus by accommodation at a 15cm distance. Despite this fact, the usefulness of the H-Stimulus is not lessened as over the age of 9-10 a co-operative patient can perform perimetry, which gives a clear indication of peripheral visual loss if it is present. The optimal use of the H-Stimulus is in the younger population aged 3-15 years, to give an indication of peripheral field loss, where perimetry is not possible. For example, in a study by Daneshvar et al (1999) children were excluded from the vigabatrin study due to the potential lack of reliability of visual field testing.

As the stimulus is specifically designed for children, normative data was collected from 3-10 years olds (Harding et al, 1999b). Responses were recorded from O2 and O1 referred to Fz as electrodes placed near the occipital pole record the most proximal neural signals occurring in the visual striate cortex. 45 subjects were tested monocularly and three binocularly with an age range of 3-10 years. The mean latency (±1 S.D.) of the central component was positive peak occurring at 157.92 ±14.42 ms and the mean latency (±1 S.D.) of the peripheral component was a negative peak occurring at 180.53±32.57ms. The mean amplitude (±1 S.D.) of the central component was 12.72 ±6.18μv and the mean amplitude (±1 S.D.) of the peripheral component was 11.05±4.5μv. According to International Standards of VEPs (Celesia et al, 1993, Harding et al, 1995a) responses can be labelled according to the polarity and latency of the response. The central response can therefore be denoted P160, although the actual latency and amplitude of this response is not of much interest. The main use of gaining a reproducible central response is to ensure the patient is looking correctly and complying with the test. If no central response can be elicited, despite good visual acuity, it may be deduced that the test cannot give accurate and interpretable results from that individual. The peripheral response can be denoted N180 (ranging from N150-N210). It should be noted that if an external trigger box were used to average the central and peripheral responses then the latencies would be P130 and N150 for central and peripheral responses respectively. The differing latency resulting from central and peripheral

responses means that there is a clear separation of the two responses. Reliable results were obtained in all but two of children (ages 2 and 3) who could not co-operate successfully with the procedure to gain reproducible responses. Right peripheral results could not be recorded in two subjects (ages 5 and 6) although a reason for this is not clear it is probably attributable to boredom with the test (Harding et al, 1999c).

The latency and amplitude of the peripheral response is more crucial, as an absent response, in the presence of a central response, may indicate a peripheral visual loss, as may a reduced amplitude response. If the patient were not co-operating with the test by looking at the periphery, other than the centre circle, then a central response would be generated as the response is being averaged to the periphery reversal (Harding et al, 2000c). A study of patients with confirmed vigabatrin associated visual field loss was necessary to determine if the H-Stimulus test could detect peripheral visual field loss. A pilot study of the H-Stimulus on young epileptic patients with confirmed vigabatrin-attributed visual field loss, shown by the peripheral 60-4 threshold program, revealed that if the visual loss was extensive no peripheral response could be recorded. However if the loss was primarily binasal a reduced amplitude response was recorded (Harding et al, 2000c). The field-specific VEP has the potential to detect peripheral field loss in young patients, who otherwise may continue taking vigabatrin without appreciating that a visual field defect had occurred as a result of the medication, since 94% of adult patients with vigabatrin associated visual field loss fail to recognise the loss (Wild et al, 1999).

Due to the complex infoldings of the occipital cortex is it difficult to establish the definitive source generation of this field specific VEP. However the P100 generated in a standard VEP is thought to arise predominantly from the central retina with the response originating near the calcarine fissure. The central stimulus used in the H-Stimulus produces a response consisting of a P100 component that is delayed due to stimulus generation. However the central retina does not generate the peripheral response as only neurones in the outer periphery are stimulated by the 30-60° radius stimulus. Indeed the response generated from this type of stimulation is inverted to that generated by the central stimulus. A more prominent negative peak can be measured and so this implies

that a separate pathway is involved. Variable polarity and broadened responses are generally found when stimulating the peripheral retina which is thought to be related to the peripheral retina being located deeper in the striate cortex and further up the calcarine fissure (Meredith & Celesia, 1982). The positioning of dipoles up the calcarine fissure makes for extremely difficult recording as conventional skin electrodes are further away and orientated poorly for recording from such a deep source.

Previous VEP studies have shown that motion VEPs generate a positive-negative-positive (P-N-P) response in which a prominent negative peak occurs at around 160-200ms (Kubová et al, 1995). It was shown that when using motion-onset VEPs at a high contrast with a low spatial frequency, the M pathway generated a response. M cells have large receptive fields and so are suitable to respond transiently to high contrast but are relatively insensitive at high spatial frequency. It is difficult to establish the exact spatial frequency of the peripheral H-Stimulus as it contains different checks sizes, which increase in size with eccentricity, but as it utilises large increasing check sizes it is assumed that it contains a low spatial frequency. In this way it is possible to assume that the peripheral response contains a motion component and hence the M pathway is activated during this stimulation.

5.2 Investigation into the sensitivity and specificity of the H-Stimulus

Pilot studies examining H-Stimulus responses in young adults with confirmed vigabatrin associated visual field loss and in normal children exhibited how the H-Stimulus is useful as a field-specific VEP but it failed to establish exactly how sensitive to peripheral field loss it was, as only patients with extensive visual field loss were used in the pilot study. If a full-field peripheral response can be established, then by stimulating half-fields and quadrants of the peripheral field it is possible to mimic visual field loss and examine such effects on the full-field response. The occlusion of the H-Stimulus was aimed to block the stimulus in various ways so that all such defects were accounted for. As a binasal defect results in the loss of the right visual field in the left eye and the loss of the left visual field in the right eye, the best model for mimicking the classic binasal defect would be to occlude the left half field stimulation when stimulating the right eye and to occlude the

right half field stimulation when examining the left eye. It is of particular interest to see the effect of this occlusion on responses, although further occlusion indicates how much visual field loss needs to occur below which no response can be elicited.

Epileptic patients exposed to vigabatrin showing vigabatrin associated visual field loss may exhibit a loss of vision ranging from, at worst an absolute field loss encroaching to within 15 degrees of fixation, to a constriction being more evident nasally than temporally (Wild et al, 1999). The pilot study results as well as the normative study indicated that the central circle of 5° radius and the peripheral circle of 30-60° radius could elicit interpretable central and peripheral responses (Harding et al, 1999c). However it did not indicate how sensitive the H-Stimulus was to a loss of peripheral vision. The main objectives of the first part of this study were to mimic peripheral visual field loss in healthy volunteers, to find the critical level below which peripheral responses are significantly affected and to establish if the latency or amplitude of the response can be used as a marker for indicating peripheral field defects.

5.2.1 Method for normative study

Local ethical approval was gained and consent forms were completed throughout this study. Electrodes were attached to the head according to the International 10-20 system of electrode placement (Jasper, 1958). O2 and O1 were referred to Fz with Cz used as the ground. Silver-silver chloride electrodes were attached to the subjects head with 10-20 conductive paste (Unimed Electrode Supplies) after rubbing with NuPrep (Unimed Electrode Supplies). Where necessary collodian adhesive (SLE Diagnostics) and blenderm tape (3M Medica) was used. Impedances were even and below 5 kohms. Electrodes were taped onto the subjects shoulders and an orthoptic eye patch (3M Medica) was placed over one eye. The subject was then positioned 15cm away from the screen to ensure the stimulating field subtended to 5^0 for the centre circle and 30-60^0 for the peripheral circle. The stimulus was generated using a VSG 2/3 (Cambridge Research Systems Ltd) and projected onto 216 white diffusion filter paper (Lee Filters) using a Hitachi Multimedia LCD projector CP-S830W/E (Matrix Display Systems Ltd).

The subjects were positioned so that the open eye was in line with the centre circle. The subject was instructed to keep looking for 50 seconds at the centre circle as the pattern alternated whilst ignoring the larger pattern. 50 sweeps were recorded with the central trigger, and then 50 sweeps were recorded with the peripheral trigger. To ensure reproducibility of the responses, at least two trials were repeated and the whole process was repeated for the second eye. The visual evoked potential to both stimuli was recorded on the Sapphire^{II} (Medelec Ltd) using a bandwidth of 1-30 Hz with a sweep time of 500 ms. Reversal rate of the central and peripheral stimulus was 0.92 Hz and 1 Hz respectively. A Minolta LS-110 photometer was used to calculate luminance levels and contrast, which was calculated using the Michelson equation. The overall mean luminance of the peripheral stimulus was 98.05 cd/m^2 and the mean luminance of the annulus was 207.02 cd/m^2. Due to the very small squares in the centre circle it was not possible to take L-min or L-max readings, so an average of recordings was taken giving a mean luminance of 115.73 cd/m^2 with a contrast of 40%.

A full-field reproducible peripheral response was gained in either one or both eyes. The hemi-fields and quadrants were stimulated separately by covering the H-Stimulus with black card. Responses were recorded from the right half field, the left half field, the upper half field, the lower half field and all four quadrants separately. Part of the centre circle was maintained throughout the testing to ensure the subject continued to focus in the centre.

All statistical analysis was performed using SPSS ®. A one-way between groups ANOVA was used to determine if the amplitude and latency significantly differed between the three groups of full field, half field and quadrant responses. Using a repeated measures general linear model, latency and amplitude responses of the H-Stimulus responses were examined to see if an interaction occurs between which eyes is stimulated, which hemisphere is recorded from, and which field is stimulated. A p value of less than 0.01 and 0.05 was accepted as statistically significant.

5.2.2 Results from normative study

Four right eyes and four left eyes were tested from healthy volunteers who were all below the age of 25 (mean age 19 years) with good eyesight. A summary of the results can be seen in table 5.1, which shows the mean amplitude and latencies of the peripheral responses recorded under the different test conditions.

A one-way ANOVA was used to determine if the amplitude and latency parameters were significantly different between the three groups of full field, half field and quadrant responses. The amplitude of the peripheral response was found to be significantly different between the three groups [$F(2,141)=46.32;p=0.000$]. The latency of the peripheral response was found not to be significantly different between the groups [$F(2,141)=2.00$; $p = 0.139$, NS]. The significant effect of the stimulus size on the amplitude of responses can be seen in graph 5.1, and the non-significant effect of the stimulus size can be seen in graph 5.2.

Figure 5.5 shows a full-field peripheral response from a normal participant. An example of how the amplitude of responses decreases as the stimulus is occluded can be seen in figure 5.6 as left and right half field responses are shown. The stimulation of upper and lower half fields also shows that the removal of a lower peripheral stimulation results in a reduction in the amplitude of the response (figure 5.7). Examples of quadrant stimulation can be seen in figures 5.8 and 5.9.

Using a repeated measures general linear model, the amplitudes of the peripheral responses were examined further. The type of occlusion had a significant effect on the amplitude of the responses recorded [$F(8,56)=9.411;p<0.000$]. A significant difference was seen between peripheral amplitude responses recorded from the right and left hemispheres. Recording of the half field responses reveals a significant difference between amplitude of responses. The recording of left half field responses are larger in the right hemisphere, whereas the recording of right half field responses are larger in the left hemisphere [$F(1,7)=11.010;p=0.013$]. Similarly, upper half field responses are larger

on the left hemisphere and the lower half field responses are larger on the right hemisphere [$F(8,56)=4.546;p<0.000$].

The summed amplitude of the peripheral H-Stimulus response recorded from O2 and O1 were 10 μv or over. When half or more of the H-Stimulus is occluded, the amplitude is reduced to below a summed value of 10 μv. Table 5.2 shows the mean sum amplitude of O2 and O1 in response to the different field stimulation. Based on the 8 subjects seen, 3/8 (37.5%) produced a summed O2 and O1 full field peripheral response below 10 μv and so if this were to be a marker there may be some false negatives from individuals that give poor responses.

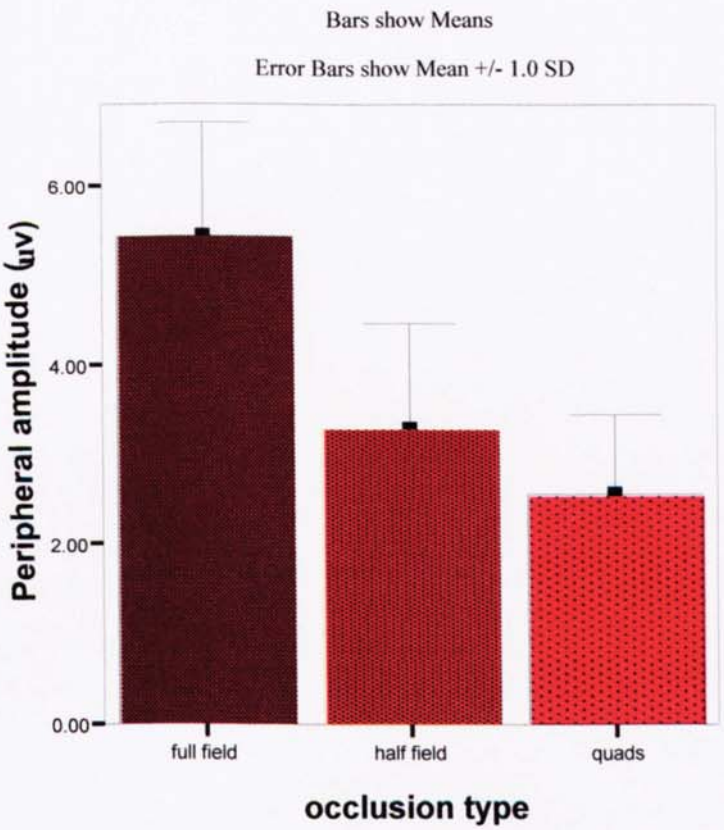
Table 5.1
Shows the mean amplitude and latency of responses from 8 eyes, under the different test conditions
μv; microvolts, msec; milliseconds

Field Stimulated	Mean average of O2 and O1 peripheral amplitude (μv ± SD)	Mean average latency of the peripheral response (msec ± SD)
1. Full Peripheral	5.43 ± 1.30	135.75 ± 14.41
2. Left half field	3.08 ± 0.74	135.13 ± 13.16
3. Right half field	3.84 ± 1.12	135.75 ± 05.05
4. Upper half field	2.72 ± 1.41	127.69 ± 13.51
5. Lower half field	3.46 ± 1.10	131.81 ± 09.32
6. Upper left quadrant	2.71 ± 0.92	140.63 ± 12.79
7. Upper right quadrant	2.54 ± 0.71	142.63 ± 09.39
8. Lower left quadrant	2.23 ± 0.81	130.13 ± 14.54
9. Lower right quadrant	2.71 ± 1.11	133.94 ± 09.66

Table 5.2
Shows the mean sum of O2 and O1 amplitudes from 16 eyes, under the different test conditions

Field Stimulated	Mean sum of O2 and O1 peripheral amplitude (μv ± SD)
1. Full Peripheral	10.86 ± 2.50
2. Left half field	6.18 ± 0.43
3. Right half field	7.69 ± 2.03
4. Upper half field	5.45 ± 2.82
5. Lower half field	6.93 ± 2.07
6. Upper left quadrant	5.43 ± 1.75
7. Upper right quadrant	5.07 ± 1.37
8. Lower left quadrant	4.47 ± 1.44
9. Lower right quadrant	5.41 ± 2.10

Graph 5.1
Shows the significant effect of the mean peripheral amplitude responses to full field, half field and quadrantic stimulation. n=16



Graph 5.2
Shows the non-significant effect of the mean peripheral latency responses to full field, half field and quadrantic stimulation. n=16

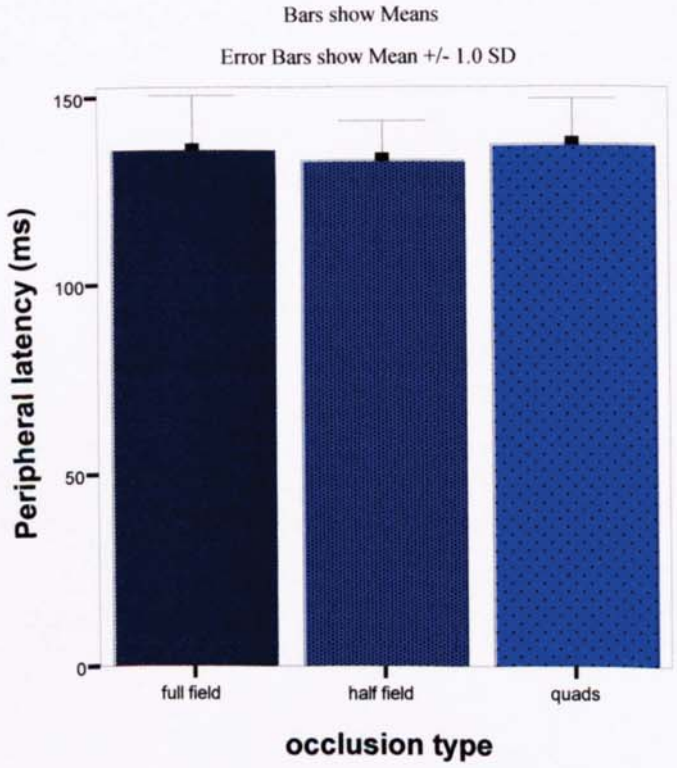
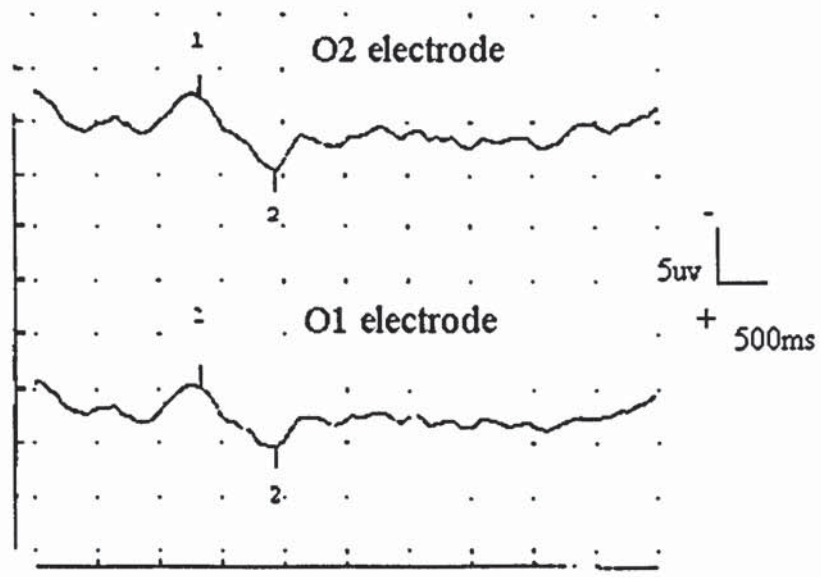


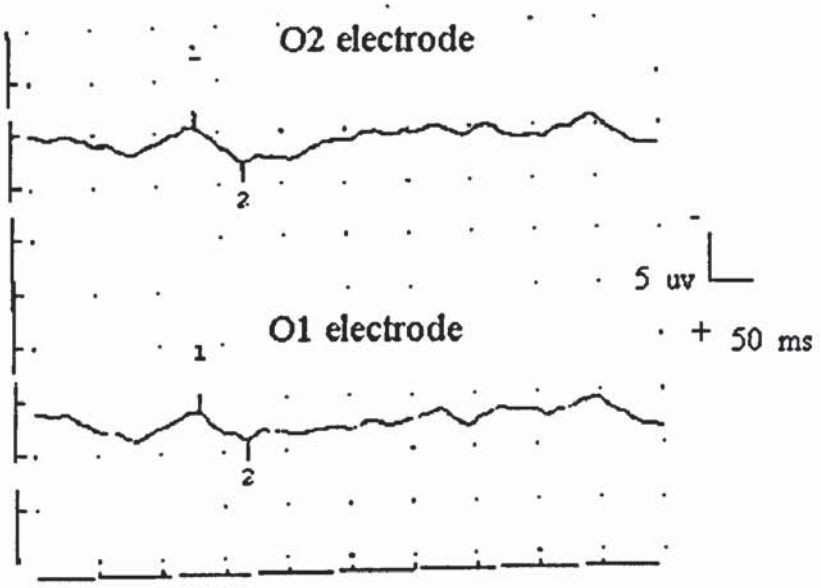
Figure 5.5
Shows a full-field peripheral response recorded from O2 electrode and O1 electrode when the right eye is stimulated from a normal participant (VK).
msec/ms denotes milliseconds, uv denotes microvolts.



	Latency1	Latency2	Amplitude
O2	133ms	193ms	6.72uv
O1	133ms	193ms	5.42uv

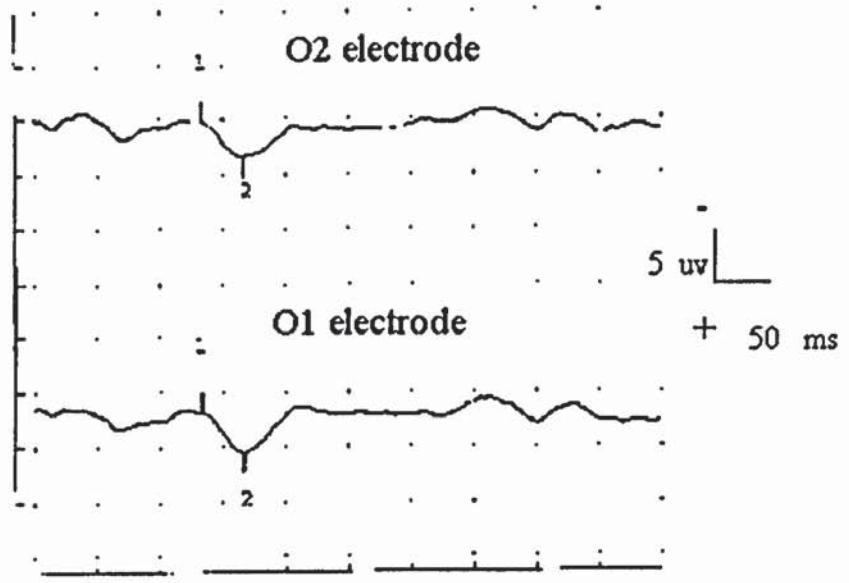
Figure 5.6
Compares an (a) left half-field peripheral response and a (b) right half field peripheral response recorded from O2 electrode and O1 electrode when the right eye is stimulated from a normal participant (VK).
msec/ms denotes milliseconds, uv denotes microvolts.

(b) Left half field response



	Latency1	Latency2	Amplitude
O2	131ms	169ms	3.38uv
O1	131ms	169ms	2.58uv

(c) Right half field response



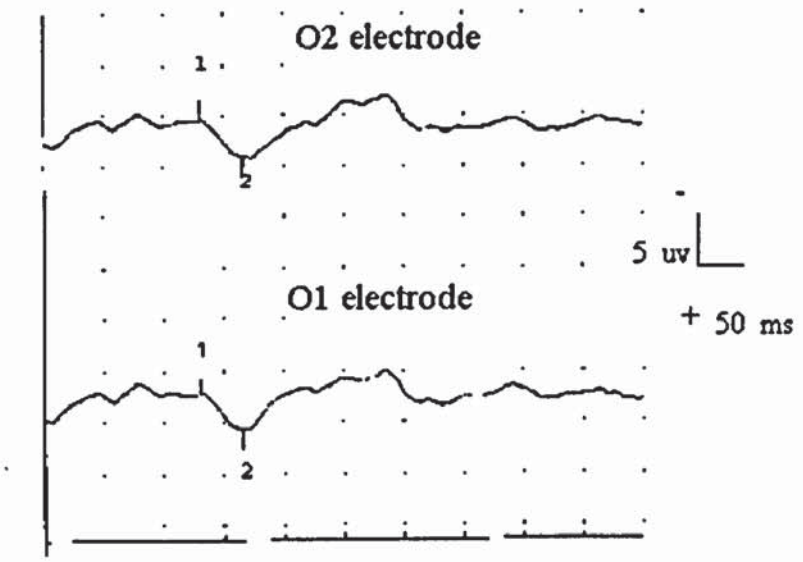
	Latency1	Latency2	Amplitude
O2	134ms	167ms	3.08uv
O1	134ms	167ms	3.52uv

Figure 5.7

Compares an (a) lower half field responses and (b) upper half field response recorded from O2 electrode and O1 electrode when the right eye is stimulated of a normal participant (VK).

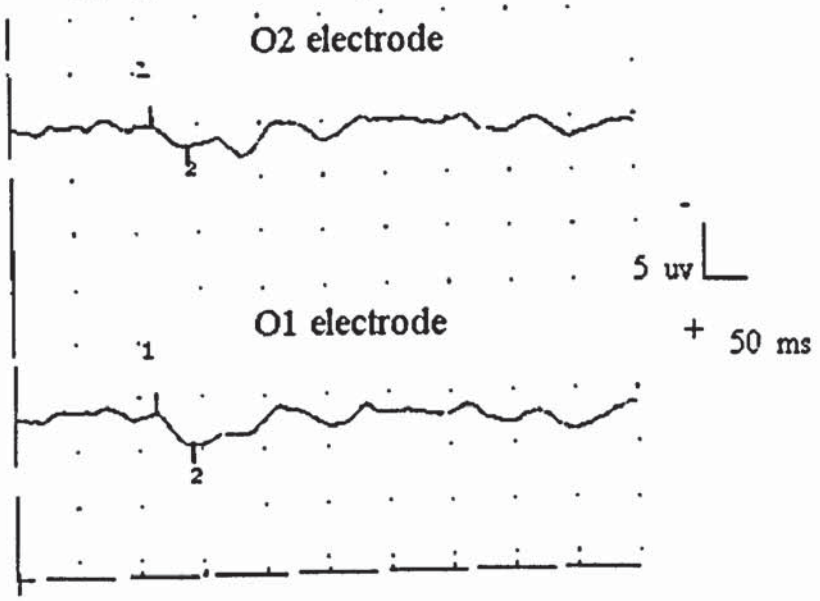
msec/ms denotes milliseconds, uv denotes microvolts.

(a) Lower half field response



	Latency1	Latency2	Amplitude
O2	130ms	166ms	3.48uv
O1	130ms	166ms	3.44uv

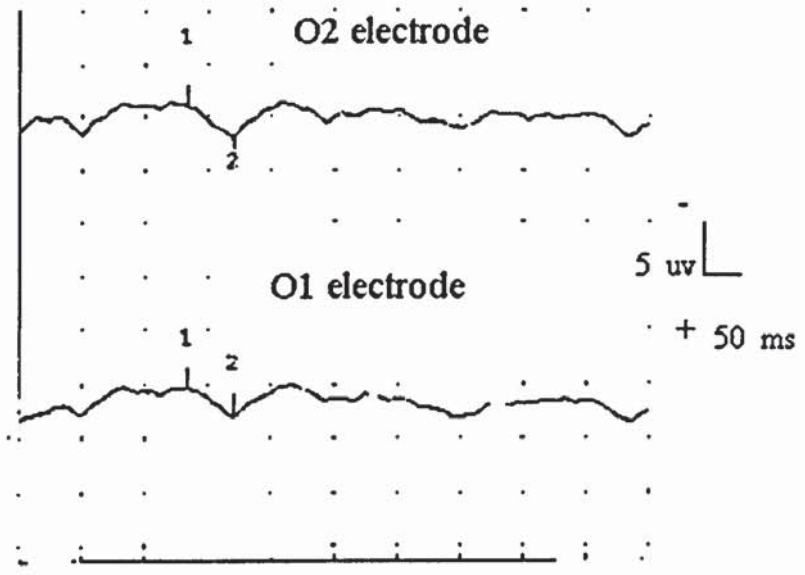
(b) Upper half field response



	Latency1	Latency2	Amplitude
O2	114ms	142ms	1.84uv
O1	114ms	142ms	2.84uv

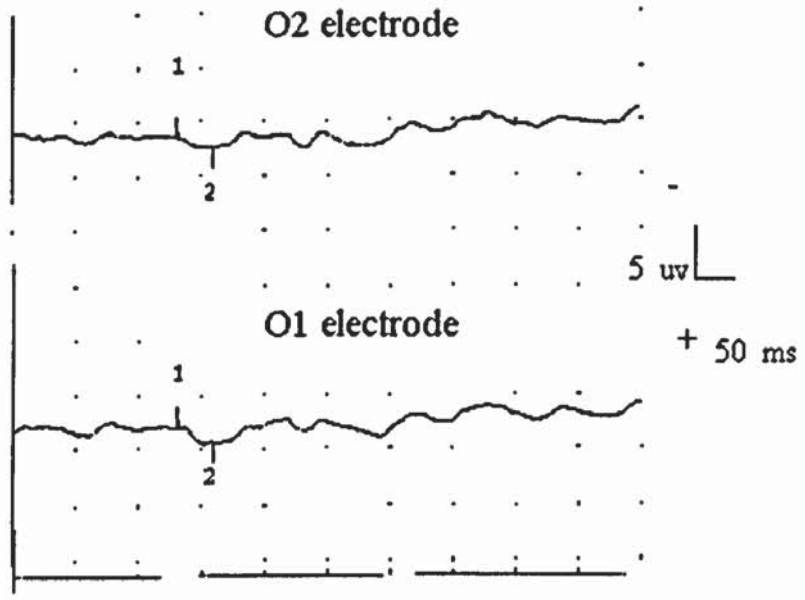
Figure 5.8
Compares the responses from O2 electrode and O1 electrode of stimulating peripheral quadrants (a) upper left (b) upper right quadrants when the right eye is stimulated. msec/ms denotes milliseconds, uv denotes microvolts.

(a) Upper left quadrant response



	Latency1	Latency2	Amplitude
O2	133ms	170ms	2.81uv
O1	133ms	170ms	2.56uv

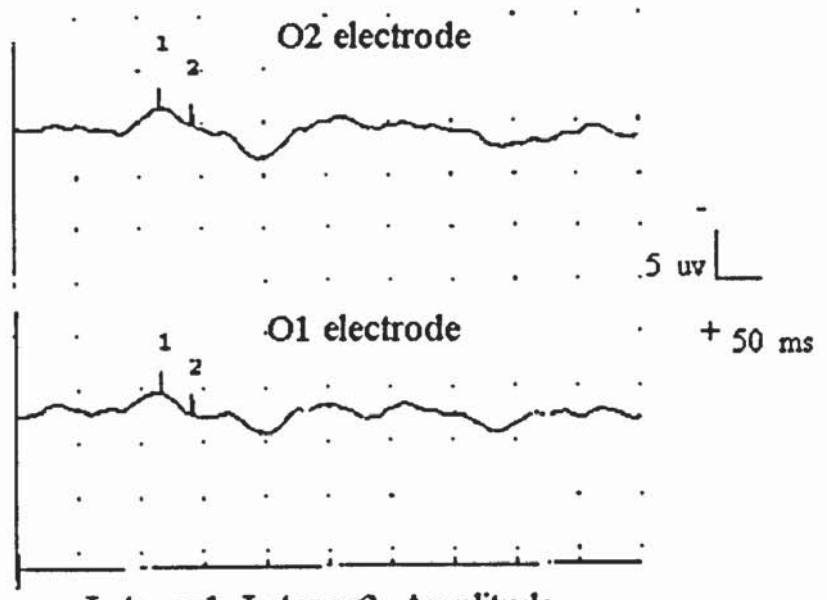
(b) Upper right quadrant response



	Latency1	Latency2	Amplitude
O2	131ms	159ms	0.83uv
O1	131ms	159ms	1.32uv

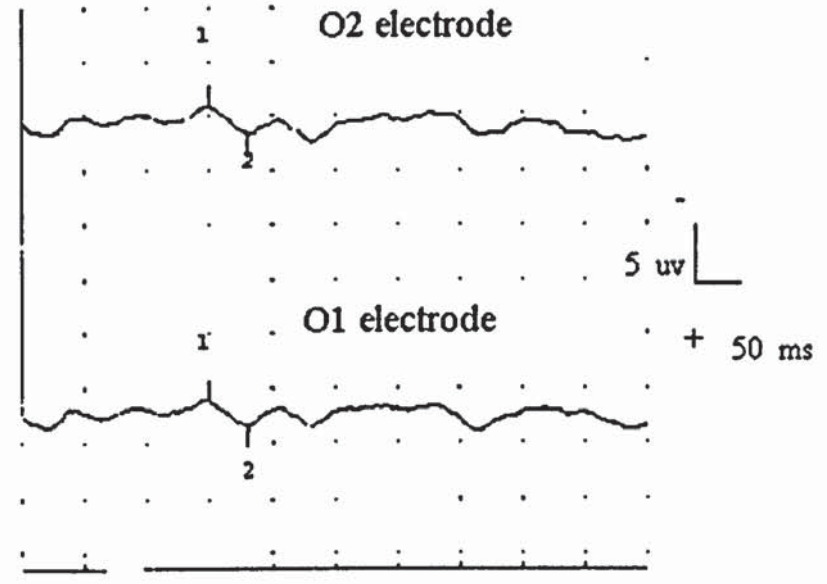
Figure 5.9
Compares the responses from O2 electrode and O1 electrode of stimulating peripheral quadrants (a) lower left (b) lower right quadrants.
msec/ms denotes milliseconds, uv denotes microvolts.

(a) Lower left quadrant response



	Latency1	Latency2	Amplitude
O2	117ms	143ms	1.58uv
O1	117ms	141ms	2.06uv

(b) Lower right quadrant response



	Latency1	Latency2	Amplitude
O2	150ms	180ms	2.47uv
O1	150ms	180ms	2.06uv

5.2.3 Discussion of normative study

The results show that when only a quadrant of the H-Stimulus is presented it is possible to generate a VEP, albeit a very small response. The amplitude of the H-Stimulus responses significantly reduces as more of the stimulus is occluded. In this way the amplitude of the response is dependent on the size of the stimulus. The full-field response produces a significantly larger amplitude response when compared to the half-field responses, which in turn have significantly larger amplitude when compared to the quadrant responses. The stimulation of half fields reveals a significant difference when recording from the right and left hemisphere and this effect is less pronounced when stimulating the full field and quadrants. The effect of occluding the stimulus had no significant effect on the latency of the H-Stimulus responses.

Half field results have shown different lateralisation of responses. Holder (1980) found patients with a bitemporal hemianopia had a VEP maximal over the hemisphere contralateral to the defect. In contrast Halliday et al (1976) found a maximal abnormality ipsilateral to the field defect. Similarly, Blumhardt et al (1977) found a localised abnormality ipsilateral to the field defect in patients with a homonymous hemianopia. A binasal hemianopia produces a similar response with an opposite laterality. Such discrepancies in results have been accounted for by the use of different recording parameters. Using a larger stimulating field with larger checks, as well as spaced out occipital electrodes results in an abnormality being recorded ipsilaterally (Harding et al, 1980). The lateralisation seen could be accounted for by the cortical representation of the macula in the occipital pole with the generator neurones being posteriorly orientated, and the peripheral retina being on the medial surface of the hemisphere with the dipoles laterally orientated.

In this study it was found that a larger response was seen from the right hemisphere when stimulating the left half field and a larger response was seen over the left hemisphere when stimulating the right half field. An actual binasal hemianopia defect would produce a contrasting asymmetric response in affected patients. Based on various studies (Barrett

et al, 1976, Halliday et al, 1976, Blumhardt et al, 1977), the asymmetry seen during full field stimulation should crossover when stimulating the other eye

When examining upper and lower responses it was evident from the data that the occlusion of the lower portion of the stimulus resulted in a reduction in amplitude of the response and this has been shown previously (Kriss & Halliday, 1978). In this way, a large majority of the peripheral response originates from the lower half of the visual field. Halliday (1982) showed that the lower half field response were generated from areas close to surface electrodes ensuring that this response recorded would be larger than the upper half field response which is generated on or near the inferior surface of the occipital lobe which generally face away from surface electrodes. However confirmed lower visual field loss has been shown to produce a reduced amplitude VEP response, whereas upper visual field loss has a relatively small effect on the VEP amplitude (Wildberger, 1984). Additionally upper visual field loss results in an earlier P100 latency and a lower visual field loss results in a later P100 latency when compared to a full field response (Wildberger, 1984). Such asynchrony between the upper and lower fields is associated with the use of large checks, as smaller checks stimulate a largely central response thus reducing the lower hemiretinal influence. In this study, the upper half field stimulation produced an earlier latency response although it should be noted that this response was more variable than the lower half field response, which had a similar mean latency response. Overall the response of the upper half field stimulation produces the largest response over the right hemisphere, and the lower half field stimulation produced the largest response over the left hemisphere.

When examining abnormal VEP responses the latency is often the parameter that is abnormal, showing an increase in time for the response to occur. Such an abnormal response is expected in such cases where a demyelination has occurred in the brain that causes the action potential generated by the stimulus to travel slower. Delayed VEP responses in cases of optic neuritis (Halliday & McDonald, 1977) and in multiple sclerosis (Mauguière et al, 1982) have been reported. However in the case of vigabatrin patients, in which peripheral visual field loss has occurred, it is less likely that the latency

would be affected as no demyelination has occurred. Instead the response may still occur at the same latency but showing greatly reduced amplitude as the response is still activated by the remaining peripheral stimulation that can be seen.

The amplitude of the peripheral response is significantly affected by the occlusion of the stimulus and so this parameter is more likely to be affected by a peripheral visual field loss caused by vigabatrin. The latency of the response does not show any consistent or significant effect following occlusion of the stimulus and so this parameter is not useful when trying to establish if a defect has occurred. All full field stimulus responses were examined and a mean summed response of over 10 μv in amplitude recorded from O2 and O1 electrodes were found. This summed response reduces to below 10 μv when the stimulus is occluded and mimicking a peripheral field defect. However when the amplitude of the full field peripheral response was examined in each normal subject, values ranged from 8.36 μv – 13.36 μv, indicating that in some cases a full field peripheral response may be deemed abnormal. The average age of the normal subjects used in this study was 19, but the average amplitude of 02 and 01 separately in the pilot study of normal control children was 12 μv. This may therefore ensure that the majority of children without a visual field defect will produce a peripheral response of over 10 μv in amplitude, ensuring that false negatives are not identified.

Using the H-Stimulus it is possible to add O2 and O1 response amplitudes together and use 10 μv as a cut-off point, below which it can be assumed that a peripheral visual field defect may be present. Additionally, an asymmetric response may also indicate a defect. This theory was tested on epileptic patients exposed to vigabatrin who could comply with the H-Stimulus and perimetry.

5.3 Paediatric patients exposed to vigabatrin

Vigabatrin is particularly effective in the treatment of infantile spasms (West's syndrome) and is also used in treatment of partial seizures in childhood (Chiron et al, 1991). Although automated static threshold perimetry produces a reliable measure of visual field loss, formal perimetry is rarely reliable below a developmental age of nine years. Consequently, there is a lack of studies concerned with vigabatrin associated visual field loss in children and it is unknown whether the prevalence in the paediatric population is the same as that found in adults, typically around 30% (Wild et al, 1999). It is clear, therefore, that an alternative method is required for children exposed to vigabatrin, which exhibits high sensitivity and specificity to visual field loss and yet can be performed quickly, with little active co-operation, and without requiring a subjective response from the patient.

The main objectives of this part of the study was to use the H-Stimulus on paediatric patients exposed to vigabatrin to determine if the test could detect vigabatrin associated visual field loss. In order to determine if the field-specific VEP can detect peripheral visual field loss, the sensitivity (responsiveness to change) and the specificity (exactitude) of the results need to be demonstrated by comparing results with reliable perimetry results.

5.3.1 Method for the vigabatrin paediatric patients

Local ethical approval was gained and consent forms were completed throughout this study, either by the patient or the guardian. The method described in section 5.3 of this chapter was used for the children exposed to vigabatrin although no occlusion of the H-Stimulus was used during testing. Additionally the paediatric patients who were able to comply with the test procedure performed a visual field examination. The test was undertaken with the Humphrey field Analyzer (HFA) 750 using the 135 point field and the age-corrected three-zone method. All reliability indices were automatically assessed. If results were normal or non-compliance occurred then no further test was carried out. If results were abnormal or contained a potential artefact then the Full Threshold strategy was used with program 30-2. The results of this latter test were then categorised as

normal, abnormal or unreliable. If the child was unable to comply with static perimetry then Goldmann perimetry was performed using the I3E and I4E isopters. JW or MC carried out all visual field examinations.

All statistical analysis was performed using SPSS ®. A Pearson' Chi-square test for independence and relatedness was performed.

5.3.2 Results from the vigabatrin paediatric patients

Thirty-nine vigabatrin patients, twenty-seven males and twelve females, aged between 3-15 years (mean age 9.6 years) were included in the study. The patient demography was noted including age, current or previous vigabatrin treatment dose and length as well as previous AEDs and concomitant medication (table 5.3). Four patients failed to comply with the H-Stimulus procedure (10.3%) and were of varying ages of three, four, seven and eleven years old. One patient aged three failed on monocular testing but managed a binocular recording (2.6%). Overall, 90% of the paediatric patients included in this study could provide reliable H-Stimulus results. This is compared to only 31% of children (12 of the 39) that could provide reliable perimetry results.

An example of the presence of a peripheral response, defined by the amplitude of the peripheral response recorded from both hemispheres being over 10μv in both eyes is shown in figure 5.10, and correctly identified seven of the eight children with normal peripheral fields giving a specificity of 87.5%. The reduction in amplitude of a peripheral response to the H-stimulus, defined by the amplitude of the peripheral response recorded from both hemispheres being below 10μv in both eyes, an example is shown in figure 5.11, correctly identified three of the four children who demonstrated a vigabatrin associated visual field loss on formal perimetry giving a sensitivity of 75%. From the four children who showed a vigabatrin associated visual field loss, three were male.

Another type of response was also noted in some cases in which an asymmetric response is recorded. As can be seen in figure 5.12, if the right eye is stimulated a larger peripheral

response is recorded over the left hemisphere and if the left eye is stimulated a larger peripheral response is recorded over the right hemisphere.

The distribution of false-positive and false-negative results was then used to estimate the prevalence of vigabatrin-related visual field defects in the population of 39 children. The marginal distribution of the H-stimulus test results were assumed to be fixed and, the results of the H-stimulus test of the 39 patients were then allocated in the same proportions as found for the 12 patients who had reliable visual fields. Using this approach, the resulting prevalence of vigabatrin-related visual field defects in these 35 patients was 25.7%.

Nine patients were still receiving vigabatrin at the time of testing with a range of doses from 1000-4000 mg/day with length of treatment ranging 2-9 years. The remainder of patients had taken vigabatrin from 3 months up to 9 years on dosages from 100-400 mg/day. A Pearson' Chi-square test for independence and relatedness found there was no relationship between vigabatrin treatment over three years and a visual field defect based on their H-Stimulus results [$\chi^2 = 0.846$, df = 1, p = 0.358, NS]. This indicated that those children who had taken vigabatrin for over three years were not at more risk to developing a vigabatrin associated visual field defect than those children that had taken vigabatrin for less than three years.

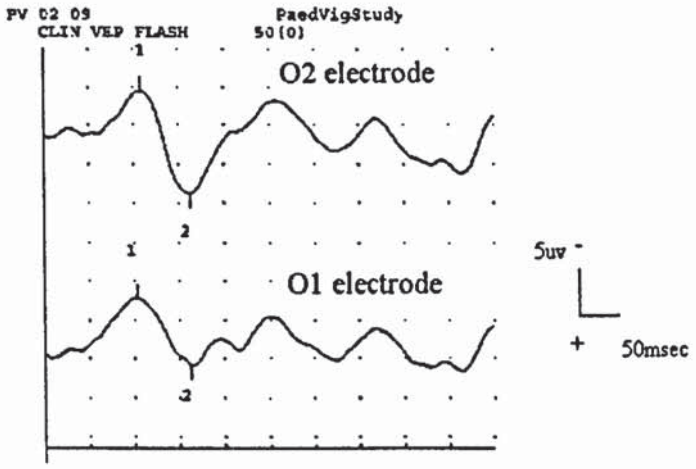
Table 5.3
Paediatric patient details and vigabatrin treatment history.

Patient Number	Gender	Age (years)	Maximum VIG dose (g)	Duration of VIG treatment (months)	Other AEDs (current or past)
101	Male	8	1.5	49	CBZ, SV, CLB
102	Male	7	1	11	CBZ, LTG, PHY, GBP
103	Male	10	1	72	CBZ, SV
104	Male	3	2	73	CBZ, PHB
105	Female	3	1	15	-
106	Male	11	1	35	CBZ
107	Male	10	1	3	CBZ, SV, LTG, CLOB, PHY, ETH
108	Male	10	4	53*	CBZ, SV, LTG, GBP
109	Female	4	1	43	CBZ, SV, LTG
110	Male	11	1	34*	CBZ, SV, LTG
111	Female	10	2.5	8	CBZ, SV, LTG
112	Female	11	3	108*	PHY, ETH, SV, PHB
113	Female	15	4	16	CBZ, SV, LTG, GBP
114	Male	13	2	73*	CBZ, SV, LTG, PHB, GBP
115	Female	5	3	24*	SV, LTG, TOP
116	Male	13	1	5	CBZ, SV, CLB, PHB
201	Male	8	1	13	CBZ, SV, PHY, CLB
202	Female	13	2	50	CBZ, SV
203	Male	8	1.5	66	CBZ, SV,LTG,GBP, PHY,CLB, TOP
204	Male	3	1	16	SV
205	Female	7	1.5	24*	CLOB, ETH, PHB, LTG, SV
206	Male	14	3	60	CBZ, SV, LTG, TOP
207	Female	11	3	61	SV, LTG, CLON
208	Male	10	1	74*	CBZ, SV
209	Female	12	2	39	CBZ, LTG, PRED
210	Male	9	2	50	CBZ, CLOB, LTG
211	Female	10	1	24	CBZ, PHY, GBP, TOP
212	Female	12	1.5	35*	CBZ, LTG
213	Male	5	1	46	CBZ, PHY, LTG, CLB, GBP
214	Male	11	3	60*	CBZ, PHY
301	Male	5	1	50	-
302	Male	8	1	29	CBZ
303	Male	15	2.5	75	SV, LTG
304	Male	14	3	108	CBZ, PHY, CLB
305	Male	11	1.5	4	CBZ, SV, LTG, ETH, GBP
306	Male	11	1	26	CBZ, SV, LTG
307	Male	7	0.5	40	CBZ, SV
308	Male	15	3	9	CBZ, SV
309	Male	7	2	19	CBZ, SV

Key: SV;Sodium valproate, CBZ;Carbamazepine, LTG;Lamotrigine, GBP;Gabapentin, CLB;Clobazam, ETH;Ethosuximide, PHY;Phenytoin, TOP;Topiramate, PHB;Phenobarbitone, CLON;Clonazepam, * ongoing vigabatrin treatment

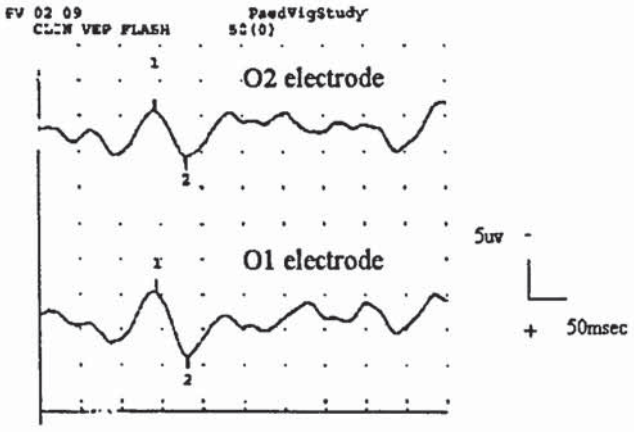
Figure 5.10
H-Stimulus results showing right eye responses from the right hemisphere (O2 electrode) and the left hemisphere (O1 electrode). (a) Shows a normal central response and (b) shows a normal peripheral response in a vigabatrin patient (PV) with normal visual fields shown by perimetry.
msec/ms denotes milliseconds, uv denotes microvolts.

(a) A normal central response



	Latency1	Latency2	Amplitude
O2	108ms	162ms	13.2uv
O1	103ms	162ms	8.56uv

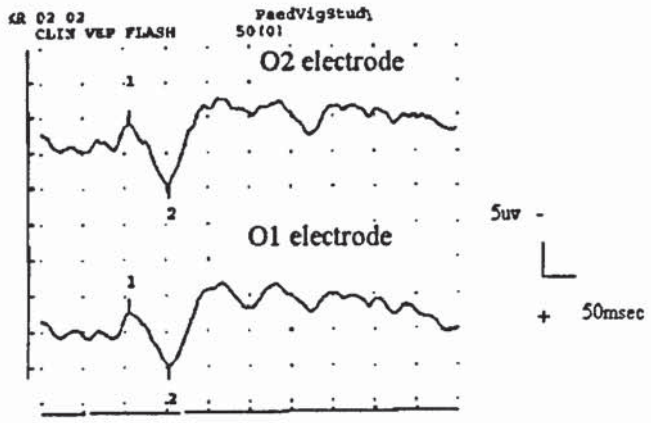
(b) A normal peripheral response



	Latency1	Latency2	Amplitude
O2	143ms	181ms	6.19uv
O1	143ms	181ms	8.75uv

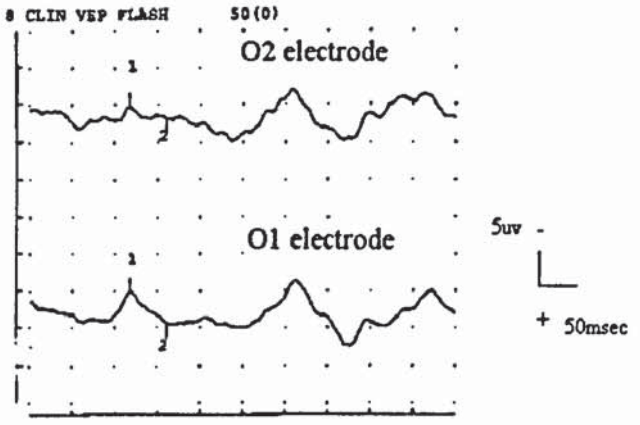
Figure 5.11
H-Stimulus results showing right eye responses from the right hemisphere (O2 electrode) and the left hemisphere (O1 electrode). (a) Shows a normal central response and (b) shows a reduced amplitude peripheral response in a vigabatrin patient (KR) with abnormal visual fields shown by perimetry.
msec/ms denotes milliseconds, uv denotes microvolts.

(a) A normal central response



	Latency1	Latency2	Amplitude
O2	106ms	153ms	8.61uv
O1	106ms	153ms	7.41uv

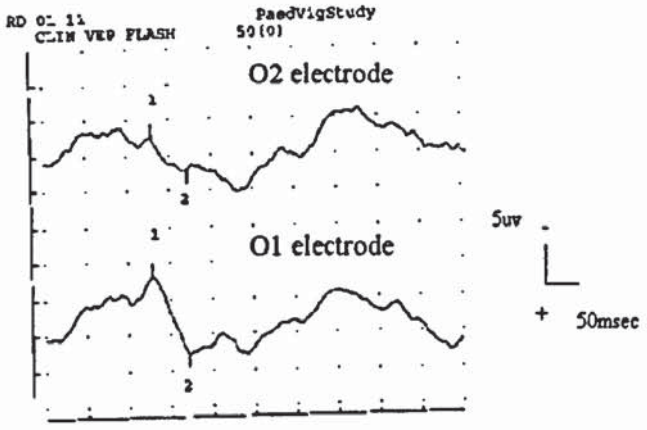
(b) A reduced amplitude peripheral response



	Latency1	Latency2	Amplitude
O2	118ms	161ms	1.63uv
O1	118ms	161ms	4.23uv

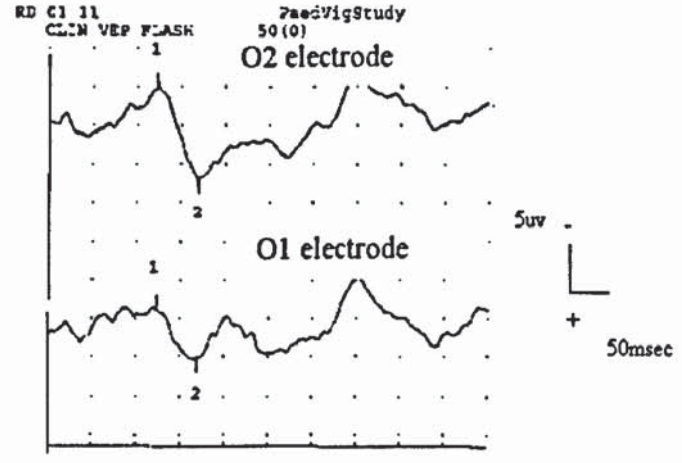
Figure 5.12
Asymmetric H-Stimulus results showing (a) responses recorded from the right eye with a larger amplitude response over the left hemisphere and (b) responses recorded from the left eye of a vigabatrin patient (RD) with a larger amplitude response over the right hemisphere.
msec/ms denotes milliseconds, uv denotes microvolts.

(a) Right eye – larger response over left hemisphere



	Latency1	Latency2	Amplitude
O2	129ms	172ms	4.42uv
O1	129ms	172ms	10.6uv

(b) Left eye – larger response over the right hemisphere



	Latency1	Latency2	Amplitude
O2	123ms	170ms	11.6uv
O1	123ms	170ms	6.14uv

5.3.3 Discussion of the vigabatrin paediatric patients

Compliance was an important factor in this paediatric study. The H-stimulus was found to be a well tolerated technique that 90% of the children participating in the study could comply with as opposed to only 31% that could comply with perimetry. Four patients of varying ages failed to provide reliable H-Stimulus results indicating that developmental age rather than chronological age is an important factor in obtaining reliable results. The test also displays a very useful sensitivity (75%) and specificity (87.5%) for identifying vigabatrin associated visual field loss in children as young as three years. Accepting the sensitivity of the H-stimulus as 75% and identifying the children whose amplitude of response failed the criteria to peripheral stimulation, but completed the H-stimulus test indicated that a typical visual field loss occurred in 25.7% children suggesting that the prevalence of the defect is similar in children to that found in adults.

Results also showed that when stimulating the right eye a smaller response is recorded over the right hemisphere compared to the left hemisphere, but when stimulating the left eye a bigger response is recorded over the right hemisphere rather than the left hemisphere. A smaller response is expected over the right hemisphere when stimulating the right eye in a binasal defect, as the fibres in the temporal retina relate to the nasal visual field which remains on the outside of the pathway and does not decussate, and it is this pathway that is affected by vigabatrin. A healthy response is seen over the left hemisphere as the nasal retina relates to the temporal visual field which is unaffected by vigabatrin and decussates to the left hemisphere.

The H-Stimulus is at present the best method for identifying visual field loss associated with vigabatrin in children with epilepsy below the age of 10 years. Since a number of these children are developmentally compromised, such a technique with high compliance represents a useful technique to be used when evaluating the risk/benefit ratio in children continuing to receive vigabatrin treatment (Spencer et al, 2001, Harding et al, 2002a; 2002b).

5.4 Paediatric patients exposed to vigabatrin in utero

Children born from mothers with epilepsy are often at increased risk of congenital malformations and such a risk is further increased if the mother is treated with AEDs (Koch et al, 1982). Often it is unacceptable to wean the mother off medication as this may result in an increase in seizures. A number of AEDs have been shown to be teratogenetic such a sodium valproate, which has been attributed to cause neural tube defects (Kaneko et al, 1999). It is therefore vital to identify a drug that is safe to be taken during pregnancy.

Investigations to determine the placental transfer and teratogenic effects of vigabatrin have been performed. It has been found to cause a deficiency in certain amino acids and so it has been speculated that the vigabatrin may have a mechanism for teratogenesis effects (Abdulrazzaq et al, 2001). The transfer of vigabatrin from maternal to foetal blood across the placenta is low (Challier et al, 1992) but it remains valuable to examine the H-Stimulus results of children exposed to vigabatrin in-utero to determine if vision is affected in these children.

5.4.1 Method for the vigabatrin in utero patients

Local ethical approval was gained and consent forms were completed throughout this study, either by the patient or the guardian. The method described in section 5.3 of this chapter was used for the children exposed to vigabatrin in utero although no occlusion of the H-Stimulus was used in any patient testing, and no perimetry testing was performed by any of the patients.

5.4.2 Results from vigabatrin in utero patients

Five paediatric patients were included in the in utero study. Three males aged three (SH), six (JH) and eight (LH) were born to the same mother who had taken vigabatrin for 13 years (1988 – present) on a dose of 4000mg a day. One male aged four (AH) and a female aged seven (AK) were born to another mother who had taken vigabatrin for 6 years (1992-1998). Dosage ranged from 2000mg a day from March 1992, reducing to 1500mg a day in February 1992, reducing to 1000mg a day in August 1998 and was

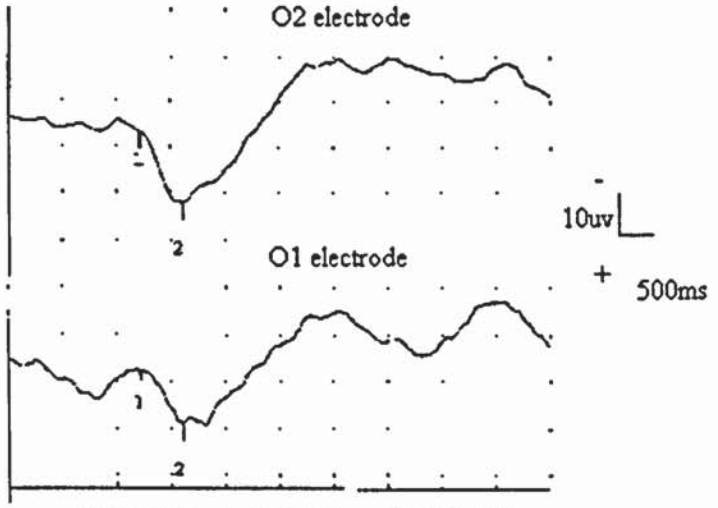
finally discontinued by the end of 1998. All five subjects complied well with the H-Stimulus procedure and clear, reproducible central and peripheral responses (an example is shown in figure 5.13) could be obtained in all cases. In each case the summed amplitude of the peripheral response from both eyes was over 10μv.

5.4.3 Discussion of vigabatrin in utero patients

Extrapolating from the previous paediatric results suggests that a summed peripheral response from O2 and O1 electrodes of over 10 μv in both eyes indicates preservation of peripheral visual fields. All five of the patients exposed to vigabatrin in utero had peripheral responses measuring over 10 μv in amplitude indicating that in all the cases vigabatrin associated visual field loss has not occurred. This study implies that any placental transfer of vigabatrin in patients exposed in utero does not produce vigabatrin retinal toxicity.

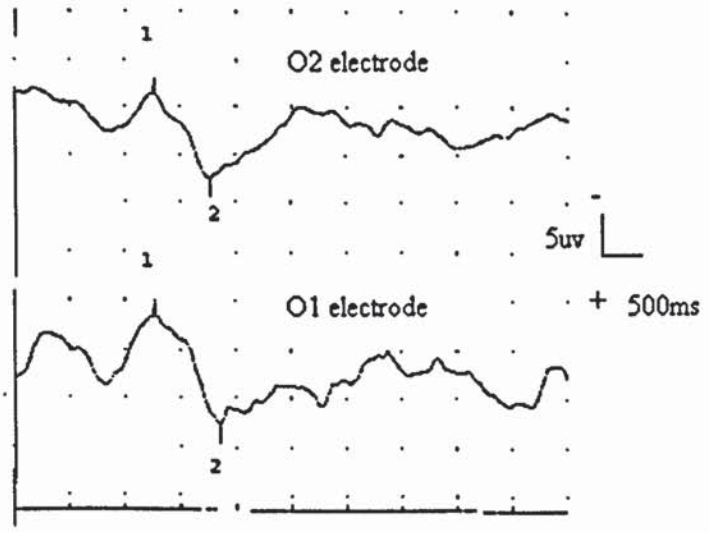
Figure 5.13
H-Stimulus results showing left eye responses from the right hemisphere (O2 electrode) and the left hemisphere (O1 electrode). (a) Shows a normal central response and (b) shows a reduced amplitude peripheral response in a patient exposed to vigabatrin in utero. msec/ms denotes milliseconds, uv denotes microvolts.

(a) A normal central response



	Latency1	Latency2	Amplitude
O2	122ms	161ms	15.3uv
O1	122ms	161ms	11.1uv

(b) A normal peripheral response



	Latency1	Latency2	Amplitude
O2	126ms	177ms	8.84uv
O1	126ms	185ms	11.2uv

5.5 Adults with learning disabilities exposed to vigabatrin

People with learning difficulties have a condition of lifelong intellectual impairment and accompanying disabilities in social functioning. Three grades of mental handicap have been established, that of mild/moderate, severe and profound learning difficulties. People with an intelligence quotient (IQ) of less than 70 are termed people with 'moderate learning difficulties', whilst those with an IQ of less than 50 are referred to as people with 'severe learning difficulties' (Fraser & Green, 1991). Often, as well as dealing with intellectual impairment, these people also suffer from additional problems including epilepsy and so are often subjected to polytherapy of AEDs, including vigabatrin.

People with a learning disability have a significantly higher visual deficiency. Ellis (1986) found that visual problems were ten times higher in this population when compared to the normal population. A variety of reasons have been suggested for the high incidence of visual problems in this group. Damage to vision may be associated with cortical damage associated with arrested brain development, a lack of motivation or ability to use residual vision or undetected visual impairments. As a consequence of this there is a greater risk of sight impairment associated with taking vigabatrin, which may result in having a profound effect on vision. Whereas an adult of normal intelligence may use their residual vision to good effect, an adult with learning disabilities may not be able to adapt in such a way.

The current method to test vision in adults with learning difficulties takes the form of a questionnaire in which questions include how the patient uses their vision as well as examining the appearance of the eyes. Additionally behaviour patterns are noted which may be related to visual problems such as individuals responses to other people, objects and movement. A non-clinical assessment is also carried out in which an eye is patched and objects placed in front of different backgrounds, both in the central and peripheral visual fields. This functional vision assessment for people with learning disabilities has been developed by Marilyn Nash (personal correspondence). The test has been developed using the Royal National Institute for the Blind (RNIB) information and practice development service on multiple disability and drawings taken from Aitken & Buultjens

(1992). However despite the thorough nature of this procedure, by definition this method is rather rudimentary and is unlikely to detect vigabatrin associated visual field loss.

A limited amount of information is available on vigabatrin treatment in patients with learning disabilities. Thalayasingam et al (2001) reported on one such patient who had mild learning disabilities, right facial paresis, right hemiplegia and right hemianopia. Generalised tonic-clonic seizures continued between the ages of 3-20 years and a total daily dose of 3g vigabatrin from the age of 20 was successful in reducing the seizures. After taking vigabatrin for five years a fundus examination revealed damaged retinal pigment epithelium and symptoms of diplopia and blurred vision were reported. The authors acknowledged that it is difficult to assume causality when there is only an association as well as highlighting the fact that no studies at that time had reported visual problems associated with vigabatrin in patients with learning disabilities. It is clear that the benefit:risk ratio for this client group needs to be more carefully examined due to the lack to reporting of visual symptoms and the impossibility of visual field testing.

It would be of obvious interest to examine H-Stimulus responses from the adults with learning disabilities but the close viewing distance of the H-Stimulus results in any person over the age of 25 being unable to accommodate to the stimulus. If a +6.50 dioptres spherical was placed in front of the eye or spectacles, then a person over 25 years of age could focus at 15cm or alternatively it is possible to use a larger stimuli size. If the stimulus size is doubled the viewing distance can be also be doubled in order to maintain the original angles subtended to the eye. The VSG 2/3 F 4MB VRAM was used to create the new stimulus but due to the triggering being altered it became necessary to use the external trigger mentioned previously. A square time locked to either the centre or peripheral stimulus was created in the top right hand corner of the stimulus. A light emitting diode was used on the box as a visual indication of the pattern alternating.

The main objectives of this study were to test the new stimulus size distance in normal adults, collect normal values for the new parameters and test the stimulus on vigabatrin patients with learning difficulties.

5.5.1 Method for normal controls and adults with learning difficulties exposed to vigabatrin

Local ethical approval was gained and consent forms were completed throughout this study, either by the subject, patient or the guardian. The method described in section 5.3 of this chapter was used for the normal subjects and adults exposed to vigabatrin except the subject was positioned 30cm away from the screen to ensure the stimulating field subtended to 5° for the centre circle and 30-60° for the peripheral circle. An external trigger was used to time-lock the stimulus with the averaging. When testing the adults with learning difficulties in order to desensitise the patients to the test procedure a number of steps were carried out. A tape measure and eye patches were shown to the patients to familiarise them with the test. Additionally a video of a subject undergoing the test procedure was shown to the patients in order to show then exactly what was involved in the test.

5.5.2 Results from normal controls and adults with learning disabilities

Seven right eyes and five left eyes were tested from healthy volunteers with good eyesight with a mean age of 27 ± 5.1 years. Using the larger H-Stimulus central and peripheral responses could be recorded at a viewing distance of 30cm. A summary of the results can be seen in table 5.4, which shows the mean central and peripheral latencies and amplitudes of the peripheral responses recorded under the different test conditions.

Based on the paediatric study, the summed amplitude of the peripheral response recorded from the right and the left hemisphere acts as a potential marker for a vigabatrin associated visual field loss. Table 5.5 shows the results of the normal values of the summed amplitudes of both the central response and peripheral response amplitudes. As in the paediatric study, the normal summed amplitude of the peripheral response is over 10μv, which means if an adult peripheral response is below 10μv in amplitude, then a vigabatrin associated visual field defect may be suspected. Figure 5.14 shows an example of a normal H-Stimulus responses taken from an adult over the age of 25 years with normal eyesight.

Nine patients with a mean age of 29 ± 7.4 years and with differing levels of learning disabilities were included in the study details of which can be found in table 5.6. Of these, one patient (NE) would not allow any part of the test procedure to be performed. A further three patients (CB, LC and PS) would allow the electrodes to be attached but were unable to maintain fixation on the central stimulus for long enough periods of time despite the best efforts of support staff. Five of the nine patients were able to provide reliable results. Three of these results (MH, DH and LG) were taken from both left and right eyes, whereas two of the results (RD and GM) were recorded binocularly. Figure 5.15 shows good left eye responses from one patient (DH) and figure 5.16 shows a good binocular response (GM). In one case an electrode was repeatedly pulled off and so results were only recorded from the right occipital lobe. The three patients who provided results from both eyes had mild learning disabilities, whereas those patients that performed the test binocularly had more profound learning disabilities. Results from patient PS in figure 5.17 show how the H-Stimulus responses in this case did not provide any additional information and results were inconclusive.

Table 5.4
Shows the mean amplitude and latency of central and peripheral responses recorded at 30cm from 12 normal eyes recorded from both eyes and hemispheres, with the average noted in italics.
ms denotes milliseconds, uv denotes microvolts.

Stimulus	Mean central latency (ms ± 1SD)	Mean central amplitude (μv ± 1SD)	Mean peripheral latency (ms ± 1SD)	Mean peripheral latency (μv ± 1SD)
Right eye O2	110.29 ± 04.82	7.31 ± 1.92	108.86 ± 08.33	5.45 ± 1.32
Right eye O1	110.14 ± 04.85	6.88 ± 1.93	107.86 ± 06.59	5.87 ± 1.66
Left eye 02	107.90 ± 11.77	7.51 ± 1.24	105.30 ± 10.57	5.65 ± 1.06
Left eye O1	107.90 ± 11.77	5.79 ± 0.88	105.30 ± 10.57	6.55 ± 1.76
Average	*109.25 ±07.86*	*6.91 ±1.66*	*107.08 ±8.42*	*5.86 ±1.43*

Table 5.5
Shows the overall mean amplitudes of the central and peripheral responses recorded at 30cm from 12 normal eyes.
uv denotes microvolts.

Stimulus	Mean central amplitude (μv ± 1SD)	Mean peripheral amplitude (μv ± 1SD)
Both eyes, O2 and O1	13.81 ± 2.73	11.73 ± 2.38

Table 5.6
Shows the summed amplitude of the peripheral responses in right and left eyes of the adult patients with learning disabilities.
uv denotes microvolts.

Subject	Right eye: summed peripheral amplitude of O2 and O1 (μv)	Left eye: summed peripheral amplitude of O2 and O1 (μv)
SH	16.26	19.71
JH	16.06	20.04
LH	12.73	18.37
AH	16.01	20.31
AK	14.72	21.41

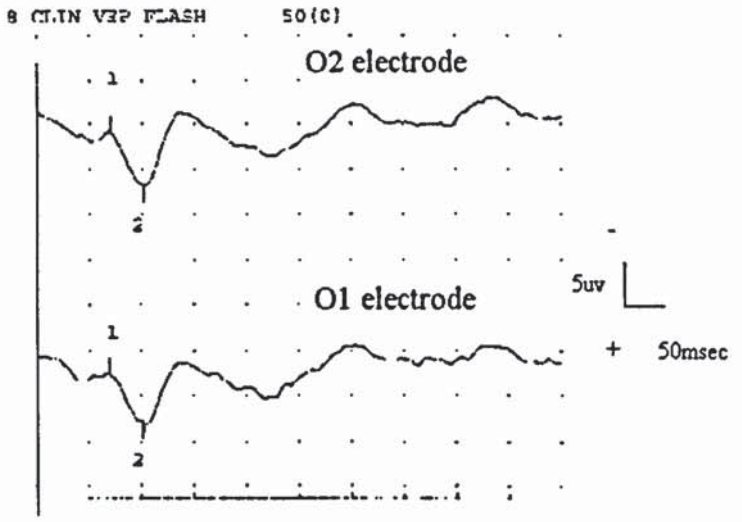
Table 5.7
Details of adult patients with learning disabilities

Patient	Epilepsy & other problems	Vigabatrin treatment history	Other concomitant antiepileptic drugs
MH	Refractory epilepsy Photosensitive Mild learning difficulties	Discontinued 10/2001	SV, LTG, CLB
DH	Refractory epilepsy Mild learning difficulties	Withdrawal ongoing	KEP
LG	Refractory epilepsy Right sided hemiplegia Mild learning difficulties	Taken for 4-5 years Discontinued 11/2001	SV, PHY, TOP
CB	Refractory epilepsy Profound learning difficulties	Taken for 4-5 years 1000mg, reduced to 500mg, discontinued 3/01	LTG
RD	Refractory epilepsy Profound learning difficulties	Discontinued 3 years ago	CBZ, CLB, CLON
NE	Refractory epilepsy Profound learning difficulties	?	?
LC	Refractory epilepsy Severe learning difficulties	Taken for 8-9 years Discontinued 8/01	CBZ, SV
GM	Sturge-Weber Syndrome Nocturnal seizures Profound learning difficulties	Currently on 2000mg, taken for over 3 years	CBZ
PS	Refractory epilepsy Profound learning difficulties	Taken for 8-10 years 1000mg	PHY

Key: SV;Sodium valproate, CBZ;Carbamazepine, LTG;Lamotrigine, GBP;Gabapentin, CLB;Clobazam, ETH;Ethosuximide, PHY;Phenytoin, TOP;Topiramate, PHB;Phenobarbitone, CLON;Clonazepam, KEP; Keppra, ?; unknown

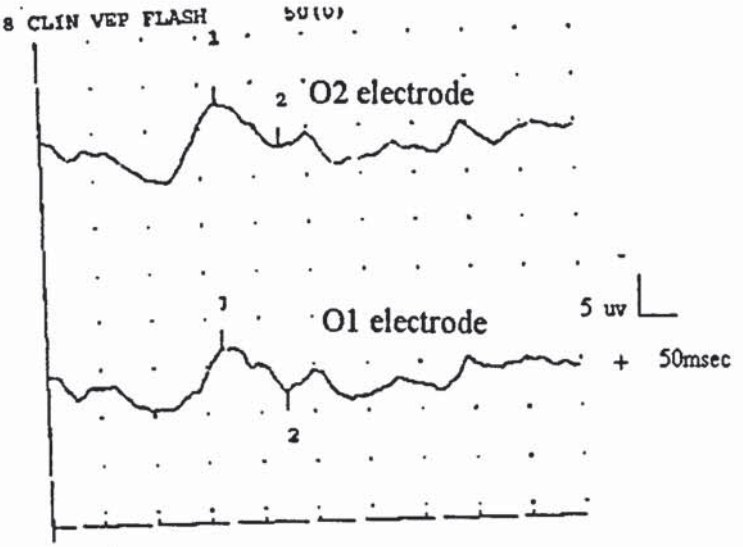
Figure 5.14
Normal data from subject RC showing right eye responses recorded from O2 and O1 electrodes with the stimulus viewed at 30cm showing (a) a central response and (b) a peripheral response.
msec/ms denotes milliseconds, uv denotes microvolts.

(a) Central response



	Latency1	Latency2	Amplitude
O2	69.5ms	102ms	5.81uv
O1	69.5ms	102ms	5.21uv

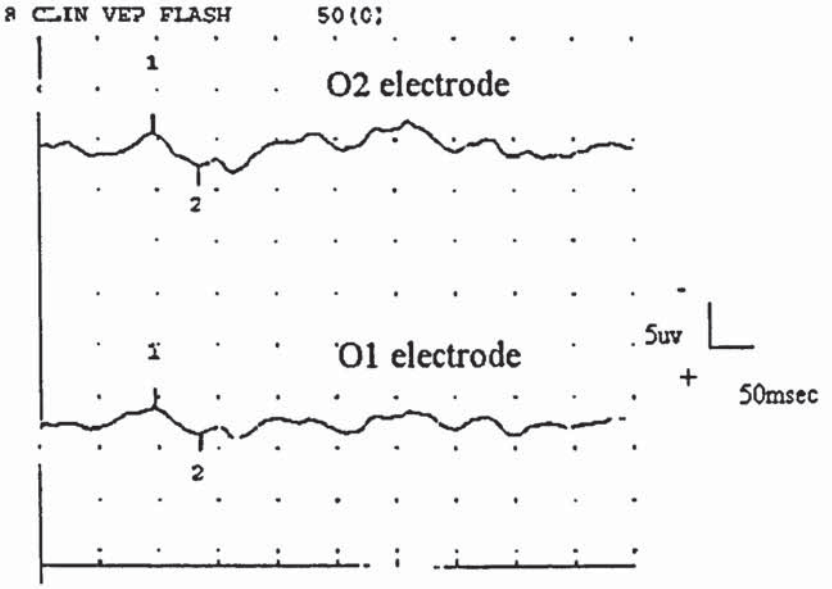
(b) Peripheral response



	Latency1	Latency2	Amplitude
O2	166ms	224ms	5.13uv
O1	165ms	224ms	5.23uv

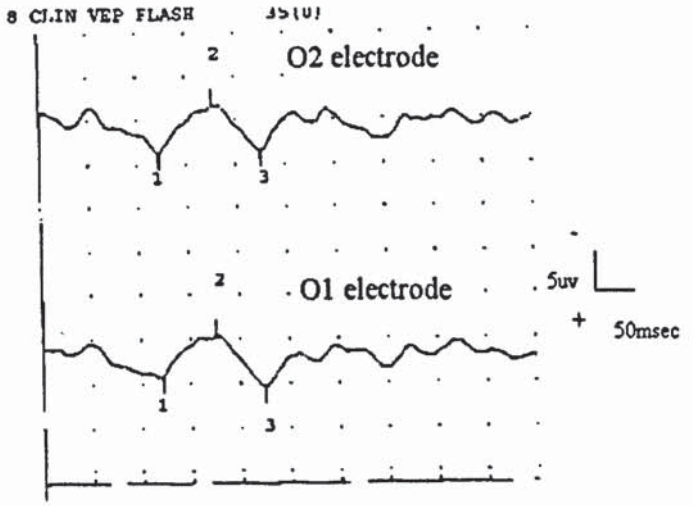
Figure 5.15
Patient DH showing left eye responses recorded from O2 and O1 electrodes with the stimulus viewed at 30cm showing (a) a good central response and (b) a good peripheral response.
msec/ms denotes milliseconds, uv denotes microvolts.

(a) A good central response



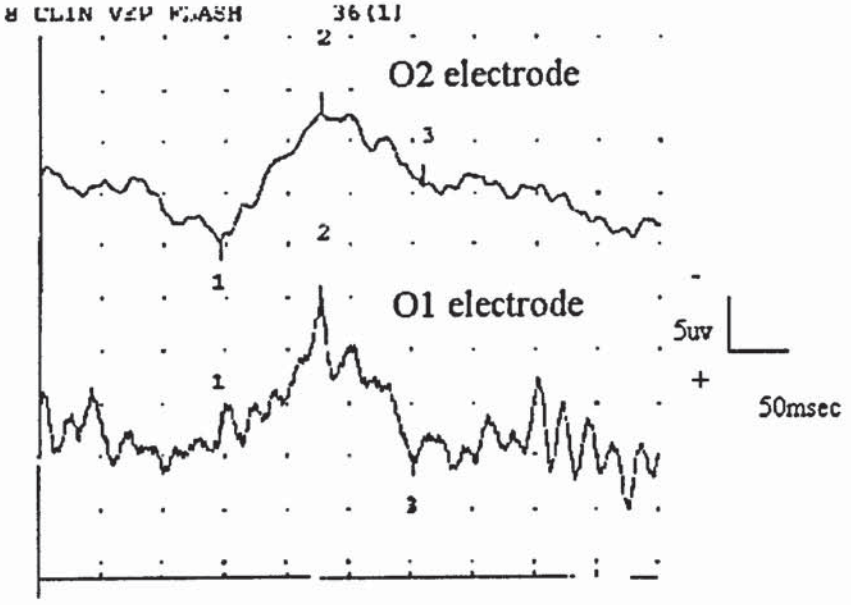
	Latency1	Latency2	Amplitude
O2	96.5ms	135ms	3.18uv
O1	96.5ms	135ms	2.40uv

(b) A good peripheral response



	Latency2	Latency3	Amplitude
O2	176ms	225ms	5.06uv
O1	176ms	225ms	5.62uv

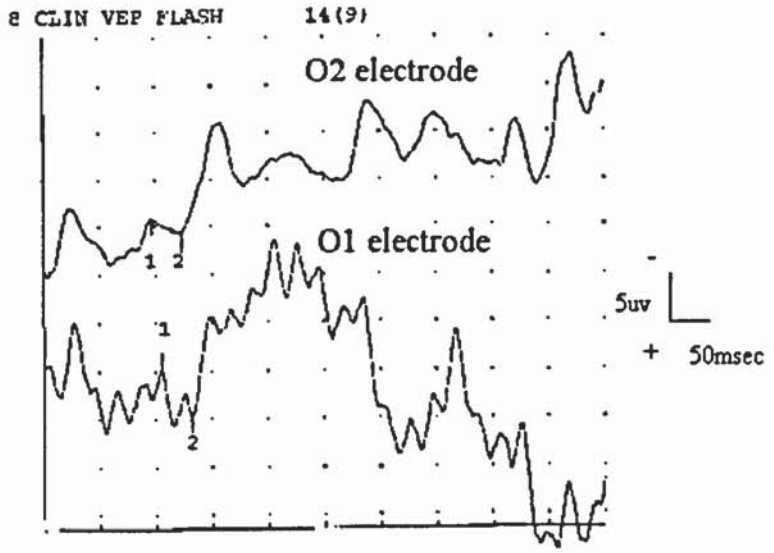
Figure 5.16
Patient GM showing a good peripheral binocular response recorded from O2 and O1 electrodes with the stimulus viewed at 30cm.
msec/ms denotes milliseconds, uv denotes microvolts.



	Latency2	Latency3	Amplitude
O2	227ms	308ms	6.91uv
O1	227ms	302ms	14.0uv

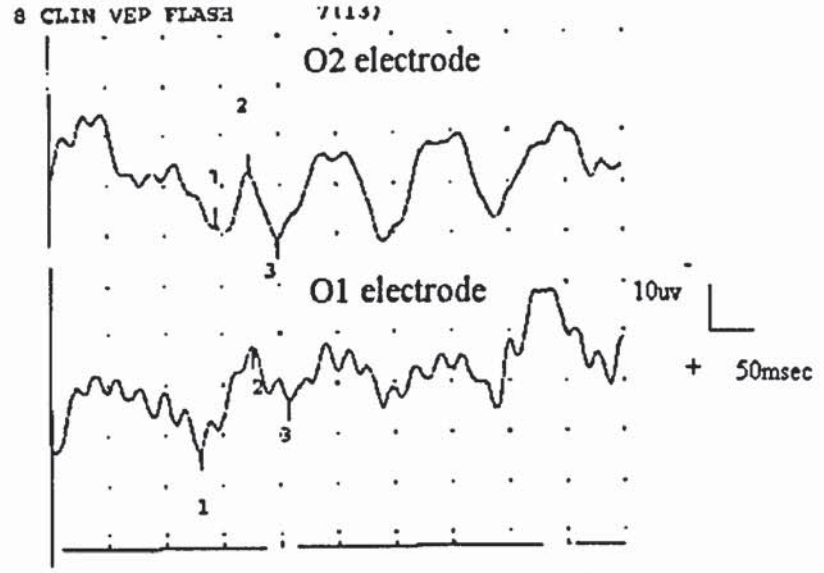
Figure 5.17
Patient PS showing binocular responses recorded from O2 and O1 electrodes with the stimulus viewed at 30cm showing (a) a poor central response and (b) a poor peripheral response.
msec/ms denotes milliseconds, uv denotes microvolts.

(a) A poor central binocular response



	Latency1	Latency2	Amplitude
O2	97ms	123ms	1.52uv
O1	106ms	132ms	4.31uv

(b) A poor peripheral binocular response



	Latency2	Latency3	Amplitude
O2	174ms	198ms	13.1uv
O1	177ms	207ms	10.7uv

5.5.3 Discussion of adults with learning difficulties exposed to vigabatrin

The H-Stimulus has been found to be a useful test in the paediatric population with a developmental age of less than nine that cannot comply with conventional visual field testing. However as adults with severe learning difficulties also cannot comply with visual field testing it is of use to determine if this procedure can be used in this patient group. The usefulness of the H-Stimulus could be expanded if this group of patients exposed to vigabatrin could comply with the H-Stimulus test procedure, and so give an indication of a possible visual field defect.

Central and peripheral responses can be gained from the normal population when the size of the stimulus is increased and the viewing distance is increased. The desensitisation of the test procedure used for the vigabatrin patients prior to the actual test date proved to be very helpful in a number of cases. The patients were confident about what was involved in the test and so complied well. However, one particular case had responded well to the desensitisation procedure but would not comply with any part of the test on the day. Although the desensitisation procedure is a very useful process to aid in the cooperation of the patients, it is clear that such patient behaviour is liable to fluctuate on a daily basis and so it is not always possible to prepare such patients for testing.

Three of the nine patients responded well to the whole test procedure and consequently provided reliable results of which to examine their peripheral vision. In the case of MH peripheral vision was clearly preserved. DH showed good central responses from both eyes but peripheral responses were better from the left eye than from the right. This does not have any significance for vigabatrin since all changes to peripheral vision seen with vigabatrin therapy are bilateral and therefore this would suggest that visual fields have not been affected by vigabatrin. Peripheral responses from patient LG were clearly present bilaterally and although responses were better from the left eye than from the right eye this may well be an order effect if the patient was less co-operative.

Two of the nine patients were unable to tolerate an eye patch and so binocular stimulation was performed. Even though the patient (RD) would not co-operate, responses do appear

to be present binocularly and peripheral responses were clearly recorded, indicating preservation of peripheral vision. In the case of patient GM the peripheral responses seemed to be markedly delayed for the age but they do appear to be consistently present on repeat and therefore this would indicate that some peripheral vision is certainly retained. Unfortunately it is not possible to be more specific in this case.

Four of the nine patients included in this study were unable to provide reliable results. All these patients suffered from severe to profound learning difficulties. In three of the cases electrodes could be attached but the patients could not focus on the stimulus for long enough periods. In these cases the electrodes where attached relatively easily as the patients were often unaware of their surroundings. The other patient was more vocal and aware of his surroundings and simply refused to partake in any part of the test. The H-Stimulus test procedure therefore does not suit all patients with learning difficulties.

Overall, the five patients that provided reliable results did not appear to have vigabatrin associated visual field loss. The remaining four patients did not provide reliable results so it is unclear as to whether any peripheral damage has occurred. Although the H-Stimulus test failed to work on all of these patients reliable results were gained from 66.6% of them and so it remains a worthwhile test to perform if peripheral visual field loss is suspected.

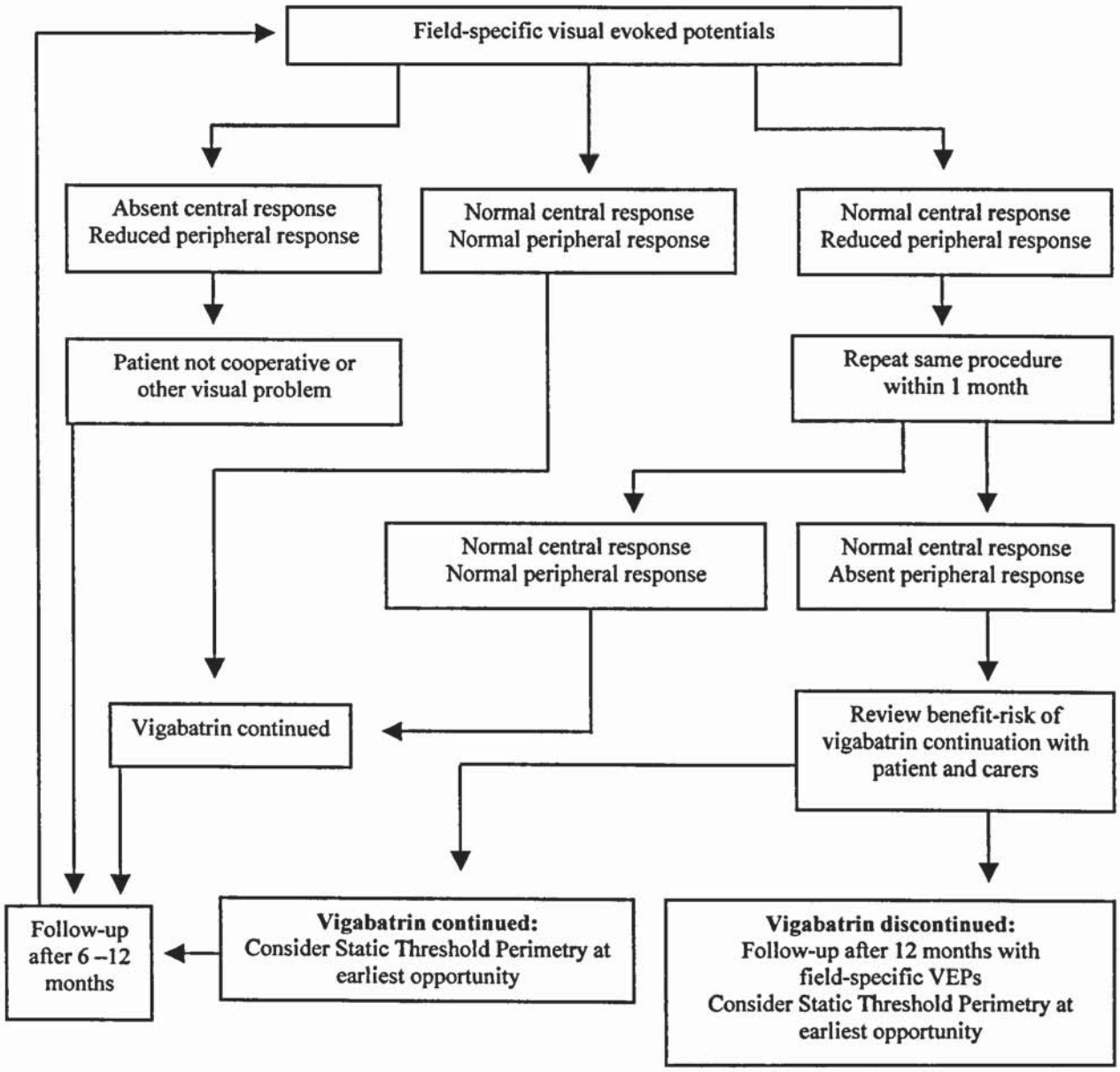
5.6 Conclusion

The field-specific VEP developed in this chapter can be utilised in patients exposed to vigabatrin that cannot provide reliable perimetry results. Figure 5.18 highlights the potential practical implications of using the H-Stimulus in a clinical setting. Using the test on patients, three scenarios are possible. If a normal central and peripheral response were recorded, vigabatrin treatment can be continued although the patient will benefit from a follow-up test in 6-12 months so vision can be monitored for vigabatrin retinal toxicity. If an abnormal central and peripheral response is recorded, the patient either cannot comply with the test procedure shown by the absence of a central response or another visual problem may be the cause of the absent responses. In such cases a follow-

up test is useful at a later date as the patient may be more cooperative and in some cases it may be necessary to test the patient when they are older. If a normal central and a reduced amplitude peripheral response were recorded ideally it would be useful to repeat the procedure as soon as possible to clarify the result. If normal responses were recorded on this occasion, vigabatrin can be continued. However if the peripheral amplitude response remains reduced, the benefit-risk of continuing vigabatrin has to be assessed. If discontinuing vigabatrin is not advisable due to the success of the drug reducing seizures where other drugs have failed it may be advisable to continue with the vigabatrin treatment but ensure a follow-up field specific VEP test is performed. At the earliest opportunity it would be useful for the patient to attempt perimetry testing, as this would provide a more conclusive indication of vigabatrin associated visual field loss. However, it may be desirable to discontinue vigabatrin treatment if another AED is accessible and this would ensure that the vigabatrin associated visual field loss would not progress. A follow-up field specific VEP as well as attempting perimetry is also advisable to monitor the visual field loss circumspectly.

Vigabatrin associated visual field loss is a particular problem in paediatric patients who are unable to have their visual fields tested accurately. Hence, it was necessary for an alternative test to be developed. The H-Stimulus fulfils such requirements in that it is both sensitive and specific to a vigabatrin associated visual field loss. The sensitivity of the H-Stimulus has been established and the test has been used on paediatric vigabatrin patients and children exposed to vigabatrin in utero. Additionally the stimulus size may be altered to allow for the examination of responses in adults with learning difficulties that cannot comply with perimetry. The H-Stimulus is at present the best method for identifying vigabatrin associated visual field loss in children with epilepsy below the age of 10 years, and the prevalence of defects of around 30%, has been found to be similar as that found in adults.

Figure 5.18
Visual field examination screening recommendations for children exposed to vigabatrin.



CHAPTER 6
THE ELECTRORETINOGRAM: EXAMINING RESPONSES FROM NORMAL PARTICIPANTS AND VIGABATRIN PATIENTS

6.1 Introduction

Electrophysiology testing has indicated that vigabatrin causes a disturbance of retinal activity by increasing GABA in the retina, which may result in the characteristic visual field loss with temporal sparing that occurs in one in three patients exposed to the drug. Studies have focused on ERG recordings to investigate the possible mechanism underlying the visual field defect, but results have varied as to which parameter is primarily affected and why. Additionally, only a few studies have focused on the ERG of children, to see if this population is affected in the same way as adults. In this study photopic and flicker ERG responses in both healthy adults as well as adults and children exposed to vigabatrin were examined to determine which parameters are affected most by vigabatrin treatment. The sensitivity and specificity of the ERG results were deduced by comparing results with central fields examined with the Humphrey Visual Field Analyser.

The retina is very rich in neurochemical substances including GABA (Kojima et al, 1958). GABA is used in the lateral pathway by subpopulations of horizontal and amacrine cells, and exerts postsynaptic effects on cones, bipolar cells and ganglion cells. Major GABAergic synapses include feedback and feedforward synapses from horizontal cells to bipolar cells, feedback synapses from amacrine cells to bipolar cells, feedforward synapses from amacrine cells to ganglion cells and amacrine-amacrine cell synapses (Wu, 1992). Nag & Wadhwa (1997) have located GABA in the axonal processes of Müller cells and $GABA_A$ receptors and uptake transporters have been located (Reichenbach et al, 1997).

Investigations into GABA activity in the retina have indicated that $GABA_A$ receptors may mediate the receptive field surround of both on and off bipolar cells as well as the inhibition between amacrine and bipolar cells being GABA mediated (Vardi & Sterling,

1994). Evidence also suggests that GABA may play a role in metabolism during retinal development, as well as in horizontal cell differentiation and maturation (Nag & Wadhwa, 1997). It is clear therefore that GABA plays a vital role in the activity of the retina and so any changes in GABA levels may alter retina function.

GABA-transaminase inhibitors, such as vigabatrin, effectively increase the amount of available GABA and have been shown to increase GABA levels in the retina of animal models. Vigabatrin was found to inactivate GABA-T and so cause an increase in GABA concentrations in the brain and the retina (Neal & Shah, 1990). In the neurones and glial cells of the rabbit retina an increase in GABA occurs following the administration of vigabatrin, but interestingly it also results in a reduction in neuronal pools of glutamate especially in the inner plexiform layer (Pow & Rogers, 1996). It is possible that the glutamate pool is derived from the GABA shunt and so vigabatrin may work by both increasing the inhibitory GABA whilst also decreasing the excitatory glutamate.

The effects of GABA on the ERG have been examined both in the nonmammalian and mammalian retina. In general, the nonmammalian retina, such as the chick retina, shows a reduction in the a and b wave amplitude following the administration of GABA (Bonaventure et al, 1974). This was further supported by the fact that picrotoxin, the GABA antagonist, reversed this depressing effect and in other studies caused an increase in b wave amplitude. Such results support the fact that GABA pathways may be involved at the outer and inner plexiform layer. Oscillatory potentials, resulting from the inhibitory feedback system initiated by amacrine cells, are as expected reduced in amplitude by the administration of GABA (Wachtmeister, 1980). Within the mammalian retina (mainly the rabbit) different results were gained. Starr (1975) showed that GABA caused an increase in the amplitude of the b-wave. Picrotoxin abolished the b wave amplitude, which is directly opposite to its effect seen in the nonmammalian retina. With all such evidence it is perhaps therefore not surprising that a GABA enhancer such as vigabatrin will affect ERG waveforms in humans.

6.2 Normative study

It is recognised internationally that each laboratory performing ERG testing should establish and confirm normal values, as ERG waveforms are prone to variation between equipment, room lighting and patient population. It is necessary to collate normal ERG data prior to testing on patients in order to develop normal range of ERG responses to which to compare with patients, and hence evaluate if the responses fall within the normal range. In this study the collection of normal data was performed on normal adult participants. Due to ethical considerations, the dilation of normal paediatric eyes was not to be performed in this study. Additionally the collection of paediatric ERG data is fraught with difficulties due to movement and poor fixation. The latency and amplitude of the ERG has been shown to reach adult levels by the age of three (Westall et al, 1999), and the ISCEV ERG standards (Marmor & Zrenner, 1995) acknowledge that young children ERG responses approach adult waveform and size and so the ERG data collected in normal adults are deemed comparable to paediatric patient results.

Descriptive statistics are necessary for summarising, and therefore calculating a normal range, of a large amount of data. The ERG data collected in this normal study is interval data, in that we know 3 is more than 2 and 4 is more than 3, but we do not know what zero represents, as a value of zero in an ERG test would indicate that the test was not performed or that the subject was dead. The mean and standard deviation is the accepted calculation for describing such interval data, as long as it is normally distributed, and so this was calculated for each ERG parameter recorded. However in the ISCEV ERG standards (Marmor & Zrenner, 1995) it is recommended that the median and 95% confidence interval limits are used as often ERG data is not normally distributed and the use of the standard deviation may be misleading. Therefore both types of descriptive statistics were to be calculated for each ERG parameter.

In addition to determining the normal range of ERG responses, the photopic a-b wave amplitude ratio was also calculated in the normal group in order to compare presynaptic (receptoral) retinal activity represented by the a-wave, and the postsynaptic activity represented by the b-wave (Ikeda, 1987).

The objectives of this normative study therefore were to develop a large database of normal ERG results. Existing ERG data (Harding et al, 2000a) was to be amalgamated to data newly acquired for the study in order to produce a more accurate normal database. Additionally, the method of analysing the normal data was to be scrutinised in order to determine the optimal method for establishing a normal range of ERG data.

6.2.1 Method for normative study

Local ethical approval was gained and consent forms were completed by the subject before the start of all testing. The ERG was performed according to ISCEV standards (Marmor & Zrenner, 1995). All subjects had pupils maximally dilated with 0.5% tropicamide (Chauvin Pharmaceuticals Ltd). Prior to dilation, all subjects and patients had an IOP within the normal range, as shown by tonometry testing. A full flash Ganzfeld Stimulator GS2000 (Medelec Ltd) was used. Flash duration was less than 5 ms, and background illumination was 22cd/m^2. DTL fibres were placed in the lower fornix of the eyelid of each eye and referred to a silver-silver chloride electrode at the lateral canthi of the respective eye. An electrode attached to the forehead served as a ground. The skin was cleaned before attaching the electrodes and impedances were less than 5 kohms. All subjects were positioned with their chins on a chin-rest with their heads at the opening of the Ganzfeld bowl and allowed at least three minutes for light adaptation. Testing was performed binocularly with a fixation point. Filter settings for the photopic and 30Hz flicker test were set at 1Hz (low frequency filter) and 200Hz (high frequency filter). Recording of the oscillatory potentials required a 100Hz low frequency filter with a 200Hz high frequency filter. A sweep speed of 100ms was used for each test. Eight to sixteen averages were gained for each response, which was repeated at least three times in order to establish repeatability. Latency measurements were taken and all amplitude measurements were taken from the preceding trough to the peak. The largest response recorded was noted.

Statistical analysis was performed using SPSS®. When examining the normal ERG results the mean, standard deviation and the median were used as the measure of central tendency, with the median being calculated by placing the scores into rank order and the

middle score being calculated. The 95% confidence interval was also calculated. A Pearson's correlation coefficient was used to determine if age had an effect on any of the ERG parameters.

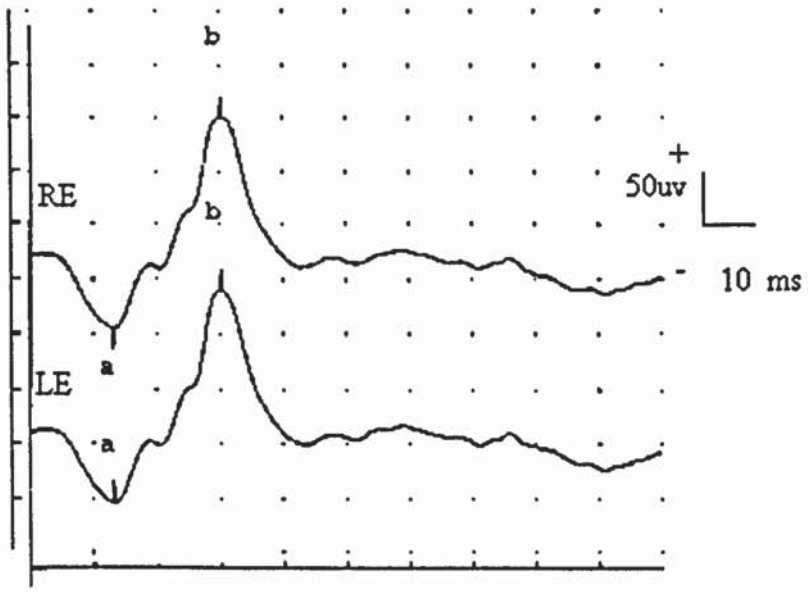
6.2.2 Results from normal participants

Normal ERG data was collected from twelve dilated eyes and amalgamated to existing normal data (Harding et al, 2000a) to construct a normal database from forty eyes with an age range of 20-38 years (mean age 24.8 ± 4.5 years). The best response from each subject was measured for latency and amplitude values. An example of a photopic, oscillatory potential and a 30Hz flicker response are shown in figures 6.1, 6.2 and 6.3 respectively. Each ERG parameter was first examined to determine if the data was normally distributed. In each case the data was normally distributed and so met the assumptions of normality of distribution, therefore the mean and standard deviation was calculated for each parameter (table 6.1). However as it is suggested in the ISCEV standards (Marmor & Zrenner, 1995) and as some of the parameters displayed a slight skew in distribution, the median and 95% confidence intervals were also calculated for each test as seen in table 6.1.

The relative proportion of the a- and b-wave amplitude was also determined in the normal control group. The mean ratio of the photopic a-b wave amplitude ratio was 2.27±0.15.

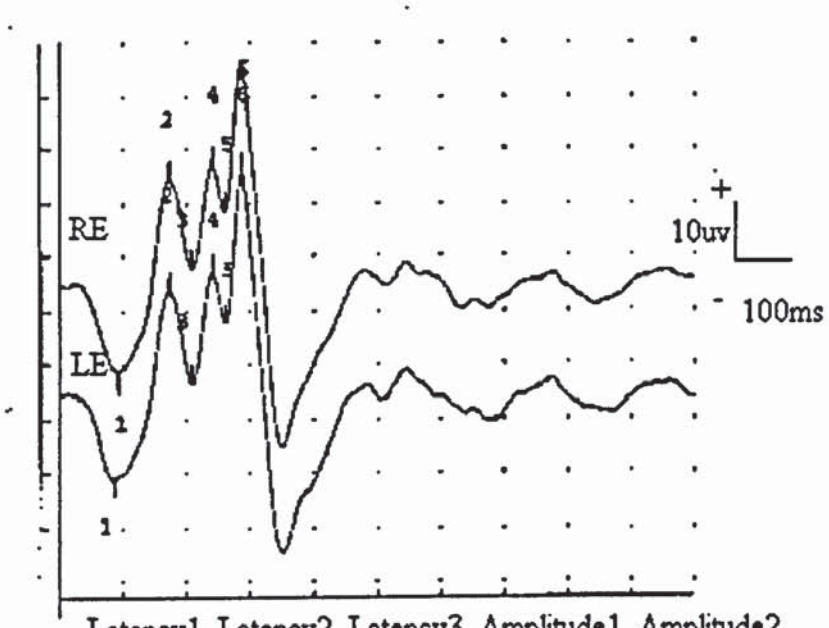
The effects of age were investigated using a Pearson's correlation coefficient. A significant negative correlation was found between age and the photopic b-wave latency [r=0.427, n=40, p=0.006], as age increased the b-wave latency decreased. A significant negative correlation was also found between age and the second oscillatory potential latency [r=0.327, n=40, p=0.039], as age increased, the second oscillatory potential latency decreased. All the other ERG parameters did not significantly correlate with age.

Figure 6.1
The photopic ERG from the right eye (RE) and left eye (LE) showing the negative a-wave and positive b-wave, and the oscillatory potentials can be seen on the ascending limb of the b-wave, recorded from a normal subject
ms; millisecond, uv; microvolts



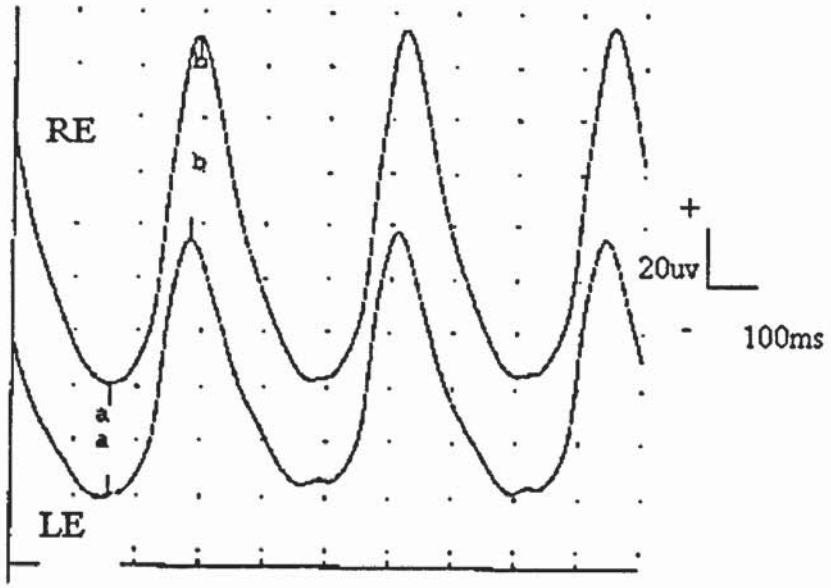
	Latency 1	Latency 2	Amplitude
RE	13.2ms	30.3ms	194uv
LE	13.2ms	30.3ms	192uv

Figure 6.2
The oscillatory potentials from the right eye (RE) the left eye (LE) showing the first, second and third oscillatory potentials, recorded from a normal subject.
ms; millisecond, uv; microvolts.



	Latency1	Latency2	Latency3	Amplitude1	Amplitude2
RE	17.2ms	24.0ms	28.5ms	35.5uv	19.2uv
LE	17.2ms	24.0ms	28.6ms	34.2uv	20.5uv

Figure 6.3
The 30Hz flicker potentials from the right eye (RE) and the left eye (LE) showing the negative a-wave and the positive b-wave, recorded from a normal subject.
ms; millisecond, uv; microvolts.



	Latency1	Latency2	Amplitude
RE	15.7ms	29.2ms	130uv
LE	15.6ms	28.1ms	94.1uv

Table 6.1
Dilated ERG normal values for 40 eyes.
CI; Confidence intervals, ms; milliseconds, μv; microvolts.

Test/measurement	Mean ± SD	Range	Median	95% CI
Photopic Latency a wave (ms)	14.6 ± 0.7	13.9-15.3	14.6	14.4-14.9
Photopic Latency b wave (ms)	33.3 ± 2.04	31.3-35.3	33.3	32.6-33.9
Photopic a-b wave amplitude (μv)	128.4 ± 41.1	87.3-169.5	117.5	115.3-141.5
OP latency 1 (ms)	17.7 ± 0.7	17-18.4	17.7	17.2-18.2
OP latency 2 (ms)	24.0 ± 0.6	23.4-24.6	24.2	23.6-24.5
OP amplitude 1 (μv)	18.2 ± 6.2	12-24.4	17.5	14.3-22.3
OP amplitude 2 (μv)	10.2 ± 4.4	5.8-14.6	11.8	7.4-12.9
30 Hz Latency a wave (ms)	14.1± 1.4	12.7-15.5	14.4	13.6-14.6
30Hz Latency b wave (ms)	27.4 ± 1.5	25.9-28.9	27.6	26.9-27.9
30 Hz a-b wave amplitude (μv)	86.8 ± 29.5	57.3-116.3	87.0	70.4-96.3

6.2.3 Discussion of normative study

The ERG results from the normal participants were collected and analysed in order to provide a normal database with which to compare vigabatrin patient responses.

The maturation of the ERG is a reflection of developing neuronal and glial cells and as the human retina is not completely mature at birth, particularly in the fovea, the ERG at birth differs greatly to that of an adult ERG. However, development is rapid and children reach adult levels of ERG measurements by the age of three (Westall et al, 1999). The effect of increasing age did not have an overall effect on the ERG, apart from the increase in b-wave latency and OP2 latency. Previous studies have found that latencies of ERG responses show no significant age correlation (Weleber, 1981) and it is unlikely that the correlations found in this study are of any significance. Ideally an age-corrected normal range would be of most use in any study, but due to difficulties testing children this was not possible in this present study.

As can be seen in table 6.1, the use of the standard deviation compared to the confidence intervals reveals a large difference in what is deemed normal. For example, the use of the mean and standard deviation in calculating the photopic amplitude normal lower limit reveals amplitude of 87.3 μv. However using the 95% confidence interval lower limit, the photopic amplitude lower limit would be set at 117.5 μv. This is obviously quite a large difference in what is deemed normal and at this stage it is unclear as to which parameter is more useful to use in vigabatrin patients.

As the majority of the ERG parameters were normally distributed it was decided to utilise the mean and standard deviation of the normal database, as also used in Harding et al (2000a), to compare with those of vigabatrin patients in the next section. However, the use of the median and 95% confidence interval would be utilised if the ERG parameter were found not to be sensitive or specific to visual field defects.

6.3 Introduction to vigabatrin study

Many studies have focused on finding an association between ERG measurements and vigabatrin attributed visual field loss in patients with epilepsy. The photopic a wave latency and the photopic a-b wave amplitude appear to be associated with the severity of visual field loss (Harding et al 1995b, Krauss et al 1998). However, the photopic b-wave latency does show a marked increase when vigabatrin is currently being taken suggesting that this is primarily affected by current blocking of GABA-T in the retina (Harding et al, 1999a). This indicates that vigabatrin may affect GABA receptors, a process which is altogether different from that which causes visual field loss. A reduction in oscillatory potentials has been noted in patients both on and off vigabatrin. In patients with a visual field defect, it appears that the latency, rather than the amplitude, of the first oscillatory potential correlates with the defect (Krauss et al 1998, Arndt et al 1999b) suggesting that amacrine cells are sensitive to the increase of GABA in the retina. ERG findings to 30 Hz flicker has revealed abnormalities of cone function to be present with vigabatrin associated visual field loss.

Due to the difficulties involved with testing the electrical activity in the retina in children, few paediatric studies investigating the effects of vigabatrin on the ERG have been performed. Westall et al (2000) performed a longitudinal pre- and on-treatment ERG examination in children with epilepsy exposed to vigabatrin. In a six-month follow-up period a significant reduction of the oscillatory potentials was found in children who were not taking vigabatrin at the initial examination. However, those children who were taking vigabatrin at the initial assessment showed no significant difference in their ERG recordings.

Further studies have indicated that the effects of vigabatrin on the retina may alter over the course of the treatment. A long term follow-up study showed a short term treatment of vigabatrin affected b-wave and flicker latencies, whereas long term treatment resulted in increases in rod- and cone-mediated a-wave latency with a decrease in the flicker amplitude being observed (Brigell et al, 2000). ERG waveforms also appear to be affected differently depending on whether the patient is currently taking vigabatrin. Rod

function appears to be spared with vigabatrin treatment although a delay to the scotopic a and b wave latency has been shown with current vigabatrin treatment (Harding et al 1995b, Harding et al 1999a).

Interestingly, ERG abnormalities may be seen in the absence of any visual field loss calling into question the significance of ERG findings. Harding et al (2000a) found an increased latency of the second oscillatory potential in vigabatrin patients compared to those patients not exposed to vigabatrin yet this defect occurred in the absence of any visual field defect. Additionally healthy volunteers exposed to vigabatrin have shown a significantly altered photopic b-wave in the absence of any visual field defect (Harding et al, 1999). This indicates that the ERG parameters may actually not be a useful harbinger of visual field defects but instead may reflect current use of the drug.

Furthermore abnormalities in ERG waveforms have been noted in patients with epilepsy that have never been exposed- to vigabatrin, and who were only serving as a control group (Schmitz et al, 1999). Harding et al (1999a) compared patients with epilepsy who had been exposed to vigabatrin with those who had never taken vigabatrin and were receiving carbamazepine and found the latter to have a reduced photopic a-b amplitude. Indeed, Wild et al (1999) found a high percentage of visual field defects in those patients taking a combination of vigabatrin and carbamezpine when compared to other patients on different drug regimes. The effects of sodium valproate on visual fields has not been extensively studied although recent findings suggest that the combination of vigabatrin and sodium valproate treatment maybe particularly toxic (Ardnt et al, 1999b). It should be noted that a study involving patients on sodium valproate monotherapy did not show any visual field loss (Schmitz et al, 1999) although Wohlrab et al (1999) found one patient taking sodium valproate and lamotrigine to have visual field defects. Such a finding implies that vigabatrin treatment, whilst causing visual field defects, may result in visual field loss and ERG abnormalities by a different mechanism of action that may involve other AEDs.

The aim of this study was to establish if any one ERG parameter is more closely associated with vigabatrin-attributed visual field loss. Such a parameter may then be used as a marker in detecting retinal dysfunction associated with vigabatrin. However, it is important to determine if any such factor differs in adult and children ERG results and therefore both these populations were examined. Based on previous ERG studies in vigabatrin patients, the use of scotopic stimulation was deemed both unnecessary, as this factor is only affected by current vigabatrin treatment (Harding et al, 2000a), and difficult to perform on children, due to a 20 minute dark adaptation being required. Additionally, Westall et al (2002) found the scotopic response to be abnormal in children with epilepsy prior to vigabatrin treatment indicating that this abnormality may be caused by a mechaism related to the neurological impairment. Therefore the tests included in this study included photopic and 30Hz flicker stimulation as well as examining oscillatory potentials. Children and adults with epilepsy who had been exposed to vigabatrin for at least three months were included in the study. A detailed AED history was to be noted for each patient to determine if a trend occurred between any particular drug regimes. Perimetry results from a number of children and the adult group where compared with ERG results in those patients showing both a visual field defect and those showing no defect, and so the sensitivity and specificity of the important ERG parameters were calculated.

6.3.1 Method for vigabatrin study

Local ethical approval was gained and consent forms were completed, either by the subject or a guardian before the start of all testing. The method described in section 6.2.1 was used when testing all the patients. Results where compared to each of the normal ERG test results. A detailed vigabatrin treatment history was recorded in all cases including length of treatment, whether the patient was still taking the drug as well as noting the highest dose received of vigabatrin. Concomitant or previous AEDs received were also noted. However, in some circumstances notes were not available and so such details could not be ascertained (Tables 6.2 & 6.3). All patients were Caucasian apart from paediatric patient 6.

Table 6.2
Paediatric patient details and vigabatrin treatment history.

Patient	Gender	Age (years)	Maximum VIG dose (g)	Duration of VIG treatment (months)	Other AEDs (current or past)
1	Male	8	1.5	49	CBZ, SV, CLB
2	Male	10	1	72	CBZ, SV
3	Male	11	1	35	CBZ
4	Male	10	4	53*	CBZ, SV, LTG, GBP
5	Female	4	1	43	CBZ, SV, LTG,
6	Male	11	1	34*	CBZ, SV, LTG
7	Female	10	2.5	8	CBZ, SV, LTG
8	Female	11	3	108*	PHY, ETH, SV, PHB
9	Female	15	4	16	CBZ, SV, LTG, GBP
10	Male	13	2	73*	CBZ, SV, LTG, PHB, GBP
11	Male	13	1	5	CBZ, SV, CLB, PHB
12	Female	13	2	50	CBZ, SV
13	Male	8	1.5	66	CBZ, SV, LTG, GBP PHY, CLB, TOP
14	Male	14	3	60	CBZ, SV, LTG, TOP
15	Female	11	3	61	SV, LTG, CLON
16	Male	10	1	74*	CBZ, SV
17	Female	12	2	39	CBZ, LTG, PRED
18	Female	10	1	24	CBZ, PHY, GBP, TOP
19	Female	12	1.5	35*	CBZ, LTG
20	Male	8	1	29	CBZ
21	Male	14	3	108	CBZ, PHY, CLB
22	Male	11	1.5	4	CBZ, SV, LTG, ETH, GBP
23	Male	11	1	26	CBZ, SV, LTG
24	Male	7	0.5	40	CBZ, SV
25	Male	15	3	9	CBZ, SV
26	Male	7	2	19	CBZ, SV

Key: SV;Sodium valproate, CBZ;Carbamazepine, LTG;Lamotrigine, GBP;Gabapentin, CLB;Clobazam, ETH;Ethosuximide, PHY;Phenytoin, TOP;Topiramate, PHB;Phenobarbitone, CLON;Clonazepam, * ongoing vigabatrin treatment

Table 6.3
Adult patient details and vigabatrin treatment history.

Patient	Gender	Age (years)	Maximum VIG dose (g)	Duration of VIG treatment (months)	Other AEDs (current or past)
1	Female	17	1.75	18*	CBZ
2	Female	19	2	72	LTG, GBP
3	Female	17	1	24	CBZ
4	Male	18	1.5	36	CBZ
5	Female	18	1	?	CBZ
6	Male	26	2	30	LTG, CBZ
7	Female	23	2.5	?	CLOB, TOP
8	Female	31	2	32*	CBZ
9	Female	29	2	37	CBZ, GBP
10	Male	45	2	24	CBZ, PHY
11	Female	51	2.5	23	LTG, PRD
12	Female	50	?	24	LTG, GBP
13	Male	45	2	120*	CBZ, LTG
14	Female	47	1	72*	CBZ
15	Male	18	1	96	CBZ
16	Male	49	2	72	PHY
17	Male	50	?	12	CBZ
18	Female	44	1	120	CBZ, SV
19	Female	43	2	120*	CBZ
20	Male	24	1.5	108*	CBZ
21	Female	28	3	156*	GBP
22	Male	46	4	96*	CBZ
23	Male	19	2	144*	LTG

Key: SV;Sodium valproate, CBZ;Carbamazepine, LTG;Lamotrigine, GBP;Gabapentin, CLB;Clobazam, ETH;Ethosuximide, PHY;Phenytoin, TOP;Topiramate, PHB;Phenobarbitone, CLON;Clonazepam, PRD;Prednisolone, ?; unknown dose,
* ongoing vigabatrin treatment

The visual field examination was undertaken with the Humphrey field Analyzer (HFA) 750 using the 135 point field and the age-corrected three-zone method. All reliability indices were automatically assessed. If results were normal or non-compliance occurred then no further test was carried out. If results were abnormal or contained a potential artefact then the Full Threshold strategy was used with program 30-2. The results of this latter test were then categorised as normal, abnormal or unreliable. If the child was unable to comply with static perimetry then Goldmann kinetic perimetry was performed using the I3E and I4E isopters. JW or MC carried out all visual field examinations.

Statistical analysis was performed using SPSS®. A multivariate between groups ANOVA was used to determine if a statistical difference occurred in ERG responses from patients with normal and abnormal visual fields determined by perimetry. For those results in which homogeneity of variance was not met, a non-parametric Kruskal-Wallis test was also performed. A Pearson's correlation coefficient was used to determine if a relationship existed between the length of vigabatrin treatment with the outcome of the flicker test and the perimetry result. A p value of less than 0.01 and 0.05 was accepted as statistically significant.

6.3.2 Results of paediatric and adult vigabatrin study

ERG results were examined from 26 paediatric patients exposed to vigabatrin with an age range of 4-15 years (mean age 10.7 ± 2.6 years). Twelve of the twenty-six paediatric patients could give reliable perimetry results of which eight were normal and four were abnormal. An additional two children gave unreliable perimetry results, and a further twelve children were unable to attempt perimetry testing.

ERG results were examined from 23 adult patients exposed to vigabatrin with an age range of 17-51 years (mean age 32.9 ± 13.3 years). Fifteen of the twenty-three adult patients could give reliable perimetry results of which six were normal and nine were abnormal, although one patient showed abnormal perimetry result due to a right homonymous hemianopia and so was not vigabatrin-related. Six adult patients gave

unreliable perimetry results, and a further two adults were unable to attempt perimetry testing.

The mean and standard deviation from the normal data collected (table 6.1) was used to determine if the ERG parameters fell within the normal range. Of the paediatric patients, all had normal photopic responses within the normal range while two of the adult patients (9%) showed a delayed response. Two (8%) of the paediatric patients showed reduced amplitude or delayed oscillatory potential response whereas nine (39%) of the adult patients showed an abnormality in this response. The 30Hz flicker response was deemed abnormal in 6 (23%) paediatric patients and in 12 (52%) of the adult patients.

When using the normal database and comparing the ERG parameters to perimetry results, the 30Hz flicker amplitude response appeared to be the most affected in the vigabatrin patients. Based on the lower mean value of the normal result generated on healthy adults, the cut-off point for the 30Hz flicker amplitude response was 57 μv, below which the response was rated abnormal. An example of an abnormal 30Hz flicker response is shown in figure 6.4. Using this criterion in the paediatric patients, one abnormal ERGs were identified from four abnormal perimetry results giving a sensitivity of 25%. Six out of seven normal perimetry results also showed a normal 30Hz flicker giving a specificity of 85.7%. However if the cut-off point was increased to 70 μv (based on the lower 95% confidence interval) so that anything below this figure was deemed abnormal, then three out of the four abnormal perimetry results showed an abnormal ERG resulting in a sensitivity of 75%, but the specificity reduced to 62.5% as five from eight had a normal 30Hz flicker response. Similarly in the adults a cut-off point of 56 μv identified five abnormal 30Hz flicker results from the nine abnormal perimetry results giving a sensitivity of 55%, and four out of the six normal perimetry responses were identified giving a specificity of 67%. When the 30Hz flicker criteria were changed to below 70 μv in amplitude the specificity remained the same but the sensitivity was increased to 100%. If adult and paediatric results are pooled together using this criteria, a sensitivity of 92% (11/12) and a specificity of 77% (10/13) is found.

In this way the cut-off points of the ERG can be adjusted to improve the sensitivity and specificity of the test. Other ERG variables in which the criteria was altered was the photopic a wave latency, the 30Hz a wave latency and the first oscillatory potential latency. Such ERG parameters failed to show a useful sensitivity or specificity, and if they are adjusted in a similar way to the 30 Hz amplitude cut-off point, then the specificity of the test would be greatly reduced, as the abnormal results would fall within the normal criteria.

Those vigabatrin patients that had a confirmed vigabatrin associated visual field loss determined by perimetry and those patients with confirmed normal visual fields were grouped separately. The means of the two groups were noted (table 6.4). A multivariate between subjects ANOVA was used to determine if a statistical difference occurred in ERG responses from patients with normal and abnormal visual fields. No significant differences were found between any of the ERG parameters and normal and abnormal visual fields. The non parametric Kruskal-Wallis test was not significant for all ERG parameters so confirming the parametric test that results were the same in the two groups of normal and abnormal visual field results. Table 6.5 shows a summary of test results for the ERG and perimetry performed in each patient.

The a- and b-wave amplitude was also determined in the vigabatrin group. The mean ratio of the a-b wave amplitude was 2.27±0.087 (range 2.10-2.44) and so remained within the normal range.

The paediatric patients had been given a mean maximum daily dose of vigabatrin of 1.88g (range 0.5-4g) for a mean duration of 44 months (range 4-108 months). The adult patients had been given a mean maximum daily dose of vigabatrin of 1.89g (range 1-4g) for a mean duration of 75 months (range 12-156 months). The duration of vigabatrin treatment was investigated using a Pearson's correlation. A positive correlation was found between vigabatrin treatment length and the 30Hz flicker result [$r=0.314$, $n=47$, $p=0.032$] indicating that an increase in vigabatrin treatment was related to an abnormal 30Hz flicker response. A positive correlation was also found between vigabatrin

treatment length and perimetry results [r=0.452, n=25, p=0.023] indicating that an increase in vigabatrin treatment was related to an abnormal perimetry result.

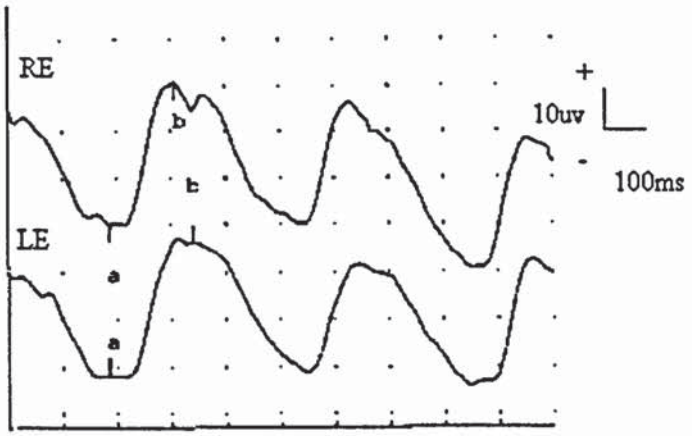
A number of the patients that were included in this study were still receiving vigabatrin at the time of testing (table 6.1 and 6.2). Six paediatric patients still received vigabatrin at the time of testing and all had a normal photopic response. Three of the paediatric patients (50%) had an abnormal 30Hz flicker response, whilst one also showed reduced oscillatory potential responses. Nine adult patients were still receiving vigabatrin at the time of testing and all had a normal photopic response. Seven of the adult patients (78%) had an abnormal 30Hz flicker response whilst four (44%) also showed delayed oscillatory potential responses. Two of the patients had normal ERG responses as well as normal visual fields and in these cases both patients had been taking vigabatrin for two and six years.

Current or past AED medication was examined in all patients and then compared to see if a trend occurred between which patients had taken certain drugs and who had visual field defects. As expected the predominance of patients had taken a plethora of AEDs but the main drug used in the majority of patients was carbamazepine, as well as sodium valproate being widely used amongst the paediatric patients. In the paediatric patients, all had been exposed to carbamazepine except for two patients. Of these two patients, neither showed a visual field defect or any change in retinal activity. Seven of the adult patients had been exposed to carbamazepine, and of these three had no retinal abnormalities. All the paediatric patients had been exposed to sodium valproate except for six patients of which four had normal visual fields and two had abnormal results. None of the adult patients had taken sodium valproate, except for one patient who had an abnormal ERG result.

Table 6.4
Shows the mean ± 1 SD of each ERG parameter in the vigabatrin patients with normal or abnormal visual fields confirmed by perimetry.
ms; milliseconds, μv; microvolts.

ERG parameter	Normal visual field	Abnormal visual field
Photopic Latency a wave (ms)	14.83±0.88	15.03±0.18
Photopic Latency b wave (ms)	33.82±1.86	34.42±2.51
Photopic a-b wave amplitude (μv)	107.97±30.1	105.93±35.3
OP latency 1 (ms)	18.48±0.88	18.98±1.28
OP latency 2 (ms)	25.34±1.79	25.63±2.00
OP amplitude 1 (μv)	15.24±10.5	16.28±7.42
OP amplitude 2 (μv)	9.81±7.39	7.20±4.66
30 Hz Latency a wave (ms)	14.17±2.81	14.89±2.36
30Hz Latency b wave (ms)	28.45±1.66	28.66±1.86
30 Hz a-b wave amplitude (μv)	77.58±38.9	66.75±31.01

Figure 6.4
ERG results showing right eye (RE) and left eye (LE) responses repeated. Shows a reduced amplitude 30Hz flicker response in a patient with abnormal visual fields shown by perimetry.
msec/ms denotes milliseconds, uv denotes microvolts.



	Latency1	Latency2	Amplitude
RE	18.6ms	30.7ms	30.5uv
LE	18.6ms	33.9ms	27.6uv

Table 6.5
Results of ERG tests compared to perimetry results.

Paediatric Patient	ERG Result	Perimetry Result
1	Normal	-
2	Abnormal	-
3	Normal	Abnormal
4	Abnormal	Normal
5	Normal	-
6	Abnormal	-
7	Normal	Unreliable
8	Normal	Normal
9	Normal	Normal
10	Abnormal	-
11	Normal	-
12	Abnormal	Abnormal
13	Abnormal	-
14	Normal	Abnormal
15	Normal	-
16	Normal	-
17	Normal	Normal
18	Normal	-
19	Normal	Normal
20	Normal	Normal
21	Abnormal	Abnormal
22	Abnormal	Normal
23	Normal	Unreliable
24	Normal	-
25	Normal	Normal
26	Normal	-

Adult Patient	ERG Result	Perimetry Result
1	Normal	Normal
2	Normal	Unreliable
3	Abnormal	Normal
4	Abnormal	Abnormal
5	Abnormal	Abnormal RHH
6	Abnormal	Abnormal
7	Abnormal	Abnormal
8	Abnormal	Abnormal
9	Abnormal	Abnormal
10	Normal	Normal
11	Abnormal	Unreliable
12	Normal	Normal
13	Abnormal	Abnormal
14	Normal	Normal
15	Abnormal	-
16	Normal	Unreliable
17	Abnormal	Normal
18	Abnormal	Unreliable
19	Abnormal	Abnormal
20	Abnormal	Unreliable
21	Abnormal	Unreliable
22	Abnormal	Abnormal
23	Abnormal	-

Key: - test not performed, RHH; right homonymous hemianopia

6.3.3 Discussion of vigabatrin study

When grouped separately, the patient group with a vigabatrin associated visual field loss had no significant difference when compared to those patients without a defect. When compared to the normal database, two adult patients had an abnormal photopic response, only one of which had a confirmed visual field defect. This therefore indicates that this parameter would not be a useful predictor of field loss in either adults or paediatric patients. However a number of studies have shown the b-wave latency and the a-b amplitude to be affected by current vigabatrin treatment (Harding et al, 1995b) although in this present study this parameter was found not to be a significant factor in predicting vigabatrin associated visual field loss as the majority of patients were no longer taking the drug.

In normal subjects the b wave amplitude should be two to three times larger than the a-wave, and indeed the ratio gained from the normal data set agreed with this fact. Toxic substances or certain drugs may affect synaptic transmission or neuronal modulation that may reduce the a-b wave amplitude ratio as the b-wave is a postsynaptic wave of the ERG reflecting both outer and inner plexiform layer transmission. However, this study showed that all the vigabatrin patients had an a-b wave amplitude ratio within the normal range indicating that the drug may not affect the pre- and postsynaptic retinal activity.

When the normal and abnormal visual field results are grouped separately no significant difference between the two groups of oscillatory potentials results was found indicating that this factor may not be useful in predicting a visual field defect. When compared to the normal database three of the paediatric patients had an abnormal oscillatory potential response and all of these patients could not comply with perimetry. Nine of the adult patients had an abnormal oscillatory potential response, four of which had an abnormal visual field although a further three could not perform perimetry. Evidently, this particular parameter is more affected in the adult population implicating that the cumulative dose of vigabatrin, adults taking vigabatrin for on average a longer period of time, has a significant affect.

In a recent study by Comaish et al (2002) a statistically significant correlation was found between vigabatrin associated visual field loss and a reduction in amplitude of oscillatory potentials. It has been suggested by Harding et al (2000) that the oscillatory potentials are affected by vigabatrin as the drug interferes with amacrine cell function, which is concerned with lateral inhibition in the retina. Such a change to inhibition and the different population of ganglion cells reducing in density from the centre to the periphery may account for the predominantly peripheral field defect seen in patients. More recently Westall et al (2002) have shown that the early oscillatory potentials are affected by vigabatrin treatment. As these early responses are elicited by the onset of a light stimulus, this result implies that the ON rather than the OFF mechanisms are affected by vigabatrin.

The 30Hz flicker appeared to be the ERG parameter affected most by vigabatrin treatment. Although no significant difference was found in the 30Hz flicker response between the vigabatrin patients with a visual field loss and those patients without a field loss, using the 30Hz flicker criteria of below 70 μv in amplitude, adult and paediatric results gave a sensitivity of 92% (11/12) and a specificity of 77% (10/13) when compared to perimetry results. The reduction of the flicker response indicates that vigabatrin has a toxic effect on the cone activity within the retina. A number of studies have supported this finding such as Harding et al (2000) and Miller et al (1999). The disruption of cone function appears to be associated with vigabatrin associated visual field loss but as yet it is unclear as to whether this has a causal relationship with the visual field loss. The majority of studies publish vigabatrin patient data in case report form making a proof of causation difficult. However both the 30Hz flicker response and field loss could be clinical signs of an underlying condition (Harding et al, 2000a; Hardus et al, 2000a; Kälviäinen et al, 1999). Only prospective studies can show if the change in one variable precedes the other.

A number of patients still received vigabatrin at the time of testing and in such cases it is useful to determine if vigabatrin treatment should be continued. In two cases the patients had normal ERG results and it is unlikely that either patient would progress to develop a

visual field defect or altered ERG parameters as vigabatrin visual field loss is not seen in two out of three patients that are exposed to the drug and generally if a defect was to occur it would do so within the first two years of taking the drug. However, it would be of use to monitor such patients ERG parameters to determine if any retinal changes occur with continued use of the drug. An abnormal photopic response does not appear to be associated with current vigabatrin treatment, which does not agree with previous data in which the photopic b-wave latency showed an increase with current vigabatrin use (Harding et al, 1999a). In this study a reduced 30Hz flicker and delayed oscillatory potential responses were found to be associated with current vigabatrin treatment. Harding et al (2000b) have reported similar findings in which vigabatrin patients still taking vigabatrin had a prolonged second oscillatory potential in the presence of a visual field defect. In this study patients that could provide reliable perimetry results and were still taking vigabatrin, showed an ERG abnormality in the presence of a vigabatrin associated visual field defect. Such results indicate that delayed oscillatory potentials and the reduced 30Hz flicker amplitude response may be more closely related to current vigabatrin treatment use rather than being related directly to the visual field defect.

The lack of correlation between visual field loss and the ERG parameters may reflect the differing mechanisms that vigabatrin acts by to cause such abnormalities. The cessation of vigabatrin treatment has been shown to result in the recovery of the EOG and ERG b-wave although the visual field constriction remains unchanged. (Graniewski-Wijnands & Van Der Torren, 2002). Such results may indicate that the two types of defect, recorded in electrophysiology and perimetry testing, occur via different mechanisms. It is possible that the visual field defect occurs as a result of an irreversible intoxicating effect on the retina, whereas the defects in the electrophysiology of the eye reflects a more direct effect on retinal glial cells level. The apparently more permanent defect on the ERG cone b-wave may share the same origin as the visual field loss, that of a permanent damage of the Müller glial cells in the peripheral retina.

This study showed that the longer period of time a patient had been exposed to vigabatrin was well correlated to an abnormal 30Hz flicker response and an abnormal perimetry

response. Wild et al (1999) examined the relationship between vigabatrin associated visual field loss and the length of treatment and found there was no significant difference in occurrence of visual field defects in those treated for greater than or less than four years. A study by Hardus et al (2000b) showed a significantly increased severity of visual field loss in patients treated for over two years compared to those treated for less than two years, although a difference was not found between those patients treated for two to four years and four to six years. Evidently the visual field loss must occur at a certain period of time and is probably a gradual process, although no significant progression of the defect occurs after around four years. Indeed both studies mentioned indicate that a plateau occurs in the condition, although Hardus at al (2000b) showed a slight progression of visual field loss in patients who continued taking vigabatrin. Furthermore Hardus et al (2001) used a linear regression to show that the total amount of vigabatrin was the most significant parameter to predict visual field loss.

Conversely, a problem exists were ERG parameters are abnormal despite a preserved visual field. This result was seen in four patients (paediatric patients 4 and 22 and adult patients 3 and 17). In all of these cases the patients had an abnormal ERG result despite visual field testing showing no vigabatrin associated defect to be present. Such a result has been supported by other work such as Duckett et al (1998) who reported 36 of 140 vigabatrin patients to have delayed latencies and reduced amplitudes in the b-waves as well as reduced oscillatory potentials in the light adapted conditions. Only one of these patients had a change in visual field although in this study focused on the paediatric population indicating that perhaps not all the subjects provided reliable visual field results. Such results calls into question the significance of the ERG test and it should be used with caution as it is remains unclear as to which parameter follows or predicts the emergence of a vigabatrin associated visual field loss, and at present it is unclear as to which parameter is affected most by current rather than past vigabatrin treatment. Despite these findings, Jensen et al (2002) argue that the presence of an abnormal ERG result puts the patient at a higher risk of going on to develop a vigabatrin associated visual field loss.

All the patients involved in this study had been exposed to at least one other AED at some stage of their treatment for epilepsy. This is expected as vigabatrin has a licence as an adjunctive therapy rather than as being used as monotherapy, except in the case of treating infantile spasms. It was interesting to find that those paediatric patients who had not been exposed to carbamazepine, did not show any visual problems thus supporting the theory that the combination of vigabatrin and carbamazepine is particularly toxic Patients exposed to carbamazpine only have been shown to have reduced b-wave amplitude responses and reduced oscillatory potential responses (Bayer et al 1991). The absence of sodium valproate from epilepsy treatment revealed a trend towards a normal visual field although this was not seen in all patients. Unfortunately as this study had no control over AED regimes it is difficult to establish a definite link between the two drugs but this study certainly supports previous findings. Indeed testing vigabatrin monotherapy patients would be of immense use in this type of study but such patients are rare.

It is clear from all previous ERG studies that vigabatrin has multiple effects on the ERG which may include an early physiological effect of elevated retinal GABA levels and a progressive effect on the outer retina which is associated with an increase risk of developing a visual field defect. Interestingly, another GABA reuptake inhibitor, tiagabine, has been investigated extensively to determine if visual field defects occur with this drug. However an absence of a visual field loss in this drug, that resembles the mode of action of vigabatrin, indicates that the mechanism of visual field constriction is not just due to an increase in synaptic GABA in a retinal layer but may be due to other factors more closely related to the drug itself, such as the physiochemical properties of the vigabatrin molecule (Spence & Sankar, 2001).

A possibility of future research into the effects of vigabatrin on the retina would be to examine the full-field bright flash ERG (flash intensity setting being far higher than that recommended in the ERG ISCEV standards). Holopigian et al (1997) have described a technique by which a light adapted ERG response can be recorded to a 'white' light as a function of light intensity. Intensity is expressed in terms of density filters, the greater the density the lower the intensity. Such a high intensity flash is capable of examining the

cone system at the receptoral level and so may provide more information as to precisely where the oculotoxic effects of vigabatrin occur.

6.4 Conclusion

In this present study the amplitude of the 30Hz flicker gave the best indication of vigabatrin-attributed visual field loss in both adults and children. The oscillatory potentials appeared to be more affected when a field loss was present in adults than in children. In conclusion to this study, the 30Hz flicker ERG response may play a role in predicting the emergence of a vigabatrin associated visual field loss and so may be of particular use where reliable perimetry results are not obtainable.

CHAPTER 7

THE MULTIFOCAL VISUAL EVOKED POTENTIAL: EXAMINING RESPONSES FROM NORMAL PARTICIPANTS AND VIGABTRIN PATIENTS

7.1 Introduction

Visual evoked responses recorded using the multifocal recording technique produce 60 traces that are derived from the 60 cortically scaled individual segments. The peak-to-trough amplitude for each wave within a specified interval, around 50-150 ms, can be determined and compared for every stimulated segment of the visual field. Reproducible results can be gained using this technique (Klistorner & Graham, 1999). However the placement of electrodes remains vital in the recording of mVEPs. Conventional occipito-frontal electrode placements used in many studies has been criticized for minimizing the upper field dipole contribution. Klistorner et al (1998) showed that monopolar electrode placement reveals the major negativity at 100 msec, being more pronounced in the lower field. Klistorner et al (1998) have therefore suggested an alternative in the bipolar occipital straddle (BOS) electrode position in which electrodes were placed at equal distances 2 cm inferior to and 2 cm superior to the inion. Using the BOS electrode placement, a negativity at 100 msec is seen in lower hemifield stimulation, whereas a positive deflection at 100 msec is seen in upper hemifield stimulation. Indeed the BOS electrode has been shown to objectively detect visual field defects (Nakamura et al, 1999). The extended BOS electrode position (3cm above, 4.5 cm below inion) has also proved a useful montage (Klistorner & Graham, 2000).

However Klistorner & Graham (2000) also showed that using the BOS electrode resulted in a decrease in amplitude along the horizontal meridian. The section of visual field that is situated along the horizontal meridian is represented deep within the calcarine banks at the fissure base. Dipoles that generate a response from this part of the field are almost tangential to a projected line between vertically situated electrodes, which therefore means no signal would be detected. Consequently an alternative montage using horizontally orientated bipolar electrodes has been proposed. On the basis of cortical topography, it was believed that electrodes straddling the inion horizontally would be

optimally placed to record from horizontally orientated dipoles from the base of the calcarine sulcus.

The rationale behind using an electrode placed below the inion is to accentuate the upper hemifield response. However due to the massive differences between individuals cortical anatomy such a fact may be true for some whilst others may give poor responses. Moreover the placing of an electrode below the inion, in the nape of the neck, can prove difficult to attach due to the curvature of the neck making this electrode position susceptible to electromyography (EMG) artefact. As a consequence of these facts, other studies have advocated the use of a montage which uses the inion as a reference site and 4cm above the inion being the active site on a number of occasions (Hood et al, 2000a, b; Hood & Zhang, 2000). This montage placement is both easier to attach and ideally placed to record responses from the lower and upper bank of the calcarine fissure.

The proposed montage of using the inion (reference) and 4cm above the inion (active) electrodes may prove to be a useful derivation in some but not all subjects. This can be attributed to the location and orientation of the calcarine sulcus. Hood & Zhang (2000) investigated this by measuring the distance from the inion to the point where the calcarine intersects the skin. Such a distance varied from 1.5cm below to 3.5cm above the inion indicating that using the extended BOS electrode placed at 4.5cm below the inion would be too low to measure the relevant brain activity. As the distance from the inion to the calcarine increases it was found that the angle the calcarine makes with the line drawn from 4cm up from the inion to the inion also increases becoming more perpendicular. The mVEP response is affected resulting in larger responses recorded from the upper field or the lower bank of the calcarine. In general, mVEP responses are larger from the lower visual field (upper bank of calcarine) and so responses from the upper visual field can increase in amplitude if electrodes are lowered with the reference falling below the inion.

7.2 Normative study

It can be seen from the literature that there is no 'best' montage to be used in mVEP studies and so it is useful to perform a study on normal controls to determine which montage produces the optimum responses. This study investigated the use of the three montages, in order to determine the optimum electrode placement for recording mVEP responses in normal participants. The chosen montage would then be utilised in a greater number of participants and a normal database created.

7.2.1 Methods for normative study

The stimulus array was produced with visual evoked response imaging system (VERIS) software (Electro-Diagnositc Imaging; EDI, San Mateo, CA). A pattern dartboard stimulus was utilized which consisted of 60 sectors, each with 16 checks, 8 white and 8 black. The sectors were cortically scaled with eccentricity to stimulate approximately equal areas of cortical surface. The sizes of the individual 16 checks were proportional to the size of the segment and so were also dependent on eccentricity. The entire display had a total subtense of 48.8°, with the central 12 sectors falling within 2.9° radius of the foveal centre (figure 7.1). Luminance of the white check was 150 cd/m^2 and luminance of the black check was <3 cd/m^2 (Minolta LS-110 photometer) producing a Michelson contrast of 97%. Background luminance of the screen was maintained at a mean level of 73.5 cd/m^2. The checkerboard of each sector had a probability of 0.5 of reversing on any pair of frame changes and the pattern of reversals for each sector followed a pseudorandom (m) sequence (VERIS Version 4.3; EDI). The stimulus array was displayed on a 21" CRT Sony G500 Trinitron colour monitor driven at a frame rate of 75Hz and the monitor had a resolution of 1024 x 768 pixels. A hood was attached to the monitor to prevent the intrusion of stray light onto the stimulus.

Data was recorded using a Grass amplifier Model 12 Neurodata (Quincy, Mass, USA). The continuous VEP record was amplified with the low and high frequency filters set at 3 to 100 Hz. The signal was amplified 100,000 times. The m-sequence had 2^{15}-1 elements requiring 7 minutes 17 seconds recording time. The recording runs were broken into overlapping segments lasting 27.31 seconds in order to improve the subjects ability to

maintain fixation. Additionally subjects were instructed to focus on a red fixation target. Monocular stimulation was performed with the contralateral eye occluded with light pressure to facilitate the suppression of blinks. Segments contaminated with a high level of noise or eye movements were rejected and repeated.

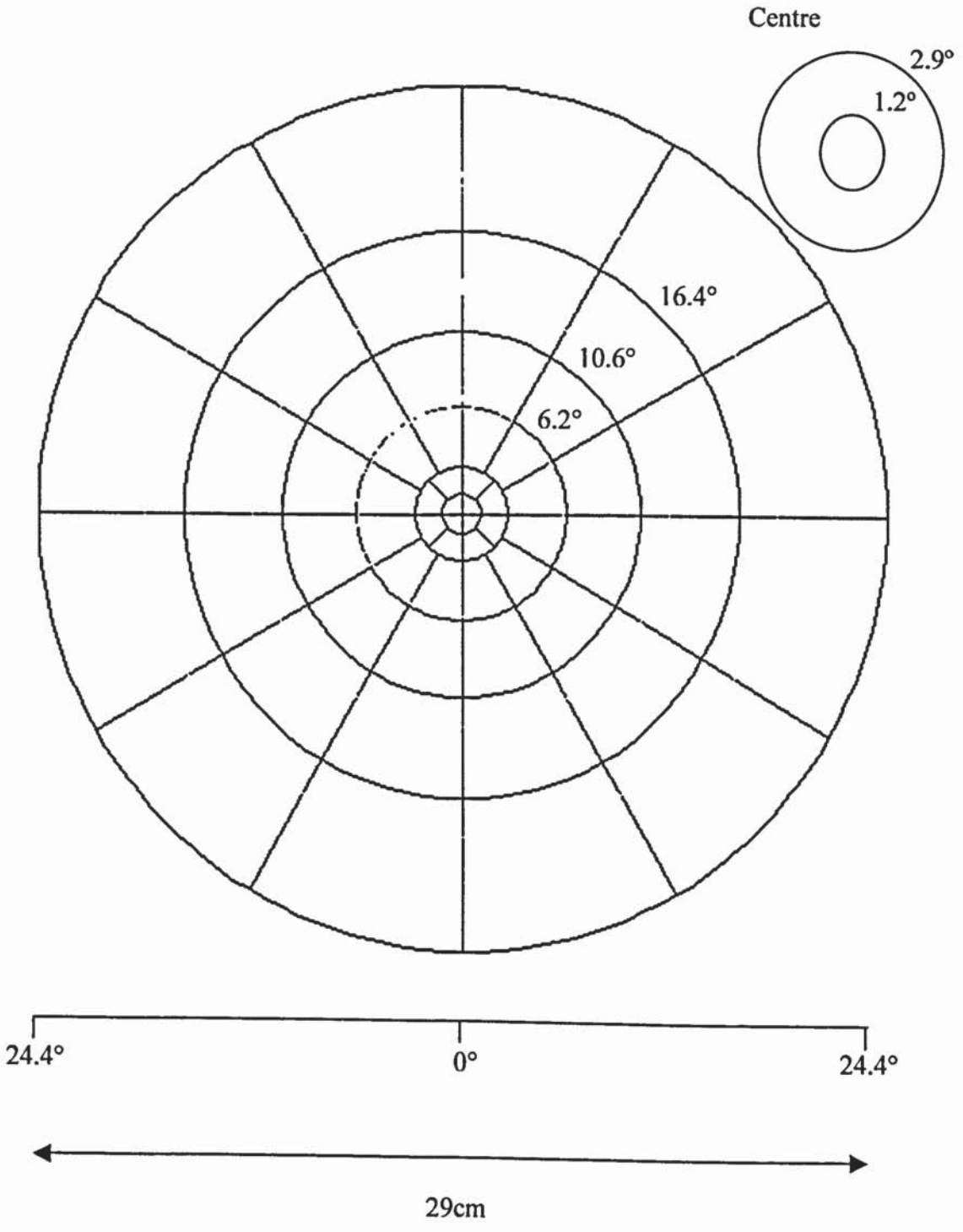
Data was recorded from one channel and three different electrode positions were compared as followed:

1. Electrode placed at Oz (active) and Fz (reference)
2. Bioccipital straddle (BOS) electrode position with electrodes placed 2cm above (active) and 2cm below (reference) the inion
3. Electrode placed at the inion (reference) and 4 cm up from the inion (active)

A further montage was used on a small number of participants termed the extended BOS (3cm above the inion and 4.5cm below the inion). An electrode placed at the forehead served as a ground. Gold cup electrodes were attached with ECG electrode gel (Dracard) and collodian adhesive glue (SLE) was used for electrodes attached to the scalp. Impedance values were maintained below 5kohms.

Twenty-five healthy volunteers age ranging from 18 to 45 years with no known abnormalities of the visual system participated in the study. Each subject was able to comfortably accommodate on the screen at 32cm distance. The display was viewed with the natural pupil, and all subjects had normal or corrected-to-normal vision. A forehead rest and a red fixation cross were used to control the position of the stimulus on the retina. Fixation was always toward the centre of the display. Local ethical approval was gained for the study in accordance with the ethical standards of the 1964 Declaration of Helsinki and informed consent from all participants was obtained.

Figure 7.1
Visual angles when stimulus viewed at 32cm, total subtense being 48.8°.



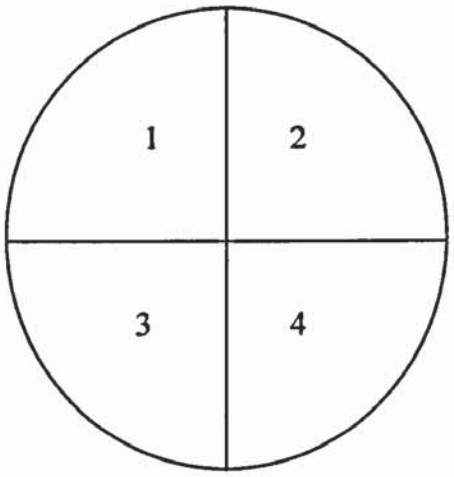
7.2.2 Analysis of normative study

The first-order and first slice of the second-order response components (kernels) were extracted and analysed in this study (VERIS Version 4.3; EDI). A number of methods for analysing the data were available. Traces represent individual responses from the corresponding stimulus area and so are proportional to that area. Sixty traces are produced in each mVEP test, which correspond to the 60 cortically scaled segments. The outputs of traces were drawn in a similar relative position as the stimulus elements that produced them. Examining this output is useful in order to gain an overall view of the responses.

When examining trace arrays it is often difficult to highlight areas of interest due to the traces being contaminated with noise or responses being small in amplitude. It is therefore useful to average together traces from areas where either response characteristics are thought to be similar or are in particular areas of interest. Any type of grouping is possible and depending on which eye is being tested, averages are examined under field view the orientation differs. Betsuin et al (2001) concluded from their study that mVEP responses summed within four quadrants can be used as an objective evaluation of the visual fields and so in this initial study quadrants were examined in field view and the orientation has been shown in figure 7.2. As well as grouping the traces in different ways it is also possible to examine the averages in different ways. 'Sum of groups' examines amplitudes as the traces for each group are added together to provide a cumulative response. This set-up is useful to compare entire retinal responses of one subject to the total response of another. 'Response density scaled' is a more accurate view of the actual response amplitude as each trace is scaled to compensate for stimulus size. 'Normalised' examines amplitude as each trace has approximately the same vertical axis using the root-mean-square (RMS) of each response. This is particularly useful to compare waveform shapes. However as the 'sum of groups' setting examined the amplitude of responses (by examining the total voltage in the area), it was decided to examine all averages using this setting.

Figure 7.2
Orientation of visual fields when examining mVEP data which is true for "field view" in averages, traces and plot

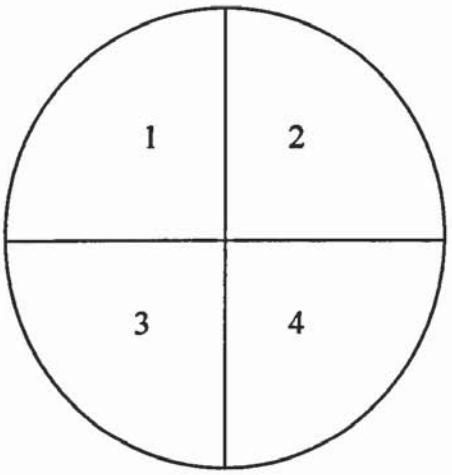
Left eye



If examine left eye responses:

1. Superior temporal
2. Superior nasal
3. Inferior temporal
4. Inferior nasal

Right eye



If examine right eye responses:

1. Superior nasal
2. Superior temporal
3. Inferior nasal
4. Inferior temporal

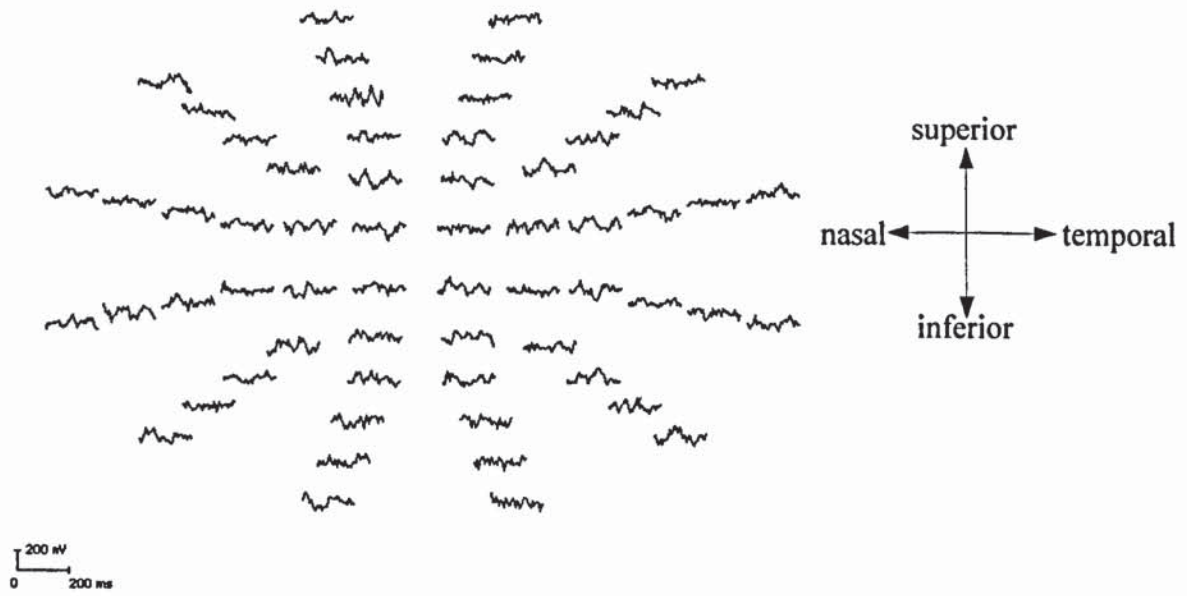
7.2.3 Results of normative study

An example of a first order mVEP trace response (figure 7.3a) shows how all the traces are relatively flat. This is expected as the nature of the pattern reversal stimulation, in which the pattern polarities are equal, results in the luminance responses being cancelled. Second-order first slice kernels show a very different picture of mVEP responses as shown in figure 7.3b, which shows such responses from the same subject. These responses represent the interaction between two consecutive frames of the monitor and are regarded as being analogous to the conventional pattern VEP. As a result of this fact, first-order responses were not examined in mVEP responses and the remaining analysis in this study is derived from the first slice, second-order responses.

Second order traces were examined from participants using the different montages. Figure 7.4 shows both good and poor responses recorded using the Oz-Fz montage. The poor response contains interference so much so that the majority of waveforms, particularly in the upper field cannot be seen. The good response does show the majority of the waveforms although interference is still present in areas. Figure 7.5 shows good responses recorded using the BOS electrode from two different participants. Both traces show clear mVEP responses particularly along the midline and in the upper hemifield. Figure 7.6 however shows the BOS electrode to record poor responses from other participants. Figure 7.6a shows almost non-existent responses whereas figure 7.6b shows responses contaminated with interference. The extended BOS was used on a small number of subjects and figure 7.7a shows a good response with large mVEPs recorded from both the upper and lower hemifields. However figure 7.7b shows traces contaminated with a high level of interference, which masks some of the responses. Figure 7.8 shows two sets of good responses recorded using the inion and 4cm up montage. The mVEP responses in both participants are of large amplitude and are particularly prominent in the lower hemifield. Poor responses were also recorded using this montage as seen in figure 7.9. Responses are of low amplitude but still remain measurable in the lower hemifield.

Figure 7.3
Comparison of (a) first order traces and (b) second order traces recorded from the right eye of a healthy subject (CB).

(a) First order traces



(b) Second-order traces

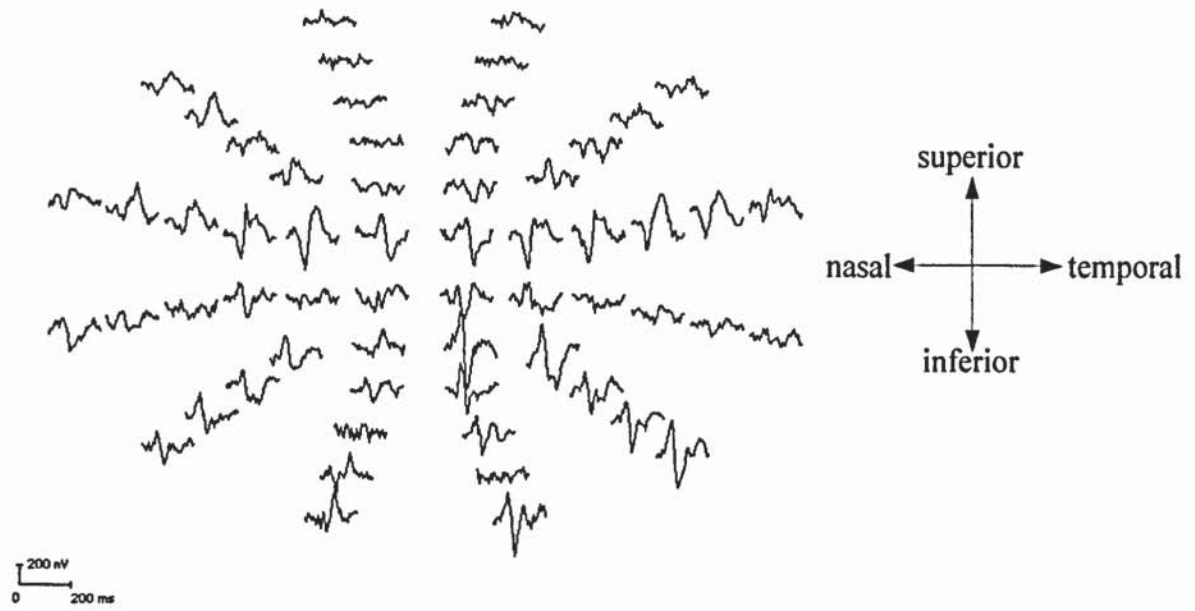
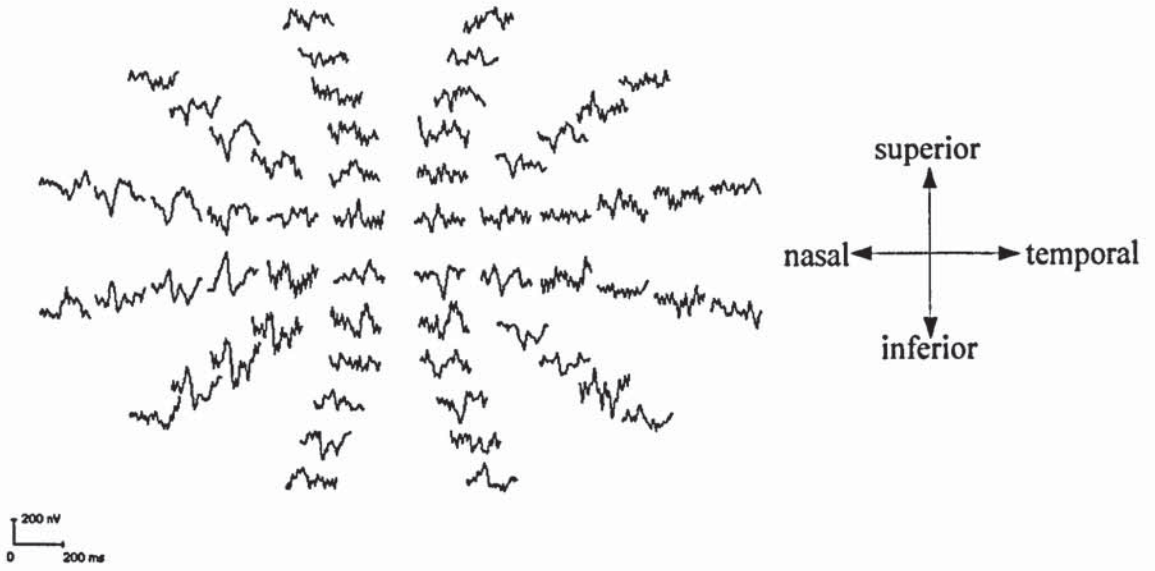


Figure 7.4
Second order traces recorded from electrodes placed at Oz-Fz showing (a) good right eye responses from a healthy subject (ZH) and (b) poor left eye responses from a healthy subject (AK).

(a) Good right eye responses



(b) Poor left eye responses

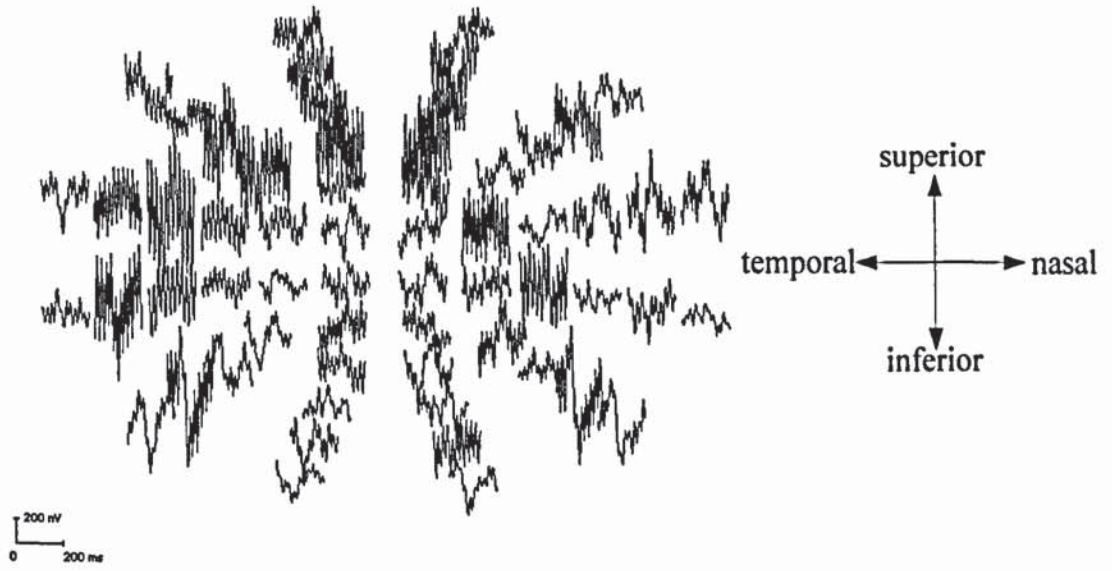
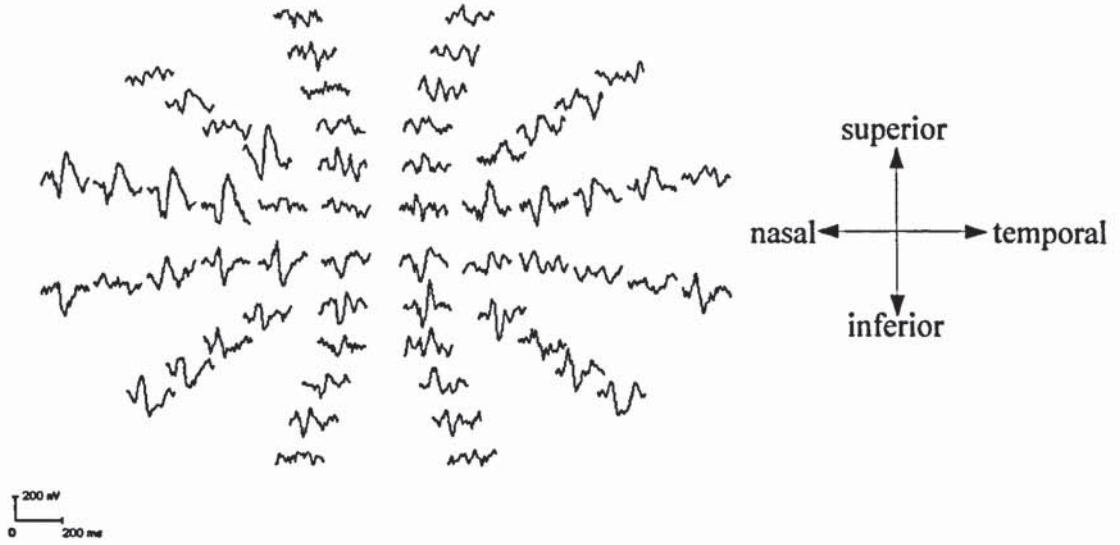


Figure 7.5
Second order traces recorded from electrodes placed 2 cm above and below the inion (BOS) showing good right eye responses from healthy subjects (a) SB and (b) AC.

(a) Good right eye responses



(b) Good right eye responses

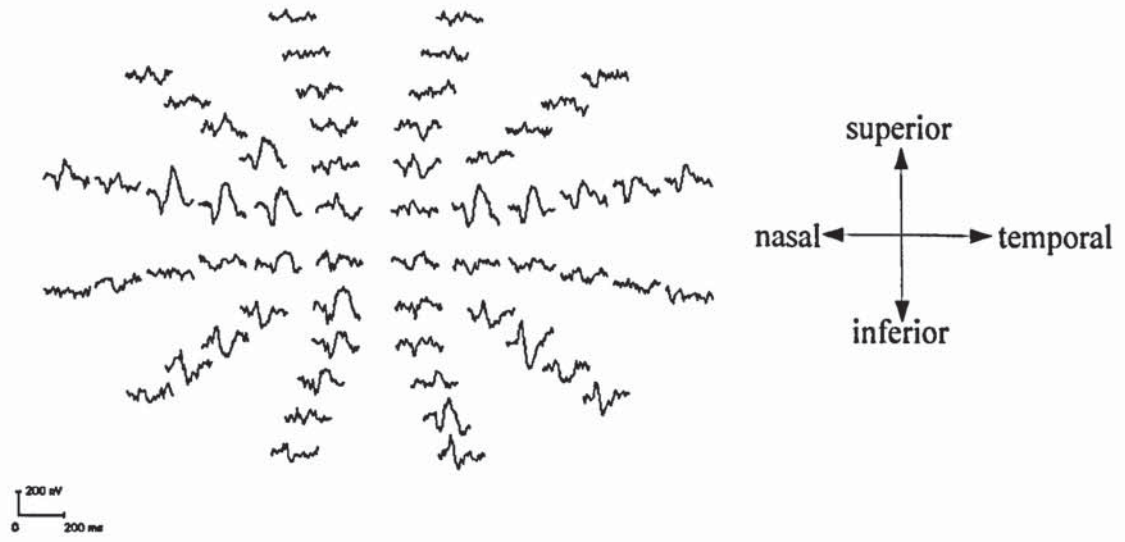
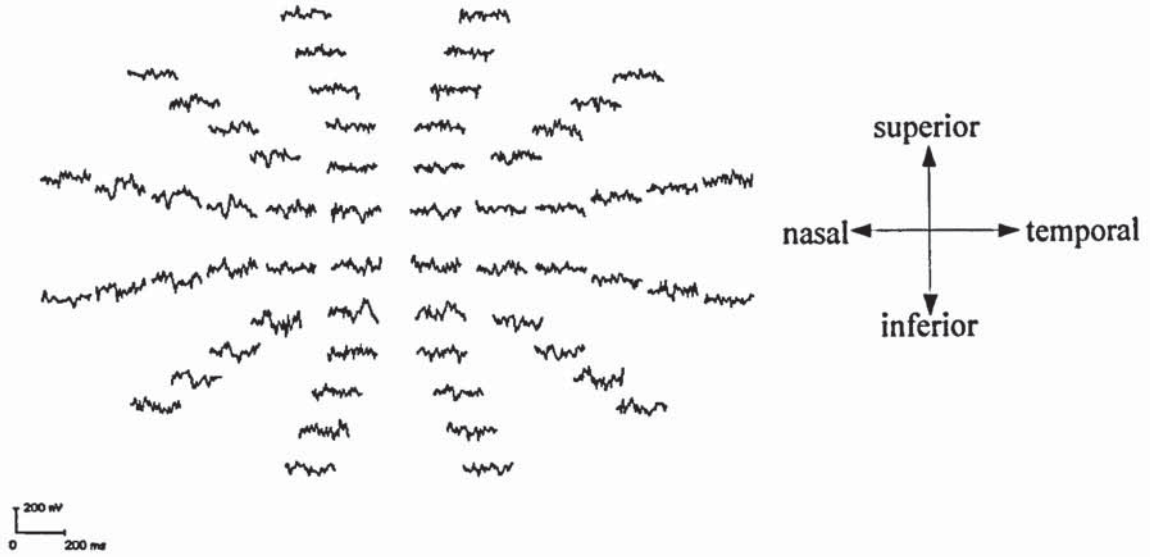


Figure 7.6
Second order traces recorded from electrodes placed 2 cm above and below the inion (BOS) showing poor right eye responses from healthy subjects (a) ZH and (b) LD.

(a) Poor right eye responses



(b) Poor right eye responses

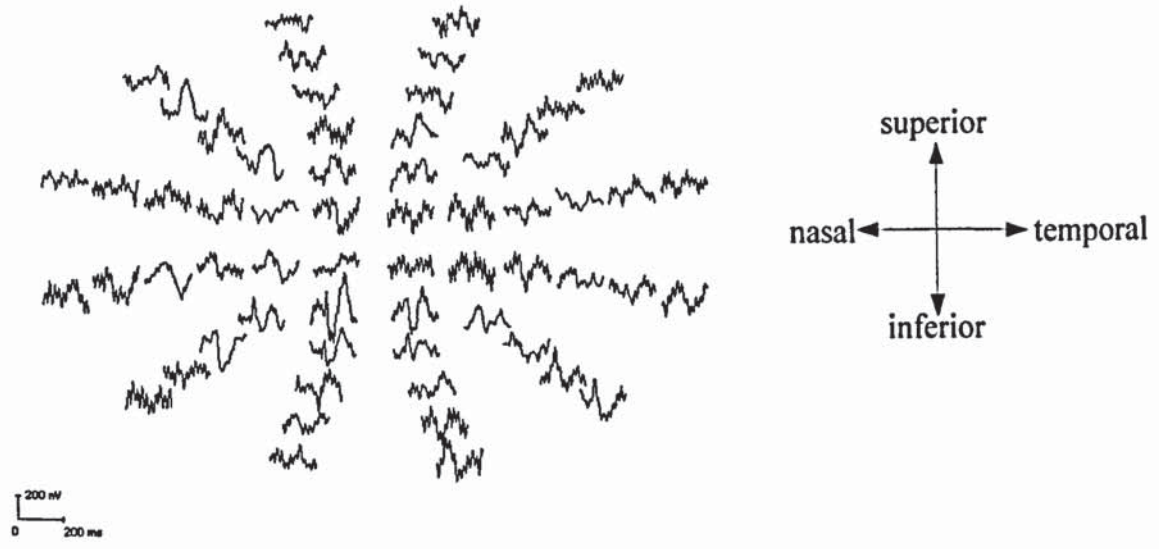
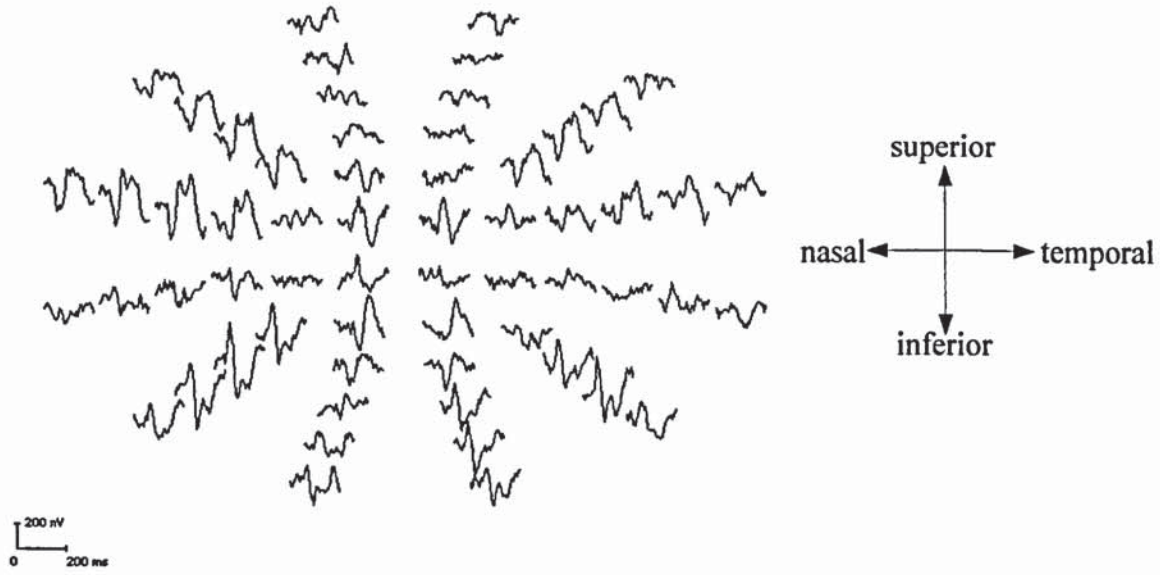


Figure 7.7
Second order traces recorded from electrodes placed at the extended BOS showing (a) good right eye responses from a healthy subject (ZH) and (b) poor left eye responses from a healthy subject (AK).

(a) Good right eye responses



(b) Poor left eye responses

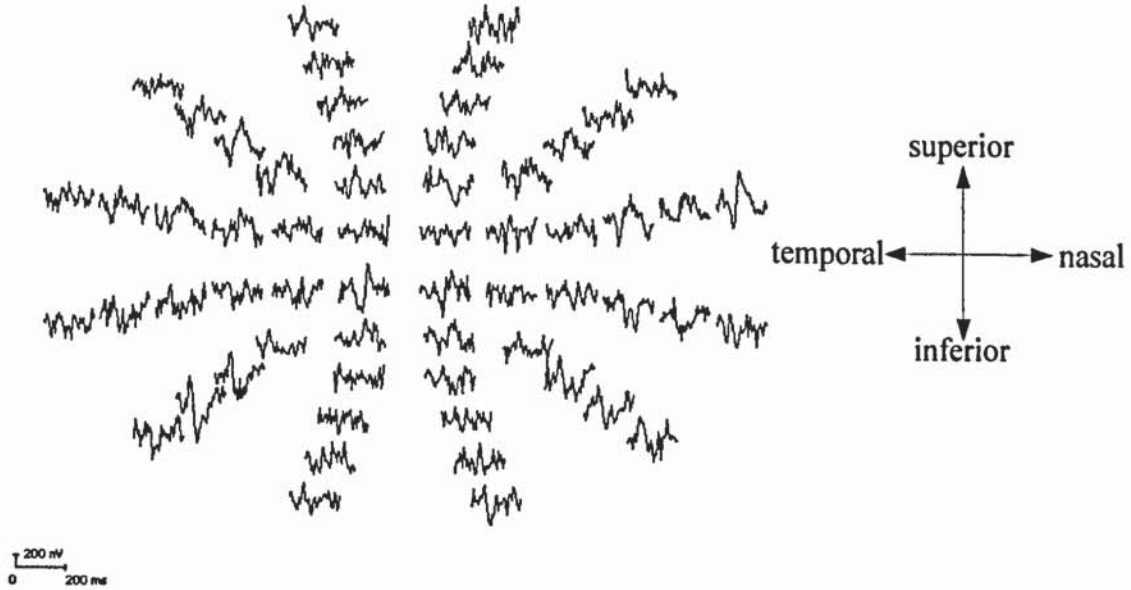
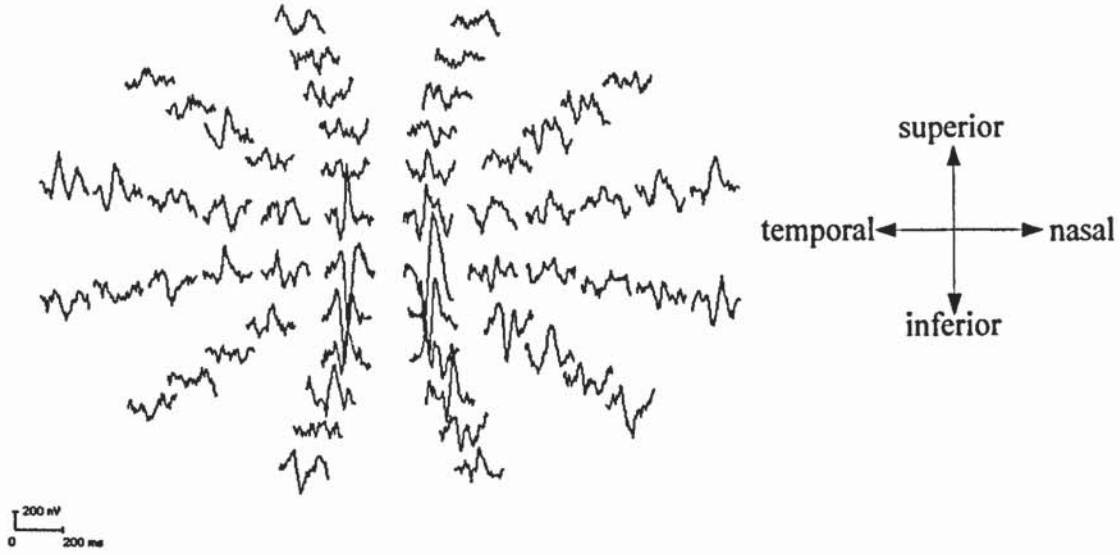


Figure 7.8
Second order traces recorded from electrodes placed at the inion and 4 cm up showing (a) good left eye responses from a healthy subject VP and (b) good right eye responses from a healthy subject AF.

(a) Good left eye responses



(b) Good right eye responses

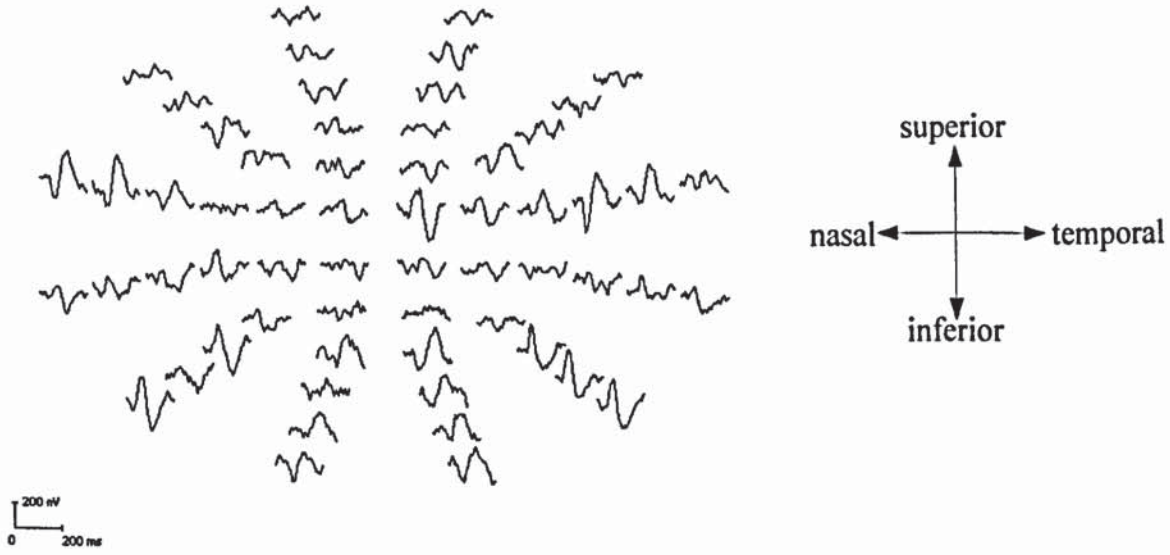
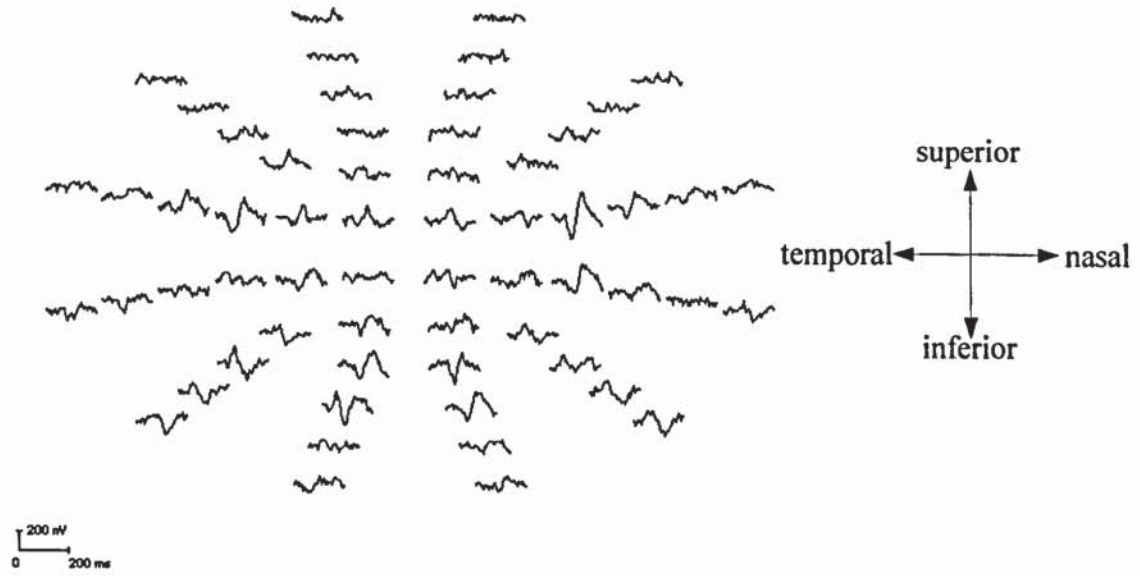
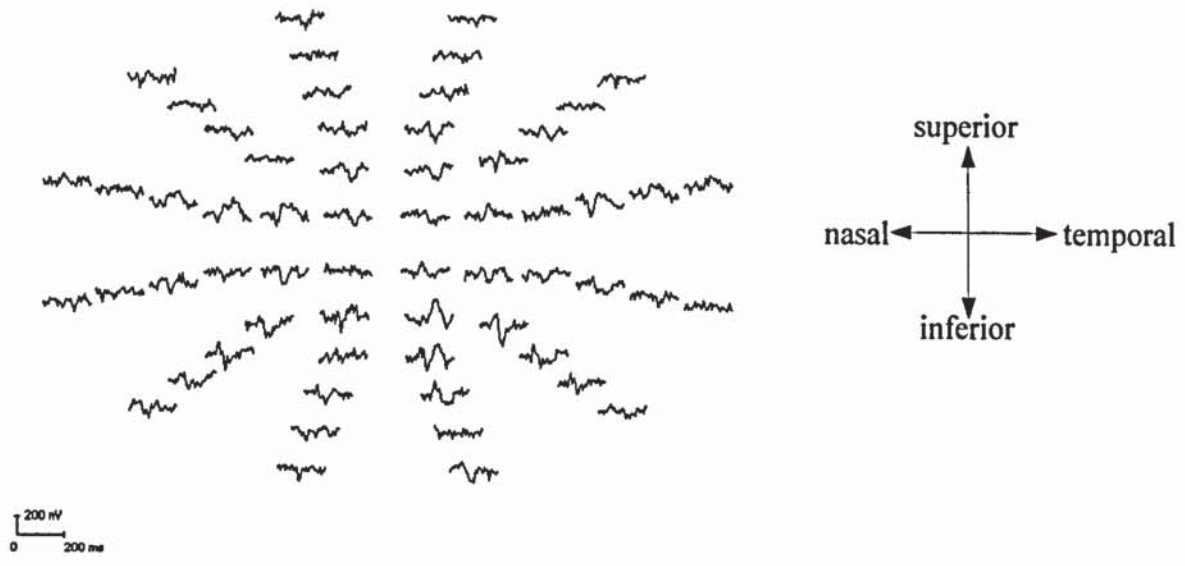


Figure 7.9
Second order traces recorded from electrodes placed at the inion and 4 cm up showing (a) poor left eye responses from a healthy subject KS and (b) poor right eye responses from a healthy subject RC.

(a) Poor left eye responses



(b) Poor right eye responses



The initial analysis of this data involved examining averages from quadrants. Upper and lower responses are mirror images of each other with the upper field response being upward going responses and the lower field responses being downward going responses. This is due to the direction of dipole vectors. Averages were examined using the 'sum of groups' setting grouped into quadrants. Figure 7.10 shows a good and bad example of the quadrants recorded from Oz-Fz. The good example shows a particularly large response from the inferior nasal field when compared to the three other areas. The poor example in figure 7.10b shows how 50Hz interference masks the majority of the responses. Using the BOS electrode position the responses from the inferior and superior fields are more clearly inverted and responses are of similar amplitude from both nasal and temporal fields. Figure 7.11a shows a clear response whereas figure 7.11b shows smaller amplitude responses contaminated by interference particularly on channel three. The use of the extended BOS electrode position served to increase the amplitude of the inferior field responses (figure 7.12a) although an increase in EMG artifact was seen (figure 7.12b). Electrodes placed at the inion and 4 cm up recorded responses of large amplitude in all areas of the visual field (figure 7.13a). A poor response was also recorded in which muscle activity is present although the response waveform can still be seen (figure 7.13b).

The reproducibility of mVEP responses was tested in a number of individuals. Figure 14 shows mVEP responses separated into quadrants recorded from the inion and 4 cm up on four different occasions from the same participant. As shown in the figure, the responses are well reproduced on each of these occasions.

Figure 7.10
Averages grouped into quadrants recorded from electrodes placed at Oz-Fz showing (a) good right eye responses from a healthy subject (ZH) and (b) poor right eye responses from a healthy subject (AK).

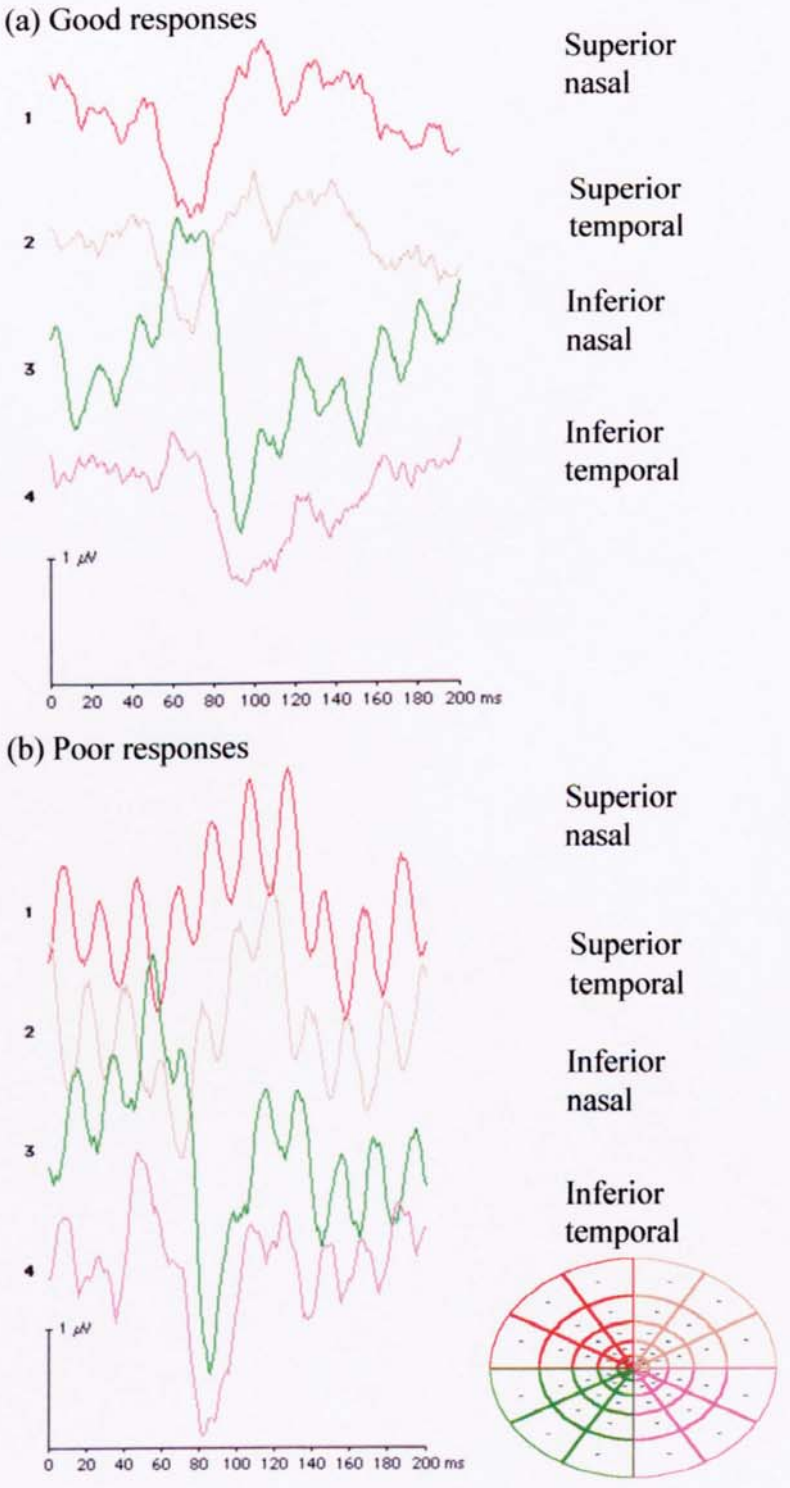


Figure 7.11
Averages grouped into quadrants recorded from electrodes placed 2 cm above and 2 cm below the inion (BOS) showing (a) good right eye responses from a healthy subject (SB) and (b) poor right eye responses from a healthy subject (LD).

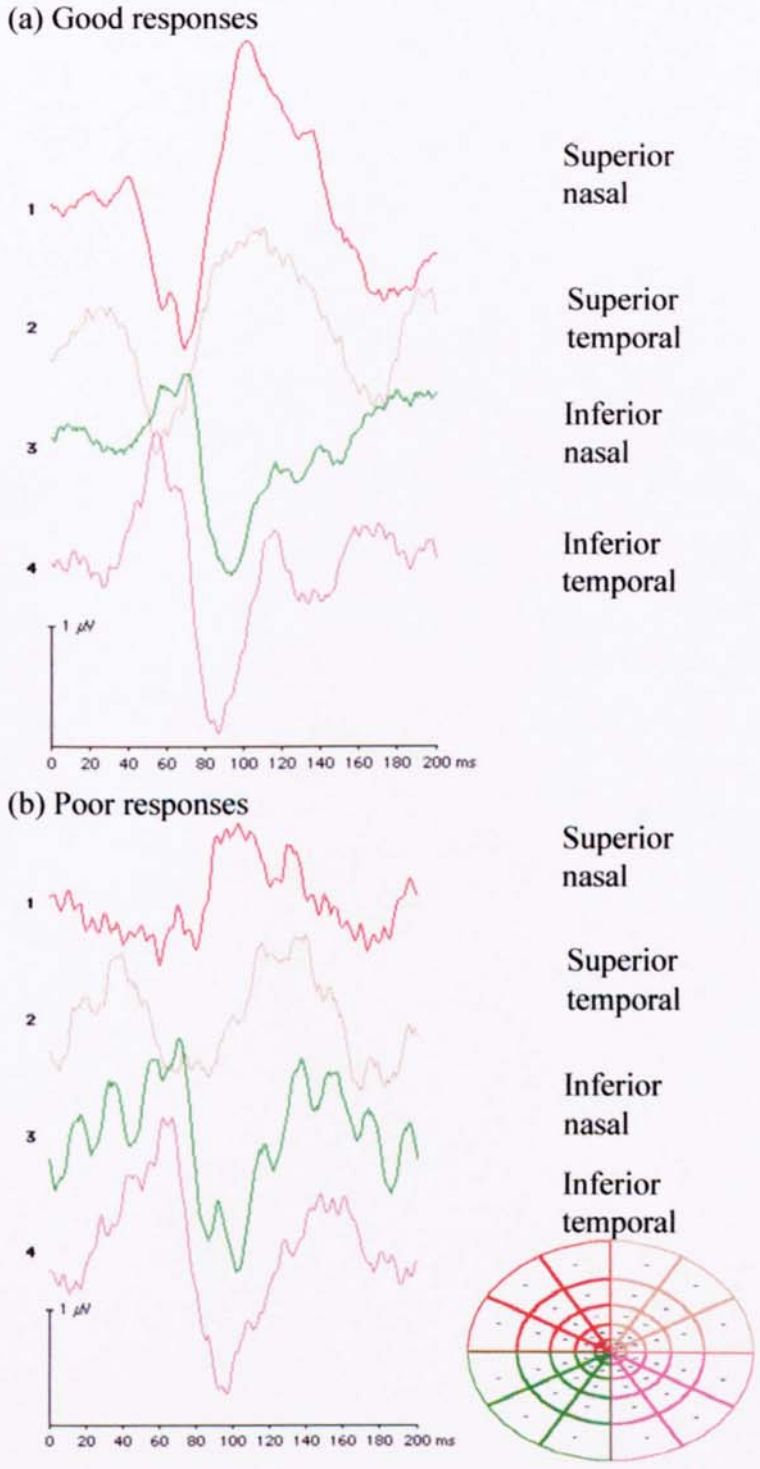


Figure 7.12
Averages grouped into quadrants recorded from electrodes placed at the extended BOS showing (a) good right eye responses from a healthy subject (ZH) and (b) poor right eye responses from a healthy subject (AK).

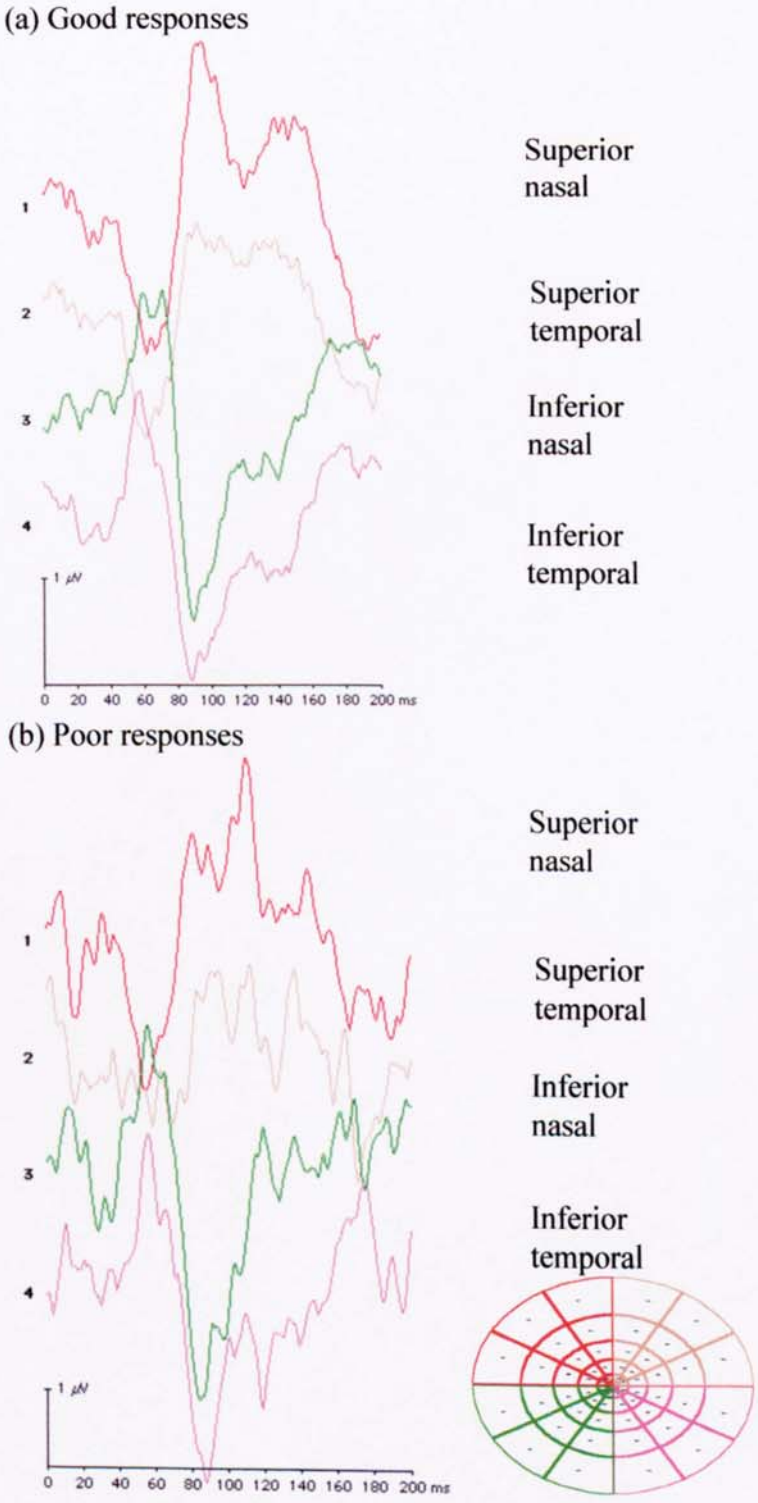


Figure 7.13
Averages grouped into quadrants recorded from electrodes placed at the inion and 4 cm up showing (a) good responses from a healthy subject (VP) and (b) poor responses from a healthy subject (KS).

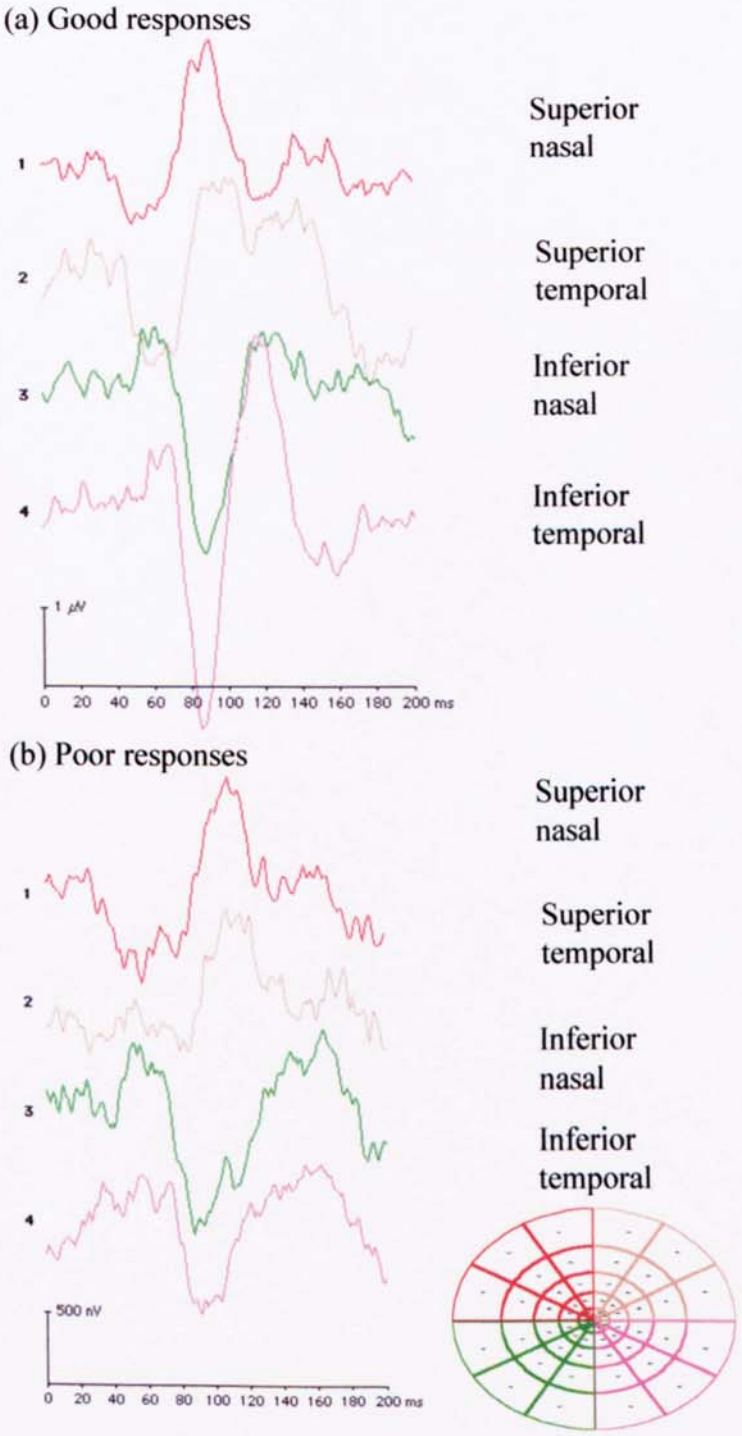
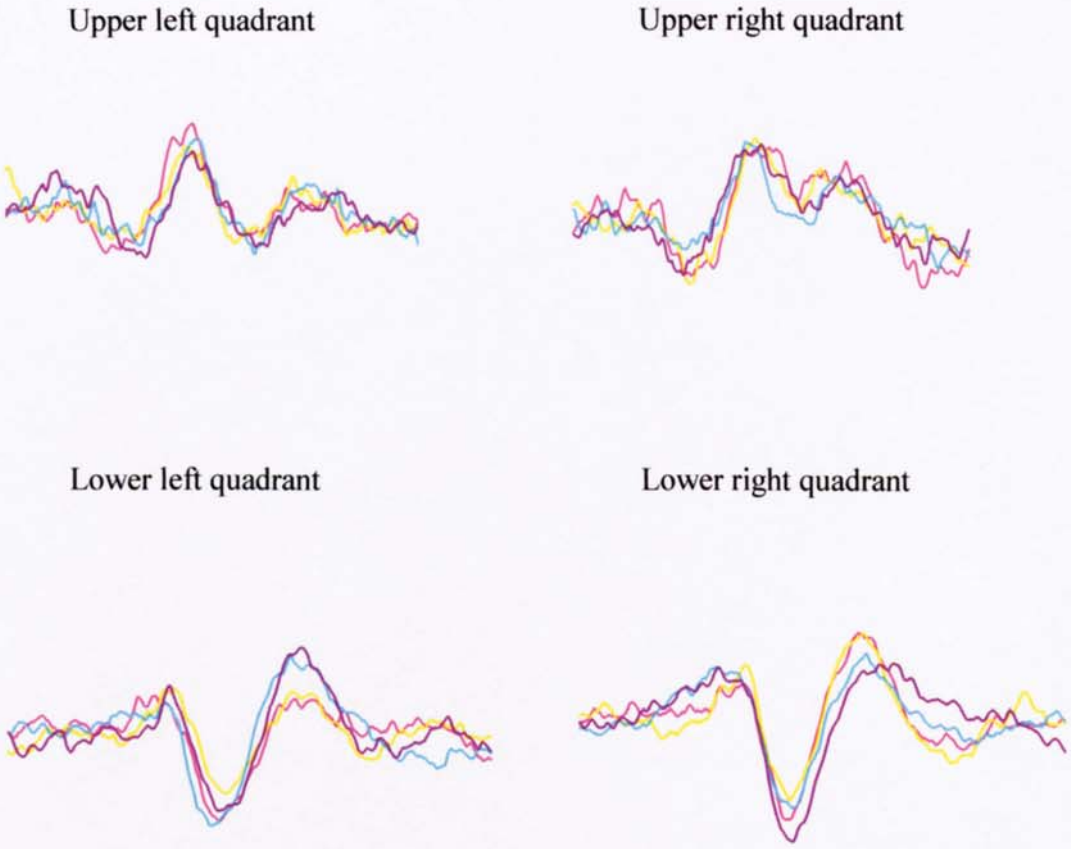


Figure 7.14
An example of the reproducibility of mVEP responses showing averages grouped into quadrants recorded from electrodes placed at the inion and 4 cm up repeated on four different occasions in subject VP (no scale shown, different colours represent a different visit).



7.2.4 Discussion of normative study

All the electrode positions are relative to the inion and are based upon the knowledge of the calcarine fissure relative to the inion and the theoretical value of all the montages needs to be appreciated. Using the Oz-Fz placement of electrodes invariably reduces responses recorded from stimulating the upper hemifield. All such responses also show the same polarity of the waveform with a negativity occurring at around 100 msec. Examining traces and averages from responses recorded from the Oz-Fz montage showed interference was prominent. This can be explained by the fact that the Fz electrode is positioned nearer to the screen than electrodes placed over the occipital cortex and so is more likely to pick up more electromagnetic radiation from the screen. Such results implicate that this montage is not as useful to use when recording mVEP responses.

Examining responses from the horizontal meridian shows a waveform that changes polarity, which can be explained by the position of the generating cortical dipoles relative to the recording electrodes. The upper part of the visual field is thought to project to the lower bank of the calcarine fissure, whilst the lower part of the visual field is mapped to the upper bank. The orientation of the upper and lower field VEP generating dipoles, which are at right angles to the cortex surface, are therefore opposite to each other (Klistorner & Graham, 1999). In order to overcome this cancelling effect and optimise the responses, the BOS electrode position is thought to produce approximately equal responses from the upper and lower hemifields. Indeed, responses from all locations of the upper and lower parts of the visual field are generated demonstrating opposite polarities in the upper and lower hemifields. In this study the BOS electrode montage was useful for recording larger responses in the upper hemifield. However the difficulty of attaching an electrode below the inion in the nape of the neck was often cumbersome and so in some cases the attachment was not secure resulting in an increase in EMG interference as well as interference due to the impedance level exceeding 5kohms. This also occurred when using the extended BOS electrode position and an increase in interference can be seen in both traces and averages.

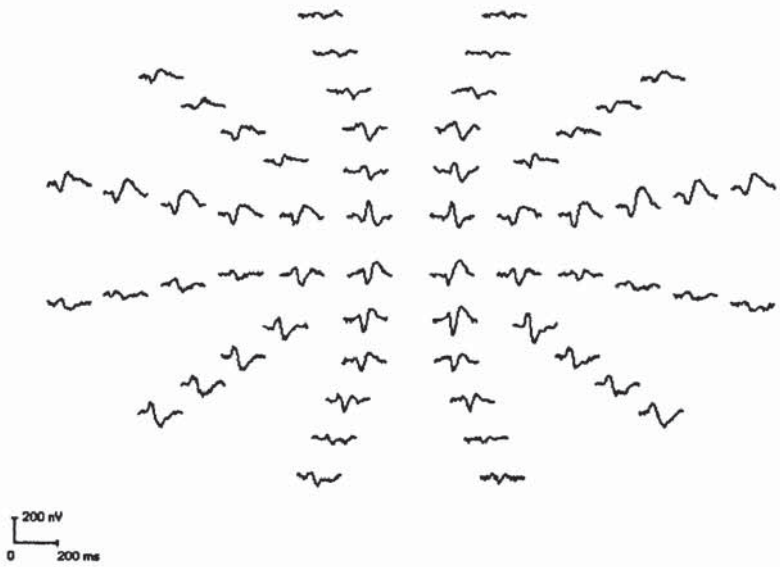
Electrodes placed at the inion and 4 cm up produced consistently good responses from the majority of participants and these electrodes were easier to attach than the electrodes below the inion. The position of the calcarine relative to the inion varies between individuals, as a line drawn from the calcarine intercepts the skin between 1.5 cm below and 3.5 cm above the inion (Hood & Zhang, 2000), an active electrode placed 4cm above the inion would therefore be above this distance in most individuals. Additionally responses recorded using these electrodes were found to be reproducible. It was therefore concluded that the inion and 4cm up electrodes placement would be used to produce a normal database.

The effects of gender on mVEP data in normal subjects has been explored by Klistorner & Graham (2001) who showed the amplitude of the mVEP in women being on average 33% larger than in men. This gender difference was removed using a technique that involved normalising mVEP responses according to the underlying EEG activity recorded using Fourier transform to quantify EEG levels. As this technique was not available in this study it was decided to collate male and female results together. The collection of age-matched controls to be used in a patient study may have provided a more useful comparison of data. However, the age range of participants available was limited. Additionally Klistorner & Graham (2000) found the age of healthy volunteers not to have a significant effect on mVEP responses giving the mVEP the advantage of not being affected by age.

Using the inion-4cm up montage, the normal database used in this study consisted of data from fifty-two eyes. It is useful to combine data from right eyes and left eyes together and this is possible due to the fact that data from right eyes are "mirrored" to be comparable to data from left eyes. It is also possible to have two separate normal files for right and left eyes although it does limit the number of subjects that can be added to the normal database. Summed traces and averages from the twenty-six left and right eyes can be seen in figures 7.15 and 7.16.

Figure 7.15
Grouped second order traces recorded from the inion and 4cm above from (a) 26 left eyes and (b) 26 right eyes.

(a) Left eyes



(b) Right eyes

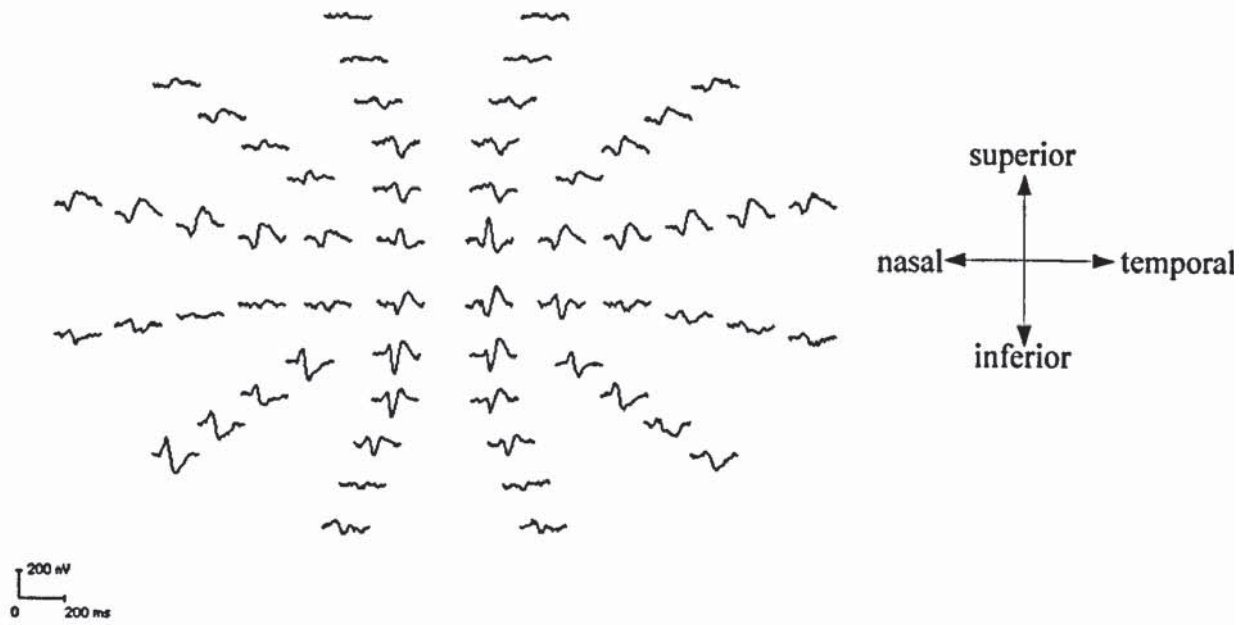
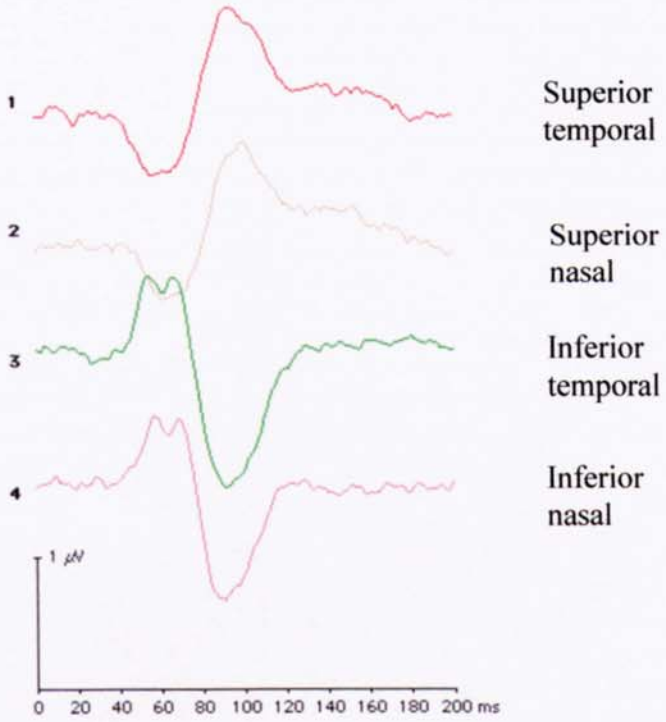
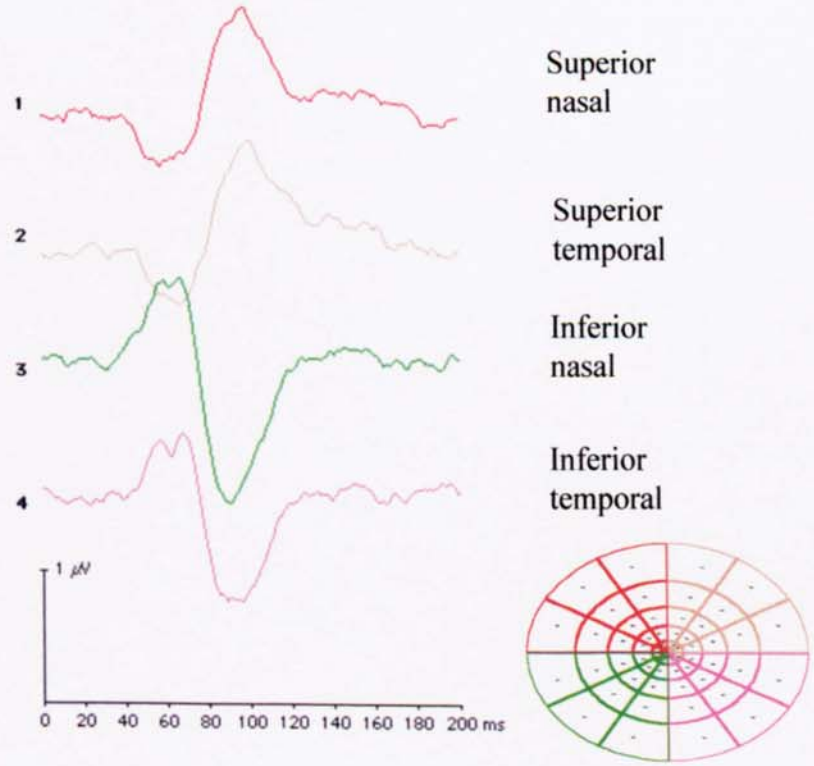


Figure 7.16
Grouped averages of quadrants from 26 (a) left eyes and (b) right eyes.

(a) Left eyes



(b) Right eyes



7.3 Introduction to Vigabatrin study

The mVEP test has a potentially vital role in diagnosing and monitoring certain visual problems. Particularly if visual field results are unreliable, inconsistent with other measures or confirmation is required, the mVEP test could prove invaluable. In multiple sclerosis patients, often visual field results return to normal limits and mVEP results may recover in amplitude yet delays in latency may remain indicating that demyelination has had an effect on vision (Odel et al, 2000). Additionally, glaucoma may be confirmed using this test particularly in the absence of reliable visual field results (Heatley et al, 2000). The mVEP test has also been shown to be useful for identifying functional non-organic problems particularly when an interocular comparison is required, such as functional bitemporal quadrantopia (Miele et al, 2000).

Following the collection of normative mVEP data it is possible that, as vigabatrin-attributed visual field loss causes such a characteristic field loss revealed by the Humphrey 30-2 perimetry test, mVEP results from vigabatrin patients may reveal this loss by differing significantly from the normal database. If the correlation between the two tests is high than the mVEP may become the preferred test for patients to perform, as it is both objective and may be easier to comply with as opposed to perimetry, which requires active concentration for longer periods of time. This test may also be useful for the paediatric population, who may not be able to comply with perimetry at all but may be able to comply with the mVEP protocol.

Often it is difficult to detect subtle or early damage in waveforms and so as well as examining the mVEP waveforms a great deal of numerical data can also be analysed. The root-mean-square (RMS) is the amplitude of responses calculated over a specified time interval and so does not depend upon a specified part of the waveform. It is determined by the square of all the values, taking the average of the squares and then taking the square root of the average. In VERIS 4.3 the RMS is defined as shown in equation 1, in which interval k to n contains the entire significant signal.

$$\text{Equation 1: } A_{rms} = \sqrt{\sum_{i=k}^{n} (r_i)^2}$$

Examining the RMS amplitude rather than the peak to trough amplitude may prove more useful as the waveforms can be contaminated by alpha or noise thus giving a false indication of a large amplitude response. An additional advantage of using the RMS is the value does not depend on identifying a particular waveform, as it only requires a specified time interval, in this study 45-150ms was used. Zhang et al (2002) showed that if two identical runs were compared, a large amplitude difference might be seen if contaminated with noise or alpha activity, giving a false indication of a response. If the RMS is used to determine the signal to noise ratio (SNR) for the two runs then the SNR would be very low for the records contaminated but high for a clean run. This shows that if a record is contaminated with noise or alpha activity, the SNR will identify this and allow for the removal of such poor recordings. A 2-run SNR (2rSNR) can be calculated using equation 2, in which Zhang et al (2002) examined the RMS amplitude of the response in the time period from 45-150ms. The 2rSNR is the ratio of the sum and difference from the two sets of responses obtained from run a and run b. Based on this equation, if no signal was present then the result of the 2rSNR would be zero.

$$\text{Equation 2: 2rSNR} = \frac{RMS(runa + runb)}{RMS(runa - runb)} - 1$$

Zhang et al (2002) also investigated using part of the record that is far from the response waveform in time. If this time window is not closely related to the pattern reversal then no response will be recorded but it is important that the time interval is not affected by kernel overlap. This noise window SNR (nwSNR) is a more conventional method (Meigen & Bach, 1999). By dividing the signal window (45-150ms) to a noise window (325-430ms) as shown in equation 3, if a waveform were present then a larger nwSNR value would be obtained.

$$\text{Equation 3: nwSNR} = \frac{RMS(signalwindow)}{RMS(noisewindow)} - 1$$

A further variation on the SNR was also developed by Zhang et al (2002) in which the mean RMS was taken from the noise window and used as the denominator (see equation 4).

$$\text{Equation 4: mnSNR} = \frac{RMS(signalwindow)}{RMS(meannoisewindow)} - 1$$

Additionally, examining the ratio of the RMS between the right and the left eye is possible. The signal window of 0-200ms between two runs can be used. On average the RMS from both eyes should be equal therefore a ratio of one would be expected. Taking the log of this ratio (see equation 5) provides a useful scale as the result would be symmetrical around a mean of zero. Hence, if the right eye responses were the same as the left eye responses then the log ratio value would be zero.

$$\text{Equation 5: 200msec} = \frac{sumruna}{sumrunb}\log$$

However, the RMS value is very sensitive to noise and so may not be the optimum method for analysing mVEP data unless the signal is free from noise. In comparison the scalar product calculated in VERIS 4.3 is an artificial number that represents the quality of correlation between a reference signal (template) and an individual signal. Consequently the scalar product is not a direct measure of amplitude or latency but instead gives a higher number depending on whether the signal corresponds in waveform, amplitude or latency.

While comparing visual field results (graded abnormal or normal) with numerical values is possible, it is preferable to use a quantitative comparison. Despite the spatial format of the visual field and the multifocal array differing, the data from the two techniques can be compared in a more scientific manner. Hood & Zhang (2000) have developed such a technique, which enables the estimation of sensitivity changes in the regions of the multifocal display. The interpolated visual field is the 30-2 Humphrey information put into the same cortical scaling that the mVEP is recorded, hence 60 sectors of information are obtained. If points from the standard 30-2 visual field are used, an estimate of

sensitivity across the region covered by a sector in the multifocal array is determined. The numerical values are expressed in dB, with 1dB being 1/10 of a log unit. A value of -18dB from a subject indicates that the response is 18dB down from normal. Depending on the question being asked, the data can be approached in a number of different ways, for example relevant sectors can be grouped together. In fact the most interesting points to be made from comparing the two types of data may be from the points of disagreement, such as delayed mVEP responses in areas of normal field sensitivity.

The aim of this study was to utilise the mVEP test in vigabatrin patients to establish if any one parameter is more closely associated with vigabatrin associated visual field loss. Such a parameter may then be used as a marker in detecting retinal dysfunction associated with vigabatrin. Children and adults with epilepsy who had been exposed to vigabatrin for at least three months were included in the study. Perimetry results from a number of children and the adult group where compared with the mVEP results in those patients showing both a visual field defect and those showing no defect. Additionally, the interpolated visual field was also examined in selected patients to determine if this analysis of comparing visual field and mVEP results could provide more information on vigabatrin associated visual field loss.

7.3.1 Method for vigabatrin study

The stimulation and recording method have been reported in the previous method section. Responses were recorded from electrodes placed in the midline 4cm above the inion referenced to an electrode placed at the inion. An electrode placed at the forehead served as a ground. Gold cup electrodes were attached with ECG electrode gel (Dracard) and collodian adhesive glue (SLE) was used for electrodes attached to the scalp. Impedance values were maintained below 5kohms. All responses in the figures are displayed with the reference inion electrode as negative.

Data from nineteen normal subjects aged between 21-45 years of age (mean age 29.3 ± 7.1 years) collected in the previous section was used in the normal database and was compared to data collected from vigabatrin patients. The normal database consisted of

data from fifty-two eyes. However, due to nature of the visual field defect associated with vigabatrin, which includes temporal sparing, the defect swaps over depending on which eye is tested and so when data from both eyes is collated together and compared a defect may be hidden. Therefore two separate normal files for right and left eyes were also created.

Twenty-three patients aged between 10-52 years of age (mean age 28.4 ± 14.7 years) with epilepsy who had been exposed to vigabatrin for at least three months were invited to take part in the study. Six patients did not provide test results with three not attending appointments and three being unable to comply with the test procedure. The remaining seventeen patients provided mVEP results. Table 7.1 shows the patient details. In ten cases both eyes were tested twice, and in five cases both eyes were tested only once. In a further two cases only one eye was tested twice, in one case due to the patient tiring after having completed three runs and in the other case the patient had no right optic nerve and so could not focus on the red fixation cross. Local ethical approval was gained for the study in accordance with the ethical standards of the 1964 Declaration of Helsinki and informed consent from all patients was obtained.

Averages were examined in quadrants (as shown in the normal mVEP study) and compared to the normal database. Additionally, outer quadrants and central versus peripheral waveforms were also compared between the groups. The 2rSNR, nwSNR, mnSNR, 200ms log and the scalar product were all compared between the normal and vigabatrin treated groups. Mean and standard deviation values of the numerical data from the group of healthy participants and the vigabatrin treated patients were calculated. The vigabatrin treated patients were split into two groups, one containing those with a confirmed vigabatrin-associated visual field defect and the other containing those showing no such visual field defect following perimetry testing. A one-way between groups ANOVA was used to determine if the numerical data values differed between the three groups and post-hoc analysis (Scheffe) determined where the differences occurred. For those results in which homogeneity of variance was not met, a non-parametric

Kruskal-Wallis test was also performed. A p value of less than 0.01 and 0.05 was accepted as statistically significant.

Estimates of visual field thresholds at arbitrary locations were made from the 30-2 visual fields in two vigabatrin patients. The interpolated visual fields were calculated by estimating the sensitivity within a stimulus patch and the antilog (dB) of the Humphrey deviation values for each test location was obtained. These values are then interpolated into a high-resolution surface using a linear algorithm. The interpolated values for the stimulus patch were calculated as the average of all the interpolated values within the patch, and the log of this value was determined. This procedure was performed in two adult vigabatrin patients (CM and NH) in both eyes separately at Columbia University, New York using the technique described by Hood et al (2000a).

7.3.2 Results of vigabatrin study

The multifocal VEP responses of the vigabatrin patients were examined as traces and as grouped quadrants. Responses were compared within the group, examining the differences between responses from patients with a confirmed vigabatrin associated visual field loss and those patients with no such defect. Additionally responses were also compared to those from the normal database.

Traces were examined from left and right eyes separately. The traces were grouped together into two groups, those with a confirmed vigabatrin associated visual field loss (figure 7.17) and those without (figure 7.18). When comparing the two sets of responses it is clear that those patients with a visual field loss have an overall depression of response in a non-specific pattern. Closer examination of the responses does reveal that the superior and nasal regions are more depressed in patients with a visual field defect but overall the reduction in responses is general.

In order to examine responses in more detail grouped averages were used. Initially full quadrants were examined but as can be seen from figure 7.19 no clear difference can be seen between those patients with a defect and those without. Central and peripheral responses were then examined (figure 7.20) as it was attempted to mirror the H-Stimulus settings of a central radius of 5° and a peripheral radius 30-60°. However due to the overall stimulus size being smaller than the H-Stimulus this was not possible. The nearest settings were used that also gave the optimum responses. Central responses were taken from the inner three sections of the stimulus (radius 6.2°) and the peripheral response was taken from the remaining outer sections (radius 6.2-24.4°). When comparing the responses from vigabatrin patients with and without a defect no difference between the two sets of patients was seen (figure 7.20).

Outer quadrants (using the outer section of the stimulus 16.4-24.4°) were then examined (figure 7.21) and a difference between patients with a defect and those without was clear. The left eye responses from vigabatrin patients with a visual field defect showed nasal responses to be absent and the right eye showed a reduced inferior nasal response and an absent superior nasal response. In comparison patients with no visual field defect had responses present in each of the outer quadrants examined.

Outer quadrant responses were then examined from the normal database. Figure 7.22 shows how the outer quadrant responses can be both well correlated with the normal database (figure 7.22a) and poorly correlated with normal database (figure 22b). Patients with a vigabatrin-associated visual field loss show nasal responses to be reduced or absent when compared to the normal database (figure 7.23). Correspondingly patients with no visual field defect correlate well with the normal database, showing no reduction of responses (figure 7.24).

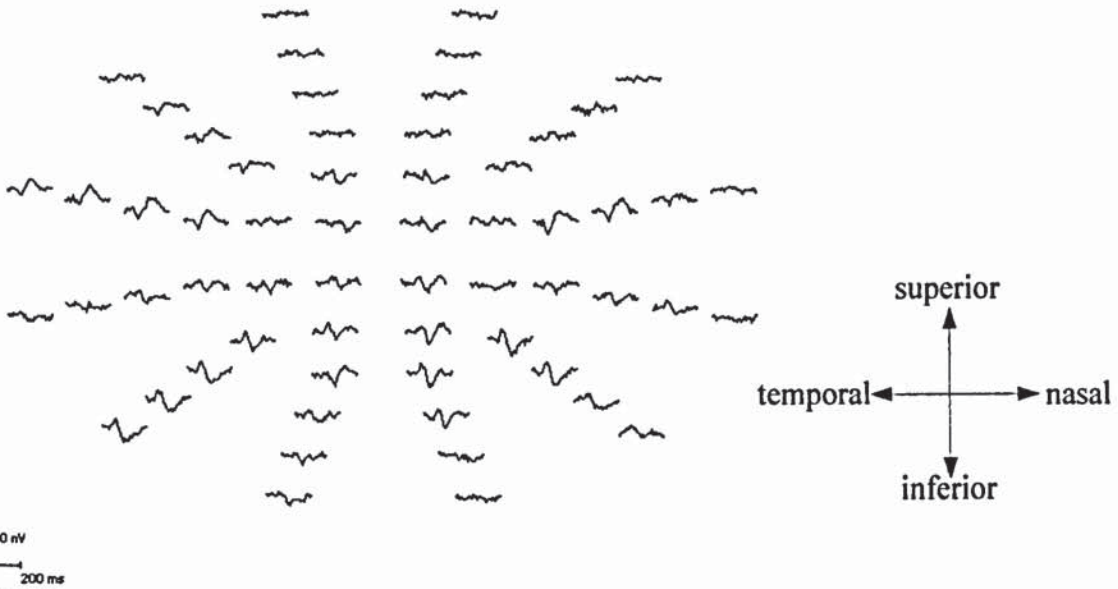
Table 7.1
Patient details and vigabatrin treatment history.

Patient	Gender	Age (years)	Maximum VIG dose (g)	Duration of VIG treatment (months)	Other AEDs (current or past)
1	Male	12	1	72	CBZ, SV
2	Male	14	1	43	CBZ, SV, LTG
3	Female	19	1	24	CBZ
4	Male	10	1.5	49	CBZ, SV, CLB
5	Male	15	2	73	CBZ, SV, LTG, PHB, GBP
6	Female	11	2.8	8	CBZ, SV, LTG
7	Female	19	2	72	LTG, GBP
8	Female	16	4	16	CBZ, SV, LTG, GBP
9	Male	25	1.5	108	CBZ
10	Female	31	?	?	CBZ
11	Female	29	3	156	GBP
12	Female	24	2.5	?	CLB, TOP
13	Female	43	2	120	CBZ
14	Male	47	4	96	CBZ
15	Female	51	2.5	23	LTG, PRD
16	Female	48	1	72	CBZ
17	Female	20	2	72	LTG, GBP
18	Male	DNA	-	-	-
19	Male	52	?	12	CBZ
20	Male	DNA	-	-	-
21	Female	35	3	156	GBP
22	Male	46	2	120	CBZ, LTG
23	Male	DNA	-	-	-

Key: SV;Sodium valproate, CBZ;Carbamazepine, LTG;Lamotrigine, GBP;Gabapentin, CLB;Clobazam, TOP;Topiramate, PHB;Phenobarbitone, CLON;Clonazepam, PRD;Prednisolone, ?; unknown dose, DNA; Did not attend

Figure 7.17
Grouped traces from (a) left eyes and (b) right eyes of vigabatrin patients with a confirmed vigabatrin associated visual field defect.

(a) Left eyes



(b) Right eyes

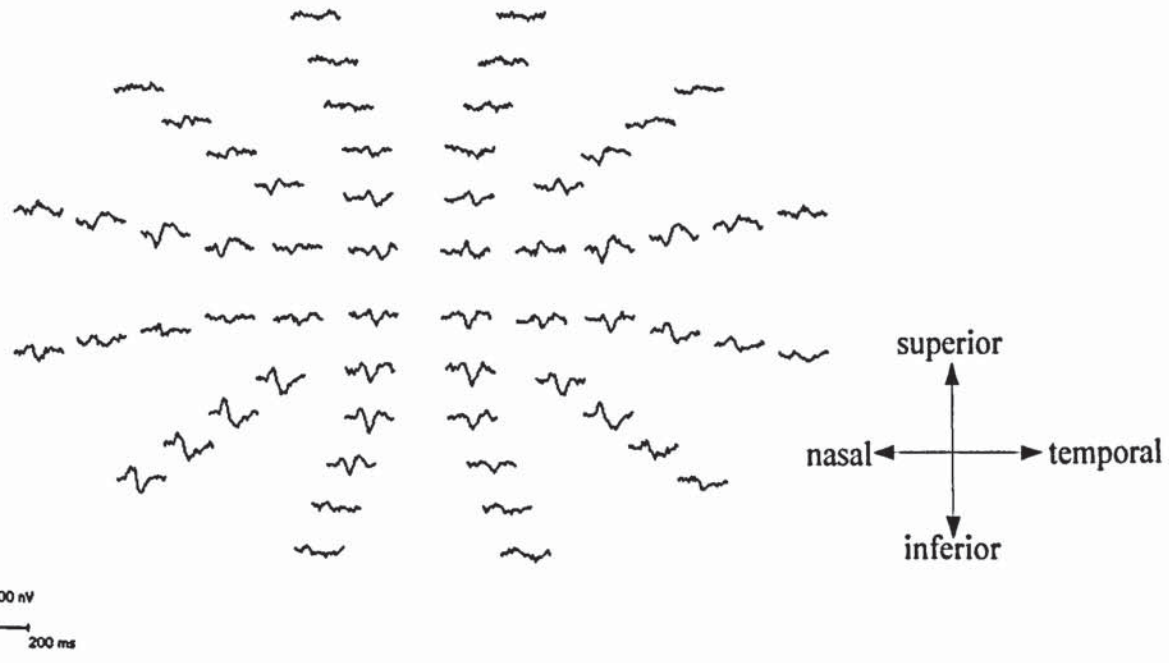
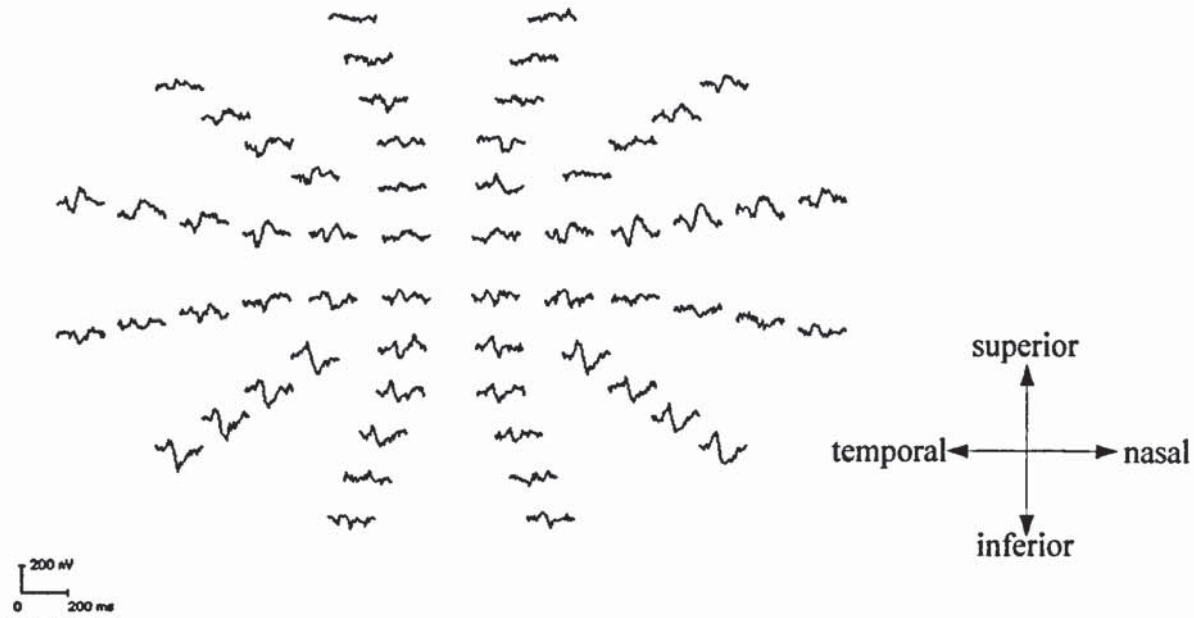


Figure 7.18
Grouped traces from (a) left eyes and (b) right eyes of vigabatrin patients with no visual field defects.

(a) Left eyes



(b) Right eyes

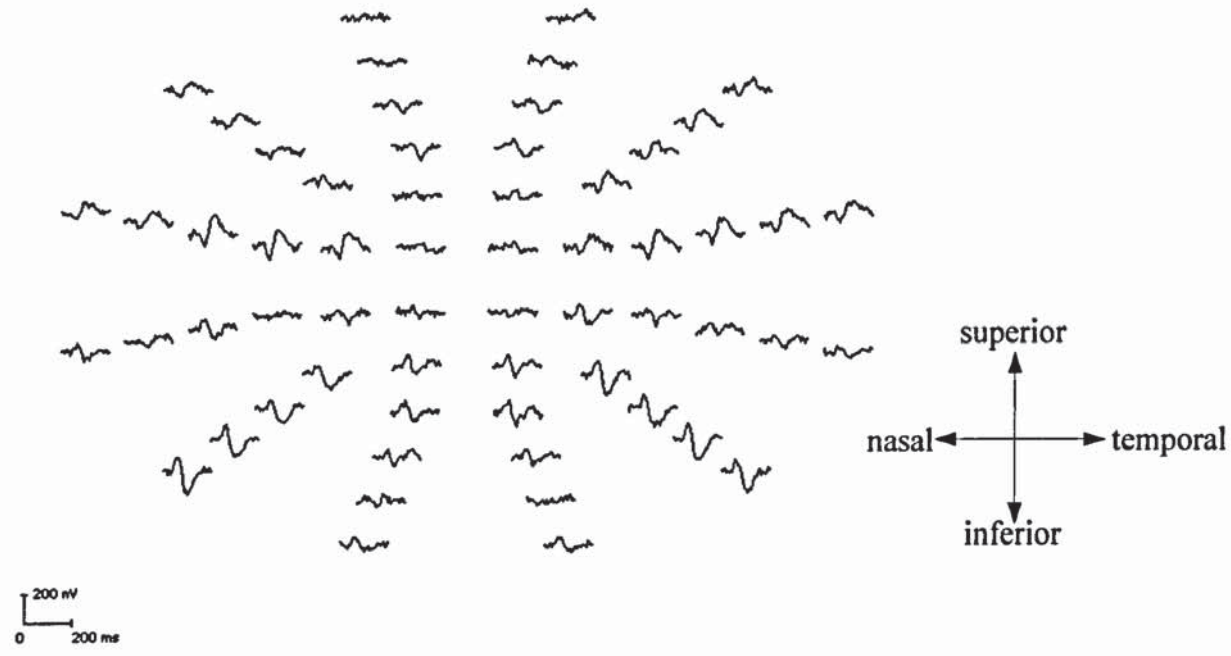
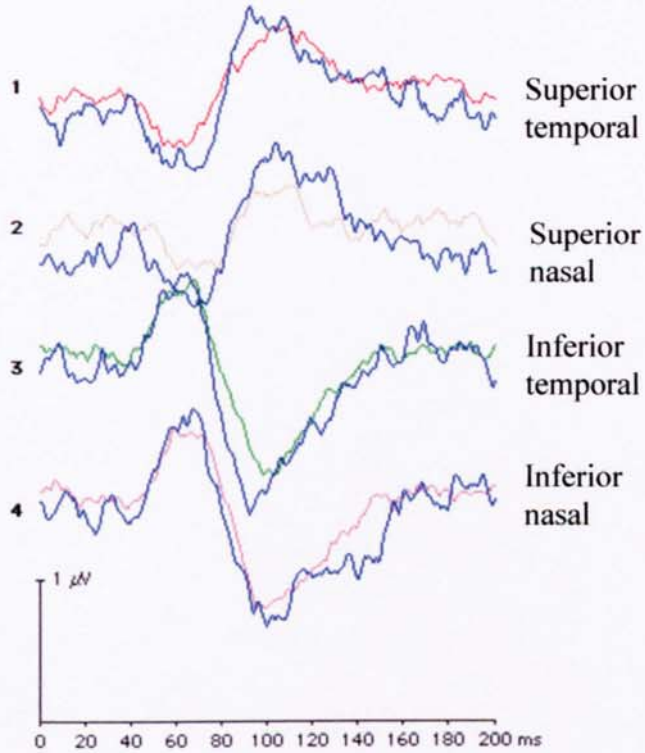


Figure 7.19
Grouped responses of full quadrants from vigabatrin patients with no defect (blue) compared to vigabatrin patients with a confirmed vigabatrin associated visual field loss.
(a) Left eye



(b) Right eye

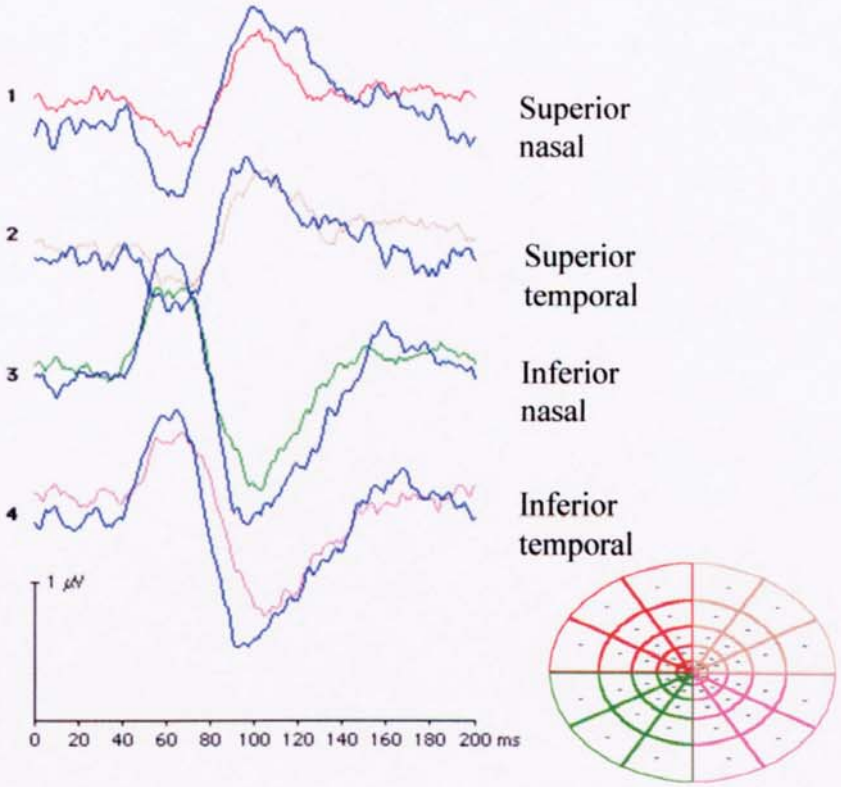
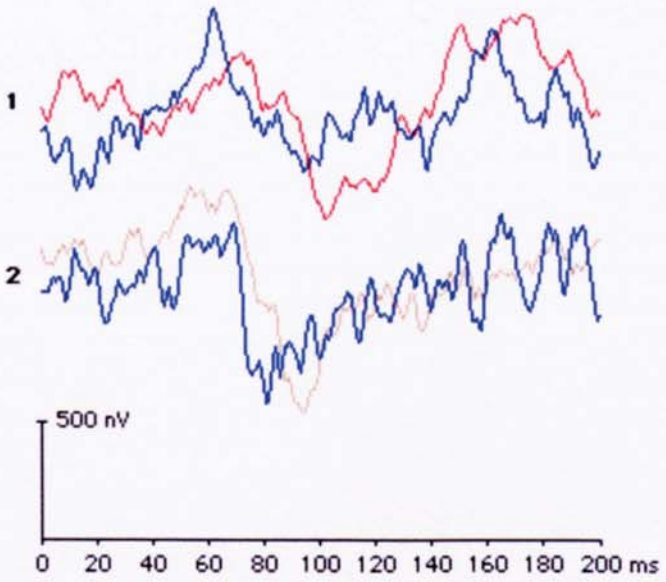
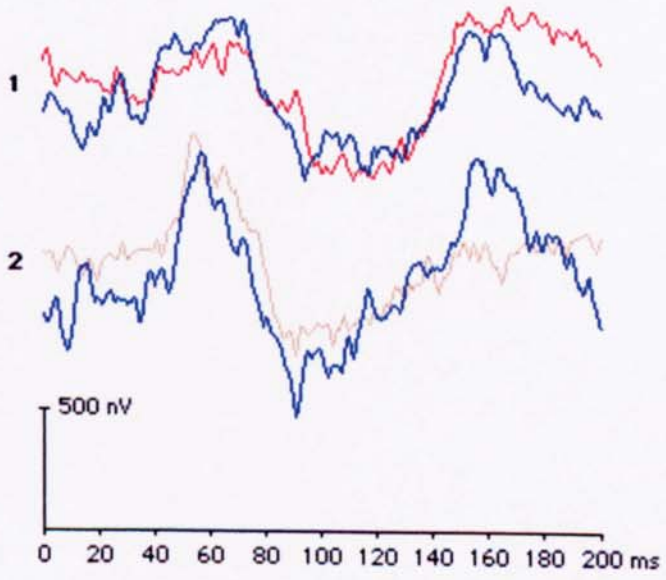


Figure 7.20
Grouped responses of central and peripheral sectors from vigabatrin patients with no defect (blue) compared to vigabatrin patients with a confirmed vigabatrin associated visual field loss.
(a) Left eye



(b) Right eye



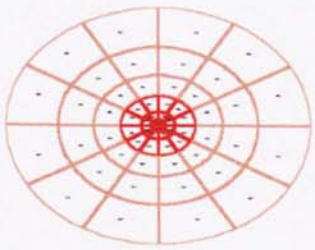
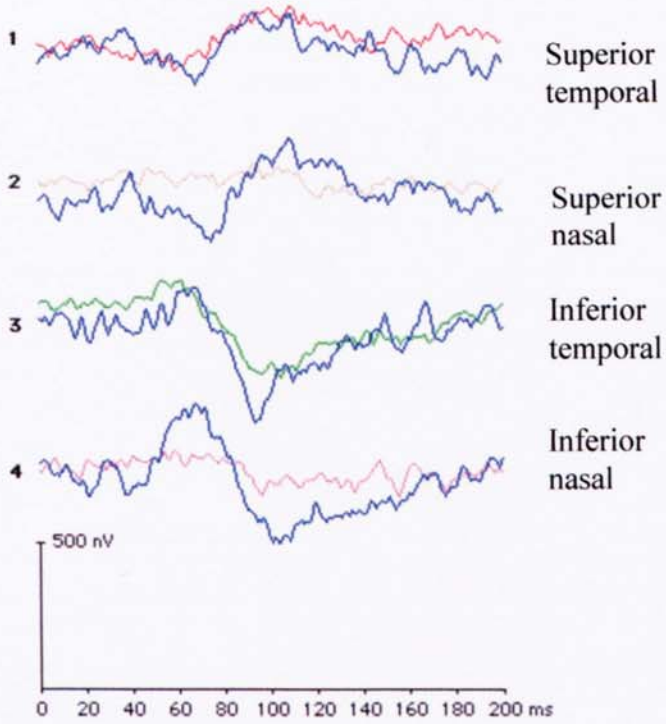


Figure 7.21
Grouped responses of outer quadrants from vigabatrin patients with no defect (blue) compared to vigabatrin patients with a confirmed vigabatrin associated visual field loss.
(a) Left eye



(b) Right eye

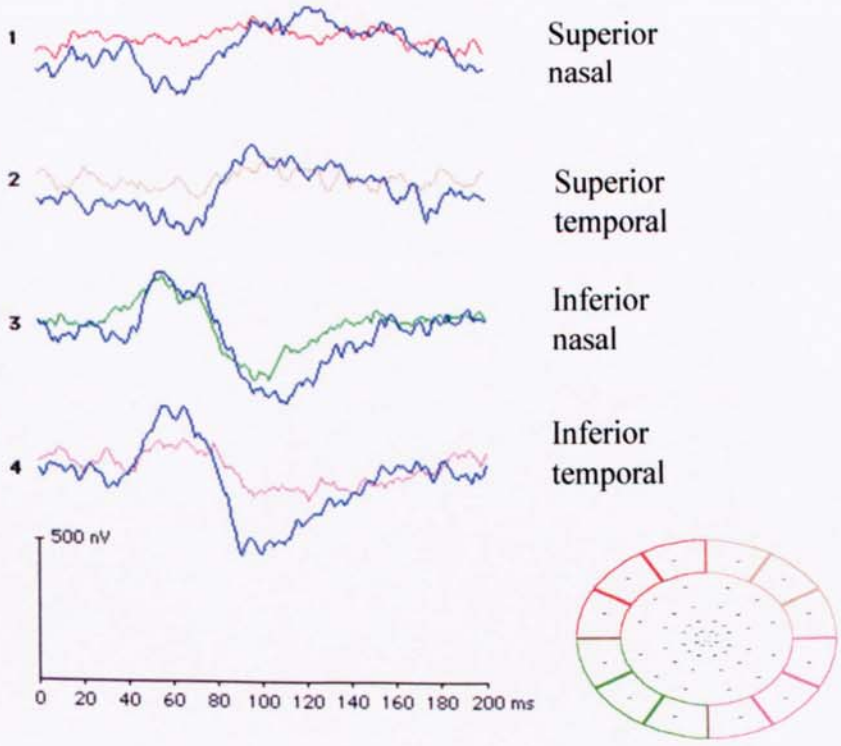
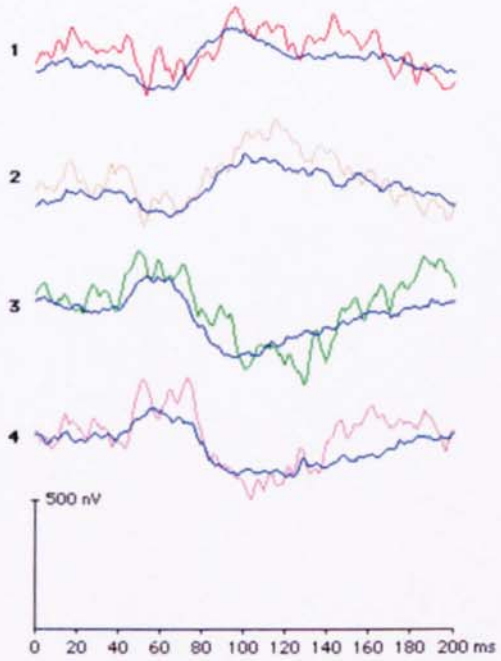
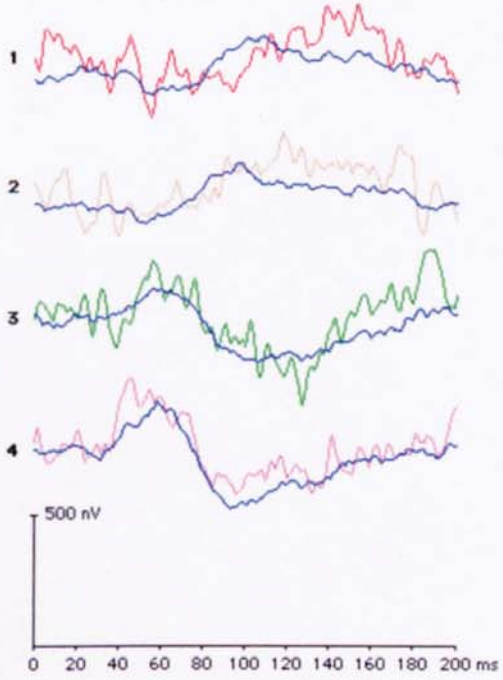


Figure 7.22
Outer quadrant averages from normal subject SW showing a good correlation and normal subject JR showing poorly correlated responses when compared to the normal database.

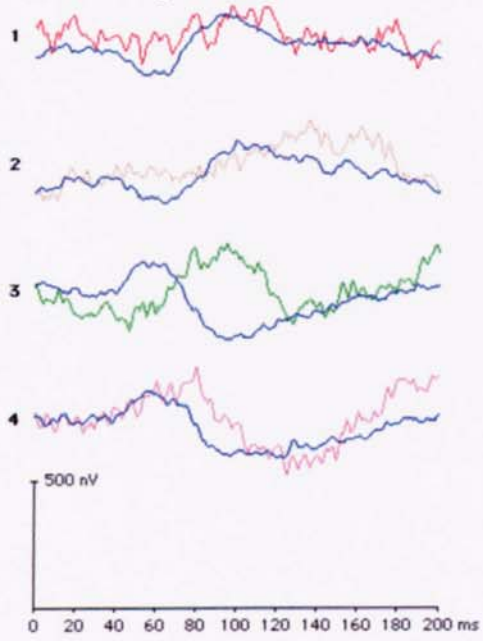
(a) SW Left eye



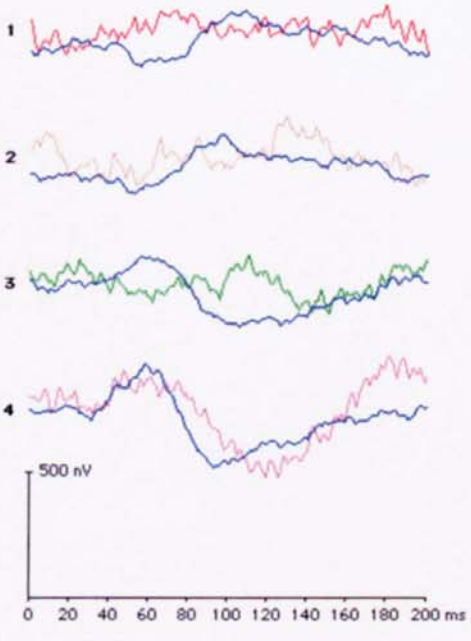
(b) SW Right eye



(c) JR Left eye



(d) JR Right eye



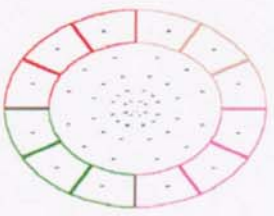
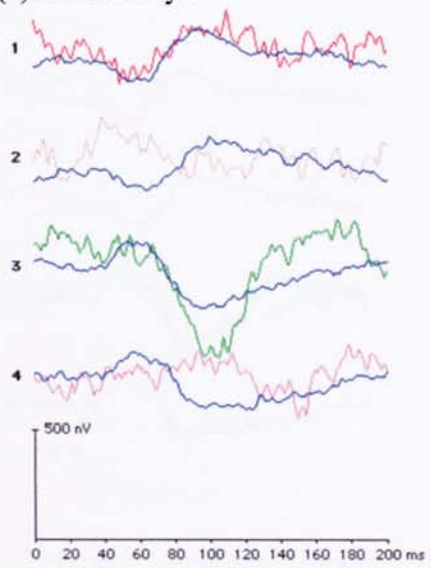
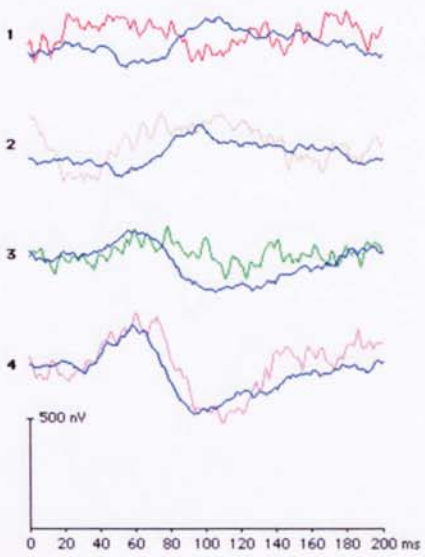


Figure 7.23
Outer quadrant averages from vigabatrin patients NH and MG (both with a confirmed vigabatrin-associated visual field loss) showing reduced responses from the nasal visual field when compared to the normal database (seen in blue).

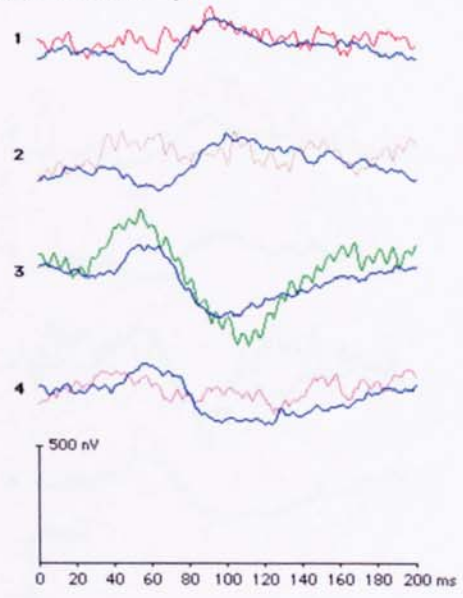
(a) NH Left eye



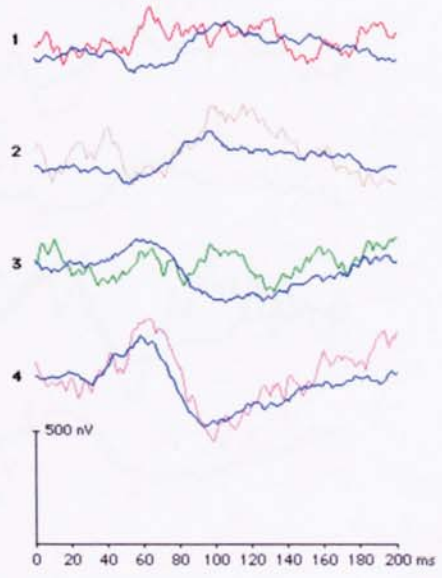
(b) NH Right eye



(c) MG Left eye



(d) MG Right eye



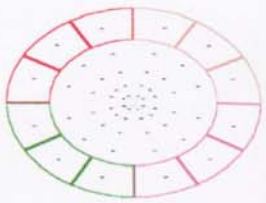
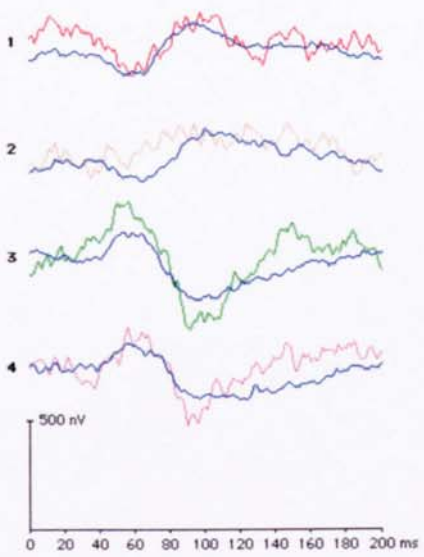
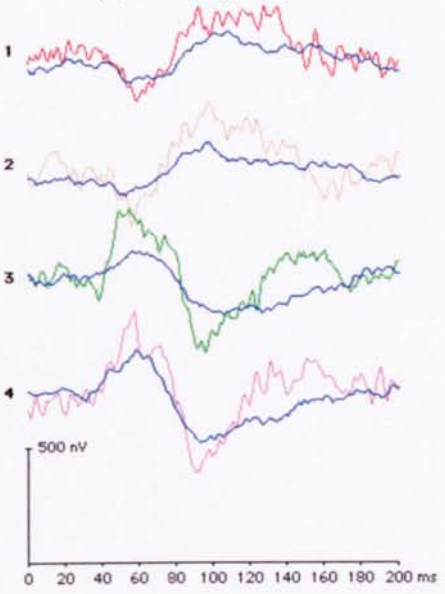


Figure 7.24
Outer quadrant averages from vigabatrin patient PA and FT (both showing no defect when tested with perimetry) showing normal responses from both visual fields when compared with the normal database (seen in blue).

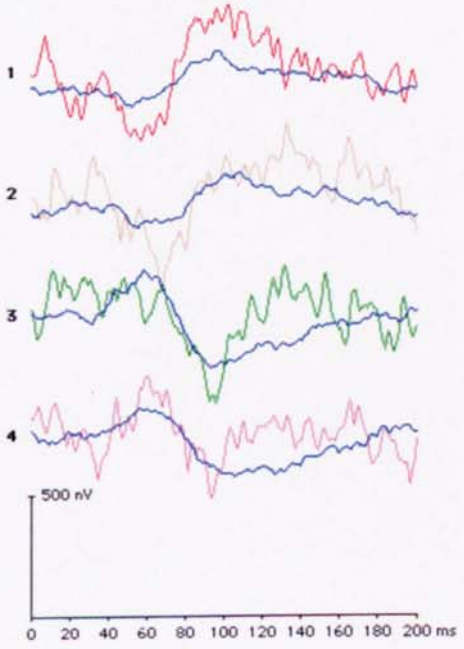
(a) PA Left eye



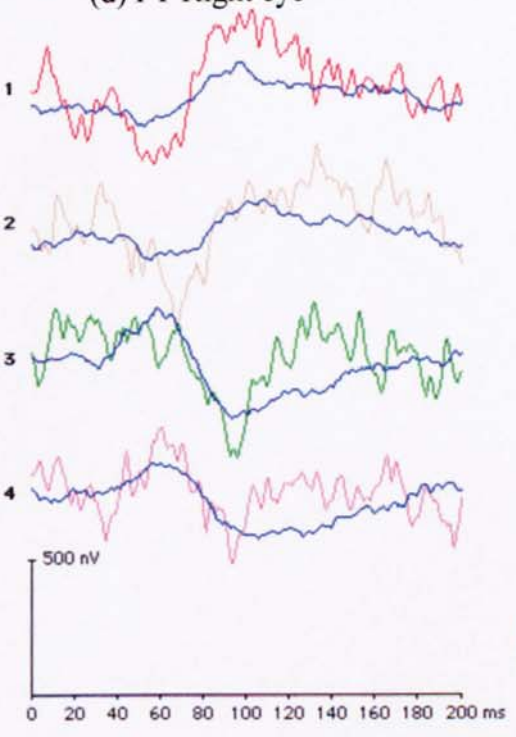
(b) PA Right eye

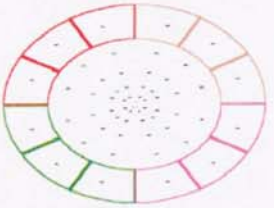


(c) FT Left eye



(d) FT Right eye





The three latency points of the triphasic waveform and the differences between the normal group, and vigabatrin patients with and without a defect were investigated. A one-way ANOVA was used to determine if the latency of the full quadrant responses were significantly different between the three groups. Table 7.2 shows the significant differences that occurred in the full quadrant latencies between the normal group and the vigabatrin group with and without a defect. The post-hoc Scheffe test revealed exactly where those differences occurred and it can be seen from table 1 that the upper left latency 1, the upper right latency 1, the lower left latency 2 and 3 and all of the lower right latencies differed in the vigabatrin group with a defect when compared to the normal control group. However, no such difference was seen between the vigabatrin no defect group and the vigabatrin defect group indicating that this measurement is not sensitive at identifying those patients with or without a visual field defect.

A one-way ANOVA was used to determine if the latency of the outer quadrant responses were significantly different between the three groups. Table 7.3 shows the significant differences that occurred in the outer quadrant latencies between the normal group and the vigabatrin group with and without a defect. The post-hoc Scheffe test revealed exactly where those differences occurred and it can be seen from table 2 that the upper left latency 1 and 3, upper right 1 and 3 and the lower right latency 1 and 3. Upper right 1 and lower right 1 latencies where found to be different between the vigabatrin patients with a defect and those without a defect.

Table 7.2
Shows the means and standard deviations of the latency taken from each full quadrant.

Quadrant	Normal mean±SD	Vigabatrin – no defect mean±SD	Vigabatrin – defect mean±SD
Upper left latency 1	67.67±5.6	68.50±7.8	71.67±7.5**
Upper left latency 2	95.72±7.33	97.08±3.8	99.05±5.7
Upper left latency 3	123.60±9.6	132.87±9.7**	126.35±10.8
Upper right latency 1	68.65±6.9	70.32±8.6	75.47±7.5*
Upper right latency 2	95.92±7.1	100.48±7.7	99.87±7.2
Upper right latency 3	124.08±10.6	130.40±15.8	125.85±8.4
Lower left latency 1	63.64±13.7	66.20±4.4	69.68±6.7
Lower left latency 2	87.59±13.2	100.09±5.7*	101.35±7.1*
Lower left latency 3	118.60±10.5	135.81±12.8*	131.91±8.1*
Lower right latency 1	65.50±11.7	68.65±6.1	72.57±8.5**
Lower right latency 2	89.18±11.4	99.91±8.7*	101.50±7.5*
Lower right latency 3	116.93±12.6	133.62±14.4*	129.44±10.8*

*significantly different from normal at 0.01 level
**significantly different from normal at 0.05 level

Table 7.3
Shows the means and standard deviations of the latency taken from each outer quadrant.

Quadrant	Normal mean±SD	Vigabatrin – no defect mean±SD	Vigabatrin – defect mean±SD
Upper left latency 1	65.87±10.1	71.18±13.1	78.07±13.6**
Upper left latency 2	98.17±10.1	104.56±13.0	104.71±21.7
Upper left latency 3	130.85±13.1	138.90±14.6	143.81±9.0**
Upper right latency 1	67.44±8.0	72.63±8.7	81.41±12.9*†
Upper right latency 2	96.23±7.8	103.74±8.8	100.37±23.4
Upper right latency 3	130.64±12.5	139.54±14.8	139.70±9.7**
Lower left latency 1	67.53±12.4	69.96±12.9	74.95±17.2
Lower left latency 2	102.3±11.5	103.1±12.5	107.62±16.2
Lower left latency 3	131.84±23.5	143.56±9.7	140.77±16.6
Lower right latency 1	64.70±10.6	67.58±6.2	78.58±16.4*†
Lower right latency 2	98.34±12.6	105.68±9.3	111.62±21.4*
Lower right latency 3	132.23±16.5	138.71±17.4	143.12±17.2

*significantly different from normal at 0.01 level
**significantly different from normal at 0.05 level
†significantly different from vigabatrin no defect at 0.05 level

In order to determine exactly were these differences occurred the latency measurements from the outer quadrants were examined between the eyes in order to establish if the differences occurred between the temporal or nasal portion of the visual field. A one-way ANOVA was used to determine if the latency of the outer quadrant responses in both eyes were significantly different between the three groups of normals, vigabatrin patients with no visual field defect and vigabatrin patients with a visual field defect. The left eye data revealed a significant difference between all the upper left latencies (Latency 1: [$F(2,35)=7.727;p<0.05$], Latency 2 : [$F(2,35)=5.338;p<0.05$], Latency 3 :[$F(2,35)=4.220;p<0.05$] the first upper right latency (Latency 1: [$F(2,35)=7.292;p<0.05$]) and the first and second lower right latencies(Latency 1: [$F(2,35)=9.715;p<0.01$], Latency 2: [$F(2,35)=4.879;p<0.05$]. Post-hoc analysis revealed the differences to be between the normal subject group and the vigabatrin group with a visual field defect. These differences revealed a significant increase in latency in the superior nasal and temporal responses as well as the inferior nasal quadrant. The right eye data revealed a significant difference between the first and third upper right latency (Latency 1: [$F(2,35)=5.403;p<0.05$], Latency 3: [$F(2,35)=7.218;p<0.01$]) and the first lower left latency (Latency 1: [$F(2,35)=3.357;p<0.05$]). Post-hoc analysis revealed the differences to be between the normal subject group and the vigabatrin group with a visual field defect. These differences revealed a significant increase in latency in the superior temporal and the inferior nasal quadrant.

Due to the small nature of the mVEP responses, the amplitude of responses where examined using the numerical data equations. A one-way ANOVA was used to determine if any of the numerical parameters were significantly different between the three groups of normals, vigabatrin patients with no visual field defect and vigabatrin patients with a visual field defect. The 2rSNR was found not to be significantly different between the groups [$F(2,33)=0.466$; $p = 0.632$, NS]. The nwSNR was found to be significantly different between the three groups [$F(2,68)=10.09;p<0.01$]. Post-hoc analysis revealed these differences to be between the normals that had a larger nwSNR value than the vigabatrin no defect and vigabatrin defect patients, whereas no significant differences were found between vigabatrin no defect and vigabatrin defect. The non-parametric

Kruskal-Wallis test confirmed the parametric test that results were not the same in all three groups [$\chi^2(2)=14.63;p<0.01$]. The mnSNR also showed a significant difference between the three groups [$F(2,69)=16.31;p<0.01$]. Post-hoc analysis revealed the differences to be between the normal group, which had a larger mnSNR value than both the vigabatrin no defect and vigabatrin defect group, whereas no significant difference was found between the vigabatrin no defect and vigabatrin defect group. The non-parametric Kruskal-Wallis test confirmed the parametric test that results were not the same in all three groups [$\chi^2(2)=22.54;p<0.01$].

Using the log parameter revealed no significant difference between any of the three groups [$F(2,32)=0.054;p=0.947$, NS]. Using the scalar product showed a significant difference between the three groups [$F(2,101)=30.74;p<0.01$]. Post-hoc analysis revealed the differences to be between the normal group which had a larger scalar product value than both the vigabatrin no defect and vigabatrin defect group, whereas no significant difference was found between the vigabatrin no defect and vigabatrin defect group. The non-parametric Kruskal-Wallis test confirmed the parametric test that results were not the same in all three groups [$\chi^2(2)=47.58;p<0.01$]. Table 7.4 shows the mean and standard deviation of each of the numerical products examined and shows which product was significantly different from the normal database.

Table 7.4
Shows the means and standard deviation of all the numerical products in the three groups examined.

Numerical product	Normal mean±SD	Vigabatrin – no defect mean±SD	Vigabatrin – defect mean±SD
2rSNR	2.0312±0.527	2.1275±0.276	2.1850±0.335
nwSNR	0.9146±0.505	0.4146±0.398*	0.4890±0.249*
mnSNR	0.9905±0.466	0.3977±0.389*	0.4880±0.249*
Log	-0.0097±0.06	-0.0018±0.05	-0.0039±0.06
scalar	$6.75\times10^{-9}\pm3.71\times10^{-9}$	$2.93\times10^{-9}\pm2.12\times10^{-9}$*	$1.97\times10^{-9}\pm1.10\times10^{-9}$*

*significantly different from normal at 0.01 level

Whilst attempting to determine just how useful the mVEP test may be identifying visual loss in vigabatrin patients, it is useful to compare such results with other measures. All patients involved in this study gave results in either some or all of the tests, including the field-specific VEP, the ERG and visual field testing (table 7.5). All subjects (with the exception of one) could provide reliable mVEP responses had a normal H-Stimulus response with abnormal mVEP measures. Similarly there was no consistency when comparing ERG results with the mVEP responses. By comparing the mVEP measures with the perimetry results it was possible to determine the sensitivity and specificity of each of the measures (table 7.6). As expected the 2rSNR values had a poor sensitivity as all of the patients had a value within the normal range regardless of whether a field defect was present or not. The nwSNR values proved to have a better sensitivity of 62.5% indicating that this measure is more useful at identifying those patients with or without a defect. The mnSNR had a similarly useful sensitivity although the specificity of the test was somewhat lessened by the fact that only 50% of patients with normal perimetry results had a value within the normal range. The use of the log values revealed both a useful sensitivity and specificity to the test as the majority of results that fell both within and outside the normal range were identified. The scalar product however, provided a useful specificity identifying three out of the four patients with normal fields although only two of the eight abnormal fields were identified.

Table 7.5
Comparison of mVEP test results compared to previous H-Stimulus, ERG and visual field results.

Patient	Gender	Age (years)	H-Stimulus	ERG	Visual Fields	mVEP measures outside the normal range
1	Male	12	Normal	Normal	Abnormal	200, scalar
2	Male	14	Normal	Normal	ND	Unable to comply with the test procedure
3	Female	19	Normal	Abnormal	Normal	200
4	Male	10	Normal	Normal	ND	200
5	Male	15	Normal	Abnormal	ND	nwSNR, mnSNR, 200
6	Female	11	Abnormal	Normal	ND	200, scalar
7	Female	19	ND	Abnormal	Abnormal	nwSNR, mnSNR, 200 (RHH)
8	Female	16	Normal	Normal	Normal	
9	Male	25	ND	Abnormal	Abnormal	
10	Female	31	ND	Abnormal	Abnormal	200
11	Female	29	ND	Abnormal	Abnormal	nwSNR, mnSNR, 200
12	Female	24	ND	Abnormal	Abnormal	Unable to comply with the test procedure
13	Female	43	ND	Abnormal	Abnormal	nwSNR, mnSNR, 200
14	Male	47	ND	Abnormal	Abnormal	nwSNR, mnSNR, scalar
15	Female	51	ND	Normal	Normal	mnSNR, scalar
16	Female	48	ND	Normal	Normal	mnSNR
17	Female	20	Normal	Normal	ND	Unable to comply with the test procedure
18	Male	52	ND	Abnormal	ND	
19	Female	35	ND	Abnormal	ND	nwSNR, mnSNR, 200, scalar
20	Male	46	ND	Normal	Abnormal	nwSNR, mnSNR, 200

Key: ND; test not performed

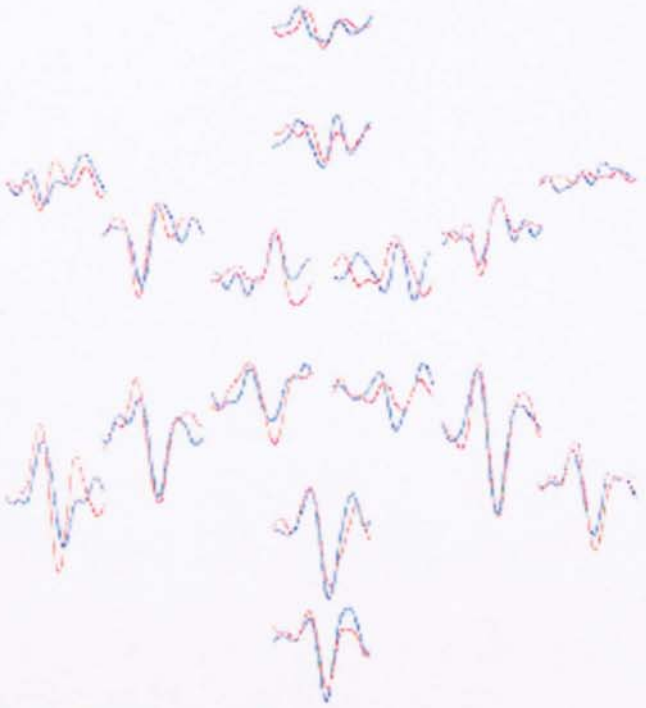
Table 7.6
Shows the sensitivity and specificity of each mVEP numerical value in vigabatrin patients when compared to perimetry results

Test		**Adults**
Sensitivity 2rSNR	$\frac{Abnormal2rSNR}{AbnormalPerimetry}$	$\frac{0}{8}=0$
Specificity 2rSNR	$\frac{Normal2rSNR}{NormalPerimetry}$	$\frac{4}{4}=100\%$
Sensitivity nwSNR	$\frac{Abnorma\ln wSNR}{AbnormalPerimetry}$	$\frac{5}{8}=62.5\%$
Specificity nwSNR	$\frac{Norma\ln wSNR}{NormalPerimetry}$	$\frac{4}{4}=100\%$
Sensitivity mnSNR	$\frac{AbnormalmnSNR}{AbnormalPerimetry}$	$\frac{5}{8}=62.5\%$
Specificity mnSNR	$\frac{NormalmnSNR}{NormalPerimetry}$	$\frac{2}{4}=50\%$
Sensitivity 200	$\frac{Abnormal200}{AbnormalPerimetry}$	$\frac{6}{8}=75\%$
Specificity 200	$\frac{Normal200}{NormalPerimetry}$	$\frac{3}{4}=75\%$
Sensitivity scalar	$\frac{Abnormalscalar}{AbnormalPerimetry}$	$\frac{2}{8}=25\%$
Specificity scalar	$\frac{Normalscalar}{NormalPerimetry}$	$\frac{3}{4}=75\%$

Superimposing the traces over each eye allows for the direct comparison of the mVEP traces from both eyes. It should be noted that the right side of the array corresponds to the right side of the patients visual field and the left side corresponds to the left side of the patients visual field. Therefore the nasal right eye lies over the temporal left eye since they both correspond to the patients left visual field. Examining 120 traces (60 traces from each eye) was difficult to assess and so traces were collated into 16 groups as described by Hood et al (2000a). Figure 7.25 shows the 16 mVEP groups from patients CM and NH. CM had normal visual fields shown by perimetry indicating that no vigabatrin associated visual field loss had occurred. When examining the 16 mVEP traces no clear difference in amplitude or latency was seen between the right and left eye traces. In contrast, the mVEP traces from patient NH who showed a vigabatrin associated visual field loss by perimetry, showed a difference in the traces from the right and left eyes. More specifically the outer left side section of the right eye which corresponds to the nasal visual field is reduced in amplitude and a shift in latency is seen compared to the left eye responses and the outer right side section of the left eye which corresponds to the nasal visual field is reduced in amplitude and a shift in latency is seen compared to the right eye responses.

Figure 7.26 shows a comparison of interocular difference fields with the left-hand column showing the 30-2 Humphrey and the right-hand column showing the mVEP results. A black square indicates that the response ratio is within 2 SD of the control values. The coloured squares indicate that the response ratio was more than 2 SD (lighter colour) or 3 SD (darker colour) from the mean of the controls. The colour denotes whether the right (blue) or left (red) had the larger response. Grey boxes denote small signals recorded. In the case of CM, the Humphrey fields are within normal limits and the mVEP responses generally support this although a number of small responses were recorded. In the case of NH, abnormal responses in the Humphrey fields were recorded in the nasal visual fields of both eyes and these changes were also seen in the mVEP fields, although a number of small responses were also recorded in this patient.

Figure 7.25
The grouped mVEPs from the right eye (blue) and the left eye (red) of two patients (a) CM who has no vigabatrin associated visual field loss and (b) NH who has vigabatrin associated visual field loss.
(a) Vigabatrin patient CM with no visual field loss



(b) Vigabatrin patient NH with visual field loss

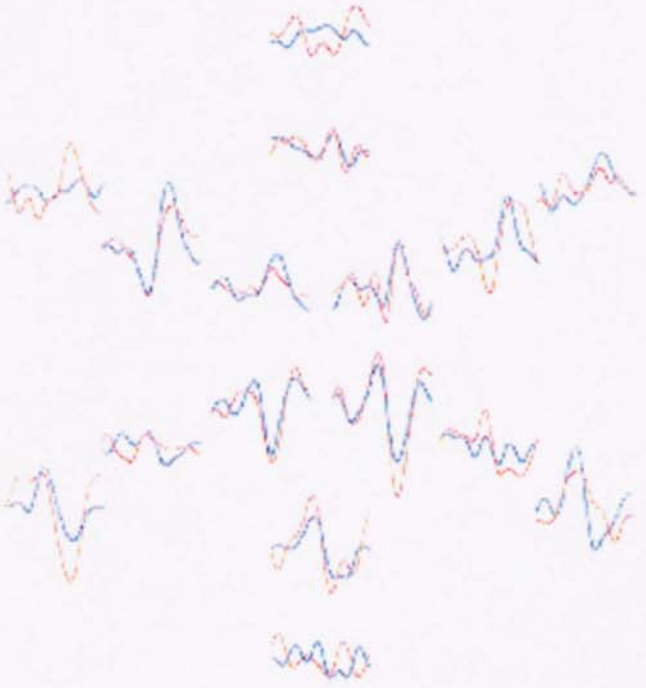
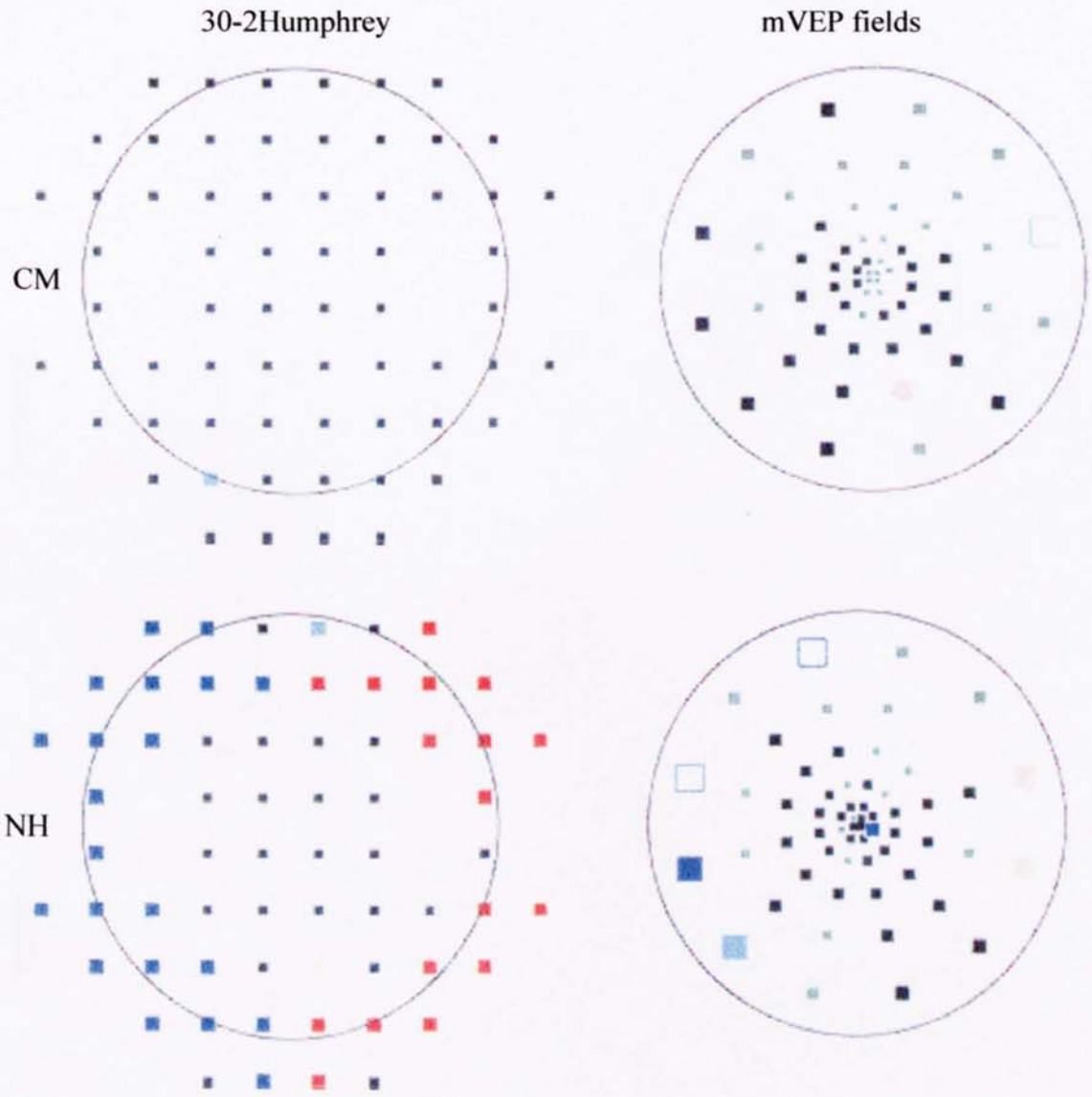


Figure 7.26
A comparison of interocular difference fields showing the 30-2 Humphrey (left hand side) and mVEP fields (right hand side) of two patients. CM has no vigabatrin associated visual field loss and NH has vigabatrin associated visual field loss.



7.3.3 Discussion of vigabatrin study

Non-compliance with the mVEP test procedure did occur with three patients. In two of the cases the patients (2 and 17) had learning difficulties and so were unable to maintain the required level of concentration for the test. In these cases the patients have also never been able to give reliable perimetry results. In the third case, the mVEP stimulus made the patient (12) feel nauseous and so the test was abandoned. This patient has performed perimetry in the past and so in this case perimetry would be the preferred test to perform for this patient.

One of the great advantages of the multifocal VEP is that the test produces a large amount of data that can be analysed in a variety of ways. However, it is clear that not all methods of analysis will be appropriate for all clinical situations. When examining the traces of vigabatrin patients it was clear that those with a confirmed vigabatrin associated visual field loss did not show a specific loss of VEP waveforms in any particular area. Indeed responses were reduced in those patients with a defect but in a non-specific manner. Clearly it was necessary to group the traces together in a manner that would accentuate the loss of VEP responses. Initially full quadrants were grouped together and these responses were shown to be the same in patients with and without a defect. Similarly trace responses grouped together into central and peripheral sections also showed no difference between the two sets of patients. It was the use of outer quadrants that revealed a difference between the responses from vigabatrin patients with and without a visual field defect. This can be explained as patients with a vigabatrin associated visual field defect generally have well maintained central vision as shown by standard VEP testing (Harding et al, 1995a). If full quadrants were grouped together the responses would remain within normal limits as the central vision would accentuate the response. When examining only the outer quadrants, central vision is not being tested and so the visual field defect, which occurs predominantly in the peripheral vision in a binasal defect with relative temporal sparing, is more likely to be revealed.

The latency of both the full quadrants and the outer quadrants were investigated. The full quadrant analysis revealed some differences occurred between the latencies of normal

subjects and vigabatrin patients with a visual field defect. In spite of this no such difference was seen in the latencies of patients with or without a defect indicating that examining the full quadrant latencies would not be sensitive enough to identify those patients with or without a defect. The outer quadrants were investigated revealing differences in latencies between normal subjects and vigabatrin patients with and without a defect. As it appeared that the outer quadrant latencies may be more sensitive to detect vigabatrin patient with and without a defect, the results were more extensively examined to establish were the differences were present. In both eyes the inferior nasal latency was significantly delayed in the vigabatrin patients that exhibited a visual field loss. The left eye also showed a significant increase in the superior nasal latency. Such results can be explained as the vigabatrin defect affects the nasal visual field predominantly. However the temporal visual field is also affected in the mVEP responses in the superior temporal quadrants of both eyes supporting the fact that the defect also displays only relative temporal sparing. The latency differences seen in the vigabatrin patients reflect a small difference in the time it takes the signal to arrive at V1 from the nasal retina of those patients affected by vigabatrin.

When examining the traces of mVEP responses it is evident that in some subjects they are very small, and in the majority of cases this is not due to abnormal vision but is related to the complicated infoldings of the visual cortex. Additionally distinguishing mVEP responses from noise can also be a difficult problem to overcome. It is therefore useful to perform an analysis, which incorporates estimating noise level and calculates a signal-to-noise ratio, rather than examining the amplitude of responses. Examining the SNR from two different runs (2rSNR) was not useful, in that results did not differ between the normal database and vigabatrin patients. Zhang et al (2002) acknowledged that the 2rSNR was perhaps not the best method for rejecting noisy records as any source of noise, such as alpha, that occurs frequently may appear in phase and so produce a large value whereas if the alpha is out of phase a smaller value would be obtained. Additionally when using the normal range of the 2rSNR values, a poor sensitivity and specificity was gained indicating that this value would not identify which vigabatrin patients had a visual field loss.

In order to overcome this problem the nwSNR utilises a period of noise to gain a value. However this value may also produce more or less false negatives, depending on if an alpha phase is in or out of phase. In this case the nwSNR values for vigabatrin patients were significantly different to those from the normal database, but the values were not different between those vigabatrin patients with a defect and those without a defect. When comparing the values with perimetry results, the nwSNR produced a useful sensitivity and specificity measure to identify visual field loss. The mnSNR, which utilises one estimate of noise level for the entire set of records, is thought to give a more reliable estimate of noise and hence a more accurate SNR value is obtained. The mnSNR values differed significantly between the normals and vigabatrin patients, but no difference was seen between those patients with a defect and those without a defect. The specificity of this measure was lessened as an abnormal value was seen in 50% of patients that had normal visual fields.

The log values calculated did not show a significant difference between the normal subjects and the vigabatrin patients. This result is perhaps expected as the loss of visual field function occurs systemically and so if a reduction in mVEP responses were seen then it would occur in both eyes hence the log value would not differ as it is calculated by the difference between the two eyes. Despite this fact using the normal range of log values identified three quarters of normal and abnormal perimetry results thus providing a useful sensitivity and specificity of the measure. Examining the scalar product did reveal a difference between the normal group and the vigabatrin group but this value was not different between those with a visual field defect and those without a defect. At present the mnSNR is thought to provide the best analysis by reducing the rate of false positives (Zhang et al, 2002) but in the cases of the vigabatrin patients it was not sensitive enough to detect those patients with a visual field defect and those without. Indeed the sensitivity of the test when compared to abnormal perimetry results was very low.

Many studies have been conducted which directly compare perimetry and mVEP responses. A good correlation between mVEPs and perimetry have been shown in subjects with visual field defects, including an upper nasal quadrantopia, optic atrophy,

glaucoma and scotoma (Klistorner et al, 1998, Hood et al, 2000a). However results from these studies indicate that hemifield responses can be more informative than full-field responses, which can give a misleading impression of conductivity delay due to a full-field cancellation effect. Klistorner & Graham (1999) expanded on this fact and concluded that multifocal recordings should be grouped as sectors along the vertical meridian and above and below the horizontal, as opposed to using hemifields or quadrants.

Further studies have compared perimetry results and mVEP results more directly. Iso degree contours can be drawn onto the results and compared to the probability points from the visual field. The interpolated visual field has also been used in which the sensitivity loss in each area is estimated. Such extensive comparison of visual field results and mVEP results was performed during this study on a limited few patients. The analysis software, described in Hood et al (2000a), allowed for the comparison of the mVEP field and the visual field. Firstly, comparing the 16 grouped traces from the vigabatrin patient with no visual field defect showed no difference between the eyes, whereas the patient with a visual field loss showed a difference between the eyes in the nasal visual field. Once the visual field and the mVEP responses are compared more directly using the interpolation technique these results are further exemplified and the two types of information displayed an agreement. However, it was also clear that in some areas the visual field showed a difference between the two eyes whereas the mVEP did not and vice versa, a result that has been shown in other studies such as Hood et al (2000b). One drawback of using this analysis technique is that damage to the corresponding points of the nasal visual field of one eye and the temporal visual field of the other eye would go unnoticed. However, in the case of vigabatrin patients the nasal visual field is affected in both eyes and so this difference is seen in the analysis.

The use of interpolated visual fields has been shown to be useful in these selected vigabatrin patients and obviously it would be of benefit to explore this technique in more patients exposed to this drug. However the mVEP data collected was not performed using the same equipment that performed the analysis and hence the exact testing conditions

were not the same as those used for the normal database making an exact comparison difficult. Additionally, the mVEP potentials recorded using the present system contained some noisy records and small VEP potentials in areas as highlighted by the interpolated analysis. Evidently the recording and analysis system described in Hood et al (2000a) is more sophisticated allowing for the recording of up to six channels using various different electrode positions. The use of additional channels has clear advantages (Hood et al, 2002). Using this technique the best channel responses are analysed and so the inclusion of small and noisy signals is reduced. Such extra features were not available during the recording of this data and so as a consequence the analysis of the results were not as robust as they could have been. However the comparison of mVEP fields with visual fields represents an objective and quantitative identification of monocular field defects and such an analysis technique may prove particularly useful for identifying vigabatrin-associated visual field loss. However, the availability of reliable visual field results is tantamount to this type of analysis being a success and so in the case of vigabatrin patients this may not always be available.

To compare mVEP responses to perimetry results is useful, as perimetry remains the gold standard test for examining visual fields. However as visual field assessment is a threshold measure compared to mVEP being a suprathreshold measure summed over numerous cells of different types, a number of factors need to be considered. Visual field changes which are measured physiologically may not always agree with the behavioural techniques of perimetry which is determined by the activity of a subset of cells, namely those that are most sensitive as compared to the activity of all cells activated in the mVEP. Additionally, the activity of damaged cells may not be detectable in the mVEP.

Unfortunately the relationship between retinal problems and mVEP responses is not clear. Previous work suggests a decrease in amplitude of mVEP responses is related to local damage to the ganglion cell and/or optic nerve (Hood et al, 2000b), as the decrease in amplitude is correlated with the degree of visual field loss in patients with glaucoma (Zhang & Hood, 2000). The relationship between latency and visual field loss is less clear as an increased latency appears to be related to the visual field obtained at onset but

does not necessarily reflect the concurrent visual field. Additionally, the nature of the stimulus, being of high contrast with a fast frame rate implies that both p-cells and m-cells are both stimulated therefore it is difficult to determine the exact cause of an abnormal result. The latency differences found in vigabatrin patients with a defect indicate a slower conduction of impulses in the inferior and superior nasal quadrant although the superior temporal impulses were also affected.

As yet, the exact relationship between standard VEP responses and mVEP responses has not been clarified. Barber & Wen (2000) used the high degree of spatial selectivity of the multifocal stimulation to differentiate the components of the standard VEP. Recording a pattern-onset/offset VEP to a checkerboard pattern using both standard and multifocal techniques, the standard VEP responses exhibited a large inter-subject variation. The mVEP responses showed that this was due to dominance of the response from a particular area of the visual field. Using the individual mVEP responses, the spatio-temporal properties of individual peak components could be charted and utilised to describe the dissimilar VEP responses. Wang et al (2001) expanded on this work by exploiting the multifocal visual evoked magnetic field (mVEF) in order to overcome the problem of recording the more tangential dipoles found in the periphery, which are not preferentially recorded using standard VEP recording. Indeed, larger mVEFs than mVEPs were recorded for more peripheral stimulation confirming that dipole orientation is an important factor in weak peripheral VEPs and mVEPs. In this manner, mVEFs is suitable for overcoming this problem in the future and may lend help to deciphering and dissecting the standard VEP in relation to the mVEP.

This study has shown that the mVEP test has the potential to be a useful clinical test that can be used for a variety of patients A possible improvement to the mVEP test procedure could include the use of a fundus-camera which allows for the precise control of the location of the stimulus on the retina and therefore monitors the patients fixation. In terms of vigabatrin patients it would be ideal to increase the stimulus size in order to examine the mVEP responses from the periphery. A multifocal test procedure has been developed with a projector to ensure a large stimulus size but at present this has only

been used for multifocal ERGs (Keating et al, 2000). Perhaps more simply, the patient could have been brought closer to the stimulus screen but then problems arise with accommodation unless a refractor is attached in order to refract the patient automatically. Future work on the mVEP test could include analysing separately the M and P pathways in vigabatrin patients by examining the second-order first and second slice responses. A high contrast black and white stimulus preferentially stimulates the M pathway, whereas a low contrast red and green stimulus preferentially stimulates the P pathway. At present the test can be used on vigabatrin patients and outer quadrants should be examined both by examining the traces themselves and by measuring the latencies. Using this information may be useful to predict whether a patient may have a vigabatrin associated visual field defect. Additionally more information may have been produced if the mVEP responses of patients with epilepsy not exposed to vigabatrin where compared with those patients receiving vigabatrin. Those receiving vigabatrin may have produced consistently different responses to those patients who had never received the drug.

7.4 Conclusion

With so many varying approaches to examining mVEP responses, it is clear that the best approach depends on the question that needs to be answered. Presently, it is unclear as to which are the optimal stimulus conditions for examining vigabatrin associated visual field loss. In this study examining the outer quadrant latencies and using the mnSNR values is the most sensitive measure of identifying those patients with a vigabatrin associated visual field loss. It is possible that the use of more patients, both exposed and not exposed to vigabatrin, may result in a more sensitive measure being developed. Additionally the progress of a field defect may be more closely followed using the mVEP responses and would be especially useful if the responses could indicate vigabatrin retinal toxicity before a visual field loss occurs. Certainly the use of interpolated visual fields and mVEP results may prove useful in future studies in which reliable perimetry results are attainable. The mVEP test procedure has the advantage of being objective, requiring minimum cooperation and no decision making by the patient and so for patients with epilepsy that need to have visual fields monitored the mVEP may be a useful alternative to standard visual field testing.

CHAPTER 8

INVESTIGATION INTO THE EFFECTS OF REMACEMIDE ON THE ELECTROPHYSIOLOGY OF VISION

8.1 Introduction

Despite a plethora of AEDs available to epilepsy sufferers, a need remains for additional safe and effective treatment of epilepsy. Indeed the search for the panaceas of epilepsy is far from over. Evidence has implicated that enhanced excitatory neurotransmission could be a useful target in epilepsy treatment. Glutamate is the major excitatory neurotransmitter in the central nervous system, which acts as the endogenous ligand for the numerous subtypes of glutamate receptors. The NMDA receptor complex has been investigated for its possible antiepileptic activity in animal models. However, such compounds have been found to be clinically unsatisfying as Upton (1994) stated that early clinical trials associated such drugs with little evidence of efficacy and a high incidence of adverse effects. Hence, focus has moved onto means of modulating NMDA function, such as using a low-affinity noncompetitive antagonist.

More promising results have been obtained from remacemide hydrochloride, which is a novel non-competitive NMDA receptor antagonist and fast sodium channel inhibitor. This mode of action results in the inhibition of the NMDA receptors located in the neuronal membrane calcium channels, which is thought to block Ca^{2+} influx mediated by the major excitatory neurotransmitters; such as glutamate and glycine. The overall results of such an action is to reduce cortical neuronal activity that has the potential of reducing seizures. Indeed, the drug has been found to be successful in treating refractory epilepsy. Additionally remacemide has also been shown to be useful in the treatment of Parkinson's disease. Consequently remacemide has been involved in a number of clinical trials in various countries.

Following on from the discovery that vigabatrin had the potential of causing visual field defects it followed that other AEDs were to be suspected of causing damage to vision. Although remacemide affects glutamate levels rather than GABA levels such as that

affected by vigabatrin, glutamate is present in the retina and so disturbances of this neurotransmitter may affect vision. Indeed, pre-clinical findings found remacemide to cause a dose-related retinal atrophy in Sprague-Dawley rats, as well as a drug-induced exacerbation of light induced ageing process of albino rats (Cano et al, 1986). Glutamate toxicity has been shown to induce apoptosis through the overaction of NMDA receptors. Amacrine cells express NMDA receptors, which are found in the inner retina and ganglion cells (Duarte et al, 1998) and so this mechanism may indicate how an increase in glutamate levels in the retina may cause a retinal toxicity.

Phase II clinical studies conducted by Astra Zeeneca, which included 770 patients with epilepsy and 440 patients with Parkinson's disease, 9% complained of diplopia and 8% complained of blurred vision. In all cases the symptoms were both mild and self-limiting. Additionally, all the patients with epilepsy had also been exposed to carbamazepine and/or phenytoin and such drugs have been linked to such problems previously. Overall, no evidence was found to indicate any systemic ophthalmological problems are associated with remacemide.

A phase III study of patients who had been exposed to vigabatrin for over nine months was deemed necessary in order to test the effects of remacemide on vision. However it was essential to utilise patients with epilepsy that had not been exposed to vigabatrin at any time, as a vigabatrin-associated visual field defect could mask other defects. In the UK this was difficult to achieve to as many took remacemide as an adjunctive or alternative to vigabatrin. It was therefore decided to find a population of patients with epilepsy that had not been exposed to vigabatrin, and these were located in Lithuania and Canada.

The main objectives of this study were to examine the electrophysiology of vision in patients with epilepsy exposed to remacemide, compare the electrophysiology results with perimetry and eye examination results and determine if remacemide affects vision.

8.2 Methods

Thirty-five patients were recruited with an age range of 19-72 years (mean age 39.8 ± 11.8 years). Informed consent from the subject, or legal representative if necessary, was obtained from all subjects. Each centre involved (Lithuania and Canada) had local ethical approval for the study in accordance with the ethical standards of the 1964 Declaration of Helsinki.

8.2.1 Ophthalmological assessment

An ophthalmologist carried out the ophthalmological assessment. Cycloplegic refraction was performed in both eyes and the conjunctivae, cornea, pupil and lens where all examined for abnormalities in appearance. The disc colour, cup disc ratio and any other disc abnormalities were noted. The macula, retinal vessels and retinal periphery were noted to be either normal or abnormal. Any patient found to have other eye problems that may have affected the electrophysiology or perimetry results were excluded.

8.2.2 Visual evoked potential

Visual evoked potentials were recorded according to the ISCEV standards for pattern reversal (Harding et al, 1995a). The pattern-reversal consisted of a checkerboard, each check of which subtended to a visual angle of 60′. The visual field subtended by the whole target was greater than 10°. A Minolta LS-110 photometer was used to calculate the luminance levels and the contrast, which was calculated using the Michelson's equation. The overall mean luminance of the white areas in the screen was 100 cd/m^2 and the contrast was 80%. The subjects head was measured according to the International 10-20 system of head measuring (Jasper, 1958). O2 and O1 were referred to Fz and Cz was used as the ground. Silver-silver chloride electrodes were attached to the subjects head with 10-20 conductive paste (Unimed Electrode Supplies) after rubbing with NuPrep (Unimed Electrode Supplies). Where necessary collodian adhesive (SLE Diagnostics) and blenderm tape (3M Medica) was used. Impedances were even and below 5 kohms. Electrodes were taped onto the subjects shoulders and an orthoptic eye patch (3M Medica) was placed over one eye. A technician monitored fixation and runs with poor fixation or lack of concentration were noted. 50 sweeps were recorded. To ensure

reproducibility of the responses, at least two trials were repeated and the process was repeated for the second eye. The visual evoked potential to both stimuli was recorded on the Synergy using a bandwidth of 1-30 Hz with a sweep speed of 500 ms. The negative N75 and the positive P100 peak latency were labelled. Amplitudes were measured from the preceding peak.

8.2.3 Electroretinogram

The ERG was performed according to ISCEV standards (Marmor & Zrenner, 1995). All subjects had pupils maximally dilated with 1% tropicamide (Chauvin Pharmaceuticals Ltd). DTL fibres were placed in the lower fornix of the eyelid of each eye and referred to a silver-silver chloride electrode at the lateral canthi of the respective eye. An electrode attached to the forehead served as a ground. The skin was cleaned before attaching the electrodes and impedances were ensured to be less than 5 kohms. A binocular mini-Ganzfeld stimulus (full-field) was used. The dome of each stimulus was visibly white and comprised of a multi LED stimulus that had a colour temperature of 7000k. Specially made goggles were used. Testing was performed binocularly with a fixation point. The stimulus strength was that of the standard flash between 1.5-3.0 cd/m^2. The standard flash had a maximum duration of 1 msec and was used for all stimulus conditions.

To record the photopic response the patient was light adapted for at least ten minutes using a background luminance of 17-34 cd/m^2 provided by the mini-Ganzfeld tubes. The response was recorded for an average of 12 standard flashes, which were delivered at a rate of two per second. The low and high frequency filters were set at 1-200Hz. Measurements were made of the latency of the a- and b-waves and the amplitude of the a-b response. The oscillatory potentials were recorded to a standard flash given under the light-adapted conditions similar to photopic responses. The low and high frequency filters were set at 100-300Hz to allow the recording of the potentials without the base-line drift of the ERG b-wave. Measurements of OP1, OP2, OP3 and the amplitude of OP1 and OP2 were made. To record the 30Hz flicker the same standard of flashes were presented at a rate of 30m per second with the same background illumination. The first few responses were discounted until stable conditions were reached. Measurements were made of the a-

and b-wave latency and the amplitude of the a-b response. The background illumination was then switched off and the patient was then dark-adapted for at least twenty minutes in total darkness. The low and high frequency filters were set at 1-200Hz. Single flashes were then delivered at a rate of less than one per second, until clear responses could be obtained without a blink artifact.

8.2.4 Electro-oculogram

The electro-oculogram measures the potential difference between a pair of surface electrodes positioned at the medial and lateral canthus of the eye while the eyes move through 30° between two points situated on the horizontal plane 15° on either side of a central fixation point. The stimulus was elicited from within a mini Ganzfeld bowl. The test was performed with the undilated eye using the Synergy machine. Amplitude measurements were taken every minute, firstly in the preadaptation phase to obtain a steady baseline. The dark trough to the 30° standard fixation movement was obtained during a 13 minute period of dark adaptation. The light peak was determined during a 12 minute light adaptation with the background intensity increased to 600cd/m^2. Original waveforms were recorded for ten seconds in each minute and manual measures of the amplitude of the potential during the period of alternating eye movements were recorded. The maximum amplitude of the light peak divided by the maximum amplitude of the dark peak was expressed as a percentage to give the Arden Index (Arden & Barrada, 1962).

8.2.5 Visual field testing

All visual field testing was carried out by one expert (JW). The Humphrey Field Analyzer (HFA) 750 was used for all visual field testing. The central visual fields were examined in both eyes using the HFA Full Threshold strategy using program 30-2. The peripheral field of both eyes was examined using the 135 Point field and the age corrected three-zone suprathreshold strategy (stimulus size III) of the HFA 750. The order each eye was tested in was designated at random and a rest period of ten minutes was allocated between eyes. All reliability indices were automatically assessed. If results were normal or non-compliance occurred then no further test was carried out. If the

results were abnormal or unreliable the test was repeated at another date. The results of this test were then categorised as normal, abnormal or unreliable.

8.3 Results

Patient details noted included remacemide history, other antiepileptic medication and relevant neurophthalmology history (table 8.1). Any patients with eye problems that may have affected the test results were excluded from the study. Twenty-four of the patients participated in all the tests, two (6849 and 4752) failed to attend for visual field examination and one (6868) failed to attend for electrophysiological examination. One patient (4768) could not perform the threshold central field examination and two patients (442 and 455) were unable to perform the threshold central field examination. One patient (446) was found to exhibit vigabatrin-associated visual field loss and was subsequently found to have received vigabatrin over a number of years.

8.3.1 Ophthalmological results

Despite many patients exhibiting abnormal ophthalmological findings, only two of these findings were inexplicable. Patient 4764 has right Sturge-Weber syndrome and most features are attributed to this although the glistening opacities in the right fundus cannot be accounted for. Also patient 4760 had a bilateral maculopathy with yellow deposits that was unexplained.

8.3.2 Electrophysiology results

VEP responses were recorded from both eyes and the N75 and P100 latency and amplitude were noted from the left and right occipital cortex. Results from three of the patients (6844, 6849 and 4753) were poor in that one or both eyes showed a reduced amplitude. However all the responses recorded were within the normal limits expected and so were deemed as normal. No patients showed abnormalities of the visual evoked potential which could be attributed to medical therapy. An example of a normal response can be seen in figure 8.1 and table 8.2 shows the mean and standard deviation of the VEP parameters measured.

Table 8.1
Remacemide patient details.

Subject	Age (years)	Remacemide current dose and length of treatment	Other antiepileptic medication	Relevant neuroophthalmology	Included in study?
4778	18	400mg, 15 months	SV		Yes
4761	40	400mg, 26 months	SV		Yes
6862	58	400mg, 15 months	SV		Yes
6866	43	200mg, 15 months	PHB		Yes
4768	55	400mg, 22 months	SV		Yes
4748	22	600mg, 20 months	TRILEP		Yes
6844	38	400mg, 16 months	SV, LTG		Yes
6849	33	800mg, 16 months	SV	Peripapillary atrophy. Peripheral lattice degeneration in left eye.	Yes – no perimetry results
6852	26	400mg, 16 months	LTG, TRILEP		Yes
4746	51	400mg, 23 months	CBZ		Yes
4741	28	400mg, 25 months	SV		Yes
4753	28	600mg, 16 months	TRILEP	Lattice inferiorly	Yes
4743	33	600mg, 16 months	SV		Yes
6850	23	800mg, 15 months	TRILEP	Left and right posterior corneal embryotoxan. Small left inferior chorioretinal scar.	Yes
4752	33	800mg, 15 months	TRILEP	Posterior corneal embryotoxan. Mild peripheral degeneration in right eye.	Yes – no perimetry results
4776	36	400mg, 25 months	TRILEP	Retrobulbar neuritis.	No
4764	21	400mg, 15 months	SV	Sturge-Weber with right secondary raised IOP and possibly glaucoma. Left homonymous hemianopia.	No
4777	53	400mg, 15 months	TRILEP	Hypermetropia outside inclusion criteria (left and right).	No
6868	38	400mg, 15 months	SV		Yes – perimetry only

Table 8.1 (continued)

4758	44	400mg, 16 months	CBZ	Left and right abnormal ocular motility.	No
4760	38	1000mg, 17 months	CBZ	Abnormal macular appearance, right and left tilted disc.	No
4750	54	1000mg, 17 months	CBZ	Right ?old CSR at macula with focal changes.	No
4744	72	600mg, 24 months	LTG, TRILEP	Anisocoria . Right sphincter and iris damage.	No
6853	40	400mg, 15 months	SV	Bilateral disc drusen.	No
449	56	1600mg, 75 months	CBZ		Yes
459	42	1600mg, 70 months	CBZ	Paraphoveal maculopathy.	Yes
446	49	1200mg, 74 months	CBZ, SV	Mild bilateral optic atrophy.	Yes
442	50	1400mg, 80 months	CBZ, SV		Yes
448	36	1200mg, 76 months	CBZ		Yes
457	35	1200mg, 72 months	CBZ		Yes
456	43	1600mg, 70 months	CBZ, PHT		Yes
455	44	1400mg, 70 months	CBZ		Yes
450	45	1400mg, 75 months	SV, PHT	Bilateral optic nerve palor.	Yes
475	29	1600mg, 70 months	CBZ, PRIM		Yes
464	39	2400mg, 80 months	CBZ, PHT	Bilateral peripapillary atrophy. Left homonymous hemianopia.	Yes

Table 8.2
Shows the mean and standard deviation of the VEP parameters measured.

	Measurement	Mean±SD
Left eye	O2 N75 latency (ms)	72.33±6.7
	O2 N75 amplitude (μv)	2.88±2.3
	O2 P100 latency (ms)	102.08±4.8
	O2 P100 amplitude (μv)	8.61±3.8
	O1 N75 latency (ms)	71.18±5.3
	O1 N75 amplitude (μv)	2.91±1.9
	O1 P100 latency (ms)	102.27±5.6
	O1 P100 amplitude (μv)	8.45±3.7
Right eye	O2 N75 latency (ms)	72.36±5.7
	O2 N75 amplitude (μv)	2.92±1.9
	O2 P100 latency (ms)	104.23±9.1
	O2 P100 amplitude (μv)	9.27±4.0
	O1 N75 latency (ms)	73.08±7.9
	O1 N75 amplitude (μv)	3.10±2.3
	O1 P100 latency (ms)	104.21±9.3
	O1 P100 amplitude (μv)	9.11±4.2

Table 8.3
Shows the mean and standard deviation of the photopic, scotopic and 30Hz flicker responses recorded from both eyes.

Test/measurement	Photopic ± SD		Scotopic ± SD		30 Hz Flicker ± SD	
	Left eye	Right eye	Left eye	Right eye	Left eye	Right eye
Latency a wave (ms)	14.4±1.9	14.3±1.8	23.1±1.4	23.4±2.1	14.4±2.1	14.0±2.7
Latency b wave (ms)	31.2±1.4	30.9±1.3	54.5±9.3	54.7±11.0	27.7±1.8	28.1±2.5
a-b wave amplitude (μv)	99.6±31.7	90.8±35.7	264.8±93	234.1±84	74.6±28.1	67.0±35.3

Table 8.4
Shows the mean and standard deviation of the oscillatory potentials recorded from both eyes.

Test/measurement	Left eye ± SD	Right eye ± SD
OP latency 1 (ms)	22.6±20.7	18.4±1.2
OP latency 2 (ms)	23.7±2.2	31.4±39.0
OP latency 3 (ms)	28.6±3.6	28.5±3.7
OP amplitude 1 (μv)	10.1±5.1	10.1±5.1
OP amplitude 2 (μv)	8.9±4.4	9.7±6.2

Table 8.5
Shows the Arden Index calculated for both eyes.

	Mean % ± SD	Minimum %	Maximum %
Left eye	261.6±72.6	154	444
Right eye	256.1±60.8	140	377

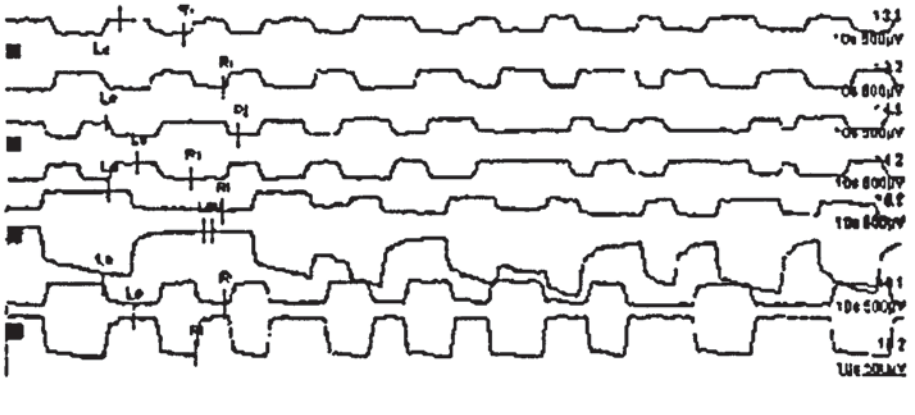
ERG measurements were recorded in 26 of the patients and the mean measurements were noted for each ERG parameter (tables 8.4 & 8.5). Of the twenty-six patients that produced ERG responses, eleven showed abnormalities of the 30Hz flicker as defined by the criteria used in the controlled study of vigabatrin (Harding et al, 2000a). A normal 30Hz flicker and photopic response can be seen in figure 8.2 and an abnormal 30Hz flicker with corresponding photopic response can be seen in figure 8.3. Seventeen patients (63%) had normal responses in all four of the tests.

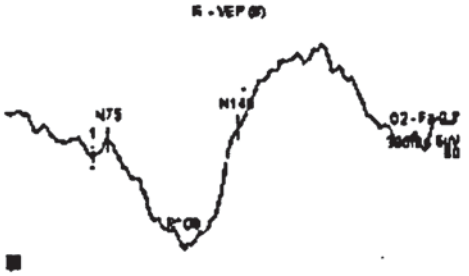
Twenty-five EOG results were obtained and the mean Arden Index, including the minimum and maximum, can be seen in table 8.6. Two patients were unable to provide responses. 20 (75%) of the patients had an Arden Index which fell within normal limits (>185%). Five of the patients had an Arden Index which was deemed abnormal, three having an Arden Index of less than 185% in both eyes (446,456,450), one having an Arden Index of less than 185% in the left eye only (455) and the other being a borderline case (patient 457 having an Arden Index of 186 and 191%). Five patients showed abnormalities of the Arden Index of the electrooculogram although the majority of results were within normal limits (figure 8.1).

8.3.3 Visual field results

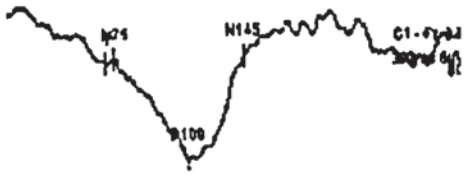
Twenty-three of the twenty-five patients who underwent peripheral field examination and twenty-two of the twenty-five patients who underwent central field examination exhibited results which lay within the accepted reliability criteria. None of the twenty-five patients exhibited a field defect which was not attributable to a known cause. One subject (446) exhibited a vigabatrin associated visual field loss, two exhibited a superior homonymous quadrantic defect secondary to temporal lobectomy (449 and 459) and one patient had an homonymous hemianopia (4764).

Figure 8.1
A normal set of results from a remacemide patient (a) Shows a normal EOG response in a patient and (b) shows a normal VEP response in a patient.
msec/ms denotes milliseconds, uv denotes microvolts.



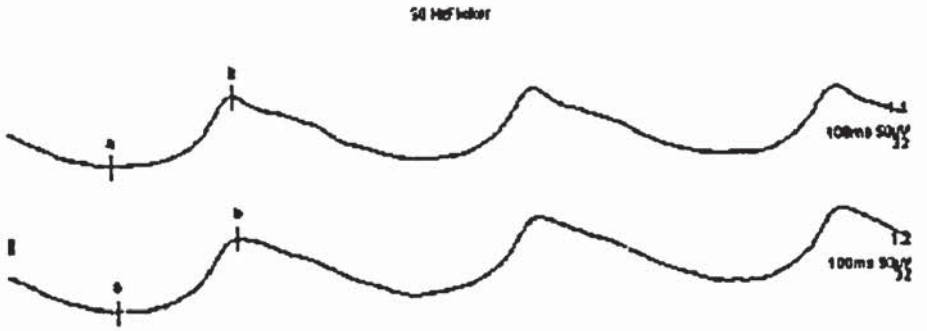


O2-Fz:
N75 latency 73.8 msec
P100 latency 116.7 msec
P100 amplitude 9.2 µv

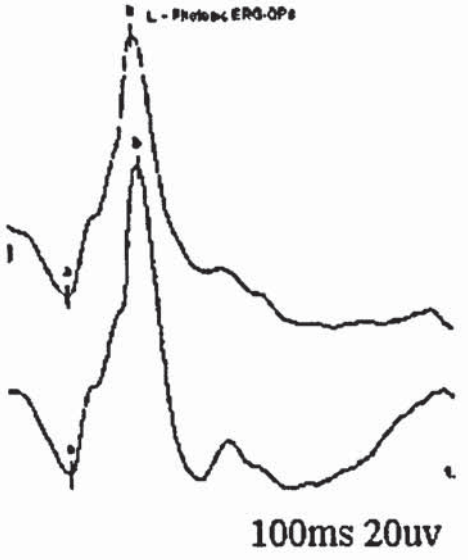


O1-Fz:
N75 latency 73.8 msec
P100 latency 116.7 msec
P100 amplitude 8.1 µv

Figure 8.2
ERG responses from a remacemide patient showing (a) a normal 30 Hz flicker responses in the right eye and left eye and (b) a normal photopic response in the same patient. msec/ms denotes milliseconds, uv denotes microvolts.

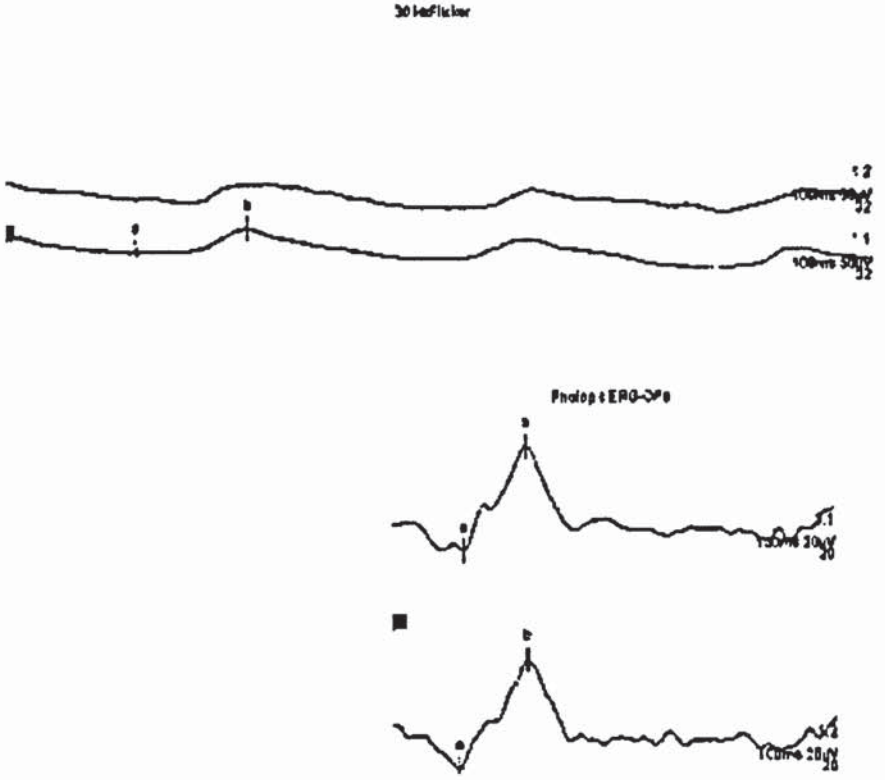


Right eye	
a wave	12.3msec
b wave	25.8msec
a-b amplitude	73.6μv
Left eye	
a wave	12.8msec
b wave	26.2msec
a-b amplitude	74.1μv



Right eye	
a wave	15msec
b wave	30.1msec
a-b amplitude	104.4μv
Left eye	
a wave	15msec
b wave	30.7msec
a-b amplitude	123.3μv

Figure 8.3
ERG responses from a remacemide patient showing (a) an abnormal 30 Hz flicker responses in the right eye and left eye and (b) an abnormal photopic response in the same patient.
msec/ms denotes milliseconds, uv denotes microvolts.



Right eye	
a wave	15.7msec
b wave	28.4msec
a-b amplitude	25.9μv
Left eye	
a wave	14.8msec
b wave	27.8msec
a-b amplitude	31.7μv

Right eye	
a wave	16.2msec
b wave	30.2msec
a-b amplitude	44.5μv
Left eye	
a wave	14.6msec
b wave	30.4msec
a-b amplitude	46.2μv

Table 8.6
Summary showing results of all the tests performed.

Subject	VEP	ERG	EOG	Perimetry
4778	Normal	30Hz abnormal	Normal	Normal
4761	Normal	Normal	Normal	Normal
6862	Normal	30Hz abnormal	Normal	Normal
6866	Normal	30Hz abnormal	Normal	Normal
4768	Normal	Normal	Normal	Normal
4748	Normal	Normal	Normal	Normal
6844	Normal – poor	30Hz abnormal	Abnormal	Normal
6849	Normal – LE reduced	30Hz abnormal	Normal	ND
6852	Normal	Normal	Normal	Normal
4746	Normal	30Hz abnormal	Normal	Normal
4741	Normal	30Hz abnormal	Normal	Normal
4753	Normal – RE reduced	Normal	Normal	Normal
4743	Normal	30Hz abnormal	Normal	Normal
6850	Normal	30Hz abnormal	Normal	Normal
4752	Normal	30Hz abnormal	Normal	ND
6868	ND	ND	ND	Normal
449	Normal	Normal	Normal	Normal
459	Normal	30Hz abnormal	Normal	Normal
446	Normal	Normal	Abnormal	Normal
442	Normal	Normal	Normal	ND
448	Normal	Normal	Normal	Normal
457	Normal	Normal	Normal	Normal
456	Normal	Normal	Abnormal	Normal
455	Normal	Normal	Abnormal	Normal
450	Normal	Normal	Abnormal	ND
475	Normal	Normal	Normal	Normal
464	Normal	30Hz abnormal	ND	Normal

8.4 Discussion

This study aimed at examining the effects of remacemide on the electrophysiology of vision. Additionally an ophthalmological examination and visual field results were examined to determine if remacemide may cause any visual problems. The results clearly show that a vigabatrin-type visual field loss is unlikely to be associated with remacemide, and undoubtedly not at the prevalence of vigabatrin-treated patients. In fact the only vigabatrin associated visual field loss seen in the study was found in the one patient that had been exposed to vigabatrin previously. The other visual field defects seen during the study were all attributed to known clinical causes which were unrelated to any AED therapy.

The VEP results were within normal limits for all the patients involved in the study. This indicates that the visual pathway, from the retina to the occipital cortex, had not been affected by remacemide in any of the patients. There were no consistent abnormalities of the EOGs, although 20% of the patients did have an abnormal or borderline abnormal Arden Index. It is at present unclear if this result is a significant finding or if the subnormal results occurred by chance. Interestingly all these abnormal results were from the same pool of patients in Canada. Two of the patients with an abnormal Arden Index had been shown to have existing eye problems, as patient 446 showed a mild bilateral optic neuropathy and patient 450 showed a possible bilateral optic nerve palor. Such problems may have accounted for their low Arden Index results.

The most consistent ERG abnormality found was that of a reduced amplitude 30Hz flicker response which was seen in 26% of patients. This type of defect implicates an abnormality of cone function and so this response tends to be dominated by macular function. In two of the cases the patient had no history of eye problems and so it is possible that drug toxicity could be the cause of the low amplitude 30Hz flicker. However in the remaining five cases eye problems had been noted which ranged from small choroidal scar (4778), early macular drusen (4746), parafoveal maculopathy (459) and posterior corneal embryotoxan (4746,4752). In this way it is possible that such problems mentioned may have caused the ERG defect. The other defects seen in the ERG

were the delayed oscillatory potentials and the abnormal photopic response but as these only occurred in one patient each this result is unlikely to be of any clinical significance.

Ophthalmological investigations revealed macular changes in a number of patients. The majority of cases could be attributed to age, congenital anomaly or previous ophthalmic conditions. In two patients, that could not be explained by any other factor. In one case, an abnormal 30Hz flicker was associated with the abnormality, but in the other case no electrophysiology was performed.

Possible future work on remacemide patients may include examining the pattern ERG response, as this test reflects cone and particularly macular function at a retinal level. Additionally, as EOG results were abnormal in four patients, it would prove useful to examine EOG results from the patients if remacemide treatment was withdrawn. A similar study in vigabatrin patients revealed EOG abnormalities to improve following the withdrawal of vigabatrin (Lawden et al, 1999a, 1999b) so it would be of interest to see if this reversal is also seen in remacemide. A larger study with a control group including patients with epilepsy not exposed to remacemide or vigabatrin, may also be of use in determining if remacemide has an effect on vision. Additionally the use of multifocal stimulation, which was not available in this country, may provide more information on possible retinal damage.

8.5 Conclusion

In conclusion, remacemide does not appear to cause any such visual field defect as seen in patients exposed to vigabatrin. The gross electrophysiology of the eye in the remacemide patients remains largely within normal limits although several cases showed an abnormality in the 30Hz flicker test and this is largely unexplained at present. The high prevalence of macular changes found in this small cohort may be attributable to remacemide. Therefore further testing is required in order to determine if a similar prevalence occurs in a large cohort of remacemide patients.

CHAPTER 9
DISCUSSION

9.1 Field specific VEP

It is clear that vigabatrin associated visual field loss is a particular problem in paediatric patients, as well as adult patients, who are unable to have their visual fields tested accurately. The field specific VEP fulfils a need for testing such patients in that it provides both sensitive and specific results that indicate if a vigabatrin associated visual field loss has occurred. The sensitivity of the H-Stimulus has been established and the test has been used on paediatric vigabatrin patients and children exposed to vigabatrin in utero. Additionally the stimulus size may be altered to allow for the examination of responses in adults with learning difficulties that cannot comply with perimetry. The H-Stimulus is at present the best method for identifying vigabatrin associated visual field loss in children with epilepsy below the age of 10 years, and the prevalence of defects of around 30%, has been found to be similar to that found in adults. Overall, this study has shown that the field specific VEP is a useful diagnostic test.

9.2 ERG

The ERG is an extremely useful test for examining the electrical activity of the retina. The amplitude of the 30Hz flicker gave the best indication of vigabatrin-attributed visual field loss in both adults and children. The oscillatory potentials appeared to be more affected when a field loss was present in adults than in children. The 30Hz flicker ERG response may play a role in predicting the emergence of a vigabatrin associated visual field loss which could be an extremely useful factor when prescribing vigabatrin as retinal function could be monitored relatively easily. This study has shown the ERG to be a useful investigative test.

9.3 Multifocal VEP

The mVEP test procedure has the advantage of being objective, requiring minimum cooperation and no decision making by the patient and so for patients with epilepsy that need to have visual fields monitored the mVEP may be a useful alternative to standard

visual field testing. Examining the outer quadrant latencies and using the mnSNR values is the most sensitive measure of identifying those patients with a vigabatrin associated visual field loss. It is possible that the use of more patients, both exposed and not exposed to vigabatrin, may result in a more sensitive measure being developed. The future role of the mVEP may be as a useful diagnostic test as well as an investigative test for future research.

9.4 Optimum test for paediatric patients

When testing paediatric patients it is essential to utilise a test, which whilst being easy to comply with, is also sensitive at detecting whatever damage has occurred. Presently, when dealing with children exposed to vigabatrin, the use of a field specific VEP is the optimum test with regards to both being able to comply with the test procedure and in detecting visual field loss. This test coupled with the 30Hz flicker ERG test would certainly indicate if a visual field loss had occurred in a paediatric patient taking vigabatrin.

9.5 Measures of retinal function

A plethora of electrophysiological studies have focused on determining how and why ocular vigabatrin toxicity affects one in three patients exposed to the drug. Standard static perimetry reveals a predominantly peripheral visual field defect with relative temporal sparing. This thesis has shown the field-specific VEP to reveal normal central responses in the presence of reduced amplitude peripheral responses indicating the peripheral visual pathway to be affected. Within the retina, abnormal photopic flicker responses indicate that the cone pathway is specifically affected by vigabatrin while the abnormal oscillatory potentials indicate that the bipolar cells and the feedback pathways initiated by the amacrine cells in the inner retina are affected. Multifocal VEP responses correlate pertinently with visual field results and have the capacity to provide more information on the visual pathway.

9.6 Conclusion

The treatment of epilepsy is crucial in influencing the quality of life for the patient. However inherent difficulties are associated with treating seizures due to problems classifying seizure types and the common practice of prescribing a combination of AEDs particularly when treating intractable epilepsy. The inevitable explosion of AEDs that has occurred recently saw many patients being treated more successfully although an increase of potential risks was seen contemporaneously.

Whilst the use of AEDs remains essential for the control of seizures, the drugs are always going to be associated with various types of side effects. This thesis has focused on examining visual problems associated with the use of AEDs. The studies described in this thesis have indicated that AEDs can have an undesirable effect on the retina. Reviewing the literature and examining electrophysiology results in this study has shown that one consistent measure that corresponds to vigabatrin associated visual field loss does not exist. Results suggest that the retinal abnormalities seen in vigabatrin patients is related to an increase in GABA, although it remains unclear as to whether this alteration has a causal relationship with the visual field defect.

Vigabatrin was found to have a particularly persistent and potent side effect causing visual field loss. However with the use of diagnostic tests and remaining vigil about who goes on the drug, vigabatrin should remain a very valuable niche drug with a particular emphasis on paediatric usage. Similarly, remacemide is a useful drug that certainly at present is not associated with a side effect as unpleasant as that associated with vigabatrin but yet the possibility of side effects should be monitored. If a future project was to be established it would be of use to examine all such responses in a specific control, that of patients with epilepsy not exposed to vigabatrin or remacemide.

One of the main problems with the vigabatrin associated visual field loss was the difficulty in detecting the visual problems in the paediatric population, and so this study examined the possibility of adapting the VEP test to suit the problem. The field-specific VEP was consequently found to be both sensitive and specific to visual field loss. Retinal

changes associated with vigabatrin were also investigated to determine exactly how the use of vigabatrin resulted in visual field loss and the ERG proved useful in such investigations. However a more specific way of testing the visual pathway and retinal function is clearly required in order to investigate this problem in more depth. The use of multifocal stimulation is currently a novel technique, which is able to utilise an alternative method of stimulation, which allows for specific areas of the retina and specific areas of the cortical pathway to be examined. Indeed the future of examining visual problems associated with AEDs undoubtedly lies with the use of multifocal stimulation in which multiple areas of the retina and the visual pathway can be examined.

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APPENDIX

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