LONG-TERM RECOVERY FOLLOWING OPTIC NEURITIS: EVIDENCE FROM SERIAL ELECTROPHYSIOLOGICAL AND PSYCHOPHYSICAL INVESTIGATIONS

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ABSTRACT

Neuropathological studies have shown that remyelination occurs after experimental demyelination in the central nervous system and in patients affected by multiple sclerosis (MS). Previous neurophysiological studies of patients affected by optic neuritis (ON), used as a model for demyelinating lesions in MS, showed a shortening of the Visual evoked potential (VEP) latency delay which suggests a remyelinating process.

Two groups of patients were studied after recovery from the acute stage of ON by means of clinical ocular examination, VEPs, Visual fields and Contrast sensitivity. The aim was to attempt to establish the extent and time course of VEP latency recovery, whether it is possible to identify any factor which could influence it and to evaluate its clinical consequences.

Fourteen subjects were studied at 6 months and at 3 years after the attack of ON. The VEP from the affected eye showed a significant shortening of latency, which was not accompanied by visual function improvement. In the clinically unaffected eye a significant increase in VEP latency was associated with deterioration of contrast sensitivity.

Thirty-one subjects were studied at 3 and 6 month intervals for two years after the onset of symptoms. In the affected eye the VEP latency shortened progressively throughout the follow-up period and functional improvement was seen for the first year. In the fellow eye

there was no evidence of VEP latency increase or visual function deterioration. No other factors were discovered to influence the rate or extent of VEP latency and visual function recovery.

The findings suggest that remyelination and insidious demyelination are both present over a 3 year follow-up. In the affected eye, although remyelination appears to assist visual function recovery only for the first year (possibly due to concurrent insidious demyelination and/or axonal loss), it is possible that serves to protect demyelinated axons against further inflammation which might result in permanent degeneration. In the fellow eye insidious demyelination is possibly continuously active but its effects become measurable only after more than 2 years. It is possible that a progressive phase of functional deterioration starts when insidious demyelination and/or axonal loss prevail over remyelination.

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LIST OF ABBREVIATIONS

4-AP	4-aminopyridine
(R)APD	(relative) afferent pupillary defect
APP	amyloid precursor protein
ATP-ase	adenosine triphosphate
BBB	blood brain barrier
BP	basic protein
C	capacitance
C	current
Ca++	calcium ion
c/deg	cycle/degree
cd/m²	candela/square meter
CEN	centre
CF	central field
cm	centimeter
CNP	2'3'-cyclic nucleotide 3' phosphodiesterase
CNS	central nervous system
CPSD	corrected pattern standard deviation
Cr	creatine
CS	contrast sensitivity
CSF	cerebrospinal fluid
Cz	vertex electrode placement
dB	decibels
EAE	experimental allergic ancephalomyelitis

Fat-Sat	Fat saturation
FGF	fibroblast growth factor
Fz	frontal electrode placement
FSE	fast spin-echo
Gd-DTPA	gadolinium-diethylenetriamine penta-acetic acid
gr	grams
HLA	human leukocyte antigen
Hz	Hertz
IGF (I or II)	insulin-like growth factor (I or II)
INF γ	interpheron γ
(iv) MP	(intravenous) methylprednisolone
(iv) Ig	(intravenous) immunoglobulin
kHz	kiloHertz
L/RHS	left/right hemisurround
LPC	lysolethicin or lysophosphatidil choline
MAG	myelin associated protein
MBP	myelin basic protein
MD	mean depression
mm	millimeter
MOG	myelin oligodendrocytes glycoprotein
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MS	multiple sclerosis

msec(s)	millisecond(s)
MT	magnetization transfer
MTR	magnetization transfer ratio
Na++	Sodium ion
NAA	N-acetyl-aspartate
NPL	no perception of light
NS	non significant
ON	optic neuritis
ONTT	American Optic Neuritis Treatment Trial
O-2A	oligodendrocytes-2A
PD	proton density
PDGF	platelet derived growth factor
PERGs	pattern electroretinogram(s)
PSD	pattern standard deviation
PLP	proteolipid protein
PNS	peripheral nervous system
Q	charge
R	resistance
RF	radio-frequency
SD	standard deviation
sec	second
SF	short-term fluctuation
SF	spatial frequencies
SPP II	standard pseudoisochromatic plates II

(LTE or STE)-STIR	(long-echo time or short-echo time) inversion recovery sequence with a short inversion time
T	tesla
TE	RF signal sampling
TF	temporal frequencies
TR	interval between RF pulses
V	voltage
VA	visual acuity
VEP(s)	visual evoked potential(s)
VF	visual field
WF	whole field
yrs	years
μV	microVolts
'	minutes of arc
°	degrees
<	less than
>	more than

1. INTRODUCTION

1.1 AIM

Neuropathological findings of the type attributed to remyelination after demyelination in the central nervous system (CNS) in patients affected by multiple sclerosis (MS) had already been described by a number of authors such as Alzheimer, Volsch and Dawson at the beginning of this century (Dawson, 1916). However, until the '60s no study supported the hypothesis that a reparative process occurs after demyelination and it was commonly believed that an established lesion could not repair itself. Since then, the evidence that a remyelinating process can take place has been given in various experimental or clinical studies, mainly histological and pathological, followed by electrophysiological studies.

The aim of this study was to establish from the electrophysiological and psychophysical points of view what is the evidence for remyelination after recovery from the acute stage of optic neuritis (ON), what are its characteristics, whether it is possible to identify any factor which could influence positively or negatively the remyelinating process (such as age, presence of active lesions etc.) and to evaluate its clinical consequences.

Patients with ON were chosen because ON represents a relatively simple model for studies of MS. One advantage of studying lesions in the optic nerve is that the lesion and its effects can be evaluated by electrophysiological, psychophysical and imaging methods. Electrophysiology makes it possible to indicate the status of

the myelin by conduction velocity in the nerve, reflected by the latency of visual evoked potentials. Psychophysical tests contribute to the study of the lesion by assessing aspects of the visual function and MRI contributes by giving morphological information.

1.2 MYELIN

The main function of myelin is to increase the speed of the nerve transmission by enabling saltatory conduction, ie the shunt of the action current from one node of Ranvier to the next. The arrangement of the insulating myelin sheath, interrupted at the nodes of Ranvier, makes it possible for an impulse to be transmitted at higher speed even in smaller diameter axons and to reduce the energy required. In particular the saltatory conduction results from the high resistance (R=V/C ie voltage/current; it quantifies the ease with which current flows through a material) and the low capacitance (C=Q/V ie charge/voltage; the amount of voltage for a given charge is determined by the physical properties of the nonconducting material and the geometry of the region) at the internodes and the low resistance and the high capacitance at the nodes of Ranvier.

Myelin is 70-80% lipid, with a high content of galactosphingolipids, cerebroside and sulfatide. Proteolipid protein (PLP), basic protein (BP) and myelin associated glycoprotein (MAG) are characteristicly associated with myelin in the CNS. Specific enzymes to myelin are 2', 3'-cyclic nucleotide 3'-phosphohydrolase (CNP) and cholesterol ester hydrolase but there are many others which are not specific such as the enzymes involved with lipid metabolism, the esterases and the endogenous neutral proteinases. The latter are known to degrade MAG and BP and it has been suggested that they might have a role in some forms of demyelination

(Norton, 1984).

It was previously believed that myelin was a stable structure: in fact different components have different rates of turnover. In particular it appears that the myelin that is formed earlier in life is also more stable than the newly formed myelin and this can be explained by the fact that the new myelin is adjacent to the cytoplasm and therefore more exposed to degradative agents (Norton, 1984). In a normal situation the stability of the structure is maintained by the expression on the cell surface of molecules which inhibit rearrangements. In particular, mature oligodendrocytes are known to express on the surface molecules which inhibit neurite outgrowth and limit axonal regeneration (Compston, 1991).

Myelin has different characteristics in the CNS and in the peripheral nervous system (PNS): the internodes are generally shorter in the PNS than in the CNS and the periodicity of the repeating unit of the myelin (on transverse sections of the axons this is given by the external cell membrane, or plasmalemma, ensheathing the axons) is higher for the PNS than the CNS (Peters et al., 1976; Ludwin, 1981; Hirano, 1981).

Myelin is produced by Schwann cells in the PNS and by oligodendrocytes in the CNS. Some differences between the two groups of cells can be noted: whereas each Schwann cell produces one myelin sheath (ie one Schwann cell myelinates one internode only), each oligodendrocyte produces 10-40 sheaths (ie one

oligodendrocyte myelinates many different internodes in different axons); the genes that encode myelin production in Schwann cells and oligodendrocytes are activated by the presence of bare axons (Ludwin, 1994), but in the case of oligodendrocytes the presence of astrocytes also appears to be important, probably because of the production of growth factors by the astrocytes. Moreover, adjacent Schwann cells are separated by the basal lamina and the collagen and reticulin fibrils of the endoneurium, whereas these structures do not exist in the CNS (Peters, 1976; Hirano, 1981).

Oligodendrocytes are very numerous throughout the central nervous system (there are almost twice as many oligodendrocytes as there are neurons), except in the cerebellum. There are several factors that in cultures cause the oligodendrocytes to increase in number such as insulin-like growth factor I (IGF I) that is a mitogen not only for oligodendrocytes but also for their precursors, insulin-like growth factor II (IGF II) and insulin (IGF I being the most active). No oligodendrocytes, or very few, can be found in demyelinated tissue when IGF I is not present in the cultures. IGF I in vitro and in vivo in transgenic mice promotes myelin production acting on proliferation of oligodendrocytes and their precursors, inducing maturation of the immature cells and regulating the myelin gene expression (McMorris et al., 1984). Platelet derived growth factor (PDGF) and fibroblast growth factor (FGF) not only induce proliferation of oligodendrocyte precursors (Compston, 1991) but are also required for the survival of

oligodendrocytes (ffrench-Constant, 1994; Grinspan et al. 1994).

1.3 DEMYELINATION

Demyelination can occur as a primary event, secondary to Wallerian degeneration or axonal damage, or in some cases as primary and secondary event at the same time.

Primary demyelination can either be due to direct damage to myelin or be related to damage to oligodendrocytes or due to both events simultaneously. Demyelination due to cuprizone is an example of toxic damage to oligodendrocytes (although a direct toxic effect on myelin sheaths cannot be excluded), whereas in the immune-mediated demyelination the most likely mechanism is direct damage to myelin (although also in this instance damage to oligodendrocytes cannot be excluded) (Ludwin, 1981).

According to Greenfield (1958) the changes observed in the myelin in MS were similar to those seen in Wallerian degeneration, with irregular swelling of myelin sheaths followed by fragmentation into particles, which are then phagocytosed by microglia. Early studies using electron microscopy agreed with this, supporting the hypothesis that the microglia did not have any role in the pathogenesis of the disease (Lumsden, 1970). Further ultrastructural studies, however, showed that macrophages had a role in initiating myelin destruction (Peters et al, 1976), and that the most frequent change seen on electron microscopy was the presence of abnormally thin internodes. Therefore it appeared that the most common pattern of myelin destruction in MS was the removal of myelin lamellae from the surface

with reduction in the thickness of the sheath, although other mechanisms such as the extracellular vesicular dissolution with splitting of myelin and the intramyelin oedema with invasion of the swollen sheaths by macrophages were possible (Prineas, 1972; Lassman et al., 1981). These were still described in 1993 (Prineas et al.) as the most likely patterns of myelin breakdown.

There are various experimental models for primary demyelination. One of the earliest was proposed in 1972 by Harrison et al. who studied, on electron microscopy, the demyelinating lesions produced by injection of diphtheria toxin in the spinal cord of cats. The myelin showed characteristic incisures followed, usually but not always, by splitting or vesiculation (demyelination). The damaged myelin only would then be ingested by phagocytes, whereas in vivo in experimental allergic encephalomyelitis (EAE), ingestion or phagocytosis of intact lamellae is also present.

Dal Canto et al. in 1982 suggested two possible mechanisms which can lead first to an autoimmune process and later to secondary demyelination following viral models experimented on animals:

1) a direct infection of the oligodendrocytes by the virus (a strain of the mouse hepatitis virus);

2) an immune-mediated process secondary to viral infection (with Theiler virus).

The immune attack could be triggered by the myelin breakdown with release of myelin material, by the insertion of viral antigenic

material in the cell membranes of the oligodendrocytes, or by the presence of common determinants between the virus and the myelin. This will be followed eventually by cell death.

Because the participation of the host immune system appears to have an important role in MS, the models where a virus-immune pathogenesis is involved are the most likely to be useful in the understanding of MS pathogenesis.

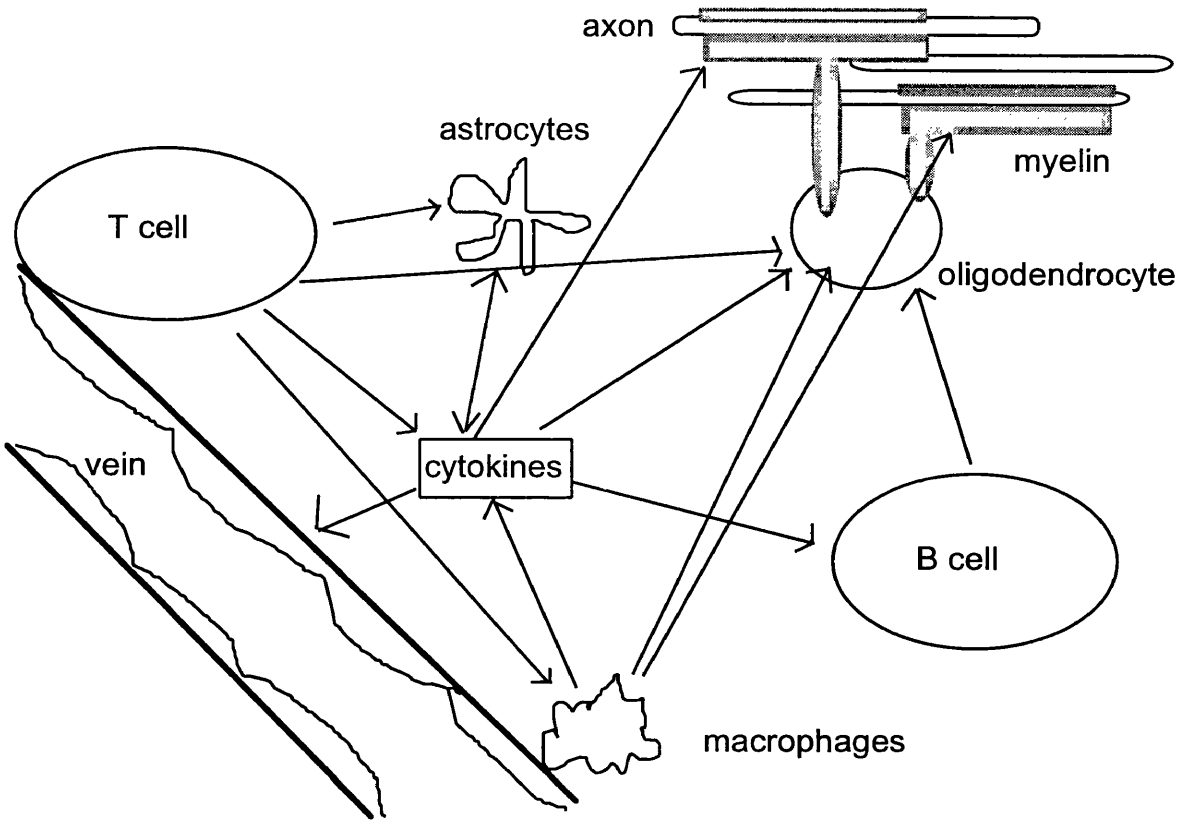
The primary immunopathological event in demyelinating disease appears to be an increased vascular permeability which occurs in the white matter and in the unmyelinated parts of the nervous system (for example the retina). A primary endothelial cell abnormality could occur or this event might depend on T-cells (as a consequence of the release of cytokines and locally active enzymes rather than of T-cell direct activity) which, however, although necessary for demyelination to occur, are not sufficient (Compston 1994). This is supported by MRI data obtained with gadolinium-dyethilenetriamine pentacetic acid (Gd-DTPA) where there is evidence that the breakdown of the blood-brain barrier (BBB) precedes the clinical symptoms and the signs of new lesions in MS (Kermode 1990).

Once the permeability of the BBB has decreased, inflammatory cells and mediators will pass through it, creating the environment for an irreversible lesion. Although it has been assessed that T-cells cause inflammation, it is not certain whether the T-cells are directly cytotoxic to the oligodendrocytes and myelin or indirectly activating the

B lymphocytes and producing gamma interferon (IFN γ) which further activates microglia and macrophages or whether demyelination is related to other factors such as complement and antibodies to myelin basic protein (MBP), to myelin oligodendrocytes glycoprotein (MOG) and to myelin lipid galactocerebroside which can reach the CNS once the BBB has been disrupted (ffrench-Constant, 1994). In 1994 Kwon and Prineas observed at autopsy that about 50% of chronic plaques showed signs of BBB breakdown (presence of fibrinogen, IgM and other serum protein), whereas it was previously believed that only the acute plaques could show them. This confirms that the BBB breakdown is not a sufficient event to determine demyelination (Fig. 1).

It has to be noted that in the majority of tissues inflammation is a protective and localized event which confines or destroys the damaged tissues and the agents responsible for the damage. This is not true in the CNS, where in the normal situation the free circulation of immune system components is prevented by the presence of an impermeable barrier. In the event of inflammation in the CNS the delicate equilibrium of the immune system homeostasis will be affected and normal structures can become the target of immunologic attack (Raine, 1994).

Fig. 1. Possible mechanism of demyelination.



Following a viral infection T-cells can directly be cytotoxic to the oligodendrocytes or indirectly damage them by the production of cytokines or T-cells can be activated in an autoimmune process: an inflammatory cascade causes the stimulation of T-helper cells and macrophage-microglial activation leading to direct or indirect myelin and oligodendrocytes injury. B cells can be activated and antibody produced leading to oligodendrocytes injury.

However, it is still unclear whether inflammation is secondary to myelin breakdown or is pathogenetically related to demyelination. Perivascular inflammation can be present without myelin damage and with normal surrounding tissues and it therefore seems plausible that a vascular event is necessary to the development of the lesion, leading

Kermode (1990) to conclude that perivascular inflammation is a primary rather than a secondary event in MS.

The damage to oligodendrocytes caused by agents like complement and perforins appears to be due to a rise of intracellular Ca++. This damage becomes irreversible when a membrane lesion occurs, although, to a certain extent, it could be repaired by vesicular shedding. The important role of Ca++ mediated cell lysis is further demonstrated by the effects of inhibition of calmodulin, which normally regulates flux and release of Ca++ (Scolding et al., 1994). Both the macrophages, which are also responsible for the phagocytosis of the myelin debris, as well as the microglia directly digest the oligodendrocytes, but their activity can be inhibited by several pharmacological agents, like high dose iv methylprednisolone (Compston, 1991). In agreement with these findings, Prineas et al. in 1984 observed that oligodendrocytes were not diminished in number or changed in structure except in those zones where macrophages were seen. Moreover, Compston in 1991 noted that when the damage occurs it appears that progenitors of oligodendrocytes which survive into adult life can repopulate areas where oligodendrocytes have been destroyed. The observation that in areas of myelin breakdown oligodendrocytes are still present, although reduced in number, would support the conclusion that myelin loss is not secondary to oligodendrocytes loss, as already suggested by Prineas and Connell in 1979. Rodriguez in 1993 also addressed this problem and showed

that although disturbance of myelin formation by oligodendrocytes might be one of the earliest pathologic features in MS, this is not due to the death of the oligodendrocytes. Raine (1994) supported the idea that, overlapping with inflammation and demyelination, there is a phase of oligodendrocyte proliferation and observed that necrosis of oligodendrocytes had never been documented.

Acute and chronic lesions in MS show several differences. The acute lesions are characterised by an inflammatory cell infiltrate of T and B lymphocytes, macrophages filled with myelin debris, oligodendrocyte loss, breakdown of associated myelin sheaths and astrocyte proliferation, while chronic lesions show little inflammatory activity, the myelin sheaths and oligodendrocytes are absent and the demyelinated axons are separated by a dense network of astrocyte processes (ffrench-Constant, 1994).

It is also important to remember that demyelination can affect both normal axons as well as remyelinated axons. No higher susceptibility to demyelination of remyelinated axons has been demonstrated (Prineas et al., 1984).

In 1996 Lucchinetti et al. added to the already proposed demyelinating models (primary demyelination or secondary demyelination) more pathological patterns which can occur in a demyelinating lesion. Different mechanisms can play a role in the formation of a demyelinating lesion and the heterogeneity of the lesions can be explained according to the prevalent mechanism at their

origin (different immunological mechanisms). Five main models have been proposed by the authors following the analysis of 82 brain biopsies, 16 autopsy of acute MS and 9 autopsy of chronic active MS:

1) demyelination with no or only minor oligodendrocyte loss

2) demyelination with concomitant destruction and loss of oligodendrocytes

3) primary demyelination with a gradient of oligodendrocyte loss towards the inactive plaque center

4) destructive plaques

5) demyelinating plaques with oligodendrocyte destruction in the periplaque white matter.

These findings, according to the authors, are supported by experimental studies where the different patterns can be reproduced by different immunological mechanisms. The implications of this classification are related to the subsequent possibility of remyelination. In those models where the destruction of the oligodendrocytes is predominant, remyelination will not occur or maybe very scanty, whereas it could be very rapid when the myelin only is primarily damaged by the pathological process.

1.4 AXONAL DEGENERATION

Axonal loss occurs to some extent in all demyelinating lesions (Lassman, 1994), but in many long-standing lesions in chronic MS (post mortem studies) axonal damage with loss of a significant number of axons can be extensive (Allen, 1991). These findings are in agreement with those studies where it is hypothesised that axonal loss occurs late in the course of the disease and that the persistence and deterioration of the symptoms in MS are mainly related to it (Ghatak et al., 1973; Ludwin, 1981; McDonald et al., 1992; McDonald 1994).

Evidence for axonal loss and its relation to persistent neurological impairment has been given in more recent years by Davie et al. (1995) by means of proton magnetic resonance spectroscopy (MRS) of cerebellar white matter. Patients affected by MS with clinical cerebellar involvement showed a fall in the level of the N-acetylaspartate (NAA) which is present only within neurons and therefore its decrease is considered an index of neuronal loss. NAA, however, was not decreased in MS patients without clinical cerebellar involvement, showing a correlation between axonal loss and persistent symptomatology. A similar study was performed by Narayanan et al. (1997), who considered the ratio NAA to creatine. Creatine is evenly distributed throughout the brain was taken into account. The ratio was reduced in patients with progressive MS as compared to relapsing MS. The hypothesis for the late occurrence of axonal loss has recently been argued against by Ferguson et al. (1997) who published data

from post-mortem studies in MS. Antibody against amyloid precursor protein (APP), which is a sensitive marker for axonal damage, was found in areas of acute inflammation and demyelination but not in areas of chronic inflammation. This means that the axonal damage is closely related to inflammation and is not a gradually progressive process as suggested in the past. Trapp et al. (1998) draw the same conclusions as Ferguson et al. (1997) although different techniques were used. Immunohistochemistry and microscopy data from brain tissue suggested once more that axonal degeneration was an event which occured to a larger extent in the early stages of inflammation/demyelination. The axonal loss could be a secondary effect of demyelination: a demyelinated axon is more vulnerable to inflammatory agents (like proteolytic enzimes, cytokines, free radicals and oxidative products) than a myelinated axon.

1.5 REMYELINATION

At the beginning of the '60s authors like Bunge et al. (1961), Bornstein et al. (1962) showed that in some animal species a remyelinating process could occur after experimental demyelination. Since then a number of studies approached different aspects (histology, pathology, clinical data, electrophysiology, imaging etc.) of the problem, using experimental models (for demyelination and remyelination) or clinical studies, although particularly at the beginning the major contribution demonstrating that remyelination could occur was given by pathology and then electrophysiology study.

In 1965 Perier and Gregoire studied for the first time by means of an electron microscope chronic demyelinating lesions. At the centre of the lesions complete demyelination was observed: the absence of oligodendrocytes and the presence of a very large interstitial space and naked axons, surrounded by astrocytes' processes, were characteristics. The axons were ensheathed by normal myelin which terminated in the proper fashion at the end of the segments, leaving them naked but without alterations. Some microglial phagocytes with lipid inclusions were also present. Moreover, thinly myelinated axons could be isolated which were thought to represent remyelinating axons.

It was difficult, however, to establish whether the thinly myelinated axons observed were remyelinating axons or degenerating/damaged axons. In the following years authors such as Prineas (1975) Prineas and Raine (1976) and Prineas and Connell

(1978) isolated internodes which varied in myelin thickness along their length as well as internodes with uniform myelin thickness. The authors thought that the internodes with non-uniform myelin were partially demyelinated, whereas the uniform internodes were remyelinating. This was confirmed by the experimental studies by Gledhill and McDonald (1977) on cats where demyelinating lesions of the spinal cord following compression were studied for 6 months in a single-fibre analysis. Prineas and Raine (1976) and Prineas and Connell (1978) in the study of the early stages of demyelinating lesions found in the vicinity of thinly myelinated axons microglial cells containing myelin debris, suggesting that thinly myelinated axons can be incompletely demyelinated axons.

In 1979 Prineas and Connell addressed this problem studying chronic plaques by means of light and electron microscopy. They isolated three types of abnormality involving the myelin sheaths of the internodes: some were uniformly thin, others were tapering so that they became thinner over their length and others showed a thin region restricted to the paranode. The characteristics of the internodes with uniform and non uniform thickness under light and electron microscope were different. Under light microscope the uniform internodes would appear as short isolated segments of myelin on naked axons or as thin short internodes separated by normal nodal gaps from contiguous thin or normal internodes. On electron microscopy they appeared as terminating in the form of normal nodal complexes composed of large,

tubule-rich lateral loops (this probably represented disrupted myelin lamellae (Harrison et al., 1972)). The non-uniform internodes appeared on light microscopy as normally thick internodes that became progressively thinner along their length. On electron microscopy the change in thickness was shown to be due to premature termination more often of superficial than inner myelin lamellae, which ended in the form of symmetrically disposed arrays of large pale lateral loops with numerous microtubules. These loops were attached to each other, to astroglial processes and to nearby demyelinated axons (the junction for the latter was similar in appearance to the junction between the myelin and the underlying axon at the normal nodes of Ranvier). The internodes with abnormally thin myelin for the diameter appeared to be newly formed internodes (this seemed to be the most common repairing mechanism rather than the extension from preexisting ones). They were, according to previous studies, shorter than normal (down to 6μm) (Blakemore et al. 1977; Gledhill and McDonald, 1977; Harrison and McDonald, 1977) and were seen at the edges of the chronic plaques, which suggested that remyelination in MS could be carried out by glial cells. On the contrary, the tapering internodes could represent original internodes that generate new myelin or new internodes of unusual type. Two features were in favour of this possibility: the myelin lamellae ended as a large cytoplasmic pocket filled with microtubules (this appearance is characteristic of myelinogenesis) and the lateral loops formed several specialized

junction with the surrounding structures which were similar to the normal junctions. Internodes with similar characteristics (incomplete destruction of the myelin and altered myelin sheaths and as a consequence non-uniform myelin thickness) have already been described not only in multiple sclerosis but in a number of demyelinating conditions and also in different animal species (Harrison et al., 1972; Gledhill and McDonald, 1977; Prineas and Connell, 1978). Areas of complete demyelination were also observed and in these areas the oligodendrocytes were absent (as described by Perier and Gregoire in 1965 in the centre of the lesions) and if present had a rather small nucleus and no processes, whereas at the margins of the plaques it was possible to find oligodendrocytes with abundant cytoplasm. In the same areas aberrant myelin formation was seen but this might also have been present in the same proportion in unaffected areas (Prineas and Connell, 1979). In experimental demyelination in animals, however, oligodendrocyte activity in totally demyelinated areas was observed. Explanations given for the difference between experimental lesions and lesions in MS were: the different size of lesions, a species-related difference in the capacity of the oligodendrocytes to divide or to be supplied and the existence of myelination-inhibiting antibodies in MS. Although the density of the oligodendrocytes might have appeared very low partly due to oedema and the high number of infiltrating macrophages that could be present in the lesion, it was difficult to explain how remyelination could occur in

the absence of the myelin-forming cells.

In 1984 Prineas et al. in a similar study on old lesions (little or no evidence of recent myelin breakdown), subacute lesions (numerous lipid macrophages at the edges of the lesion) and acute lesions (presence of lipid macrophages and macrophages with undigested myelin debris) established that the oligodendrocytes were normal and only marginally reduced in number in the white matter, apart from the zones infiltrated by macrophages. Moreover, he found by means of electron microscopy axons with uniform thinning of the myelin sheaths not only at the edges of old plaques but also in new plaques and even in active demyelinating zones. In other cases the thinning appeared to be non-uniform and therefore these axons were regarded possibly as incompletely demyelinated axons or as axons which were going to lose their myelin sheath (as already noted by Prineas and Connell in 1978). This suggested that remyelination and demyelination could coexist although it was not clear from these observations whether they could occur simultaneously. The balance between these processes would determine the size of the lesion and both new and old myelin could be destroyed at plaques' margins. From Prineas' study it was concluded that signs of remyelination were observed within 10 months of the onset of symptoms and the process could be massive.

In 1987 Prineas et al. found that in humans although oligodendrocytes and myelin were destroyed during the demyelinating process, within weeks or months oligodendrocyte proliferation, and

consequently myelin formation in the presence of inflammatory cell infiltrates occurred. The repairing process was now regarded as happening in the early stages of the disease and as being extensive. Signs of a repairing process were present in higher number in plaques seen in patients who died between 3 and 10 months after clinical onset of the disease, whereas it appeared that remyelination was blocked in long standing plaques. In the same year the authors published the interesting observation regarding remyelination, that in fresh lesions undifferentiated cells with the same phenotype as oligodendrocytes were seen inside some astrocytes. The same observation was made by Ghatak et al. in 1989 and again by Prineas et al. in 1990 and according to these authors it was not due to true phagocytosis but represented a phenomenon called emperipolesis. This is a process previously described by which lymphocytes may enter other cells, but which was later documented for different types of cell (Anzil, 1990). The phagocytosis of new oligodendrocytes by astrocytes in the demyelinated plaques can be accounted for by emperipolesis. According to Prineas et al. (1990) this phenomenon might represent a limiting factor to remyelination or a consequence of failure of remyelination, as no similar process is seen in tissues where remyelination is occurring. Anzil (1990) suggests that this phenomenon is possibly neither tissue nor disease specific but could indicate that where it is present there is an inappropriately high level of cell growth with subsequent tissue remodeling, as is seen in

demyelinated lesions.

In 1988 Ludwin analyzed the two main criteria proposed to identify remyelinated axons morphologically: abnormally short internodes and myelin sheaths inappropriately thin for the size of the axons. He concluded that as it is technically more difficult to obtain longitudinal sections of the nerves rather than transverse sections, the recognition of internodes with inappropriately thin myelin sheaths for the size of the axons would be a more crucial criterion than the identification of internodes short for the thickness of the axons. Using these morphological criteria, however, it can still be difficult or even impossible to distinguish the remyelinating axons from those which are partially demyelinated and it is therefore important for the distinction to take into account the differentiation made by Prineas and Connell (1979) between uniform and nonuniform myelin thickness. As far as the timing of the reparative process was concerned, it was noted that following experimental demyelination signs of remyelination were present within a week: oligodendrocytes insinuated their processes around the axons displacing the astrocytic processes and then wrapped the axons in clusters so that one oligodendrocyte would remyelinate several neighbouring axons (as suggested by Waxman and Sims in 1984 and Kandel et al. in 1991). Within 3 weeks after demyelination following compression abnormally thin myelinated fibres could be found in animals.

Ghatak in 1989 observed signs of remyelination in tissues from a

biopsy only 15 days after the onset of symptoms in the case of a young boy. It was pointed out, however, that the diagnosis of MS was not confirmed, although the nature of the process was demyelinating.

In 1993 Prineas studied at autopsy patients with MS who died between 27 days and 5 years from the onset of the first symptom. Because it is almost impossible on histological criteria to establish the age of a lesion, the author examined the plaques for evidence of remyelination. Those patients who died within the first month showed only signs of demyelination with loss of oligodendrocytes, whereas those who died later showed large numbers of oligodendrocytes in the areas which were presumably previously depleted. This would mean that the time interval needed for remyelination to commence in a new plaque is at least of the order of a month and that after the initial destruction of the oligodendrocytes there is a new colonization of the demyelinated area. The oligodendrocytes' responses to demyelination is therefore characterized by two phases (initial destruction and new colonization) and this would explain the different findings by various authors (depletion or presence of oligodendrocytes and their precursors). In 1994 Ozawa et al. did a quantitative study of oligodendrocytes which showed that oligodendrocyte loss is highly variable depending on the type of demyelination and the timing of the lesion: in acute lesions there was still a sufficient number of oligodendrocytes for remyelination; in the early stages of chronic lesions there was almost complete preservation of oligodendrocytes,

whereas in the later stages there is extensive loss of oligodendrocytes. On this basis Prineas and his colleagues (1993) described 4 kinds of plaques: clearing plaques (circumscribed areas with reduced numbers of normally myelinated axons), plaques remyelinated by Schwann cells (spinal cord), newly forming lesions and plaques remyelinated by oligodendrocytes. The areas of thinly myelinated axons described by the author were also defined as "shadow plaques". However, the definition of "shadow plaques" included either areas of incomplete myelin loss (fresh lesions with ongoing myelin destruction, absent oligodendrocytes and presence of infiltrates of macrophages) or areas where remyelinating fibres were associated with the presence of numerous oligodendrocytes (Prineas, 1987). The oligodendrocytes seen in these plaques were more likely precursors of oligodendrocytes that matured rather than oligodendrocytes which survived the noxious event (Prineas et al., 1987 and 1993; ffrench-Constant, 1994). From experimental data it is known that mature oligodendrocytes have little or no proliferative capacity within demyelinating areas whereas oligodendrocytes' precursors are numerous and proliferate (ffrench-Constant, 1994).

In 1991 Compston published a summary of the factors that were believed to limit the damage and promote the repair in multiple sclerosis: exclusion of inflammatory mediators from the CNS; manipulation of the intracellular consequences of oligodendrocytes' membrane permeability; inhibition of macrophage activity;

enhancement of oligodendrocytes' precursors 2A (O-2A) or oligodendrocytes' regeneration; reconstitution of defective cell populations in critically placed lesions. This means that it is necessary to ensure the presence of growth factors which will make the progenitors differentiate into oligodendrocytes and to cause the disappearance of the pathological condition which produced the disruption of the oligodendrocytes in the first place.

Ludwin in 1994 described in more detail some of the required conditions for remyelination. The most important factor would be to have oligodendrocytes in adequate numbers. These could derive from immature cells or from surviving mature cells but the first possibility appears to be more likely (ffrench-Constant, 1994). Secondly the oligodendrocytes or the precursors should be able to migrate, extend their processes to reach the axons, adhere to the axons and that the axon itself should send signals to the oligodendrocytes to activate them to start the repairing process (this was shown in vivo and in vitro). The axons should also be denuded of damaged myelin (Harrison and McDonald, 1977). It appears, therefore, that axons play an important role in remyelination, because the cells producing myelin (oligodendrocytes or Schwann cells) reexpress their myelination protein once exposed to the naked axons. It has also been suggested that demyelination caused by oligodendrocyte destruction is less likely to recover and to undergo remyelination. This is not always the case, because it is known that in particular situations remyelination by

Schwann cells can take place, rather than by oligodendrocytes, in the CNS as in the PNS. It does not appear that the signal sent by the axons is different either in remyelination produced by oligodendrocytes or by Schwann cells, although the myelin produced by Schwann cells shows a lamellar periodicity 10% greater than central myelin and also has a basal lamina around the cytoplasm is present. Schwann cells can enter the CNS when a disruption in the glial limitants occurs, some of them might move along glial bridges and this seems to be the common factor in experimental compressive lesions where Schwann cell remyelination is seen. This type of remyelination is more frequently observed around the dorsal root entry zone and is less frequent towards the middle of the cord (Harrison and McDonald, 1977). Remyelination in spinal cord lesions in MS due to the activity of Schwann cells was already reported in 1972 by Harrison et al. and in 1973 by Ghatak, who, however, commented that it did not appear to affect the clinical symptoms. According to Felts and Smith (1996), when remyelination is carried out by Schwann cells the properties of the BBB are fully restored (although astrocytes are lacking) in experimental demyelination and this may possibly apply to MS in humans.

Axonal loss might also contribute together with the following gliosis to the failure of remyelination, reinforcing the idea of a direct role for axons in the repairing process. According to some authors, however, astrocytic gliosis seems not to be an obstacle but to be

necessary to promote remyelination in demyelination due to cuprizone or experimental allergic encephalomyelitis (Blakemore, 1978 and 1984; Ludwin, 1980; Raine and Traugott, 1985; Brosnan and Raine, 1996). This is in keeping with further experimental observations where it is suggested that these cells produce neuronotrophic and neurite growth factors (Reier, 1988). Their role might be that of a guide for the myelinating oligodendroglial processes (the axo-glial recognition and contact appear to be aided by changing in the distribution of particular molecules, like for example the Ca++-ATPase (Felts and Smith, 1996). It was also suggested in the past that astrocytes abnormalities may inhibit oligodendrocyte proliferation and as a consequence the remyelinating process (Bunge et al., 1978). It is difficult to reconcile these two theories but it might well be that the role of astrocytes varies under different conditions as suggested by Ghatak (1989). It has also been observed that when astrocytes are damaged together with oligodendrocytes, remyelination is performed by Schwann cells rather than oligodendrocytes even though the latter are still present (Blakemore, 1984; Felts and Smith, 1996). The newly formed myelin sheath's integrity, however, depends on the oligodendrocytes which in turn need the presence of growth factors. The latter, however, might be lacking in demyelinated areas because of damage to the cells providing them, resulting in disintegration of the myelin following the oligodendrocytes' death (ffrench-Constant, 1994). It has also been established (Kwon and Prineas, 1994) that plasma-derived

components are able to make O-2A precursors differentiate into astrocytes rather than oligodendrocytes, preventing normal remyelination. The entire mechanism is not known, but an increased vascular permeability of the BBB in long-standing lesions is necessary.

More general factors (Ludwin, 1988) which might influence the remyelinating process after experimental demyelination could be the age of the subject and the animal species. Age could be important as immature cells as it is associated with the functional immaturity of the tissues, which means that in younger subjects there are still immature cells and the reparative process is more successful than in older animals (although good results could be seen also in adult animals). Some species, such as rodents, however, maintain even in adulthood a "germinal matrix" from which immature cells are derived that can transform into mature oligodendrocytes (this is not, however, the only source of oligodendrocytes, as remyelination occurs also in higher species woithou a “germinal matrix”) which will be able to produce new myelin.

It needs also to be pointed out that recurrent episodes of demyelination, or the longer a demyelinating factor acts, may cause failure of the repairing process and in chronically demyelinated lesions remyelination is not very effective. When demyelination is recurrent remyelination will not be complete as it would occur in the first instance and this is probably what occurs in the typical relapsing and remitting course of MS. Some remyelination may still occur although it will

probably be poor due to the depletion of proliferating oligodendrocytes, inflammatory and glial cells. The migration of the oligodendrocytes' precursors from areas which have not been affected by the pathological process may also be inhibited.

The morphological (e.g. lamellar periodicity) and immunochemical (e.g. content of myelin basic protein and myelin-associated protein) characteristics of the remyelinated myelin sheaths, with the exception of their thickness, did not differ from those of the normal myelin. The electrophysiological properties can be restored quite early after conduction block of the central nerve fibres although the characteristics of the remyelinated internodes remain different from the normal ones, as already described. In particular, it has been shown that in experimental demyelination of the spinal cord of the cat, following lysophosphastidyl coline (LPC) injection, conduction starts to recover within 2 weeks after the noxious event and becomes stable within 3 months (Smith et al., 1981). One explanation for the presence of thin myelin in remyelinating axons might be the failure to generate myelin at a normal rate in MS, although some oligodendrocytes still have the capacity to produce new myelin (Prineas and Connell, 1979). Blakemore (1984) also found that in animals after chemically induced demyelination the axons have lost at this point of their ontogenesis the ability to establish the particular axon/myelin ratio common to normal tissues. For the production of new myelin the absence of demyelinating agents and the presence of astrocytes is necessary. It

is not clear why the axons are remyelinated at different times, whereas they are demyelinated at the same time as has been demonstrated by experimental studies. This is possibly related to the fact that oligodendrocytes reach the axons at different times and, as mentioned before, remyelinate the axons in clusters (Ludwin, 1988).

1.6 ELECTROPHYSIOLOGY

Demyelination can be experimentally caused by various agents and each of them will affect the myelin in a different way, so that for example, following LPC injection segmental demyelination is more common, whereas after diphtheria toxin injection paranodal and segmental demyelination occur (Smith et al., 1981).

Electrophysiological studies have shown (Mayer and Denny-Brown, 1964; Ochoa et al., 1972; Smith et al., 1981) that the most common abnormalities after experimental demyelination by chemical or mechanical agents in the peripheral and central axons are

1) conduction block

2) slowing of conduction

3) failure to transmit high-frequency trains of impulses.

These phenomena appear to be due to the reduction of the excitability and the high capacitance of the demyelinated segment of the axon after demyelination. In particular, conduction block, appears to be caused by inexcitability of the axon membrane, due to a decrease in current density through excitable membrane which makes it impossible to reach threshold level (conduction block can be frequency related so that high frequency trains of stimuli do not propagate but low frequency trains are reliably transmitted) and reduced conduction velocity occurs when a more prolonged outflow of outward membrane current is necessary to reach the threshold.

Conduction block is present when the demyelinating process is

extensive and is more frequently seen when demyelination has a segmental (rather than paranodal) distribution (Smith et al., 1981). It is restricted only to the site of the lesion, while in the surrounding areas conduction remains normal as shown in experimental studies by McDonald (1963) and McDonald et al. (1970). Demyelination alone does not necessarily cause conduction block and the degree of demyelination is only one of the factors which can influence the presence or the absence of conduction block. For example it is important to take into account factors such as the time elapsed since the episode of demyelination and consequently the characteristics of the axolemma and the temperature. The axons are able with time to adapt to the demyelinated status, so that conduction may be restored in the absence of myelin, and conduction block can be present when the temperature is raised but not at normal body temperature (Smith, 1994).

A phenomenon seen following demyelination is the reduction in amplitude of the compound action potential which appears most likely to be due to conduction block in a number of otherwise intact fibres. According to Smith et al. (1981) further possible explanations for the reduction in amplitude would be a simple temporal dispersion of the impulses or the degeneration of the fibres. They showed that some fibres can be lost after demyelination, but later, even in the absence of regeneration, an increase in amplitude of the responses is found which suggests that degeneration cannot be the main cause for amplitude

decrement. They also did not find any delay which would exclude temporal dispersion as the cause of the amplitude reduction. Conversely conduction block of a number of fibres and later its resolution would explain the recovery of the amplitude of the compound action potential in a short period of time.

That conduction could be restored as a consequence of the adaptation of the fibres to the new situation which may lead to continuous conduction or to a saltatory conduction was first shown in the PNS (Bostock and Sears, 1976; Bostock et al, 1978; Smith et al., 1982; Rasminsky, 1984). In particular Bostock and Sears (1978) showed that continuous conduction along demyelinated axons following application of diphtheria toxin in the ventral roots of rats is possible. This was seen at a time too early (the earliest was 6 days) to possibly have involved remyelination and would suggest excitability of the internodes due to the redistribution of the Na++-channels along the demyelinated segment of the nerve. In this situation conduction may still be blocked because of the impedance mismatch at the junction of normal and demyelinated axon. In support of this hypothesis it has been shown that in experimental studies and in patients with MS lowered calcium concentration (reduces the threshold for the excitation) or lowered temperature (prolongs the duration of the action potentials) can improve the transmission (Smith et al., 1982; Bostock et al., 1978). These authors showed that continuous conduction can occur in peripheral nerve fibres only to an internodal distance of 850

μm at body temperature and therefore in small diameter fibres. Changes in the body temperature would allow conduction at a longer internodal distance and therefore in fibres with larger diameter. The expected velocity in continuous conduction would be 1/20th to 1/40th of the normal conduction velocity. In the study of Bostock and Sears (1978) it was suggested that, although their findings applied to the peripheral nervous system, it would be plausible that similar phenomena may occur in the central nervous system: continuous conduction could account for the delays seen in VEPs in patients affected by optic neuritis and may explain the lack of symptoms and/or the recovery of symptoms following central demyelination at a time when remyelination has not yet occurred. McDonald in 1977 had already suggested that delays as those seen in VEP recordings could be related to the focal slowing in the demyelinated fibres. Although aware of the fact that the peripheral nervous system and the central nervous system could have different properties and that what applies to one species may not apply to a different species (data available from the PNS of the cat) he calculated that conduction velocity in the normal optic nerve would be in the order of 10 m/sec which means that it will take 1 msec to cross a 1 cm segment: when demyelination occurs 25 msec will be necessary to travel along the same segment. The delay seen on VEPs, however, according to Waxman (1988) could be due to saltatory conduction in partially demyelinated fibres or to continuous conduction in completely demyelinated fibres for example,

but VEPs do not provide helpful information for this distinction.

It is known that the membrane of myelinated axons shows a high degree of regionalization, with sodium-channels present in a high number at nodes of Ranvier and potassium-channels present at the internode or paranode membrane. In a normal situation the high regionalization does not interfere with conduction because the myelin shunts the action current, but it becomes important in the demyelinated fibres, where the low density of Na++-channels at the internodes will cause a reduction or a lack of excitability. It has been shown experimentally that in the ventral roots of cats after LPC injection the Na++-channels can be redistributed in clusters and foci of inward membrane current in demyelinated axons before remyelination is detected (Bostock et al., 1980) and that the Na++-channels apart from remaining associated in clusters, can also be redistributed singularly along the axon or new channels can be added to the demyelinated axon membrane, so that there is a continuous rearrangement and distribution of the NA++-channels (Foster et al., 1980). The particular arrangement of the Na++-channels in clusters (phi-nodes) is similar to the normal nodes of Ranvier. The phi-nodes are described as excitable regions of the axolemma (100 µm in length), separated by regions which are not excitable, and are considered as precursors of the nodes of Ranvier, having the same spacing and the same electrical characteristics (Smith et al., 1982). In 1991 Moll et al. showed for the first time that the rearrangement of the NA++-channels and their

increase in number is also present in human demyelinating diseases, such as multiple sclerosis. The demyelinated axons show characteristics similar to those of the unmyelinated axons where the distribution of the Na++-channels is more homogeneous.

It has already been established (Morgan-Hughes, 1968; Smith et al., 1981) that conduction can be securely restored to normal or close to normal values in demyelinated fibres of the peripheral nervous system following remyelination. Smith et al. in 1981 confirmed these findings also for the central nervous system. In particular they showed that conduction in the spinal cord of cats after demyelination by LPC was restored and secure within 3 months although the remyelinated internodes were abnormally thin and shorter than normal. When remyelination occurs oligodendrocytes proliferate and produce new myelin in the CNS, but Schwann cells, which generally produce myelin in the PNS, can also contribute to the process and spontaneously remyelinate the central nerve fibres, as shown in experimental demyelination (Felts and Smith, 1992). Smith et al. (1981) suggested that, at first conduction is restored by continuous or saltatory conduction whereas the remyelinating process occurs only later in time. Although the morphological characteristics of the new internodes differ from the original ones, in that they are still abnormally thin and short, it seems that remyelination promotes the restoration of conduction to values close to the normal range. However, in their experiment (intraspinal injection of LPC in cats) it was difficult to

establish whether the normal velocity findings were real or related to the shortness of the lesion as compared to the length of the entire pathway.

1.7 OPTIC NEURITIS

1.7.1 Clinical features

Optic neuritis is an episode of inflammation/demyelination affecting the optic nerve which could present itself as an isolated event, a recurrent event or in the context of the diagnosis of a more complex demyelinating process, such as multiple sclerosis. Patients complain of sudden or more gradual onset of impairment of visual acuity (VA) (in some cases down to counting fingers or even to no perception of light, however, without prognostic implications), colour vision deficit and retrobulbar pain, particularly on eye movements. The presence of an afferent pupillary defect (APD), a pale disc (although 1/3 of cases have normal discs) and/or a swollen disc (present in rather less than 50% of patients) and a visual field defect (at the beginning a central scotoma is most commonly seen, but then the residual defect is more frequently an arcuate scotoma) is assessed by the clinician (McDonald and Barnes, 1992). Most of the time visual function recovers to normal or nearly normal in a few months, although the time course for recovery varies for the different aspects of vision in different patients (Hoyt et al., 1972; Miller, 1982) which may suggest a patchy pattern of recovery and the presence of selective channels for different functions (Bodis-Wollner, 1979). A slow recovery can continue up to 12 months from the onset of symptoms, although in a small number of patients the deficit to various visual functions might

remain severe and become permanent (Celesia et al., 1990).

Optic neuritis is more commonly seen in young adults, predominantly females, the percentage of the latter varying between 65% and 80% in various studies (Perkin and Rose, 1979; Beck et al, 1992; Söderstrom, 1995).

At our present level of knowledge it is impossible to establish whether the three kinds of presentation of optic neuritis (isolated, recurrent or ON associated with MS) represent different points of the same spectrum or are truly separate entities (Frederiksen et al, 1992; Städt et al, 1990). An important observation in this matter is that a patient affected by unilateral optic neuritis has a very much greater risk of developing MS as compared with the normal population. In the 5 years following the onset of symptoms up to 50% of patients with unilateral optic neuritis will develop MS as defined by clinical criteria (Nikoskelainen and Riekkinen, 1974; Kinnunen, 1983). The percentage would be approximately 75% in 15 years according to Francis et al., (1987), 45% according to Sandenberg-Wollheim et al. (1990) and 58% according to Rizzo and Lessell (1988). The different proportions found by these authors appeared to be related to patient selection, geographical location and ethnic origin of the subjects. The risk factors identified were: young age, female sex, abnormal CSF at onset and early recurrence of optic neuritis (although Rizzo and Lessell in 1988 did not agree on the latter finding). It is still debated whether there is an increased risk associated with particular human leukocyte antigen (HLA): according to Hely et al. (1986) DR2 and B7 increase

the risk, whereas Francis et al. (1987) found that DR3 increases the risk. The evidence of brain lesions on MRI in isolated optic neuritis is also associated with a poor prognosis (Ormerod et al., 1986). The rate of ON as the first symptom of multiple sclerosis varies between 15% to 75% when different studies are analyzed and the percentage of patients who develop ON in the course of the illness varies between 60 and 90% (Shibasaki et al., 1981; McDonald, 1983). The wide ranges can be explained by differences in the length of the follow-up, in the patients' selection criteria and in the criteria used to define MS. The recurrence rate of optic neuritis varies between 10% and 20% within a year in different studies (Hutchinson, 1976; Perkin and Rose, 1979; Miller, 1982 and Celesia et al., 1990). The chances for vision to return to normal decrease with recurrence as observed by Nikoskelainen (1975), Miller (1982) and Celesia et al (1990).

1.7.2 Visual evoked potentials

1.7.2.1 General characteristics

The effects of demyelination and inflammation on the optic nerve, with the consequent deficits in visual function can be studied using a number of tests, among them the visual evoked potentials to flash or pattern stimulation (-onset, -offset or reversal). Until the '70s responses to a flash stimulus were the most frequently recorded (Gastaut et al.,1967; Ciganek, 1969; Halliday, 1972), while pattern

VEPs started to be performed in patients affected by optic neuritis in the '70s by authors such as Halliday et al. (1972, 1973 and 1976), Matthews et al. (1977), Shahrokhi et al. (1978) etc.

The flash VEP responses are recorded to an unstructured stimulus at the occipital region, above the inion and in particular at the midline. They are characterized by amplitude, latency and morphology when responses are recorded to a "transient" stimulus (stimulation frequency:1-2/sec, i.e. the stimuli are well separate) and consist of a series of positive and negative peaks recognized by arabic or roman numerals or by their polarity, latency and distribution according to different authors (Ciganek, 1969; Gastaut et al., 1967). The "steady state" responses are obtained at a stimulation frequency higher than 5/sec, so that the stimuli are closely spaced, and are characterized mainly by the amplitude and the phase relationship to the stimulus. In this case there is some overlap of the EP components and within certain frequencies the responses will consist of a long train of identical repetitive waveforms. They can be analyzed by means of Fourier analysis, but they are not frequently used in the clinical setting (Halliday, 1993).

Nowadays also the use of flash VEPs to a transient stimulus in the clinical setting is quite limited, due to the high variability of the responses in latency, morphology and amplitude (the flash response is not well localized, although its earliest components are recorded best at electrodes placed on the midline a few centimeters above the inion) and to their relative lack of sensitivity and specificity to the aetiology of

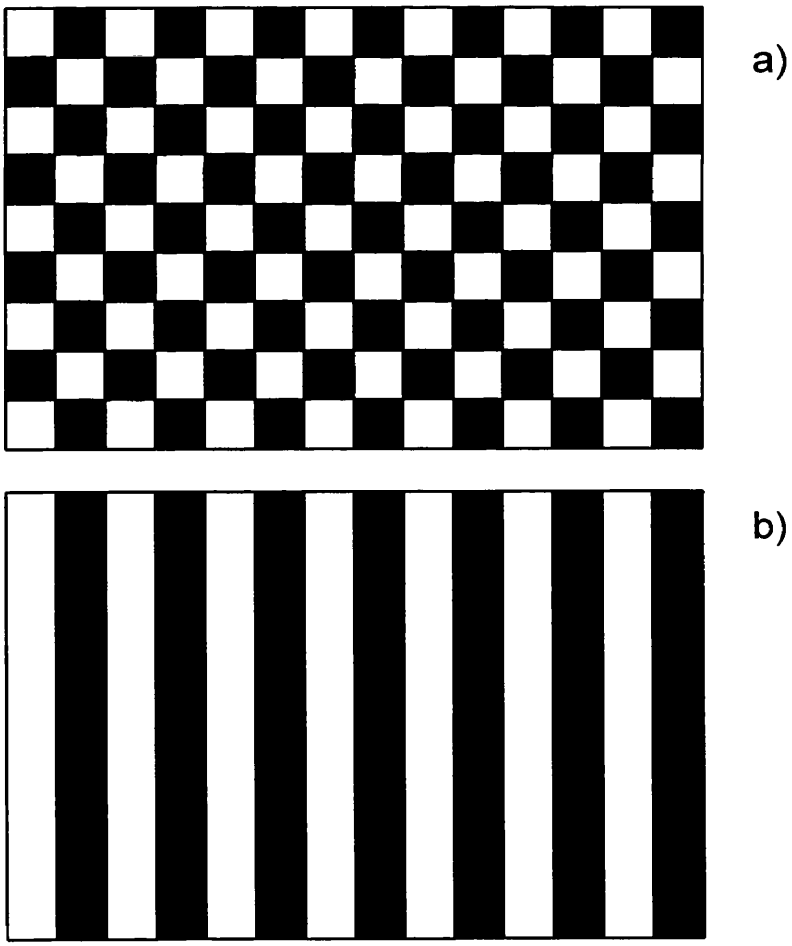
the visual loss. On the contrary, one of the advantages of flash VEPs is that a response is obtainable when the VA is very poor and pattern-evoked responses are unrecordable. Although flash VEP responses in demyelination can show a delay, this is marginal as compared to pattern responses and the overlap with data from the control group is great (Halliday, 1993). The difference between flash and pattern VEPs suggests that the two stimuli are processed by two separate pathways, possibly reflecting the M (detection of rapid changes in luminance) and the P (detection of the changes in a pattern) systems. In particular the M system predominantly detects differences in luminance, is insensitive to colour, has a high contrast sensitivity and high temporal resolution, whereas the P system deals with colour vision, has a much lower contrast sensitivity but high spatial resolution (although in reality the temporal and spatial characteristics are not so sharply separated between the two systems). According to Tobimatsu et al. (1995) it is possible to study the two systems separately by means of VEPs, appropriately changing the spatial and temporal characteristics of the stimulus, its contrast and its colour: the M system can be preferentially stimulated by low contrast and high temporal frequency stimuli, whereas low temporal and high spatial frequencies and chromatic contrast stimuli, mainly stimulate the P system (an isoluminant chromatic stimulus will stimulate its chromatic component).

As far as the stimuli in the clinical settings are concerned photopic stimulation (flash VEPs) and checkerboard pattern are generally used. Photopic stimulation is associated with a net change

in luminance, whereas the pattern stimulus is a structured stimulus rich in contrast and sharp contours, where, however, the overall mean luminance does not change. Therefore the differences of the responses to flash and checkerboard pattern reversal may be due to the differences in the system predominantly activated by the two stimuli, the M and the P, respectively (Shahrokhi et al., 1978).

Most commonly checkerboard patterns are used in clinical testing, but sinusoidal or square-wave gratings can also be of use (Fig. 2).

Fig. 2. A checkerboard pattern (a) and grating (b).



A checkerboard pattern may be shown in a reversal mode (so that

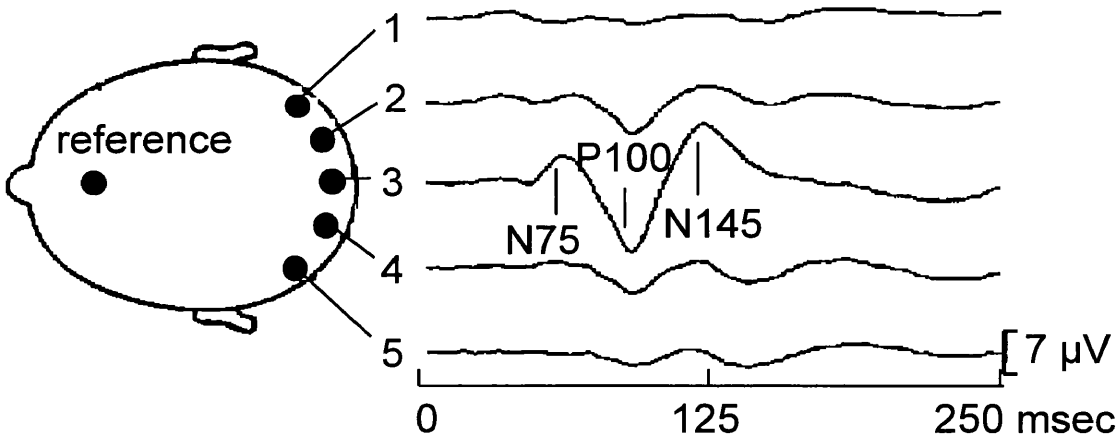
the white checks become black and the black checks reverse to white) or in a pattern -onset/-offset mode, where a transient black and white checkerboard is replaced by a neutral grey background of equal mean luminance. Specific responses can, therefore, be recorded to the reversal mode or to the appearance of the stimulus and to its disappearance when the stimuli are adequately apart (600 msec) (Kriss and Halliday, 1980; Halliday, 1993).

Clinically the checkerboard pattern reversing at a rate of 1-2/s, a "transient" stimulus, is more frequently used than a "steady-state" stimulus (presentation rate higher than 5/sec) or a grating. The main characteristics of the responses to a pattern stimulus are, as for the flash responses, the amplitude, the latency, the morphology and the distribution. The response to a transient stimulus shows a triphasic complex where the main component, the P100, is preceded and followed by the N75 and the N145, respectively (they are named according to their polarity and their mean latency). The amplitude of the pattern reversal responses, although very variable between subjects, is very similar from the two eyes of the same subject, whereas the latency of the responses is much less variable even between individuals. Factors which can affect the amplitude and the latency of the responses are sex and age (Allison et al., 1984; Halliday, 1993). The responses are picked up by electrodes attached to the scalp in the occipital area, and according to Halliday et al. (1972) the placement of the electrodes 5 cm above the inion on the midline and laterally in a chain at an interelectrode distance of 5 cm and 10 cm

from the midline on either side would be appropriate ("Queen Square montage").

The "whole field" stimulus is characterized by a checkerboard of various size and shape, according to the various laboratories, but which is symmetrical about the fixation point. To whole field stimulation the N75-P100-N145 complex (Fig. 3) is maximal on the midline but it spreads laterally and can be picked up with decreasing amplitude but similar latency by electrodes 5 and 10 cm laterally to the midline on either side.

Fig. 3. Whole field response and its distribution on the scalp.



It has been established in the past that it is also possible to record responses to various sectors of the visual field and that the sum of the constituent fields should be approximately equal to the response obtained by the direct stimulation of the whole area (Blumhardt et al. 1979; Halliday, 1993). This means that the size of the response depends on the size of the stimulated field. The most frequently

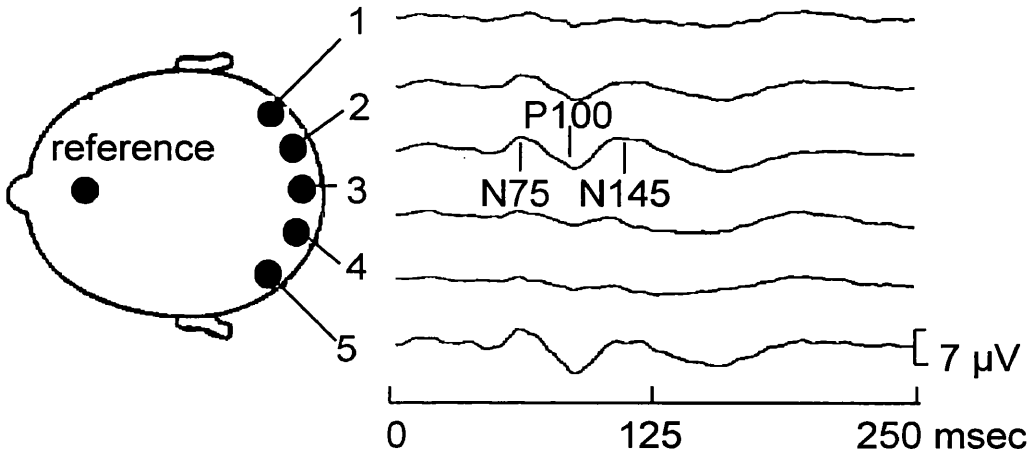
stimulated areas are the central field and the right and left hemifield but quadrant or upper and lower field stimulation may also be performed (responses obtained from the lower field are larger as compared to those obtained from the upper field, possibly because of the distinct sites where the visual pathway subserving the two areas ends at the visual cortex, and their position with respect to the recording electrodes). The importance of the stimulation of different sectors of the visual field separately is related to the possible presence of small lesions affecting some optic nerve fibres or retinal elements which may not be detected when stimulating large areas of the visual field: in this case the contribution of fibres which still conduct normally, i.e. are not affected by the pathological process, may mask the abnormality (Riemslag et al., 1985). Several studies (Hennerici et al., 1977; Diener and Scheibler, 1980; Hammond and Yiannikas, 1986) have shown a higher sensitivity of central field or foveal stimulation (subtending a visual angle up to 4°-5° degrees in diameter) as compared to whole field and hemifield stimulation to detect abnormalities following demyelination. This was thought to be related to a more common involvement of the fibres subserving the central field during an attack of optic neuritis (Lumsden, 1970; Frisen and Hoyt, 1974; Edgar et al., 1990) but, as proposed in more recent years, this may alternatively be explained by a higher susceptibility of the small axons (mostly subserving the central field) to conduction block caused by demyelination as compared to the larger axons (Dain et al., 1990).

The central field response (Fig. 4), characterized by a similar

triphasic waveform, the N75-P100-N145, is also largest at the midline electrodes, but it will be generally of lower amplitude than the whole field response, because the area stimulated of the visual field is proportionately smaller. However, it is also known that the evoked response mainly comes from the central part (approximately up to 10° in radius) of the visual field and less from the periphery. An explanation suggested in the past was that the electrodes placed on the scalp could "see" better the responses coming from the fibres subserving the central field (these fibres terminate at the pole of the occipital lobe) rather than those subserving the periphery (Yiannikas and Walsh, 1983). A second explanation proposed by the same authors was that of a higher concentration per unit of cone receptors in the foveal region of the retina: the cone receptors appear to be more sensitive to pattern stimulation, whereas transient movement detectors (rod system) appear to predominate in the more peripheral areas. A more plausible explanation, however, is that of cortical magnification: larger cortical areas are devoted to the projections coming from the central fibres of the retina and particularly from the foveal and parafoveal regions, as compared to the periphery. In addition the central field response shows a relatively longer latency as compared to the peripheral responses (Yiannikas and Walsh, 1983), possibly on account of the presence in the macular region of smaller fibres which conduct at a slower velocity (Novak et al., 1988). However, Blumhardt et al. (1989), on the basis of hemifield P100 data, suggested that the latency difference between central and peripheral responses is only

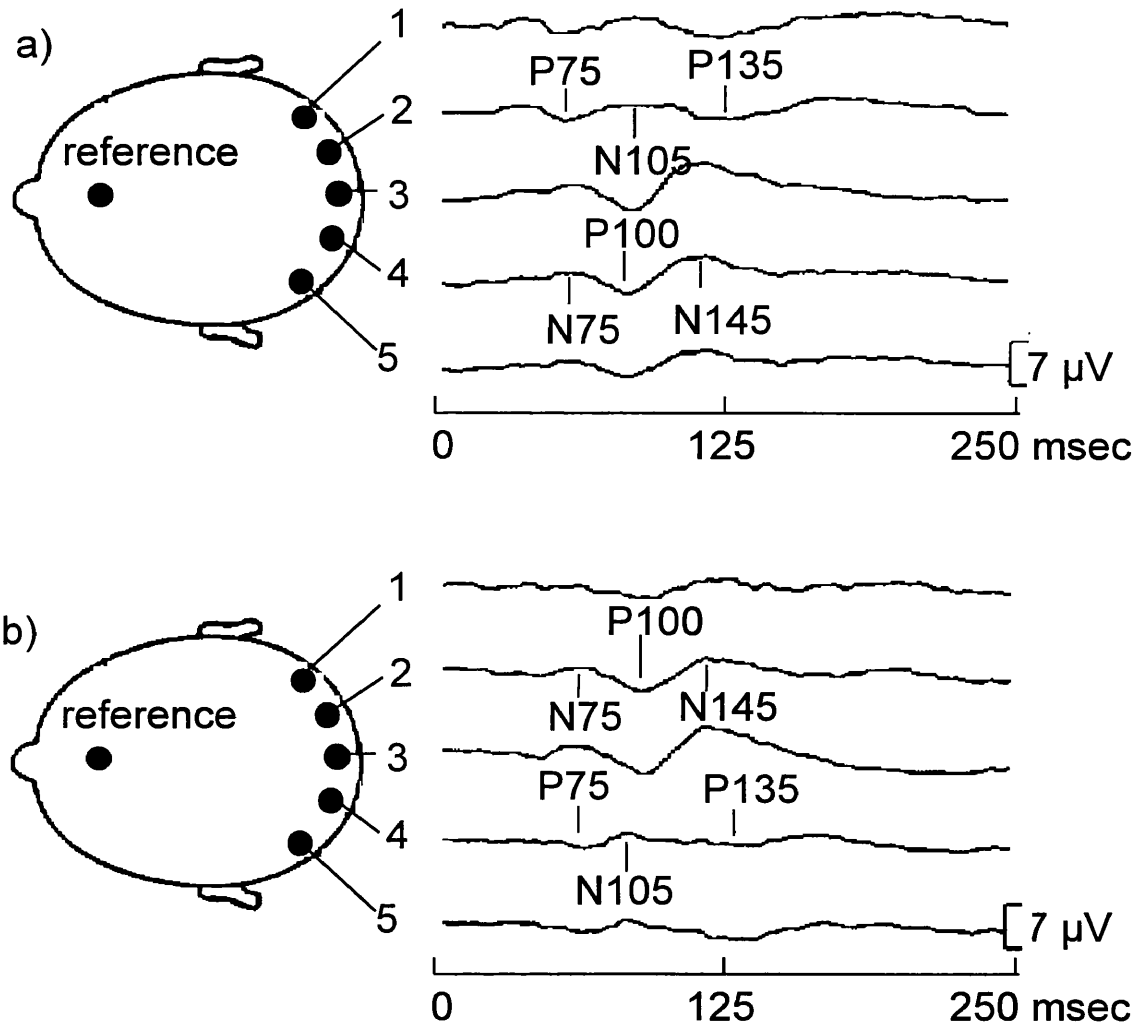
apparent and probably due to the variation in topography and therefore of waveforms of the responses with eccentric stimulation.

Fig. 4. Central field response and its distribution on the scalp.



The left and right hemifield stimulation (Fig. 5) produces the characteristic triphasic waveform at the midline and at the electrodes ipsilateral to the stimulus (N75-P100-N145 complex, or ipsilateral waveform), but at the contralateral electrodes a triphasic response of lower amplitude, opposite polarity and approximately the same latency is also recorded (P75-N105-P135 complex, or contralateral response).

Fig. 5. Left (a) and right (b) hemifield responses and their distribution on the scalp.



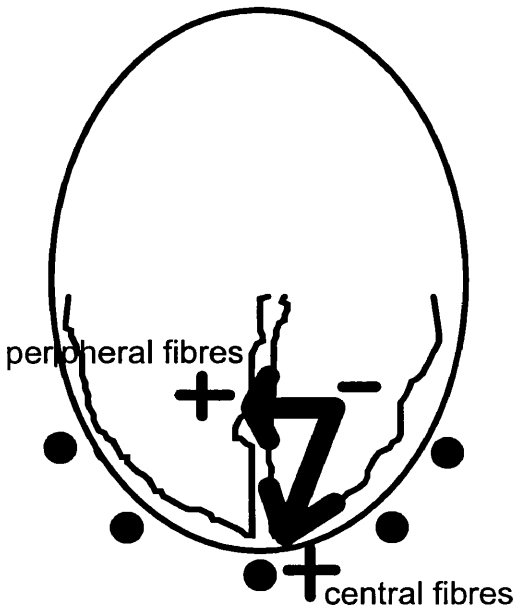
The ipsilateral response is usually larger at the electrode 5 cm lateral (on the same side as the stimulus) to the midline, although this is usually recorded also 10 cm laterally and on the midline. The contralateral response, conversely, is usually largest 10 cm laterally to the midline, on the contralateral (to the stimulus) side of the head. The response at the contralateral electrode 5 cm from the midline is most

frequently a transition between the ipsilateral and the contralateral responses (and therefore relatively variable), although sometimes a transitional waveform can be seen at the electrode on the midline or the electrode 10 cm laterally to the midline.

The effect of varying the field size and introducing central "scotomata" shows that the ipsilateral waveform is mainly recorded when the field subtending the central 0° to 6° (radius) from fixation point is stimulated (and therefore called macular response), whereas the contralateral response arises from the stimulation of fields subtending 10° or more (paramacular response) (Blumhardt et al., 1978 and 1989). Although the response is generated in the hemisphere contralateral to the stimulated hemifield (according to the anatomy of the visual pathway) the fact that responses are recorded over both hemispheres (Barrett, 1976) is due to the position of the generators, the cortical columns, and as a consequence to the orientation of the dipoles with respect to the recording electrodes: the foveal and central field fibres reach the pole of the occipital lobe so that the dipole will be directed towards the midline electrodes. Conversely, the fibres arising from more peripheral areas of the visual field end on the mesial surface of the hemisphere in and around the calcarine sulcus: therefore their dipole will be orientated perpendicular to the mesial surface. The resultant vector of the activity arising from the central areas and from the more peripheral areas will be orientated postero-medially and directed towards the electrode on the contralateral side (Fig. 6). There is, however, a high variability in the

distribution of the foveal and central fibres at the cortex: instead of terminating at the pole of the occipital lobe, these fibres can reach more lateral or more medial areas of the occipital cortex in different individuals and sometimes also in the 2 hemispheres of the same individual. It can occur that all central and foveal fibres end on the mesial surface in the calcarine fissure (like the peripheral fibres) or that these fibres end on the lateral side of the occipital lobe. Conversely, the peripheral fibres will end more constantly on the mesial surface of the brain. Therefore the orientation of the resulting vector will change according to the distribution of the central and foveal fibres (Blumhardt et al., 1989).

Fig. 6. Example of the orientation of the vectors for the central and peripheral fibres ending at the right occipital lobe.



As previously mentioned the sum of the constituent field responses will approximately equal that of the whole field (Halliday, 1993). Therefore the distribution of the ipsilateral and contralateral responses to hemifield stimulation can clarify the morphology and distribution of the whole and central field responses. In whole field responses the amplitude decreases from the midline to the laterally placed electrodes: this is partly due to the contralateral response which tends to cancel out the ipsilateral response from stimulation of the opposite hemifield, to the greatest degree at the 10 cm electrode. Sometimes the contralateral response may exceed the ipsilateral response in amplitude, and this may result in intrusion of contralateral components (chiefly the P135) into the whole field waveform: asymmetrical responses, bifid responses or "apparently delayed" responses can then be produced to whole field stimulation. Conversely, for the central field response, because of its being "central" it is more unusual to have paramacular responses intruding. The analysis of the macular and paramacular responses can, therefore, be of use in interpreting abnormalities occurring in the whole field response (Blumhardt et al., 1982). In particular, when the response recorded at the midline electrode is of bifid morphology (W-shaped, with 2 positive peaks) it is important to establish whether the later deflection represents a P135 (the contralateral response generated from the more peripheral areas of the visual field) of normal latency or a truly delayed P100 (ipsilateral response, generated in the more central areas of the visual field). The importance of the correct

interpretation of responses "apparently prolonged" in latency was described in 1978 (Blumhardt et al.) and in 1979 (Blumhardt and Halliday). However, it was in 1984 (Carroll et al.) that hemifield stimulation was used for the distinction between a P100 of truly prolonged latency and a P135 of normal latency in patients affected by optic neuritis. In 1985 Halliday and in 1986 Hammond and Yiannikas confirmed the above findings and showed also the usefulness of hemifield stimulation in the detection of postchiasmal lesions (an homonymous defect, ie affecting the right or left hemifields, suggesting a lesion in the post-chiasmal visual pathway of the contralateral hemisphere). In 1996 Onofrj et al. studied 20 patients affected by optic neuritis and distinguished between "real delays" and "pseudodelays", as had previous authors, on the basis of the distribution on the scalp of the VEP responses and the identification of the ipsilateral and contralateral components to hemifield stimulation. Onofrj et al. also suggested that "real delays" indicate a good functional prognosis, whereas "pseudodelays" indicate a poor prognosis for visual function. This view was explained by the fact that a delay indicates that conduction, however slow, is occurring, whereas in "pseudodelays" there is reduction in the P100 amplitude due to block of conduction which has not been overcome.

One of the parameters which influences VEP recording is the contrast which is close to 90-100% in most studies. Low contrast stimuli generate responses which are of lower amplitude and broader in morphology (Spekreijse et al., 1973). A second parameter, the

overall luminance, will affect latency and amplitude of the response, showing an increase in latency and a decrement in amplitude when the stimulus luminance is decreased (Halliday, 1993).

In normal subjects no effect of check size (described in terms of the angle they subtend at the eye) on VEP latency was shown by Oishi et al. (1985), but a significant prolongation of the latency with smaller checks has been reported by other authors (Plant et al., 1983 and Novak et al., 1988). The difference between these studies has been explained by the size of the screen and the size of the checks (larger in the study by Oishi, so that retinal ganglion cells outside the fovea were also stimulated). It has also been previously reported that smaller checks produce the largest response from the central field, whereas larger checks produce the largest response in the periphery according to the sizes of the receptive fields, which are respectively smaller for the central field and larger for the periphery (Oishi et al., 1985). In the diagnosis of patients the use of various check sizes increases slightly the yield of abnormal responses according to Oishi et al. (1985) and Novak et al. (1988). However, the use of various check sizes for each patient would be time consuming. Most laboratories use check sizes ranging between 20' and 50' which produce the largest whole field response and control for accomodating defects (it is important to remember that if VA is suboptimal due to accomodation error the check size should be appropriate for the VA in that eye).

Other factors which influence the evoked responses are sex and age of the subjects. In particular it has been reported that VEPs show

a longer latency for males but a higher amplitude for females (Allison et al, 1984; Halliday, 1993). Even more important are the changes with age. This aspect has been studied by a number of authors and although the methods used for the stimulation were different the overall agreement is that the latency decreases with maturation of the central nervous system up to approximately 20 years of age and increases in patients over 60 years of age (Allison et al., 1983; Celesia et al., 1987; Halliday, 1993). There are contrasting findings on what occurs in the intermediate years so that for example according to Allison et al. (1983) latency would be stable and according to Sokol et al. (1981) there is steady increment of the latency which is more evident (twice as fast) with small check size.

1.7.2.2 VEP characteristics after optic neuritis

In the initial phase of optic neuritis, when vision is most markedly impaired (decreased VA, impaired colour vision, etc.), it may be impossible to record any visual evoked response. The characteristics of this phase (duration, severity, etc.) predominantly depend on the ongoing inflammatory process (characterized by oedema and swelling of the optic nerve), responsible for reversible conduction block of the nerve fibres. However, the demyelinating process might also contribute to the findings: demyelination causes conduction block and it has been demonstrated to occur at about the same time as inflammation, although the correct order of events is not yet entirely

clear (Youl et al., 1991).

Once the acute phase, mainly related to inflammation, has subsided VEP recording becomes possible and the classical pattern of the responses is characterized by:

1) prolonged latency (in absolute or relative terms),

2) usually reduced amplitude,

3) relatively preserved morphology.

The early amplitude changes seems to be mainly attributable to the effects of reversible conduction block (possibly affecting most of the fibres of the optic nerve) and it has been suggested that the initial visual loss (and maybe part of the delay in latency) is also related to conduction block and inflammation. In particular Youl et al. (1991) found that low amplitude and delayed latency in evoked responses as well as symptoms and signs of acute optic neuritis such as visual loss, impaired colour vision, afferent pupillary defect and pain on eye movement occur at the same time as Gd-DTPA leakage on MRI. The latter is believed to be an index of inflammation and it has been shown that with its resolution (about a month after the initial symptoms on the basis of MRI data) and therefore resolution of conduction block, VEP amplitude and symptoms and signs of ON improved, but the latency did not (which suggests a cause of the latter distinct from inflammation).

During the recovery phase a positive correlation between the impairment of visual acuity and the decreased amplitude of the visual evoked responses was found and later it was observed that they could

improve to nearly normal values. These findings may suggest that the same pathological mechanisms may be at their basis. At present there is no evidence of a causal connection between amplitude changes and inflammation, but it is likely that inflammation determines the reversible element of conduction block, whereas the residual amplitude abnormalities are probably due to demyelination and its effects such as conduction block and dispersion. However, it has to be noted that a low amplitude response in the recovery phase of ON may also be due to axonal loss. Until recently axonal damage was shown only in chronic lesions in multiple sclerosis (McDonald et al., 1992; Barnes et al., 1991) but Ferguson et al. in 1997 and Trapp et al. in 1998 gave immunochemical and immunohistochemical evidence in MS for axonal loss in the acute lesions, as if the axonal damage was an episodic phenomenon and closely related to inflammation.

However, the most important feature of the VEPs in demyelinating lesions is the delay in latency of the responses. Because of its low variability among control subjects, changes in latency enable us to distinguish between abnormal and normal responses providing useful information to the clinician.

A number of experimental studies in the '60s and '70s had established that demyelination was mainly characterised by a latency delay, whereas wallerian degeneration was characterised by loss of amplitude of the responses (McDonald, 1963; McDonald and Sears, 1970). In particular the authors showed experimentally in the cat that conduction velocity following axonal degeneration in the posterior

columns did not decrease (although minimal latency delay may occur following the loss of fast conducting fibres), but the amplitude of the responses was markedly reduced. On the other hand, demyelination in the cat following diphteria toxin injection in the dorsal root ganglion and spinal nerve root could block transmission in some fibres, reduce the conduction velocity and not affect yet other fibres. In the '70s it was shown that VEP latency was prolonged after an episode of optic neuritis (Halliday et al, 1972). In more recent years it has been observed that the slowing of conduction in the CNS seems to occur at an early stage of the pathological process (Halliday, 1981; Youl et al., 1991). Because of the tendency of the delay in latency to persist and to be stable unless a further attack of ON occurs, it was rightly thought that a measure such as delayed VEP would provide objective evidence of past optic neuritis also years after the acute episode and therefore help the clinician in the diagnosis of multiple sclerosis (Halliday et al., 1977). The amount of the delay varies among individuals but it has been reported in the past to be on average about 30 msec (=affected eye -unaffected eye) (Halliday et al., 1972; Jones, 1993). However, although subsequent evidence has substantiated the notion that VEP abnormalities tend to persist (Walsh et al., 1982; Celesia et al, 1990;) it has become increasingly clear that the degree of VEP delay is not fixed and permanent. There is enough evidence at present for a normalization of the delay in latency to occur in some 10-20% of patients (Matthews and Small, 1983; Hely et al. 1986; Jones, 1993) months or years after the optic neuritis. The rate of normal responses

is much higher in children (55% at on average 8.8 year interval after the acute episode of optic neuritis) possibly due to a greater potential for remyelination in younger than in older subjects (Kriss et al., 1988).

The last feature to take into account is the morphology of the VEP responses which can also be affected: bifid morphology (although this is not a definite indication of pathology when the latency of both peaks is well within the limit of normal) or responses degraded in chronic phase due to axonal loss (dispersion). The bifid morphology may indicate the intrusion of a P135, the contralateral potential, as a consequence of the attenuation of the ipsilateral (macular) responses. In these cases the first peak represents the real P100 and the second one a P135, possibly of normal latency. Additionally a bifid morphology may also indicate the presence of fibres which conduct at different velocity, either normal or abnormal as for example in the case of a lesion affecting only some of the fibres which produce the macular responses.

The VEPs, measuring the conduction velocity along the visual pathway, have become important tools in the confirmation of the clinical diagnosis of optic neuritis and in the detection of asymptomatic optic neuritis, revealing in a number of cases delayed responses which are clinically silent. Visual evoked responses were abnormal in 83% of cases with a clinical diagnosis of ON, with or without a diagnosis of MS, in the study of Matthews et al. (1977), in 91% in the study of Shahrokhi et al. (1978) and in 88% according to Chiappa (1980). According to Halliday (1993), only 5-6% of patients with symptomatic

ON will show a normal latency response probably because of very short plaques and therefore minimal delay. The percentage of the patients, without visual complaints but with symptoms involving other sites of the CNS (classified as definite, probable and possible MS), who show an abnormality to pattern visual evoked responses is very variable: for example Shahrokhi (1978) found 36% of abnormalities and Matthews et al. (1977) 52% abnormal cases. In a summary of 26 clinical series including 1950 patients with all MS classifications (with or without symptomatic ON), Chiappa (1997) found that the average abnormality rate was 63%. When 744 patients in all MS classifications, but without clinical history of optic neuritis were taken into account the average of abnormality was approximately 50%. He suggested that the wide range in the various studies was possibly due to the different preponderance of one group of MS patients (ie definite, possible or probable) with respect to the others and also to the different techniques used to record VEPs. From the clinical point of view the groups of interest are the possible and probable MS because they represent often a difficult diagnostic problem. The usefulness of VEP recordings in these cases where the diagnosis of MS is still uncertain is evident: detecting silent abnormalities or confirming an episode of ON in dubious cases (Halliday, 1993) adds further important information for the diagnosis of MS. In summary, to make a diagnosis of MS it is necessary to show on examination or in the history of the patient the involvement of 2 or more sites of the CNS each lasting at least 24 hours and at least a month apart (or gradual step-wise

progression over 6 months) (Schumacher criteria). However, it is not always possible to show a second lesion on clinical or historical grounds and therefore further methods like laboratory examinations, evoked potentials (and neuroimaging) have been used to help the clinician in this task (Poser criteria).

The role of VEP and MRI is, however, distinct: VEPs provide functional information about the central nervous system, whereas MRI provides morphological data. Therefore they are both useful in the diagnosis and investigation of MS because they explore the nervous system from different points of view and they are not mutually exclusive.

1.7.3 Psychophysical examination

1.7.3.1 Contrast sensitivity

Other than the VEPs, which analyze the neuronal functions and the organization of the visual pathway, additional methods for the study of optic neuritis are the psychophysical tests which mainly give information about the sensitivity of vision (threshold tests). In a pathophysiological study, the importance of performing different types of tests (VEPs, psychophysics etc.), in addition to the standard clinical tests, resides in the fact that the latter are often inadequate to demonstrate subtle but significant defects.

It has been noted in fact that although the examination of the visual acuity may be normal, other functions of vision may be abnormal (Zimmern et al., 1979; Regan, 1981; Fleishman et al., 1987; Budning et al., 1991). The ability to see an object does not depend only on its size (studied by use of the Snellen charts), but also on other factors, such as its relative brightness or contrast (defined as the difference between the luminance of the bright and the dark elements divided by the sum of their luminance) with respect to the background. It is evident that the Snellen chart is not a suitable test for contrast sensitivity, as the letters shown have generally a high contrast and also a predominant association with the higher spatial frequencies (Zimmern et al, 1979). The contrast sensitivity testing gives one information about the visual sensitivity to perceive contrast at specific spatial and temporal frequencies.

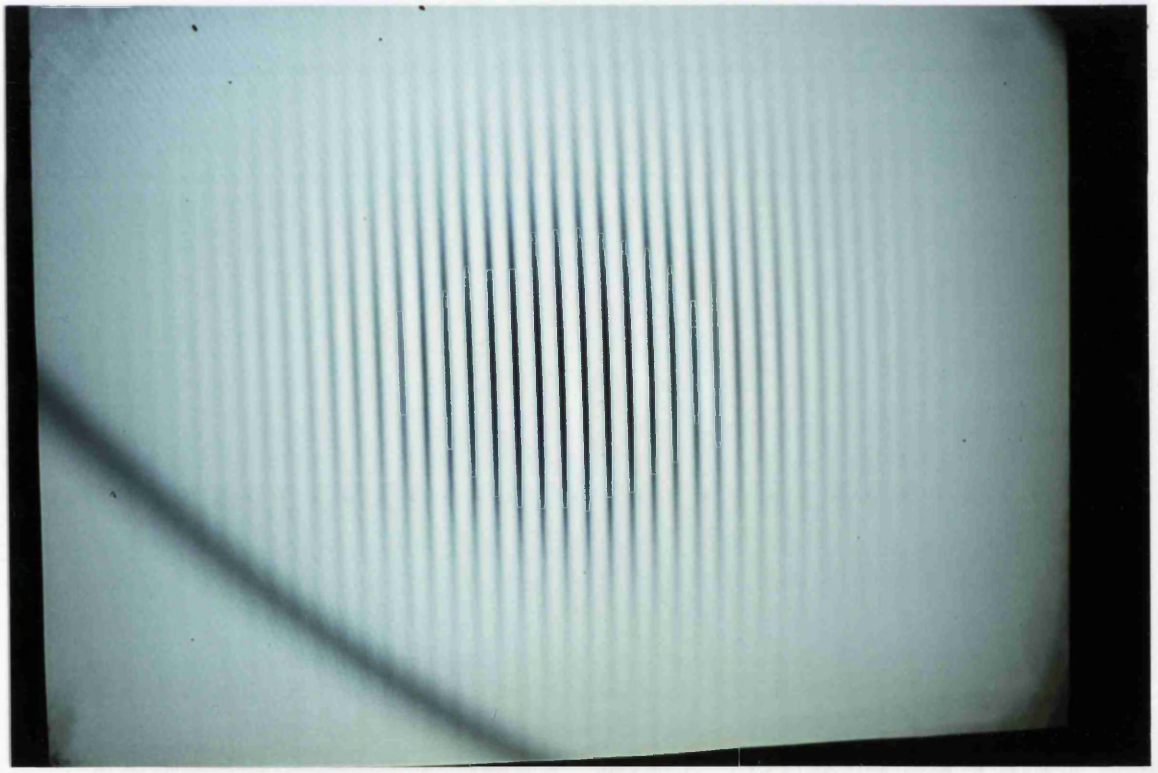
On the other hand, it is important to remember that contrast sensitivity testing is of use only when performed in patients with normal or near normal visual acuity (Moseley and Hill, 1994): the abnormalities in this test might correspond to the common complaint of "washed out" vision in MS patients with normal visual acuity (Regan et al., 1977; Regan, 1981; Hess, 1983; Beck et al., 1984).

To perform the test the target (gratings, filtered letters etc.) can be shown on a hard copy or on a screen. The former, more frequently used in clinical testing, enables one to examine a limited range of spatial frequencies only (the charts need to show small enough changes in contrast to evaluate the correct threshold), whereas the latter, more frequently used in experimental studies, allows the examiner to test a wide spatial and temporal frequency range (Moseley and Hill, 1994) and the effects of temporal modulation can be measured (Regan et al, 1977; Hess and Plant, 1983 and 1985; Medjebur and Tulunay-Keesey, 1985) (Fig. 7).

The contrast sensitivity can be analyzed as the perception of contrast above threshold or the sensitivity to contrast changes. The first type of analysis is performed using a contrast matching technique (the patient, generally with a monocular defect, has to match a grating of fixed contrast shown in the affected eye to the gratings presented to the unaffected eye), whereas the second type of analysis is performed using a contrast discrimination technique by means of a two alternative forced choice paradigm (the subject has to decide for example which gratings have the higher -or lower- contrast, or whether the screen is

blank or shows the pattern) (Hess and Plant, 1986). The contrast sensitivity in the latter case is generally evaluated for various spatial frequencies (measure of the size of the retinal images, mainly studied by means of sinusoidal gratings and hence also defined as the number of bars of the grating within a degree of visual angle) and temporal frequencies (the stimulus can be stationary or temporally modulated), although in some studies (e.g. Regan et al., 1980) the orientation of the gratings is also taken into account.

Fig. 7. Example of grating for contrast sensitivity.



Sinewave grating at 0.5 c/deg spatial frequency: it fades away to avoid sharp edges.

The eccentricity of the stimulus is a major factor determining the responses of the subjects, so that the highest sensitivity is recorded in the central regions (Hess and Plant, 1986). Other parameters which

affect the contrast sensitivity are:

- the size of the stimulus, in the sense of a reduction of the contrast sensitivity when the size of the stimulus is reduced;

- the spatial frequency

- the temporal frequency (stationary and temporally modulated stimuli). To a stationary stimulus the sensitivity is higher for medium spatial frequencies (around 4 c/deg), whereas it falls off to lower and higher spatial frequencies. When the stimulus is temporally modulated (in this case a spot of light is used to avoid the effect of spatial frequencies) the sensitivity peak is at 15 Hz, with a fall off on either side (at 60 Hz a flickering light is perceived as stationary). The fall off at lower spatial frequencies (when the stimulus is stationary) and at lower temporal frequencies (when the effect of the spatial frequency on the stimulus is not taken into account) is due to an adaptation phenomenon which enables us to detect change in luminance rather than absolute luminance. The fall off to higher spatial frequencies is due to neural and optical factors when the stimulus is stationary and the fall off to higher temporal frequencies, when only temporal characteristics are taken into account, is due to neuronal factors alone.

- interaction between the temporal and the spatial characteristics of a stimulus: at low spatial frequencies the sensitivity is highest at about 15 Hz temporal frequency, falling off on either side (same pattern as before), whereas at high and medium spatial frequencies the contrast sensitivity fall off is seen quite abruptly above 15 Hz temporal frequency, but not below it (Hess and Plant, 1986). These findings

suggest that there may be a number of discrete spatial "channels" but only a few temporal "channels" which determine the ability to detect spatial and temporal characteristics, respectively, of a specific stimulus.

Although there are differences among the various studies, most of the authors agree on the fact that in optic neuritis there is more frequently a patchy deficit as shown by the use of stimuli of various spatial and temporal frequencies at different eccentricities to the visual field. Nevertheless cases where there may be a more extensive involvement are also seen. The selective involvement in some cases of specific spatial frequencies (the least commonly involved are the low spatial frequencies and the most frequently involved the high spatial frequencies) at particular temporal frequencies would suggest a spatial frequency dependent deficit (Regan, 1977; Hess and Plant, 1986). However, Hess and Plant (1986) showed by means of simulation experiments that losses at selective spatial frequencies may also be explained by a retinal locus specific defect: a deficit maximal in the centre of the visual field causes a contrast sensitivity deficit maximal at high spatial frequencies, a parafoveal deficit predominantly affects medium spatial frequencies, a peripheral deficit (with sparing of the fovea) gives a predominant low frequency loss and finally when the visual field loss is generalized the reduction of contrast sensitivity will also be generalized, with involvement of all frequencies. From these data it is therefore uncertain whether the contrast sensitivity loss is truly spatial frequency dependent, retinal locus specific or whether both

factors play a role in the deficit (Plant and Hess, 1987). A greater vulnerability to the effects of demyelination of the small fibres in optic neuritis was suggested in 1990 by Edgar et al.: he studied patients with recovered optic neuritis, testing for deficit in the temporal domain. He found contrast sensitivity (stimulus: 1° in diameter) deficits to al, but mainly to medium and lower temporal frequencies, at 0° and 2.5° eccentricity, to medium temporal frequencies at 5° eccentricity and no abnormalities at 10° eccentricity. This pattern of abnormalities was explained by the different distribution of small and large fibres across the retina, with the highest concentration of small diameter fibres (the more vulnerable to the effects of demyelination) in the macula and their decrement with eccentricity as shown in the past in primates (Potts et al., 1972; Sanchez et al., 1986).

In the case of temporally modulated stimuli, Medjbeur and Tulunay-Keesey (1985) reported that the introduction of temporal modulation produced 3 different patterns in different patients: 1) it reduced the deficit at all spatial frequencies, 2) increased the deficit at most spatial frequencies, 3) reduced the deficit at low spatial frequencies (below 2-3 c/deg) and increased it at high spatial frequencies. They commented that these abnormalities could not be predicted by stationary stimulation alone. According to Hess and Plant (1983 and 1985) the contrast sensitivity loss to low spatial frequency would be dependent on the temporal frequencies (the sensitivity loss was more severe at low temporal frequencies and it diminished with their increment), whereas the threshold contrast sensitivity loss at high

and medium spatial frequency was unaffected by changes of the temporal frequencies. As previously mentioned, the findings led the authors to conclude that high and low spatial frequencies information is possibly analyzed by separate "channels" and noted that the non-uniform functional involvement is in agreement with the findings of non-uniform lesions at histological examination: areas of complete and/or patchy demyelination are in fact present in the same nerve (mainly inferred from neuropathological findings) possibly causing patchy defects in contrast sensitivity (Regan et al, 1977; Prineas and Connell, 1978; Riemslag et al., 1985; Plant and Hess, 1987).

Regan et al. (1980) and Regan (1981) have shown that the contrast sensitivity loss in patients with MS depends also on the orientation of the stimulus. From experiments in cats it appears that true orientation specific structures are first found in the cortex as demonstrated anatomically and neurophysiologically by Hubel and Wiesel (1974) so that selective orientation loss was thought to be evidence of cortical involvement rather than optic nerve lesion. However, in more recent years it has been suggested that similar properties may be present in the retinal ganglion cells of the cat but the presence of selectivity for orientation has not yet been shown in primates (Thibos and Levick, 1980). Kupersmith et al. (1984) confirmed Regan findings by means of evoked potential contrast sensitivity to 3 spatial frequencies (1, 4, 8 c/deg) and 4 orientations (0°, 45°, 90° and 135°). Twenty-four out of 26 cases showed abnormal responses to multiple orientations, whereas only 14 cases had

abnormal responses to checkerboard pattern reversal stimulation. It was also observed that patients with optic neuropathy and macular lesions showed contrast threshold elevations which were not orientation-specific, whereas patients with MS (with or without symptomatic ON) showed orientation-specific losses. Orientation-specific loss was also shown by Camisa et al. (1981) by means of electrophysiology rather than psychophysics: VEP recordings to checkerboard pattern and to gratings (vertical, horizontal and oblique) in patients with MS were compared and the authors found orientation-specific abnormalities, but all orientations could be affected in MS.

It was generally believed that the abnormalities seen in contrast sensitivity threshold were non-specific for optic neuritis and were common also to other pathologies, such as lesions of the visual cortex, glaucoma and amblyopia (Regan et al., 1981). However, Hess and Plant (1983 and 1985) found that the pattern of abnormality in optic neuritis might show some specific characteristics: it appeared that the finding of a spatio-temporal dependence of the contrast sensitivity deficit when low spatial frequency stimuli were used applied to mild optic neuritis and was not shown for more severe optic neuritis or in other pathologies of the optic nerve, when contrast thresholds would be affected in the same way at all temporal frequencies (detection experiments). Abnormalities as severe as those found in threshold testing (detection experiments) were also seen in suprathreshold testing (matching experiments), whereas in other optic nerve pathologies deficits in the latter were only mild.

Zimmern et al. (1979) believed that the test, although the abnormalities of the contrast sensitivity in their patients were always correlated with the clinical picture or the electrophysiological data, could add further information for the clinician. Conversely, other authors, such as Regan et al. (1977) and Medjbeur and Tulunay-Keesey (1985) showed that this technique was sensitive to subclinical defects when a stationary stimulus alone was used. Additional testing with a temporally modulated stimulus may increment even more the yield of the test and add information about the spatio-temporal processing, not predicted by the stationary testing. The functional abnormalities detected with this test may also be at the basis of the subjective complaint of some patients affected by optic neuritis. The Optic Neuritis Treatment Trial (Cleary et al., 1997) tried to compare the patients' subjective assessment of visual impairment with measurements of visual acuity, contrast sensitivity (the test was performed with a Pelli-Robson chart so that results may not be totally comparable with the previous ones), visual fields and colour vision and found that of those patients who perceived their vision as worse than before optic neuritis at 6 months, 20% had all the tests abnormal, 14% 3 out of 4 tests, 23% 2 out 4, 23% 1 out of 4 and 20% had all the tests normal, which means that visual tests may show normal results although the patients perceive a defect in his/her vision. In addition, whereas in the acute phase all vision tests were often abnormal, at 6 months from the onset of symptoms the contrast sensitivity defect was the most frequent as compared to VA or visual field defect. It was

proposed that these findings suggest a differential vulnerability of visual functions after optic neuritis or a different sensitivity of the tests performed (Trobe et al., 1996).

1.7.3.2 Visual fields

The most frequently used method to study the visual fields in recent years is the automated field analyzer, but in the past other techniques like the Goldmann perimetry have been used. The automated field analyzer is a static quantitative method where certain points of the visual field are explored one at a time (different from the kinetic perimetry where the stimulus is shown where it cannot be seen and moved towards the fixation point till it can be seen). It is mainly used to study the central 30° of the visual field where the changes in sensitivity with increase or decrease of eccentricity are more marked. One of the advantages of the automated method is the higher reliability in the comparison of follow-up tests. The parameters taken into account in visual field investigation are the background luminance, which establishes the retinal adaptation during the test, the stimulus duration, which should be short enough not to alter the retinal adaptation, the stimulus interval (which in some cases is determined by the subject responses), its size (different sizes have been used by different authors) and its luminance (the sensitivity to luminance can be measured only if the background luminance is kept constant and the retinal adaptation does not vary) (Bedwell, 1982). When the technique is used in patients with ocular symptoms a visual field defect is detectable in almost all patients: the overall agreement is that at presentation after an episode of ON the most common deficit pattern is a central defect, but this may evolve and leave an enlarged blind spot

with an arcuate scotoma (fragments of this may be permanent) (McDonald, 1992). In particular Nikoskelainen and Riekkinen in 1974 using a Goldman perimetry found that the most common abnormality seen at presentation (retrospective analysis) was a diffuse field defect (approximately 65% of eyes) while at re-examination (on average 10.2 yrs later) a paracentral defect (mainly in the blind spot region) was more common (34%). Normal visual fields at presentation were approximately 5% and abnormalities persisted at follow-up, with only 34% of patients with normal visual field after ON. In 1979 Perkin and Rose found at presentation a field defect in almost 80% of their patients studied less than 3 weeks from the onset of symptoms. A central defect alone was seen in 30% of patient and in 24% associated with a peripheral defect. Other defects encountered were generalized depression, altitudinal, peripheral and arcuate defects. Only 9% of asymptomatic eyes showed a field defect. Patterson and Heron in 1980 (using a tangent screen) studied patients with MS (definite, probable and possible) and patients with past ON. They found visual field defects in 94% of eyes with a history of visual symptoms (time range: 0.1-9 yrs) and 67% of those without a history of visual symptoms (time range:0.1-25 yrs). The most common abnormality was an arcuate scotoma, but generalized depression or paracentral scotoma were also sometimes present. Celesia in 1990 reported that all 20 patients affected by optic neuritis and tested by means of a Goldman perimetry had a field defect at presentation but at 3 to 12 month follow-up the deficit persisted in only 20% of them. A similar

pattern was also found in the American Optic Neuritis Treatment Trial (ONTT) (Keltner et al., 1993 and 1994), where automated static visual field analysis was performed on 448 patients on 9 occasions in a year. All patients had abnormal visual fields at presentation as required by the criteria of entrance in the study. Recovery occurred between presentation and 6 month follow-up in 51% of cases which increased to 56% at one year follow-up. It is generally believed that a central or a centrocaecal scotoma is very common in patients with optic neuritis but according to this study only 8.3% of the patients had such a defect, while 20.1% had a localized defect (altitudinal, arcuate etc.), 23.2% had a variety of other field defects and 48.2% had a diffuse defect at presentation. Asymptomatic visual field defects at presentation were a common finding also in the fellow eye (68.8%). At 1 year approximately 30% were still abnormal (Keltner et al., 1993 and 1994). Rizzo and Lessell (1991) made a list of the type of defect they found retrospectively in 81 patients: 59% had a central defect, 10% altitudinal, 7% arcuate scotomas and 10% other types. The authors concluded that there was no pattern of visual field loss characteristic of this condition and therefore the visual field findings could not be considered as diagnostic. In 1995 Söderstrom found that 84% of his patients, tested by means of Goldman perimetry, had a visual field defect, in the majority of cases a central scotoma. Some of the differences encountered among these studies were probably due to the variable interval elapsed between the onset of symptoms and the time when the test was performed, to different patient selection criteria and

also to the different techniques used .

1.7.4 Imaging of the optic nerve

A further method to study the optic nerve and the effect of demyelination is magnetic resonance imaging (MRI).

Magnetic resonance imaging of the brain was introduced in the '80s as a diagnostic tool (Young et al., 1981) which, although very sensitive, is not specific for demyelination (Paty et al., 1988). In the clinical setting it is mainly used to localize the demyelinating lesions (morphological information), but by means of particular contrast agents, like gadolinium, it can give information concerning changes in a particular lesion with time. Because of these characteristics, it is an important tool in research in an attempt to understand the pathophysiology of MS (Paty, 1988 and 1993). The use of MRI as a method to monitor the effects of treatment in multiple sclerosis has also been proposed (Paty, 1993).

Nuclear magnetic resonance imaging depends on the density and velocity of the hydrogen ion and their T1 and T2 magnetic relaxation times after applying a radio frequency (RF) pulse to a subject in a strong magnetic field. T1 indicates the time constant for the magnetization of the sample, when exposed to the magnetic field (it reflects the interaction of the hydrogen ion with its molecular environment) and T2 indicates the time constant of the decay of the signal emitted following sequential radio frequency pulses (it reflects the magnetic interaction between nuclei) (Lukes, 1983). Because the signal from a normal brain is mainly derived from free water protons

and T1 and T2 are influenced by the macromolecular environment, abnormalities in the images will derive from changes in the water quantity and the macromolecular environment. It has to be noted that it is impossible to image the myelin directly, because it is mainly composed of lipid protons and water associated with it which have a very short T2 relaxation time. However, following the loss of myelin part of the tissue may be occupied by water molecules with a longer T2 relaxation time which are therefore detectable and make the abnormalities evident. In summary, although the MRI does not allow the direct study of the myelin, it may give us information regarding phenomena which occur along with demyelination, such as oedema, where the water molecules have a long T2 relaxation time (it depends on the elemental composition and the molecular structure), or gliosis with an increment of the water content (gliosis alone in fact does not prolong the T2 relaxation time and therefore cannot be responsible for MRI changes) (Ormerod et al., 1987; Barnes et al., 1991). Further information can be obtained by means of gadolinium-DTPA, a paramagnetic contrast agent which reflects the presence of inflammation with the "enhancement" of areas of breakdown in the blood-brain barrier in the first month from the onset of symptoms (Gd-DTPA does not cross the normal blood-brain barrier but an alteration of its permeability is thought to be the first event in autoimmune demyelination). Once it has passed the BBB this agent causes a marked decrement of the T1 relaxation times of hydrogen ion resulting in a bright signal on T1-sequences (McDonald et al., 1992). Contrast

agents are generally used to distinguish new and active lesions from old ones, although some studies on long-standing lesions have also shown an increased vascular permeability which may reflect an incomplete repair of the blood brain barrier due to recurrent damage (Barnes, 1991). The same effects as in the brain with the administration of contrast agents can be seen in the optic nerve where a blood-optic nerve barrier is present (Guy et al., 1990 and 1992). Gd-DTPA leakage has been shown in experimental optic neuritis to occur before demyelination, which supports the hypothesis that increased permeability of the barrier is probably the initial event in autoimmune demyelination (Guy et al., 1990 and 1992).

To improve the imaging of specific tissues and depending on the aim of the scan, it is possible to modify some of the characteristics of the acquisition sequences, such as the radiofrequency (RF) pulse duration and frequency (TR), the magnetic field gradients (the net direction of the magnetization will be in the same direction of the field) and the RF signal sampling (TE). In particular the variation of the TR or interval between RF pulses (msec) and TE or time between the original excitatory RF pulse and the detection of the signal (msec) will give origin to the so called T1 and T2-weighted images. The former, where the TE is smaller than 90 msec, is mainly used for the anatomical details and the distinction between white and grey matter (high resolution), whereas the latter, where the TE is greater than 90 msec, is most useful in the detection of abnormalities (improved contrast). The proton density sequences have intermediate

characteristics, with high resolution and improved contrast (Ramsey, 1987; Brant-Zanadzki and Norman, 1987; Orrison, 1989). The T1-weighted images are also called inversion recovery images and the T2-weighted images spin-echo images. It was Lukes et al. in 1983 who showed the superior sensitivity of the spin-echo sequences as compared to the inversion recovery sequences in the study of multiple sclerosis. The lesions in inversion recovery imaging appear as areas of decreased signal intensity but were not visualized as well as on spin-echo images.

It is well known that the imaging of the optic nerve raises two major problems: the chemical shift (a light or dark band due to the difference in resonant frequencies of the hydrogen ions when bound to water molecules or to lipid molecules) between the optic nerve and the orbital fat and the high signal from the fat with the T1 and T2 sequences (this is due to the high proton density and the shorter T1 relaxation time for fat than the surrounding structures so that the image seen on the MRI is brighter for the fat, while the other structures appear of darker colour). Moreover, the signal to noise ratio is low, particularly where the nerve lies at a distance from the surface, it has a relatively small size and it is surrounded by bones and the cerebro-spinal fluid.

The short inversion time inversion recovery sequences (STIR; time inversion of 100-150 msec) which have been introduced by Bydder and Young in 1985 suppresse the orbital fat signal (it appears dark) and reduce the chemical shift artefact, without reducing the

contrast between the nerve and the surrounding tissues. The demyelinated areas, however, because of their long T1 relaxation times, should appear brighter than the normal optic nerve with this sequence (Miller et al, 1986). Miller et al. in 1988 showed that the STIR sequences were very useful in the visualization of the effects of demyelination of the optic nerve (in the acute phases the presence of oedema and in the chronic phases also the presence of gliosis can be detected). It has to be noted, however, that the detection of lesions in the intracranial portion of the nerve or the optic chiasm is still difficult. When STIR sequences are used the characteristic phenomenon of the enhancement after administration of Gd-DTPA is reversed so that the signal is decreased rather than enhanced and this is therefore called "leakage" (Youl et al, 1991). This means that the contrast between the nerve and the surrounding structures after gadolinium administration is reduced (Miller at al. 1993).

In addition to the conventional short-echo time STIR (STE-STIR) sequences, long-echo time STIR (LTE-STIR) sequences have been used and the two techniques were compared in 1996 by Onofrj et al.: the latter was more sensitive in the detection of optic nerve lesions and the lesions appeared longer with LTE-STIR either in acute cases or in cases of previous optic neuritis.

A different technique which suppresses the fat signal by means of a frequency-selective saturation pulse is that of Fat saturation (FatSat). T1, proton density and T2 weighted images can be performed (Guy et al., 1992 and Miller et al., 1993). This technique has been shown to be

advantageous as compared to the STIR sequences because it enables the measurement of T2 relaxation times (characteristic values of the latter have been found for oedema, gliosis and axonal loss so that it would be possible to distinguish the 3 events) and secondly because T1 weighted FatSat images show gadolinium "enhancement" rather than areas of reduced signal and therefore there is no reduction of contrast between the nerve and the surrounding tissues as occurs with STIR sequences (Miller et al., 1993).

More recently fat suppressed fast spin-echo (FSE) sequences have been introduced which enable one to record higher resolution images in a shorter time as compared to standard spin-echo sequences. The comparison with STIR sequences has revealed that FSE has a greater resolution and a higher sensitivity in the detection of lesions in optic neuritis (Gass et al., 1996).

A recently developed technique, which may be able to detect changes of the myelin directly, is that of magnetization transfer (MT). The basic mechanism is the exchange of magnetization between free water protons (which can be seen on conventional MRI) and protons that are part of large and poorly mobile macromolecules, such as the myelin (which cannot be seen on conventional MRI) after administration of an off-resonance RF pulse. The latter causes the saturation of the bound water protons, creating a new equilibrium between the two pools of protons. Therefore the MR signal after an on-resonance RF pulse will be reduced and the magnitude of this reduction is the magnetization transfer ratio (MTR), which is therefore

an indirect indicator of the amount of macromolecular structures present per unit volume. The MTR is greater in the white matter than in the grey matter (Thorpe et al., 1995). It is reduced in multiple sclerosis and it has been found to be abnormal even in patients where a standard MRI is normal (Grossman, 1994). A low MTR is an indication of loss of tissue structure; it is difficult to establish at present whether this reduction is due to demyelination, axonal loss or oedema, although it has been suggested that the former appears more likely (Gass et al., 1994, Thorpe et al., 1995).

The majority of patients with optic neuritis have an abnormal MRI of the optic nerve and between 47% and 79% of the patients with clinically isolated optic neuritis have additional silent lesions in the brain on MRI (the rather wide range is probably attributable to different inclusion criteria and different methodology), according to various authors (Jacobs et al., 1986; Ormerod et al., 1986; Städt et al., 1990; Christiansen et al., 1992 and Beck et al., 1993). An abnormal MRI, however, does not mean definite development of clinical MS in a few years time, in the same way as a normal MRI does not mean protection against the development of MS (Jacobs, 1991). It is also true that some patients with definite clinical involvement of the optic nerve have normal MRI of the optic nerve: one possibility could be that the lesion is too small to be detected and a second possibility would be that the lesion may be in a particular stage of its evolution at the time of the scan so that its detection is not possible.

The attempt to correlate in a statistical analysis the length

(number of slices) of optic nerve the lesion as seen on MRI (STIR sequences) and the latency (msec) or the amplitude (μV) of VEP responses in the acute stage of ON has been made in the past. In 1991 Youl et al. did not find any correlation between these two parameters, possibly because the measurable signal on MRI is due to oedema and gliosis rather than directly to demyelination. Conversely, at follow up a month later the amplitude of the VEP responses was inversely correlated to the residual lesion length. In agreement with these findings was the study by Kakisu et al. (1991). They imaged (STIR sequences) the optic nerve of acute and chronic patients and found no correlation of amplitude or latency with lesion length in the acute group (possibly because of oedema); conversely, the chronic group showed a correlation between VEP amplitude (μV) and the MRI lesion length (number of slices) and also between VEP latency (msec) and lesion length. A correlation between VEP latency and MTR has also been found, supporting the hypothesis that the MTR reflects at least partially the myelin loss (Thorpe et al., 1995).

MRI of the optic nerve was also thought to have a prognostic value for the recovery from an episode of optic neuritis. Miller et al. in 1988 showed that with STIR sequences a longer lesion of the optic nerve or the involvement of the intracanalicular portion of the nerve were more frequently associated with a poor clinical recovery. In 1996 Dunker et al. again used STIR sequences and reached the same conclusions, so that patients with shorter lesions (<17.5 mm) had better visual recovery than those with longer lesions (>17.5 mm) or

lesions involving the intracanalicular portion of the optic nerve.

1.8 FOLLOW-UP STUDIES (VEPs)

In the past only a few studies have tried to establish the changes of the characteristics of the visual evoked responses over time in ON and no multiple follow-up by means of VEPs has yet been performed. A small number of authors have followed up single cases or larger groups, but at very variable intervals from the onset of symptoms. The patients studied were affected by isolated ON or MS.

The first accounts that a recovery of the VEPs could occur were made by Asselman et al. (1975) who observed a recovery of the latency to normal values in two patients who previously had delayed responses.

In 1979 Matthews and Small studied 51 patients (definite MS, probable MS or isolated ON) who underwent VEP recordings on two or more occasions over a period varying from 2 to 42 months and at intervals ranging from a few days to many months. The aim of the research was to find a measure which might correlate with clinical indices of MS. VEP recordings were obtained from 97 eyes, whereas for 5 eyes no response could be measured at any time. Forty eyes had normal responses at the initial recording; of them 26 had normal responses (latency) at follow-up, whereas 14 became abnormal (ie prolonged in latency). Fifty-seven responses were abnormal at presentation; of them 21 did not show any change, 12 became more abnormal (ie the delay in latency increased), 15 less abnormal

(shortening of the delay) and 9 returned to normal latency values. The amplitude of the responses was on average lower at follow-up as compared to the initial recording. The authors noticed that the changes in visual acuity were accompanied in 61% of eyes by appropriate changes of VEP latency and in 69% by the appropriate change of VEP amplitude. Because of the persistence of the abnormalities for a long interval after clinical remission in most cases it was concluded that VEPs were important for diagnostic purposes but not for monitoring the course of the disease.

A study by Walsh et al. (1982) comprised 56 patients with definite MS who were recorded at the beginning and at the end of a 2 and a half year period by means of visual, brainstem and somatosensory evoked potentials. The aim of the study was to determine the frequency of abnormalities and the changes in latency of the responses in that interval. From their data it appeared that it is uncommon over a long interval to have a decrement in latency (the changes in amplitude of the responses were not described): a significant deterioration in the latency of the evoked responses in the overall group for all modalities was the rule and only exceptionally a reduction in latency was noted in individual patients. The authors noted that the changes in the electrophysiological data (latency delay) were correlated with the increase in the patients' clinical disability, but a clinical improvement was not accompanied by a shortening of the EP latency.

In a later study (1983) Matthews and Small described a patient in

whom serial VEP recordings were performed: a month after the onset of the attack VA was 6/9 and the VEP clearly prolonged in latency and of low amplitude as compared to the fellow eye (167 msec and 6.3 μV); no change in the responses was seen in the following 3 years, but 6 and a half years after the onset of optic neuritis VA was 6/5 and VEP latency and amplitude were back to normal values (although a small peak at 144 msec was recorded). Following this observation the authors studied 21 patients affected by MS, who had bilateral or unilateral VEP abnormalities and followed them up between 6 to 8 years after the first recording. The findings were described in the same paper, Matthews and Small, 1983. Amplitude changes were regarded as significant when the difference between the recording at presentation and at follow up was at least 50% and latency changes were regarded as significant when the difference was at least 10 msec. VEPs were initially normal in 6 eyes (amplitude and latency) but 1 was prolonged in latency at follow up; responses were initially not recordable in 5 of the 42 eyes, but were then present at follow-up. In the remaining 31 eyes the amplitude of the responses improved or did not change, except in one case who suffered from a new attack of optic neuritis (absent response at follow up). The response amplitude was abnormal (<4μV) at presentation from thirteen eyes but it increased (>50%) at follow-up. Out of 26 eyes who showed a response prolonged in latency 10 eyes improved (latency shortened >10 msec). None of the 21 patients showed the long term normalisation in the VEPs and the visual acuity seen in the first case; nonetheless, it was

suggested that, if new lesions did not occur, a slow healing process might take place and moreover that in patients with progressive disease the recovery process may still occur except that it might not be possible to detect it.

Hely et al. (1986) followed up 80 patients affected by optic neuritis by means of VEPs. These patients were analyzed either to assess the risk of developing MS in the future or to evaluate the outcome of the lesion in the optic nerve. Whole field recordings were performed on average 4 months after the onset of symptoms on the first occasion and 98 eyes showed abnormal VEPs. On average 46 months later the abnormal eyes were followed up and responses to whole and central field stimulation were recorded: 79 eyes were still abnormal, whereas the remaining 19 responses returned to normal values. In an overall view (98 eyes) the latency of the responses did not change significantly in 40 eyes, latency increased (>10 msec) in 19 and the latency decreased (>10 msec) in 39 eyes. The patients followed up more than 4 years after the first recording showed more frequently significant changes in the VEPs (increase or decrease in latency). In particular in 42% of eyes seen before 4 years latency had decreased (10/59 eyes were normal), whereas the percentage was 51% in those seen after 4 years (9/39 were normal). It has also to be noted, however, that only 7% of the eyes seen before 4 years showed an increase in latency whereas after 4 years the percentage was 36%. Ophthalmological examination data at presentation were not reported but at follow-up were completely normal in 9/98 eyes; the residual

abnormalities were those of impaired colour vision, pale discs, APD, visual field defects and reduced visual acuity. The latter was abnormal in 38/98 eyes but only in 9/98 cases was it 6/60 or less. It is important to notice that 3/79 eyes with abnormal VEPs had normal ophthalmological examination and that 13/19 eyes with normal VEPs had abnormal ophthalmological examination. The authors stated that VEP recovery is consistent with a remyelinating process involving a significant proportion of the optic nerve fibres; they did not comment on the increase of latency in some eyes.

In 1988 Kriss et al. followed-up 39 children with optic neuritis (29 had bilateral simultaneous or sequential attacks and 10 unilateral attacks), on average 8.8 years after the onset of symptoms. At follow-up 78% of the 53 eyes examined had normal visual acuity. Twenty children were studied by means of pattern VEPs and the percentage of abnormal responses at follow-up was 45% (prolonged latency). The improvement inferred and also directly observed in 11 cases where serial recordings were available was explained by a recovery of conduction in the surviving nerve fibres. Moreover because the frequency of normal responses at this interval after optic neuritis appears to be much higher in children than in adults, it was concluded that the potential for remyelination is greater in the young than the old population (the authors took into account the longer duration of their follow-up as compared to other studies on adults).

Celesia et al. in 1990 studied 20 patients affected by optic neuritis over a period of 12 months to monitor the course of the disorder and

establish whether psychophysical or electrophysiological data could be used as prognostic indicators of visual recovery or permanent visual loss. Visual acuity, colour vision, contrast sensitivity, visual fields, VEPs and pattern electroretinograms (PERGs) were performed on 6 occasions and an MRI scan at presentation. The authors found that at follow-up a high percentage of VEPs remained prolonged in latency (90 to 95%) whereas approximately 20% of the patients had abnormal visual acuity, colour vision, contrast sensitivity and visual fields. From this data they concluded that VEPs, because of the persistent P100 delay, are useful to document the occurrence of optic neuritis in the past.

In 1993 Jones studied 353 patients divided in 6 groups according to the time interval since the onset of symptoms (between 1 week and 19 years). He found that the rate of abnormal VEP responses was 89% in group 1 (1-4 weeks since the onset), increased to a maximum (92-94%) in groups 2-4 (5-26 weeks) and then declined to 79.7% in group 5 (27-104 weeks) and 71.4% in group 6 (105-990 weeks). Abnormal or absent responses in terms of amplitude ratio with the unaffected eye decreased from group 1 (23.4%) throughout group 5 (6.7%) to increase slightly in group 6 (8.9%). The amplitude ratio was significantly lower in group 1 than in groups 3-6 but not than group 2. Absent responses appeared to decrease and normal amplitude responses to increase throughout the 6 groups, although no trend in the mode was seen. The relative delay in latency of the affected eye as compared with the fellow eye did not change significantly for the first four groups

(although it was noted that from group one many latency values were missing because of the absence of the responses), whereas in group 5 the delay was significantly less than in groups 1 to 3 and in group 6 than in groups 1 to 4. The mean delay of more than 30 msec in group 1 was reduced by about 37% in group 5 and 46% in group 6. Visual acuity was most impaired in group 1 and significantly less so in groups 3 to 6. The author concluded that shortening of the latency, which could indicate a reparative process, was apparent over the 2 years following the onset of symptoms. This process was possibly lasting for a longer period especially in young people (as shown in this study by the correlation, although weak, between individual latency values and patients' age at the time of the attack), but according to Jones' data it appeared that after 2 years the latency of the responses did not change significantly in the majority of patients.

The data from the literature on electrophysiology are not at all unequivocal, but the general view is that there is evidence for a "recovery process" to occur after an episode of optic neuritis which is represented by a shortening of the delay of the VEP responses. The recovery process, however, was not shown in all the studies or in all patients: the reason for this observation is still not clear neither is it clear why some patients recover to normal values and others only to a limited extent.

Although with our present knowledge remyelination is the most likely candidate to cause the shortening of the VEP latency, it is still not known whether other phenomena such as for example a reorganizing

process at different neuronal levels could also contribute to it. It is common belief that the prolonged VEP latency is due to demyelination and it is therefore plausible to think that remyelination, the opposite process, could cause a shortening of the delay. It is more difficult to explain how cortical reorganization could shorten the latency of the VEPs (when it would probably be more likely an increase in latency). Reorganisation of sodium-channels in the axon membrane may be responsible for restoring continuous conduction (non-saltatory), but it has been experimentally proved that conduction in demyelinated fibres is slower than in myelinated axons.

Many other questions are still unanswered, such as: what is the ultimate extent of the recovery process, is there any factor which can influence it, how does this recovery process affect the clinical recovery and in general the course of the disease?

In the present study the attempt to assess the extent of the recovery process was made for the period between 3 and 24 months. Although it has been reported in single cases that latency can still recover after 6-8 years, patients were followed-up for 2 years. In addition 12 patients were followed up over an average period of 3 years. The findings from Jones' study in 1993 (latency did not change significantly in patients who had an episode of optic neuritis more than 2 years prior to VEP recordings as compared to those who had an episode of ON 2 years prior) would corroborate our choices.

Some factors which may influence remyelination were analysed such as the age of the patient at the time of the ON, the type of

disease at presentation (isolated ON or ON in the context of MS) and the activity of the disease during follow-up on the basis of clinical and MRI data. There is still considerable debate about the best index of disease activity on MRIs (for example number of new lesions, lesion load etc) but for the purpose of the present study the lesions at presentation and at the end of the follow-up were numbered. It has been proposed over the years that early age of symptoms' onset (McDonald, 1983), a mild optic neuritis and an isolated optic neuritis as opposed to optic neuritis in the context of multiple sclerosis (Shahrokhi, 1978) were factors in favour of a normalisation of the VEPs. Conversely, from Jones' study (1993) it appeared that in patients with clinically active disease, although the electrophysiological deficit was generally more severe to start with, the recovery was faster than in isolated optic neuritis. This, however, was not a serial study but one in which different groups were compared.

The patients of the present study were also tested by means of visual fields and contrast sensitivity to assess some aspects of the visual function. Together with the ophthalomological examination this will make it possible to establish whether remyelination has any effect on visual function, as assessed by these methods.

The fellow eye data were useful as controls for the affected eye, but the analysis of these results would also allow the comparison of the findings in the affected eye with the more general course of the disease also looking for evidence of subclinical delay, common in established MS. It is not yet known whether this always occurs in

association with other episodes or is insidious.

2. PATIENTS AND METHODS

Two separate studies were performed:

1. Optic neuritis treatment trial

Fourteen subjects affected by optic neuritis were studied 6 months after onset of symptoms and followed up at on average 3 years. They were part of a larger group who participated in a double blind study to assess the effects of iv methylprednisolone treatment as compared to placebo at 6 months (Kapoor et al., 1998).

2. Follow-up study

Thirty-one subjects were tested 3 months after an episode of optic neuritis and followed up every 3 months for a year and every 6 months in the second year.

The methodology used to record VEPs differed in the two studies. In the treatment trial the stimulus (the checkerboard) was back projected via a mirror onto a translucent screen. Since the initial treatment trial study we introduced a new technique to record VEPs (Brusa et al., 1995), which was used in the follow up study. The stimulus was produced via a computer onto a monitor CRT screen.

In the treatment trial, MRI of the optic nerves was performed at presentation and at 6 months, in order to assess the length of the optic nerve lesions and their evolution at 6 months. The MRI were not repeated at 3 years. In the follow-up study MRI of the brain at the

beginning and the end of 2 year period were performed in order to check whether new lesions had developed in that interval.

2.1 PATIENTS

2.1.1 Optic neuritis treatment trial

Fourteen patients (mean age: 40 yrs, range 30 - 48; 12 females, 2 males), part of a larger group who participated in a double blind treatment trial (Kapoor et al., 1998), were studied at 6 months and followed-up on average 3 years from the onset of symptoms by means of VEPs and psychophysical testing (contrast sensitivity (CS) and visual fields (VF)).

For the initial study, 66 patients (aged between 18 -50 years) with unilateral optic neuritis, a visual acuity of 6/9 or worse at presentation, colour vision deficit and a relative afferent pupillary defect were recruited within 30 days of onset of symptoms. Exclusion criteria were the following: improvement of vision at the time of entry into the study, bilateral involvement, previous ocular pathology, previous episodes of optic neuritis, psychosis, significant systemic disease including active infection, diabetes mellitus and systemic hypertension, a history of tuberculosis, and other contraindications to steroid treatment. Patients with MS but no previous history of optic neuritis could be included.

The initial aim of the study was to assess the effects of treatment with iv methylprednisolone in a 6 month period on the basis of clinical, VEP, MRI and psychophysical data. The patients were therefore randomly selected to receive the drug (in a dose of 1 gr. daily for 3 days) or the placebo (iv saline). Clinical examination comprising visual

acuity (VA) using Snellen chart, fundoscopy and colour vision (Ishihara plates) was performed at presentation and then at weeks 1, 2, 4, 8, 13 and 26. VEPs were recorded at presentation, at 2 weeks and at 6 months. The optic nerve was imaged at presentation and again at 6 months. Psychophysical tests (contrast sensitivity and visual fields) were performed at 6 months.

For the present study patients were clinically examined (ocular examination) and VEPs and psychophysical testing were repeated on average 3 years from the onset of symptoms (Fig. 1).

Fig 1. Timing of the clinical examination and tests performed.

Of the 14 patients one patient remained with no perception of light (NPL) in the affected eye and 1 patient had had an attack of optic neuritis in the unaffected eye during the interim period. Both patients were excluded from the analysis. Moreover 1 patient withdrew from the study before performing perimetry so that only 11 patients were analysed for the latter.

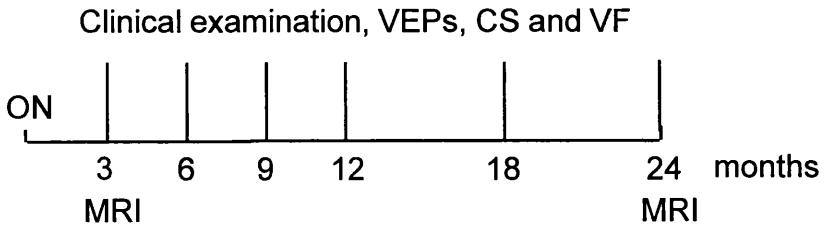
2.1.2 Follow-up study

Thirty one patients affected by optic neuritis were recruited within 3 months of the onset of symptoms at The National Hospital for Neurology and Neurosurgery and at Moorfields Eye Hospital over a period of 18 months. The age range was 24 to 51 years (mean: 33.5 yrs) and there were 17 females and 14 males. They presented with optic neuritis as an isolated symptom (15) or recurrent (7) or with signs of additional CNS involvement (9).

The protocol of the study was approved by the Ethical Committee and all patients gave informed consent to the research procedures.

All patients were first tested 3 months after onset of symptoms (mean 90.3±12.5 days) for the first visit and then at 3 month intervals for a year (6th, 9th and 12th month). After the first year follow-up, patients were seen every 6 months for the following year (18th and 24th month) (Fig. 2).

Figure 2. Timing of the clinical examination and tests performed.



Patients were studied by means of VEPs, contrast sensitivity, visual fields and MRI. All tests were performed at each visit except the MRI which was done at the beginning and at the end of the study. A clinical ocular examination was performed at each visit comprising visual acuity using Snellen chart, fundoscopy, colour vision (Standard pseudoisochromatic plates, part II - SPP II) and visual fields to confrontation.

2.2 METHODS

2.2.1 VEPs

2.2.1.1 Optic neuritis treatment trial

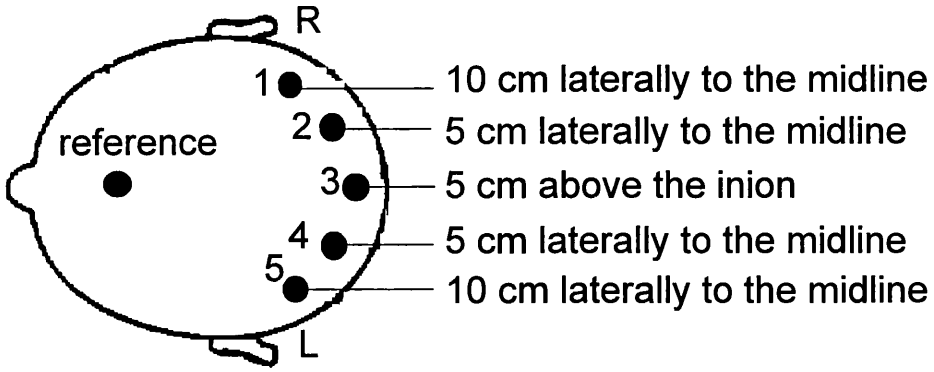
Visual evoked potentials to circular whole field (16° in radius) and to central field (4° in radius) stimulation were recorded by means of 6 occipital electrodes (five electrodes in a lateral chain 5 cm above the inion at an interlectrode distance of 5 cm and a sixth one at the inion were attached with collodion after scarification) referred to Fz. The earth was at Cz. The checkerboard was back-projected via a mirror onto a translucent screen and fixated at a distance of 1 m. Once every 510 msec the mirror rotated abruptly (10 msec) through a small angle, resulting in the horizontal displacement of the pattern through one check (50' in size). In the central area of the visual field the intensity of the light checks was 227 cd/m² and of the dark checks 8 cd/m², while in the periphery the luminance was slightly less. The recording bandwidth was flat from at least 1 Hz to 1 kHz. Signals were digitised at 0.8 kHz for 320 msec after each stimulus and 200 responses were included in each average. Amplitude (from the preceding negative peak) and latency of the P100 responses were measured at the electrode which was on the midline 5 cm above the inion. The test was carried out in a dark room. The patient was comfortably seated and wore spectacles or contact lenses when appropriate.

2.2.1.2 Follow-up study

Visual evoked potentials were recorded to stimulation of the whole field (WF) and interleaved right hemisurround (RHS) + left hemisurround (LHS) + central (CF) fields (Brusa et al., 1995). The test was carried out in a dark room with the patient comfortably seated and the head supported. The patients wore their spectacles or contact lenses when appropriate. Consistency of fixation and alertness were monitored throughout the recording session via a mirror.

Five recording electrodes were placed in a lateral chain 5 cm above the inion at an interelectrode distance of 5 cm and a sixth one 2.5 cm above the inion. All electrodes were referred to Fz and the earth was fixed at Cz (Fig. 3).

Fig. 3. Electrode placement.



An additional electrode (number 6) was fixed 2.5 cm above the inion.

The electrodes were attached by means of a cream (Elefix) and impedances were reduced to less than 5 kOhm by skin preparation

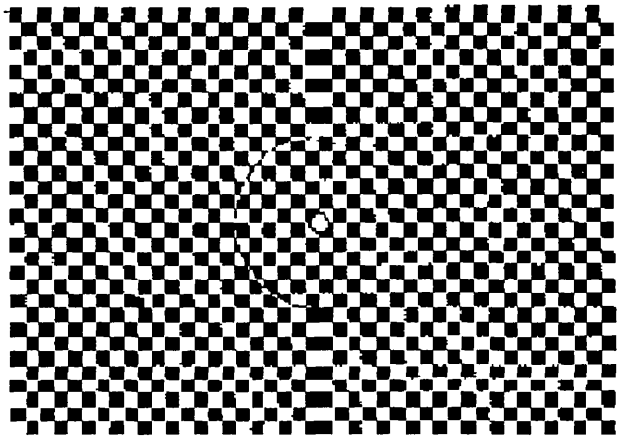
with Skinpur. The bandpass filter was 0.16 - 1000 Hz and sampling occurred at a rate of 2 kHz for 250 msec. Sweeps containing deflections greater than 165 μV were automatically rejected.

At an eye-screen viewing distance of 88 cm the angle subtended at the eye by the screen was 28° (horizontal) x 20° (vertical). The screen monitor had a 99 Hz frame rate and 619 x 560 pixel resolution. To minimize the artifacts associated with the screen monitor the average trigger was evenly spread with respect to the raster beam. The check size was 40'. The brightness of the light checks was 60 cd/m² and of the dark checks 4 cd/m². For whole field and interleaved LHS+RHS+CEN stimulation the overall cycle was 1818 msec (ie checks changing from black to white and back to black), such that each field was reversed every 909 msec. The interstimulus interval for the 3 interleaved stimuli was 303 msec. The whole field was represented by the entire screen, whereas for the interleaved stimuli the screen was split into 3 areas: the central field was a circular area whose radius subtended 4° at the patient's eye while the remainder of the screen was split vertically into 2 halves, representing the left and right hemisurrounds (Fig. 4).

The responses to the interleaved stimuli were concurrently recorded and stored separately. Two repetitions of 100 responses for the whole field and three for the interleaved stimuli were recorded. If judged to be consistent repetitions were then averaged together and latency and amplitude of the P100 response were measured (the latter

from the previous negative peak) at the electrode which was on the midline 5 cm above the inion.

Fig. 4. Checkerboard pattern reversal: the left/right hemisurround and the central fields are shown (after right hemisurround and central field reversal).



Results were then compared to those of a control group of 16 subjects (8 females and 8 males; age range 21 to 58 years). The responses were considered abnormal if the latency or the interocular latency difference exceeded the mean value +2.5 SD of the control group. Because of the high variability and occurrence of missing data responses to hemisurround field stimulation will not be used in the statistical analysis.

2.2.2 Contrast Sensitivity

2.2.2.1 Optic neuritis treatment trial and follow-up study

Contrast sensitivity was performed to vertical sine wave gratings at 0.5 and 4 c/deg spatial frequency, temporally modulated sinusoidally at 0, 8, 32 Hz. The eye-screen distance was 114 cm and the screen size was 13° x 9°. The patient had the head supported at the chin, the fellow eye was occluded and lenses were worn when necessary. Stimuli at the 3 temporal frequencies were randomly interleaved in order to equalise the effect of practice and presented on a screen with a background luminance of 90 dB. The stimulus was represented by a symmetrical Gaussian weighted grating to avoid sharp edges. The stimulus faded away at about 2.5 cm from the midline for the 4 c/deg (80 pixels and each pixel is 0.3 cm) and at 4.5 cm for the 0.5 c/deg spatial frequencies (150 pixels). The stimulus duration was 1 sec and the stimulus decay was 0.25 sec. Two situations could occur: gratings on the screen followed by a blank screen (with the same overall luminance as the previous one) or a blank screen followed by gratings (see Fig. 7 in Introduction). At the same time as the gratings and the blank screen (or viceversa) a first and a second beep, respectively, were heard. The patient had to call out "first" or "second" according to which of two beeps was associated with perception of a grating, in a two-alternative forced choice staircase. The subject was instructed to guess the answer when uncertain. The contrast was automatically

doubled when the answer was wrong and decreased by a fixed percentage (15%) when the answer was correct (fixed ratio protocol). For the measure of the threshold the reciprocal of the contrast values to which the patients made a mistake were multiplied by 100, transformed into logs and then averaged together. Standard deviations and standard errors were also calculated. The results were then compared to those of a control group of 27 subjects (20 females and 7 males; age range: 21 to 52 years).

In the follow-up study one patient did not perform the contrast sensitivity at 3 months.

2.2.3 Visual Fields

2.2.3.1 Optic neuritis treatment trial and follow-up study

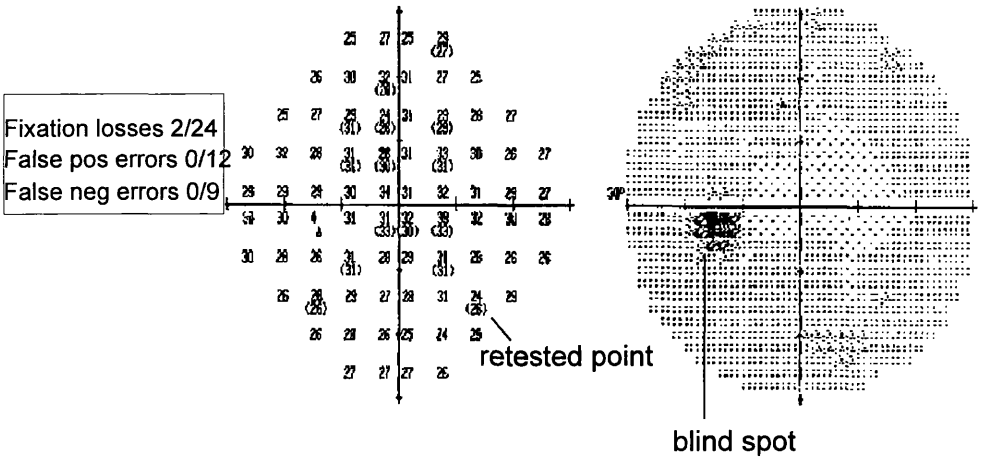
Visual fields were performed with a Humphrey automatic field analyzer using the central 30-2 threshold protocol (i.e. the central 30° of the visual field were tested starting at 2° eccentricity from the fixation point) (Fig. 5). The field analysis was performed in a dark room and the patient, with the head supported at the chin, was instructed to press a button when he or she could see the test target, while concentrating on an illuminated fixation spot. The test targets differed in brightness and were produced by the computer via a projector, which was capable of rotating to project the light in different directions. The alignment of the tested eye was checked directly throughout the test by means of a telescope. Wide angle lenses were used to correct refractive errors. The fellow eye was occluded by a patch.

The strategy followed was that of a full threshold: the intensity of the stimulus first presented was one which is expected to be seen by the patient; this was then decreased in 4 dB steps until the patient could not see the stimulus and then increased in steps of 2 dB until the patient could see it again and therefore pressed the button. The latter value was identified as the patient's threshold at that particular point. The same procedure was used for all tested points of the visual field. At the beginning of the test the blind spot was localised: this was the

area where a bright stimulus could not be detected by the subject around 13° eccentricity in the temporal field. The visual field was tested at 76 points in total (6° interval) and when one of them showed a threshold value more than 4 dB away from the expected value, that point was retested, and the mean between the two values was used in further analysis. For the analysis of the data, the mean deviation (STATPAC computation, derived from the results of the test, which gives an index of the deviation of the visual field of the patient as compared to a reference field) or the mean threshold (as dB of attenuation of the stimulus) were taken into account (Fig. 5 and Fig. 6) (Owner's manual. Automatic field analyzer. Allergan 1992). The data were also analysed by means of a software for Windows, PROGRESSOR, which performs linear regression of sensitivity on time for each test location (Fitzke and McNaught, 1994; Viswanathan et al., 1997).

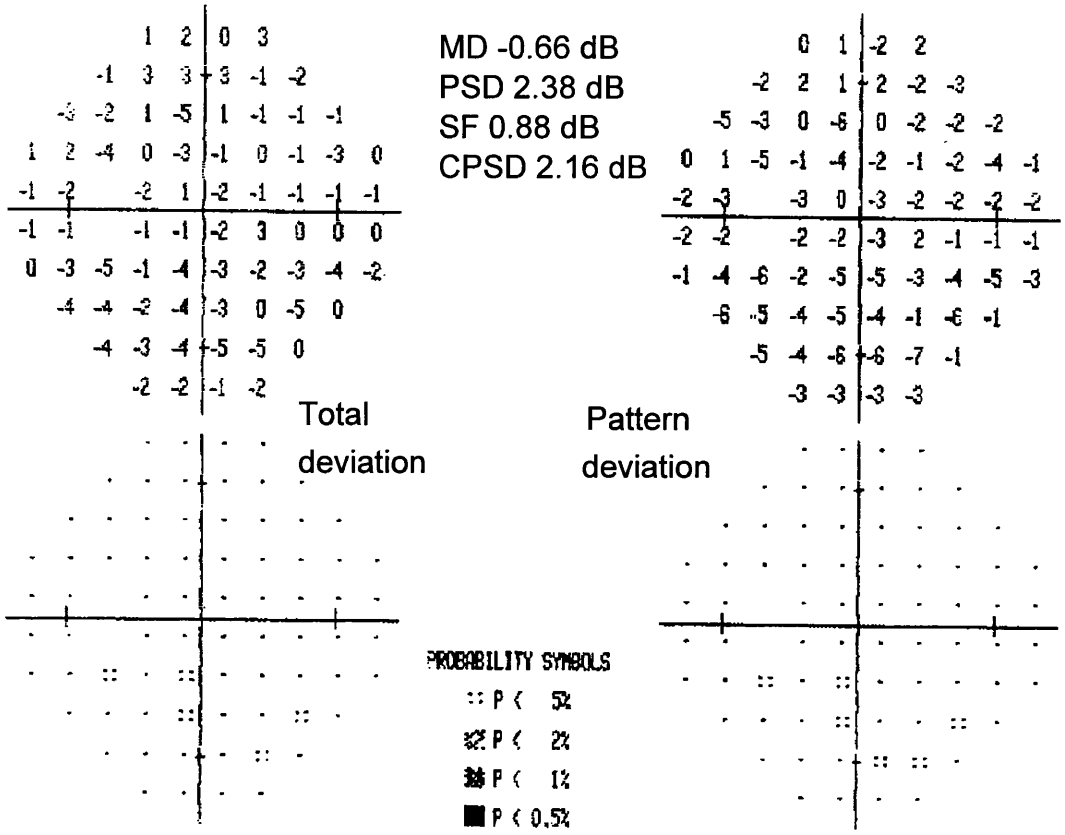
In the follow-up study one patient did not perform the Visual field test at 3 months.

Figure 5. Example of a normal visual field to central 30-2 protocol for the left eye.



On the left is reported point by point the threshold in dB of attenuation of the stimulus; on the right the same values are converted to a greytone scale (black means <=0 dB and white means between 41 and 50 dB). Fixation losses: due to variable fixation the patient pressed the button when the stimulus was presented in the blind spot. False positive errors: once every 40 stimuli the projector was redirected but the stimulus was not produced. A false positive error was recorded if the patient pressed the button in this situation. False negative errors: were recorded when a very bright stimulus in an area previously assessed as of normal sensitivity was not reported.

Figure 6. Graph representing the "total deviation" and "pattern deviation" of the field from normal values.



The top graphs show the difference in decibels between the subject values and those obtained from a population of the same age and sex (on the left) and taking into account the possible presence of cataract and small pupils or "supernormal" vision (on the right). The lower graphs indicate the probability of the deviations (the darker the area the less likely the findings are seen in the normal population).

Mean deviation (MD): of the overall field compared to the normal reference field. If the deviation is outside the normal limits a p value will be given in addition; 5% for example will indicate that less than 5% of the normal population will show a larger MD value. Pattern standard deviation (PSD): measurement of the degree to which the patient's field differs from the normal age-corrected reference field. A high value means that there are irregularities in the field or variability in the responses. Short-term fluctuation (SF): indication of the consistency of the responses, testing 10 preselected points twice. Corrected pattern standard deviation (CPSD): same as PSD but corrected for intra-test variability.

2.2.4 MRI

2.2.4.1 Optic neuritis treatment trial

The MRI of the optic nerve was performed with a 1.5 T Signa scanner. Short inversion recovery inversion time sequences (STIR) were used and imaging extended from the globe of the eye to the chiasm, using slices 5 mm thick, contiguous and in a coronal plane. STIR 2500/40/175 was performed with a 16 mm field of view and 256 by 128 matrix with two excitations. The optic nerve lesions were classified as long when 3 or more slices and the intracanalicular portion of the optic nerve were involved (24 patients: long lesion subgroup) and short in all other cases (42 patients: short lesion subgroup). This was done because the initial hypothesis was that patients with longer lesions should have benefited from treatment more than those with shorter lesions. The MRI was not repeated at 3 years and therefore the data were not relevant to the present study.

2.2.4.2 Follow-up study

MRI of the brain was performed by means of a 0.5 T Ge Vectra Scanner (for 1 patient a 1.5 T Signa scanner was used). Imaging of the brain (slices 10 mm thick) was obtained in 28 patients, but only 23 patients were followed-up at the end of the study (at least 2 years

apart).

Spin-echo sequences (T2-weighted and proton density - PD) were used. The field of view was 25 cm, with a matrix 160 x 192.

It was previously observed that patients with clinically active disease recovered from optic neuritis (VEP data) at a faster rate than those affected by isolated ON (Jones, 1993). The MRI of the brain was therefore performed in order to check whether the activity of the disease, as measured by counting the number of new lesions at follow-up, could affect the VEP data in this study. Patients were divided into two subgroups: those with MRI changes and those without changes in the interval analysed.

2.2.5 Statistical analysis

2.2.5.1 Optic neuritis treatment trial and follow-up study

Statistical analysis was performed using Borland QuattroPro, Microsoft Excel and SPSS packages. In particular, all data from the VEPs (amplitude and latency), contrast sensitivity and visual fields were analysed by means of Repeated measure ANOVA. Polynomial, simple and repeated contrasts were also performed to establish what kind of trend was present, the difference between the first visit and the following ones and the difference between adjacent visits, respectively. Because this type of analysis can be performed only for constant intervals, Visits I, II, III and IV (3 months apart) were first studied and then Visits II, IV, V and VI (6 months apart). Paired T-test analysis was performed between Visits I and VI by eye.

Paired t-tests were also mainly used for the "Optic neuritis treatment trial". The Chi-square test was performed to examine the association between variables. The visual fields data were also analysed separately with a specific program (Progression). Correlations among various parameters obtained were checked (regression analysis).

Patients with missing data were excluded from the analysis limited to the test where the data was missing.

The statistical analysis was performed on the whole group except missing data (Group A - 31 subjects), then excluding those patients

who had a further episode of optic neuritis affecting the affected and/or the fellow eye (Group B - 18 subjects). It was not possible to analyse those patients who had a single episode of optic neuritis in the fellow eye (prior to recruitment) as a separate group because they were too few. Furthermore the group was divided into 2 subgroups according to the clinical history or the MRI findings: with or without symptoms affecting other sites of the CNS (at presentation and at 2 year follow-up) and with or without changes on MRI.

Regression analysis for the affected eye was performed to establish whether it was possible to predict the outcome at 2 years from data at presentation VEP (amplitude and latency) and whether there was any relation between VEP amplitude (or latency) at Visit I and VI and indices of the visual function like Contrast sensitivity and Visual field data.

For the fellow eye a regression analysis was performed to exclude the possibility that an increment in VEP latency was related to the aging process and for the affected eye to check whether the VEP latency recovery was related to the aging process.

A regression analysis between the number of new lesions and VEP latency and amplitude changes at 2 years was also performed.

For all statistical tests p values ≤ 0.05 were considered as significant, unless otherwise stated.

3. OPTIC NEURITIS TREATMENT TRIAL

3.1 INTRODUCTION

The initial aim of this study was to establish the effect of treatment with iv methylprednisolone (ivMP) on the clinical outcome of an episode of optic neuritis incorporating VEPs, MRI and psychophysical data at 2 week and 6 month follow-up (Kapoor et al. 1998). These patients also represented a suitable group for a long term follow-up: we were able to reassess 14 patients on average 3 years from the onset of symptoms to establish whether there was any clear evidence of further VEP recovery and whether this was accompanied by improvement in visual function. The data from the "unaffected" eye were also taken into account.

3.2 RESULTS

3.2.1 Summary of the results up to 6 months

At 6 month follow-up no significant effect of iv methylprednisolone treatment was found on clinical examination or psychophysical tests. This was also the case when the patients were split into short and long lesion subgroups according to MRI findings in the optic nerve. As far as the VEPs are concerned the P100 was on average larger in amplitude (whole and central field) at 2 week follow-up in the treated group (borderline significant for all patients and the short lesion subgroups) but no significant differences in latency were found. At 6 month follow-up there were no significant differences in amplitude or latency between treated and placebo groups (Table 1). The whole field latency difference approached statistical significance, but the finding was not confirmed by the central field responses which are generally considered to reflect the myelin status more accurately. MRI data did not show any significant effect of treatment at 6 months. An inverse correlation between VEP amplitude at 2 weeks and lesion length on MRI at 6 months suggested that the extent of optic nerve damage was related to the severity and/or duration of the initial inflammation (Kapoor et al., 1998).

Table 1. VEP data (mean ± SD) at 6 months for the affected eye.

	amplitude			latency		
lesions	***ivMP***	***placebo***	**p-value**	***ivMP***	***placebo***	**p-value**
WHOLE FIELD						
all	8.9±4.8 (26)*	8.3±4.6 (23)	NS	123±15.0 (25)	131±19.0 (23)	NS
long	5.2±3.2 (7)	7.4±4.2 (8)	NS	129±18.2 (6)	130±11.3 (8)	NS
short	10.2±4.6 (19)	8.8±4.9 (15)	NS	121±13.9 (19)	131±22.7 (15)	NS
CENTRAL FIELD						
all	5.2±2.9 (26)	5.5±3.6 (21)	NS	135±20.6 (24)	136±18.6(22)	NS
long	3.4±2.4 (6)	5.4±2.7 (6)	NS	148±19.4 (6)	133±15.9 (8)	NS
short	5.8±2.9 (19)	5.5±3.4 (15)	NS	130±19.6 (18)	139±20.2 (14)	NS

* number of patients

3.2.2 Three year follow-up data

3.2.2.1 Clinical examination

As compared with the 6 month visit VA in the affected eye at 3 years had improved in 5/11 (45%) patients and deteriorated in 4/11 (36%), with no change in 2. Five patients (45%) had a VA of 6/9 or worse (one developed cataracts since treatment). This was somewhat greater than at 6 months (2/11, 18%) but the difference was not significant. In the fellow eye there was no significant change in VA. Eight patients had pale optic discs at both assessments and the number of patients who had an RAPD diminished from 6 to 2 between 6 months and 3 years (Table 2).

Table 2. Clinical data at 6 months and 3 years for the affected eye.

Subjects	Age*	Sex	Visual acuity			Disc		RAPD	
			presen	*6 mths*	*3 yrs*	*6 mths*	*3 yrs*	*6 mths*	*3 yrs*
1	36	F	CF	6/5	6/18	pale	pale	-	-
2	32	F	6/36	6/5	6/4	N	N	-	-
3	45	F	6/24	6/12	6/4	pale	pale	+	-
4	39	F	HM	6/36	6/18	pale	pale	+	+
5	33	F	HM	6/5	6/4	pale	pale	-	-
6	35	F	6/60	6/6	6/6	N	N	+	-
7	38	M	6/36	6/5	6/4	N	N	-	-
8	27	M	6/12	6/6	6/36	pale	pale	+	-
9	40	F	6/60	6/5	6/9	pale	pale	+	-
10	42	F	6/36	6/5	6/9	pale	pale	+	+
11	42	F	6/12	6/5	6/5	pale	pale	-	-
12	36	F	6/18	6/4	N/A	N	N/A	-	N/A

* at presentation

3.2.2.2 VEPs

The treated (7 patients) and the placebo (5 patients) groups were combined for statistical analysis on the account of the findings of the previous study showing there to be no significant effect of treatment.

The mean P100 latency from the affected eye (Fig. 1a and 2) was significantly shorter at 3 years than at 6 months and no significant difference in amplitude between the two visits was found for both central and whole field responses (Table 3).

Table 3. VEP data (mean ± SD) at 6 months and 3 year follow up: affected eye (12 subjects).

Field	***6 months***	***3 years***	***6 months***	***3 years***
	amplitude (μV)		**latency (msec)**	
Whole	8.5±4.7 NS	7.2±2.7	131±16.2 p=0.04	123±14.9
Central	5.4±3.4 NS	4.7±2.5	136±10.6* p=0.005	125±15.9*

* 11 subjects (a central field response was not identifiable at 6 months)

Fig. 1. Latencies changes between 6 months and 3 years for whole and central field responses (affected and "unaffected" eyes).

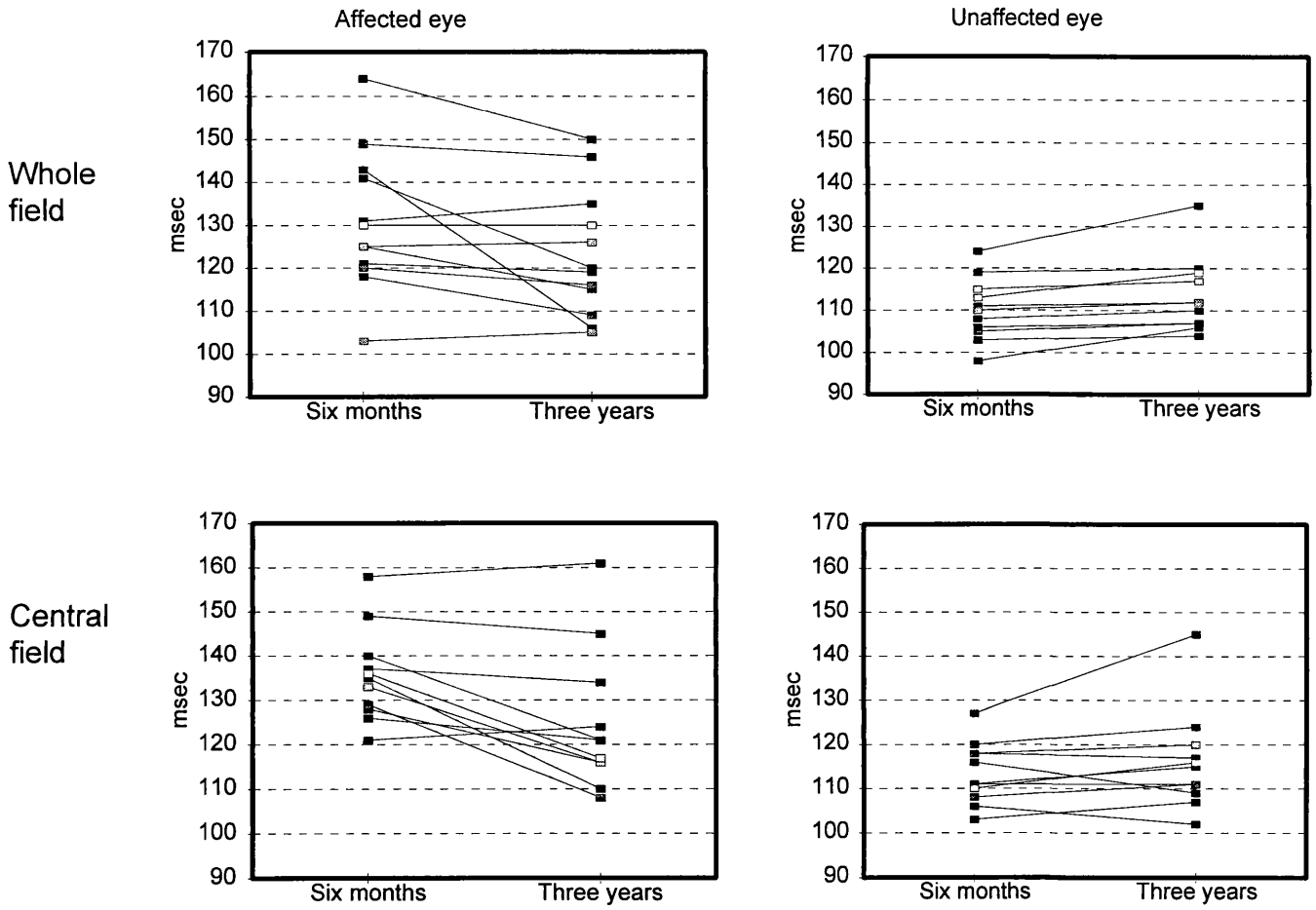
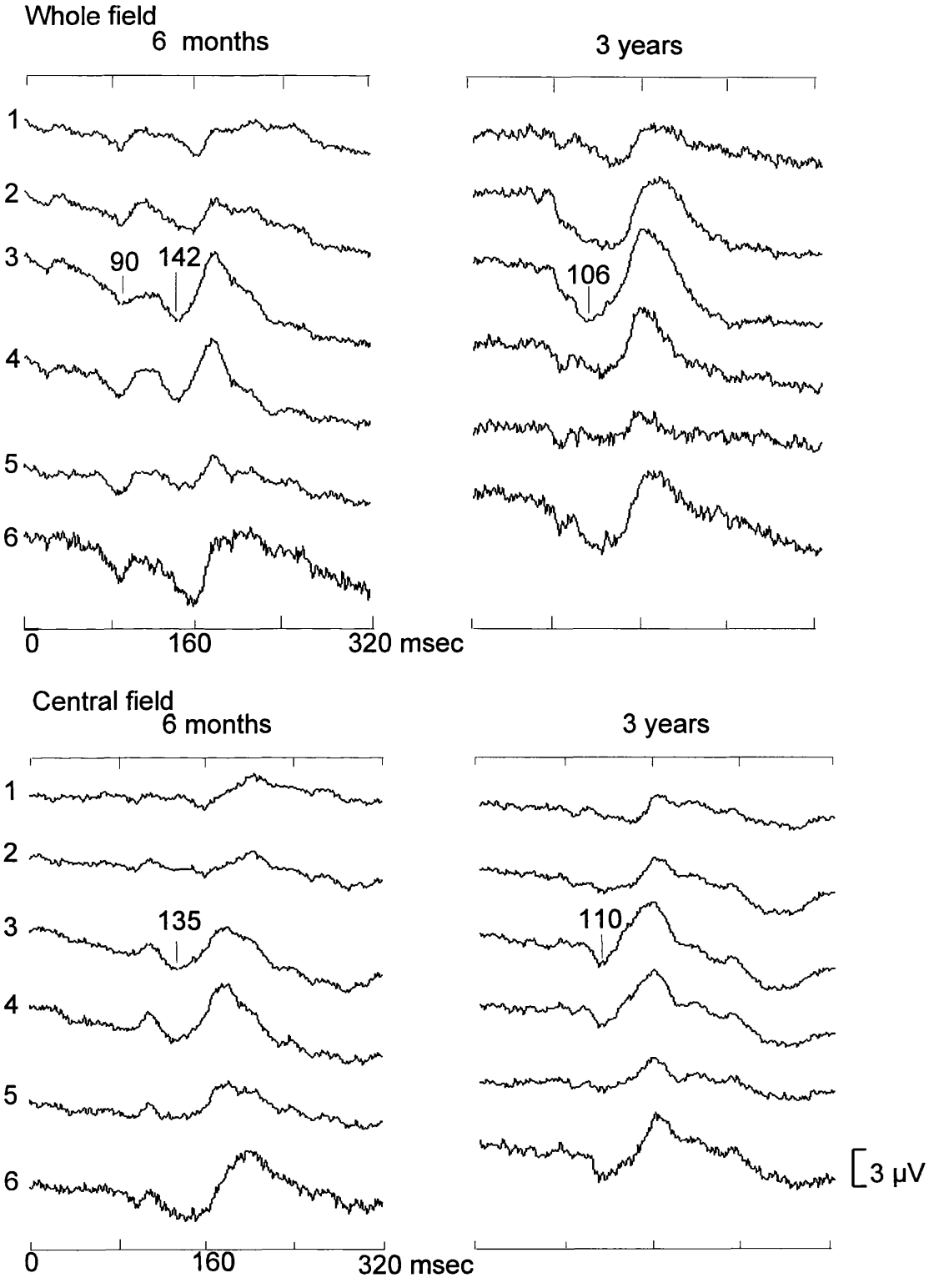


Fig. 2. VEP responses at 6 months and 3 years for a single patient.



It cannot be entirely excluded that the later peak in the whole field response at 6 months represents a P100 intermixed with a P135 intruding on the midline rather than a P100 alone.

A contrary finding came from the analysis of the "unaffected" eye, where whole and central field responses had longer latency at 3 years as compared to 6 months, significantly so for the whole field responses only. No significant difference in amplitude was found for the whole and the central field responses between the two visits. (Fig. 1b, Table 4).

Table 4. VEP data (mean ± SD) at 6 months and 3 years: "unaffected" eye.

Field	*6 months*	*3 years*	*6 months*	*3 years*
	amplitude (μV)		**latency (msec)**	
Whole*	11.9±4.7	10.6±5.0	110±7.0	113±8.5
	NS		p=0.005	
Central°	7.4±3.2	6.7±3.6	113±7.0	116±11.4
	NS		NS	

* 12 subjects; °11 subjects (one central field response was not recorded at 6 months)

From the affected eyes 9/12 whole field responses and 10/11 central field responses were reported as borderline or abnormal at 3 year follow-up, whereas from the "unaffected" eyes 4/12 whole field responses and 5/10 central field responses were abnormal. Four of the abnormal "unaffected" eyes responses were already abnormal or borderline at presentation. At 3 year follow-up these showed a similar tendency for latencies to increase as did the eyes with the initially normal responses.

3.2.2.3 Psychophysical tests

At 3 year follow-up the contrast sensitivity values obtained from the affected eyes were on average slightly worse as compared to 6 months for all spatial and temporal frequencies tested, except for the 4 c/deg spatial frequency when a temporal frequency of 0 Hz was used. The difference, however, did not reach statistical significance for any of the frequencies (the 0.5 c/deg spatial frequency at 8 Hz temporal frequency was close to significance) (Table 5).

Table 5. Contrast sensitivity data (log): affected eye (12 subjects).

Temporal frequency	***6 months***	***3 years***	**p-value**
	Spatial frequency: 0.5 c/deg		
0 Hz	1.64±0.3	1.52±0.1	NS
8 Hz	2.11±0.2	1.87±0.3	p=0.08
32 Hz	1.38±0.5	1.27±0.2	NS
	Spatial frequency: 4 c/deg		
0 Hz	1.93±0.4	1.99±0.3	NS
8 Hz	1.90±0.3	1.89±0.3	NS
32 Hz	1.02±0.7	0.91±0.2	NS

A more striking pattern of deterioration was seen for the "unaffected" eyes, where the difference was significant or close to significance for the 0.5 c/deg spatial frequency at 0 Hz and 8 Hz temporal frequencies and for the 4 c/deg spatial frequency at 8 Hz

and 32 Hz temporal frequencies (Table 6).

Table 6. Contrast sensitivity data (log): "unaffected" eye (12 subjects).

Temporal frequency	***6 months***	***3 years***	***p-value***
	Spatial frequency: 0.5 c/deg		
0 Hz	1.84±0.2	1.63±0.2	p=0.01
8 Hz	2.35±0.1	2.23±0.2	p=0.08
32 Hz	1.50±0.2	1.36±0.2	NS
	Spatial frequency: 4 c/deg		
0 Hz	2.33±0.3	2.26±0.2	NS
8 Hz	2.31±0.2	2.15±0.2	p=0.05
32 Hz	1.39±0.3	1.15±0.1	p=0.06

For the visual fields there was no significant difference between the mean threshold (expressed as dB of attenuation) in the central 10° between 6 months and 3 years (Table 7).

Table 7. Mean threshold (dB of attenuation) at 6 months and at 3 years (11 subjects).

	6 months	***3 years***	***p-value***
affected eye	24.9±5.7	25.2±5.6	NS
unaffected eye	30.3±1.3	29.5±2.3	NS

3.2.2.4 Correlations

No correlation was found between lesion length on MRI at presentation or at 6 months and VEP latency or amplitude at 3 years. Furthermore, the degree of change in VEP latency between 6 months and 3 years were not significantly correlated with the MRI lesion length at 6 months. VA at 2 weeks and at 6 months were not correlated with VEP latency or amplitude at 3 years. Contrast sensitivity measures at 6 months were not correlated with VEP latency or amplitude at 3 years.

3.3 DISCUSSION

The principle conclusion from the findings from this small study is that there is evidence for recovery of the VEP latency in the affected eye, suggesting that a reparative process takes place between 6 months and 3 years from the onset of symptoms. Interestingly this phenomenon, did not significantly correlate with possible modifying factors such as treatment, lesion length or initial visual acuity and was not accompanied by improvement in visual function.

Conversely, over the same interval there was a slight prolongation of the latency in the "unaffected" eye (significant for the whole field responses). As the single patient who had a history of ON involving the previously "unaffected" eye in the interval studied was excluded, the mild deterioration of the responses from the fellow eye is an important finding which suggests additional episodic or ongoing subclinical demyelination in that eye. It seems unlikely that the aging process alone could account for the delay seen in the "unaffected" eye (3 msec in the whole field), because over the age interval analyzed (3 years for a mean age of 40 yrs) the latency difference would be in the order of 1 msec as shown by data from other centres (Chiappa, 1997). From further analysis it was also noted that only 4 subjects had a prolonged latency in absolute terms for the "unaffected" eye and that eye was already involved, possibly subclinically, at presentation (as

demonstrated by a prolonged latency of the responses in that occasion), whereas for the patient who had an attack in the previously "unaffected" eye (excluded from statistical analysis) the latency markedly increased in the interval we analyzed. Our data are very similar to those obtained in a previous study (Jones, 1993) in which cross-sectional VEPs were performed at various intervals from the onset of symptoms. Although the P100 latency data from the affected eye were published in a different form the mean was: 137 msec at on average 2.5 weeks, 133 msec at on average 4.5 months and 127 msec at on average 15 months from the onset of symptoms. Allowing for time, these means are in accordance with the present study when 11 patients were recorded on 3 occasions: 134 msec at 2 weeks; 129 msec at 6 months; 121 msec at 3 years. Interestingly, the data from the "unaffected" eye were also very similar when patients who had an episode of optic neuritis or had abnormally delayed VEPs were excluded from the present study as they were from the previous study; the whole field responses were significantly longer in latency at 3 years as compared to 2 weeks (present study: $p<0.006$) and at 15 months as compared to 6 weeks (previous study: $p<0.01$).

It is also important to notice that according to the cross sectional study (Jones, 1993) patients with clinical evidence of disseminated disease were more likely to show a lesser delay in the affected eye between 2 months and 2 years suggesting that factors associated with active lesions could accelerate the VEP recovery. This was not seen in this group of patients, although the number of those who had

new symptoms was very low (3 patients) and therefore not entirely suitable for statistical analysis.

In contrast to our findings, a previous study reported a reduction in the amplitude of the VEPs recorded on average 18 months after the initial recording (Matthews and Small, 1979). The majority of these were patients with MS rather than isolated optic neuritis, who may therefore have had a greater number of lesions affecting the visual pathway. Also, patients who experienced symptomatic optic neuritis in the interim were not excluded. More recent studies showed no significant changes or an increase in VEP amplitude at various intervals after an episode of ON (Matthews and Small, 1983; Jones, 1993).

The contrast sensitivity data did not show any significant difference between 6 months and 3 years for the affected eye, but from the "unaffected" eye there was a significant or close to significant deterioration for most of the conditions tested. No clear pattern of changes was found for the visual fields in either eye. In accounting for these differences it is necessary to keep in mind that the overall trend for the two eyes may still be similar, but it is more difficult to show a deterioration from a pathological state than from normality. However, it is plausible to think that, whereas the prolongation of VEP latency and deterioration in contrast sensitivity for the "unaffected" eye may have been due to subclinical episodes of demyelination and axonal degeneration, respectively, similar processes in the affected eye may have been prevented or their effect counteracted by the ongoing

process of remyelination after the clinically overt episode. On the other hand it might be argued that remyelination truly has no direct effect on visual function, because the visual deficit is caused by irreversible axonal degeneration.

Recently published data (Ferguson et al., 1997; Trapp et al., 1998) have shown that axonal degeneration occurs in acute demyelinating lesions to a substantial extent. The role of remyelination might therefore be to protect the axons from further damage (demyelinated axons being more vulnerable than myelinated ones) and as a consequence from further clinical deficit, but not to reduce the existing clinical deficit. The fact that some patients diagnosed with MS fail to develop a phase of progressive deterioration may be attributable to more successful remyelination. However, progressive visual deterioration is relatively uncommon in MS, and it may not be coincidental that a shortening of evoked potential latency is not widely observed in other sensory modalities (Matthews and Small, 1979; Walsh et al, 1982). Although in many respects useful as a "model" of MS, it is possible that the long-term reparative processes demonstrated in the optic nerve are not typical of the disease as it affects other parts of the central nervous system.

In a recent treatment trial (ONTT) (Beck et al., 1993) the authors evaluated the fellow eye status on the basis of clinical examination, contrast sensitivity and visual field testing. The most common abnormalities were: reduced visual acuity, impaired colour vision, visual field defects, although these were generally mild, and

abnormalities in the contrast sensitivity testing. At follow-up the authors found an overall improvement of the psychophysical data. This finding would suggest that the abnormalities detected at presentation may be due to acute inflammation rather than long-standing lesions. In support of the recent onset of the fellow eye abnormalities was also the observation of a better recovery in those patients who had the ivMP treatment as compared to the placebo group or the oral treatment group. Our findings are not in agreement with those reported by Beck et al. (1993), in that we found an overall deterioration of the responses from the "unaffected" eye. As noted by Beck and colleagues, also in our study some of the abnormalities were due to previous episodes (prior to recruitment) of optic nerve demyelination, but there is also evidence in our patients for new lesion formation. The VEP responses from the "unaffected" eye did not show any shortening of the latency in the time interval we analysed, suggesting that the recovery process, if it ever happened, was already over when the patient was recruited for the study. An explanation for the contrary findings would be the longer period of follow-up for our study (3 years) as compared to the ONTT (6 months).

In an overall view the fact that the unaffected eye showed a deterioration of the neurophysiological data contemporaneous with the improvement of the affected eye gives us confidence that these results were not spurious and in excluding the occurrence of technical problems.

From the VEP data of this study there is evidence for a

reparative process in the affected eye taking place between 6 months and 3 years from the onset of symptoms of optic neuritis. As previously mentioned, in view of the fact that, once inflammation has subsided, the VEP delay observed is considered to be an index of the degree of demyelination, it seems plausible that any shortening of this delay may be due to remyelination, although other possibilities need to be considered (see general discussion). If it is true that axonal degeneration is the cause of the persistent visual deficit, it may be that a beneficial function of remyelination is to prevent more axons from degenerating, in accordance with the findings that a demyelinated axon is more vulnerable to inflammatory agents than a myelinated one.

At the same time a continuous subclinical demyelinating process appears to occur, suggested by the deterioration of the data (VEP latency and contrast sensitivity) obtained from the previously "unaffected" eye. The latter, quantitatively documented for the first time in this study, may account for the frequent finding of delayed VEPs in patients with no history of acute visual impairment.

Our data suggests that demyelination, contrary to what is generally believed, is not a stable condition but a dynamic process where it is possible to observe lesions undergoing repair while new lesions are occurring, clinically symptomatic or asymptomatic. These findings are in agreement with the histopathological literature where it has been shown that demyelinating lesions in MS can undergo repair process, even leading to the conclusion that new lesions naturally

tend to remyelinate, unless a further demyelinative event occurs (Prineas et al, 1993). However, the VEP data seem also to suggest, particularly from the results obtained from the "unaffected" eye, that the recovery process is limited in time independently from new lesion/s occurring at the same site.

In conclusion, after acute optic neuritis, reparative processes appear to predominate over subclinical demyelination for a period of at least 3 years, although the failure of old lesions to show improvement suggests that repair eventually slows or stops. Even before 3 years, it is possible that remyelination may not convey any beneficial effect on visual function if the functional deficits are the result of previous axonal loss. This appears to be supported by the psychophysical indices which suggest that optic nerve remyelination on one hand is unlikely to lead to long-term functional improvement but on the other hand may counteract progressive deterioration due to subclinical demyelination and axonal degeneration.

4. FOLLOW-UP STUDY

4.1 INTRODUCTION

The data from the literature regarding VEP recovery and in particular the shortening of the latency delay are not unequivocal. Most of the authors showed a latency recovery over various time intervals (Matthews and Small, 1983; Hely et al., 1986; Kriss et al., 1988; Jones, 1993;) but there are also some studies where the occurrence of recovery was not thought to be common (Matthews and Small, 1979; Walsh et al., 1982).

The aim of this study was to assess whether there was any evidence for a shortening of the latency delay in 31 patients over a period of 2 years after an episode of optic neuritis starting 3 months after the acute episode in order to avoid the effects of the initial inflammatory process. Patients were examined by means of electrophysiological and psychophysical tests, the latter in an attempt to evaluate the effects of the recovery process on visual function. Recordings were repeated at 3 month intervals for a year and every 6 months in the second year to establish whether there was any trend over time for the various parameters analysed.

Other factors which could have influenced the recovery process such as age of the patients at presentation, MRI changes over a 2 year period and the clinical diagnosis (ON or MS) at presentation and at two years were also analysed.

4.2 RESULTS

4.2.1 Clinical findings

Fifteen patients out of 31 presented with isolated optic neuritis, 7 also had a history of optic neuritis in the fellow eye, 2 had a previous history of optic neuritis in the affected eye and 9 had a history of symptoms related to involvement of other sites of the CNS (1 patient had ON in the fellow eye and in the affected eye prior to recruitment and also had MS symptoms). Details of the clinical findings at presentation are described in Table 1. In summary there were 22 ON patients and 9 MS patients at presentation, whereas at 2 year follow-up there were 16 ON patients and 15 MS patients. According to MRI criteria, 19 subjects out of 28 were classified as affected by MS at presentation and 17 out of 23 at 2 year follow-up.

During 2 year follow-up 4 patients experienced symptoms of new episode/s of optic neuritis in the affected eye, 7 patients experienced symptoms of optic neuritis in the fellow eye (two of them already had a history of ON in that eye), 6 new patients developed symptoms related to other sites of the CNS, apart from the optic nerve.

One patient out of 31 fulfilled the criteria for isolated optic neuritis at the end of the follow-up (no other symptoms apart from those due to the optic neuritis and normal brain MRI on both occasions). A second patient had recurrent episodes of optic neuritis but no other symptoms and a normal MRI.

Table 1. Clinical findings at presentation and at 2 year follow-up for each subject.

Subjects	**Age**	**Sex**	**VA**		**RAPD**		**Disc**		**Colour vision**	
			3 mths	*24 mths*	*3 mths*	*24 mths*	*3 mths*	*24 mths*	*3mths*	*24 mths*
1	51	F	6/9	6/9	N/A	-	N/A	N	N/A	10/10
2	35	F	6/5	6/5	-	-	N	pale	N/A	10/10
3	27	M	6/6	6/5	-	-	pale	pale	N/A	9/10
4	35	M	6/5	6/4	-	-	N	N	N/A	10/10
5	24	M	6/5	6/5	-	-	pale	pale	10/10	10/10
6	31	F	6/6	6/4	+	-	pale	pale	10/10	10/10
7	37	M	6/5	6/4	-	-	pale	pale	10/10	10/10
8	27	F	6/9	6/9	-	-	pale	pale	N/A	10/10
9	25	M	6/12	6/6	+	+	pale	pale	9/10	10/10
10	30	F	6/6	6/5	-	+	N	N	10/10	10/10
11	32	F	6/5	6/4	N/A	-	N/A	N	N/A	10/10
12	34	M	6/9	6/4	+	+	pale	pale	10/10	9/10
13	44	F	6/5	6/5	-	-	N	N	6/10	10/10
14	32	M	6/9	6/9	+	+	pale	pale	1/10	5/10
15	38	F	6/6	6/6	+	-	N	pale	6/10	7/10
16	31	M	6/4	6/4	-	-	N	N	4/10	10/10

17	32	M	6/6	6/6	-	-	pale	pale	10/10	10/10
18	39	F	6/5	6/6	+	+	pale	pale	10/10	9/10
19	37	M	6/6	6/4	+	-	pale	pale	9/10	10/10
20	29	F	6/5	6/6	+	+	pale	pale	6/10	10/10
21	40	F	6/6	6/4	-	-	pale	pale	6/10	9/10
22	30	F	6/5	6/4	+	-	N	N	10/10	10/10
23	30	F	6/6	6/5	+	-	pale	pale	6/10	10/10
24	36	M	6/5	6/4	-	-	pale	pale	10/10	10/10
25	32	M	6/9	6/6	+	-	N	pale	5/10	7/10
26	37	F	6/9	6/6	-	-	N	N	6/10	10/10
27	29	M	6/6	6/9	+	-	pale	pale	10/10	8/10
28	34	F	6/5	6/4	-	-	pale	pale	7/10	10/10
29	30	F	6/4	6/5	+	-	pale	pale	10/10	10/10
30	28	M	6/5	6/5	+	+	pale	pale	2/10	10/10
31	42	F	6/60	6/60	+	+	pale	pale	0/10	0/10

Table 2. Clinical data breakdown

Visual acuity

Eye		3 mths	6 mths	9 mths	12 mths	18 mths	24 mths
Affected	6/6 or better	23 (74%)	24 (77%)	24 (77%)	26 (84%)	25 (81%)	26 (84%)
Fellow	6/6 or better	27 (87%)	25 (81%)	27 (87%)	27 (87%)	28 (90%)	26 (84%)

Visit I-VI: 31 subjects

mths = months

Discs

Eye		3 mths	6 mths	9 mths	12 mths	18 mths	24 mths
Affected	Normal	9 (31%)	8 (26%)	8 (26%)	8 (26%)	8 (26%)	8 (26%)
Fellow	Normal	24 (83%)	23 (74%)	23 (74%)	22 (71%)	22 (71%)	22 (71%)

Visit I: 29 subjects; Visits II-VI: 31 subjects

APD

Eye		3 mths	6 mths	9 mths	12 mths	18 mths	24 mths
Affected	Absent	14 (48%)	21 (68%)	24 (77%)	25 (81%)	26 (84%)	23 (74%)
Fellow	Absent	28 (97%)	29 (93%)	29 (93%)	29 (93%)	29 (93%)	30 (97%)

Visit I: 29 subjects; Visits II-VI: 31 subjects

Colour vision

Eye		3 mths	6 mths	9 mths	12 mths	18 mths	24 mths
Affected	Normal	13 (52%)	21 (68%)	23 (74%)	25 (81%)	26 (84%)	26 (84%)
Fellow	Normal	20 (80%)	26 (84%)	24 (77%)	26 (84%)	27 (87%)	27 (87%)

Visit I: 25 subjects; Visit II-VI: 31 subjects

As expected, the fellow eyes had normal VA, normal colour vision, normal disc and no APD in a higher percentage of cases at 3 months than the affected eyes. The abnormalities could be explained by a previous history of ON in 1 case of reduced VA, in 5 cases of pale discs, in 1 case of APD and in 4 cases of impaired colour vision.

At 2 year follow-up, the difference between the two eyes was less marked. In particular, the number of subjects with normal VA at 2 years as compared to presentation increased for the affected eye, but decreased for the fellow eye (Chi-square test between normal/abnormal responses for each eye: p=NS); the number of normal discs at Visit VI decreased in either eye (Chi-square test: p=NS); the number of subjects with absent APD or normal colour vision increased at Visit VI for either eye (Chi-square test: p=0.004 and 0.004, respectively, for the affected eye; p=NS for the fellow eye). When the chi-square was performed for all visits the results were as above except that the level of significance for the affected eye was slightly lower.

4.2.2 Incidence of abnormal VEP, Visual field, Contrast sensitivity and MRI data

Table 3. Incidence of abnormal VEP (latency), Visual field, Contrast sensitivity and MRI data

VEP latency (31 subjects)

Eye	*Field*		3 mths	6 mths	9 mths	12 mths	18 mths	24 mths
Affected	*whole*	normal	1 (3%)	2 (7%)	2 (7%)	4 (13%)	6 (19%)	6 (19%)
	central	normal	1 (3%)	7 (23%)	4 (13%)	4 (13%)	9 (29%)	9 (29%)
Fellow	*whole*	normal	21 (68%)	21 (68%)	19 (61%)	19 (61%)	19 (61%)	20 (64%)
	central	normal	22 (71%)	21 (68%)	19 (61%)	21 (68%)	19 (61%)	20 (64%)

The absolute latency limit for normal was 109 msec for the whole field and 111 msec for the central field.

Visual fields

Eye		3 mths	6 mths	9 mths	12 mths	18 mths	24 mths
Affected	normal	15 (50%)	13 (42%)	20 (64%)	16 (52%)	24 (77%)	20 (64%)
	abnormal	15 (50%)	18 (58%)	11 (36%)	15 (48%)	7 (23%)	11 (36%)
Fellow	normal	18 (60%)	22 (71%)	23 (74%)	24 (77%)	23 (74%)	22 (71%)
	abnormal	12 (40%)	9 (29%)	8 (26%)	7 (23%)	8 (26%)	9 (29%)

Visit I: 30 subjects; Visits II-VI: 31 subjects

Contrast sensitivity

Eye			3 mths	6 mths	9 mths	12 mths	18 mths	24 mths
Temporal frequency			***Spatial frequency (0.5 c/deg)***					
Affected	*0 Hz*	normal	20 (65%)	26 (83%)	25 (81%)	24 (77%)	27 (87%)	24 (77%)
	8 Hz	normal	22 (71%)	25 (81%)	24 (77%)	22 (71%)	22 (71%)	24 (77%)
	32 Hz	normal	7 (23%)	13 (43%)	17 (55%)	13 (42%)	10 (32%)	14 (45%)
Fellow	*0 Hz*	normal	27 (90%)	30 (97%)	28 (90%)	28 (90%)	30 (97%)	22 (71%)
	8 Hz	normal	27 (90%)	27 (87%)	23 (74%)	25 (81%)	26 (84%)	22 (71%)
	32 Hz	normal	19 (63%)	19 (61%)	16 (51%)	22 (71%)	13 (43%)	18 (58%)
Temporal frequency			***Spatial frequency (4 c/deg)***					
Affected	*0 Hz*	normal	14 (47%)	16 (51%)	17 (55%)	16 (51%)	13 (43%)	14 (45%)
	8 Hz	normal	14 (47%)	20 (67%)	21 (70%)	18 (57%)	20 (67%)	18 (58%)
	32 Hz	normal	7 (24%)	9 (30%)	13 (43%)	16 (51%)	14 (45%)	14 (45%)
Fellow	*0 Hz*	normal	19 (63%)	23 (74%)	20 (67%)	20 (65%)	20 (65%)	21 (70%)
	8 Hz	normal	24 (80%)	26 (84%)	24 (77%)	23 (74%)	24 (77%)	24 (77%)
	32 Hz	normal	13 (57%)	17 (55%)	17 (55%)	17 (55%)	15 (48%)	15 (48%)

Visit I: 30 subjects; Visit II-VI: 31 subjects

MRI findings (brain)

		3 months*	2 years°
Normal		2 (7%)	2 (9%)
Abnormal	non specific	4 (14%)	3 (13%)
	demyelination	19 (68%)	17 (74%)
	1 lesion	3 (11%)	1 (4%)
Not performed		3 (10%)	5 (18%)

* 28 subjects; ° 23 subjects

At presentation, all affected eyes showed a prolonged latency in absolute terms except one whole field response (the central field latency was abnormally prolonged), which was normal throughout the study, and one central field response (the whole field response in this case was apparently delayed) which was borderline normal (110 msec) at 3 months, became abnormal at 1 year and remained abnormally prolonged until the end of the follow-up. The number of patients with normal responses (latency) from the affected eye increased significantly throughout the study: at 2 year follow-up 6 whole field responses (one was normal also at presentation. Chi-square test: p=0.04) and 9 central field responses (Chi-square: p=0.007) were normal in latency (a Chi-square for all visits was just not significant for whole field responses and just significant for central field responses). Latency reduction occurred in a total of 24 out of 29 eyes for the

central field and 27 out of 30 eyes for the whole field. Conversely, 7 patients (5 central field responses and 4 whole field responses) showed further delay in VEP latency: 4 of them experienced symptoms of new episode/s of ON and 3 were asymptomatic.

From the fellow eye 21 patients had normal whole field responses and 22 normal central field responses at 3 months. The latency delay was possibly explained by previous history of optic neuritis in 6 out of 10 whole field responses and in 4 out of 9 central field responses. The number of normal responses (latency) from the fellow eye decreased to 20 for whole and central field responses at 2 year follow-up (Chi-square: p=NS for whole and central field, also when all visits were analysed).

As for the visual fields (analysing the overall field) 15 (50%) responses from the affected eye were abnormal or borderline at presentation. Two out of 12 (40%) abnormal or borderline responses from the fellow eye could be explained by the previous history of optic neuritis. The number of normal Visual fields increased in 2 year follow-up in either eye (a Chi-square test was close to significant, p=0.06, for the affected eye when all visits were analysed but p=NS when visits I and VI were analysed and for the fellow eye).

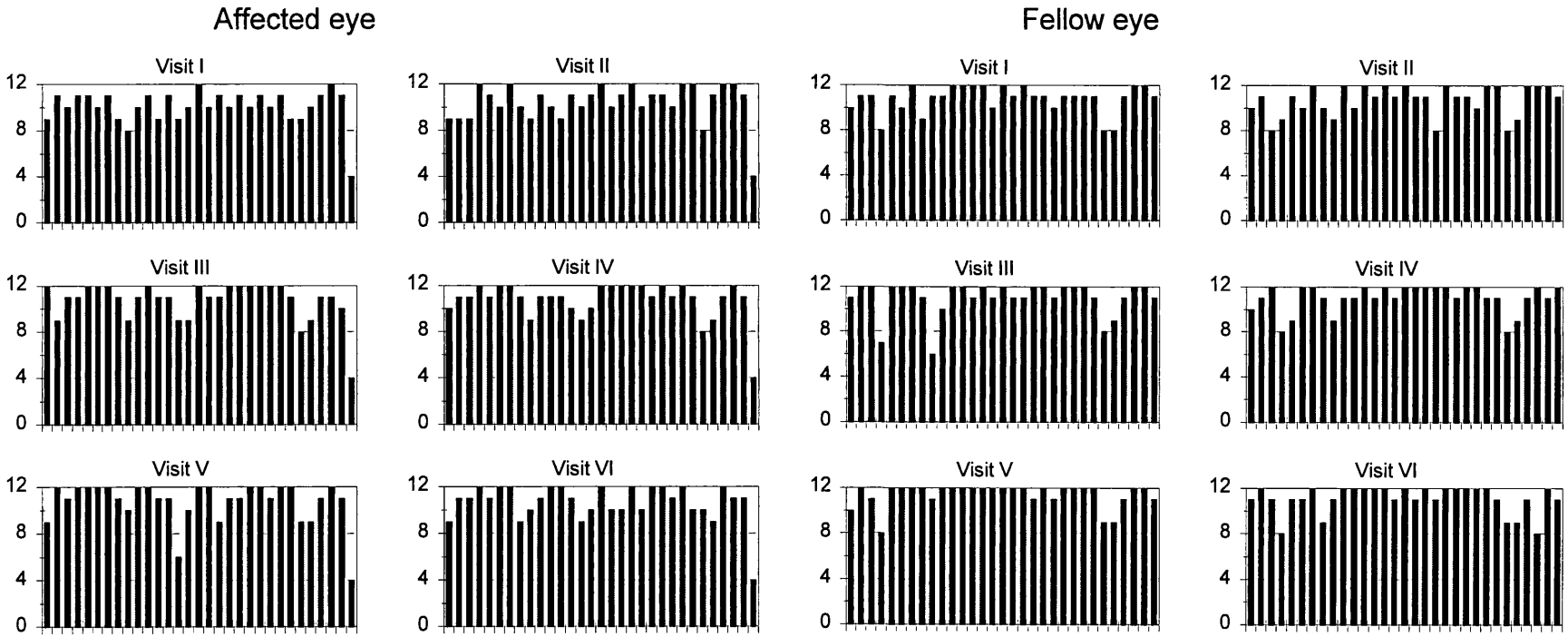
It was more difficult to discern a general pattern for the contrast sensitivity: the 32 Hz temporal frequency to both spatial frequencies, however, appeared more often abnormal than the other temporal frequencies in either eye. Visit II showed the highest number of normal responses. For the affected eye, the percentage of normal responses

increased except for the low spatial frequency (SF) at 0 Hz (Chi-square test: p=NS for all conditions when all visits were analysed together, but when Visit I and Visit VI were analysed, the contrast sensitivity (CS) to both spatial frequencies (SF) at 32 Hz was significant, p=0.05 in either case). For the fellow eye the percentage of normal CS decreased to low SF at all temporal frequencies (TF) but increased or remained stable for CS to high SF when all visits were analysed together (Chi-square: p=NS, except for low SF at 0 Hz: p=0.01 - fewer normal responses). Also when Visit I and VI were analysed the number of normal CS data to low SF at 0 Hz decreased significantly and the change was close to significance at 8 Hz. At Visit I, abnormal CS from the fellow eye was possibly explained by previous history of optic neuritis in 5/14 cases for 0.5 c/deg spatial frequency and 7/21 cases for the 4 c/deg spatial frequency.

Ninety-three percent (26/28) of the MRI scans at presentation were abnormal and 91% (21/23) were abnormal at 2 year follow-up. Seventy-eight percent of the scans (18/23) showed MRI changes at follow-up, indicating the expansion of old lesions and the formation of new lesions.

4.2.3 Visual acuity, VEP, Visual field and Contrast sensitivity graphs of data changes.

Fig. 1. Visual acuity changes for individual patients during 2 year follow-up.

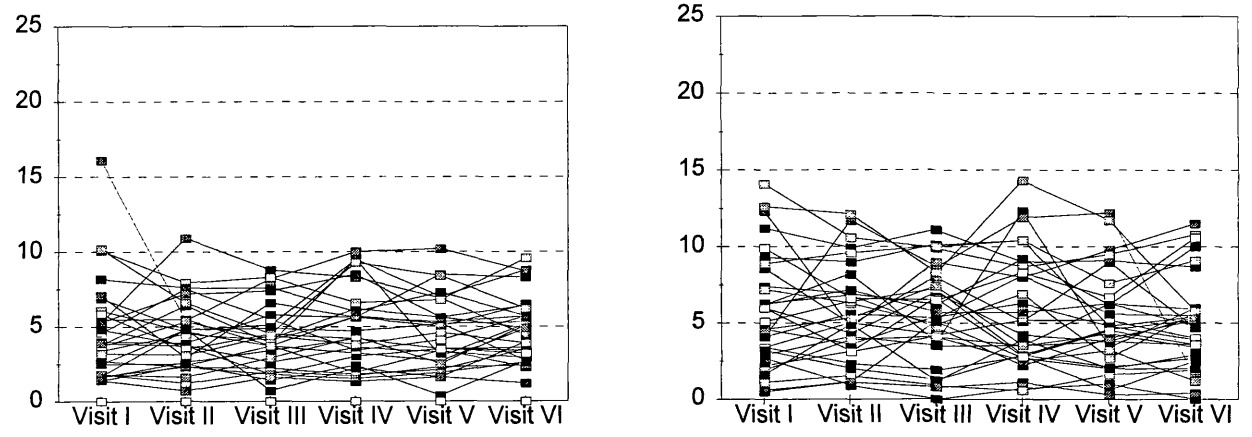


VA: 6/6=10

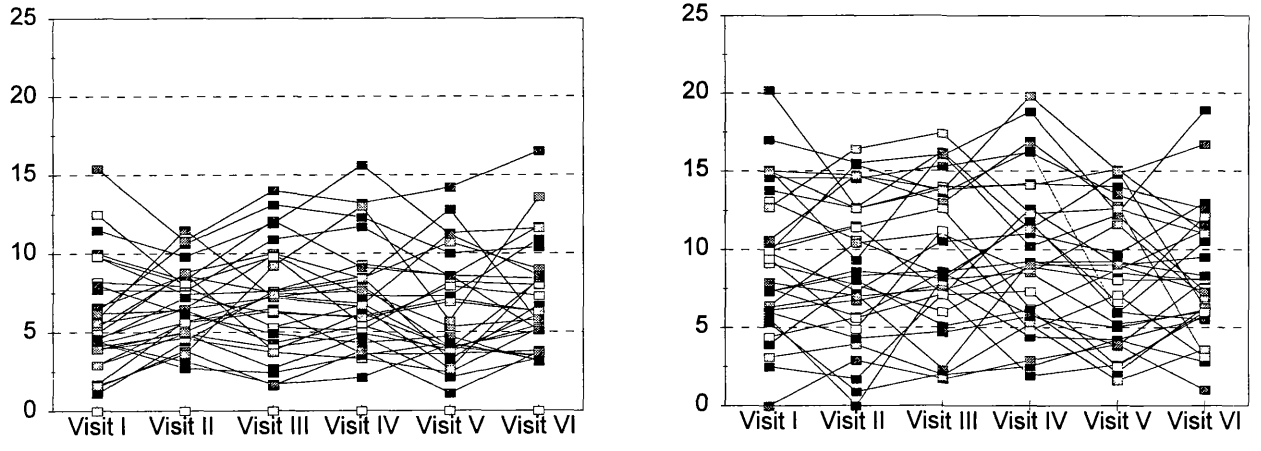
Fig. 2. VEP amplitude (μV) changes for individual patients during 2 year follow-up.

Affected eye | Fellow eye

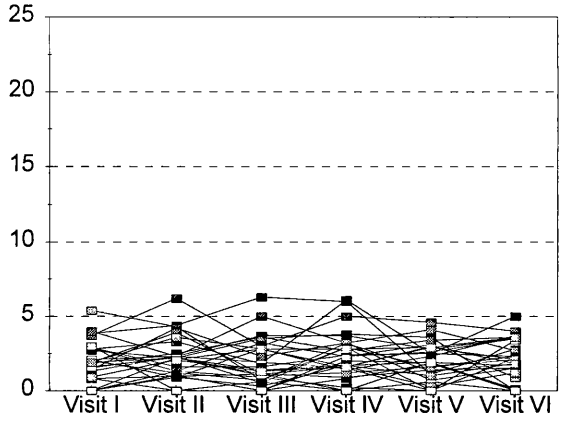
Central field

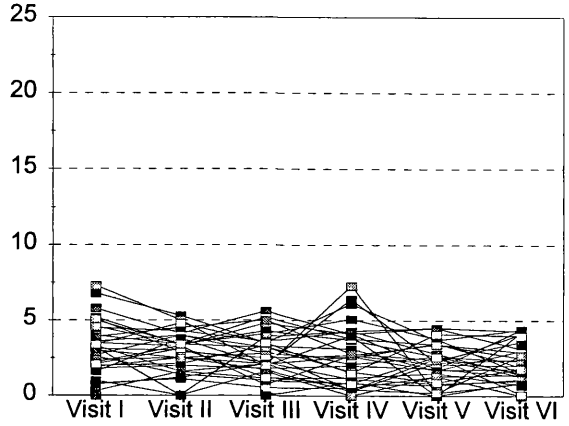


Whole field

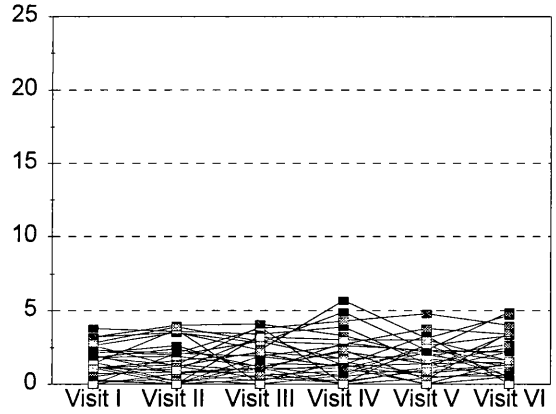


Right hemisurround field





Left hemisurround field



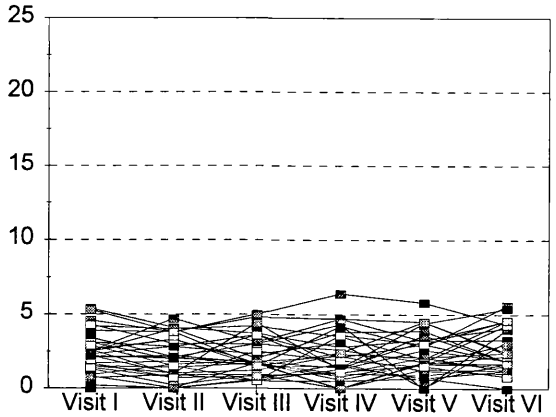
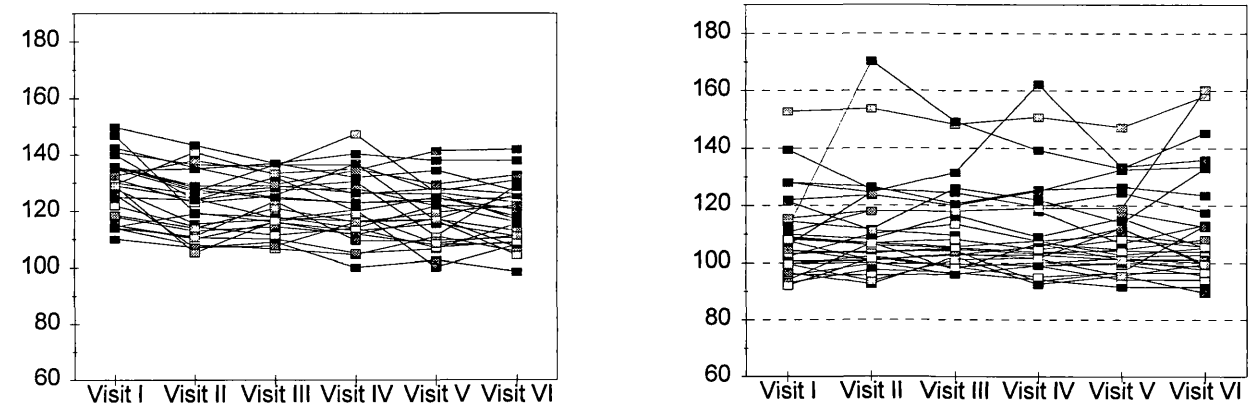


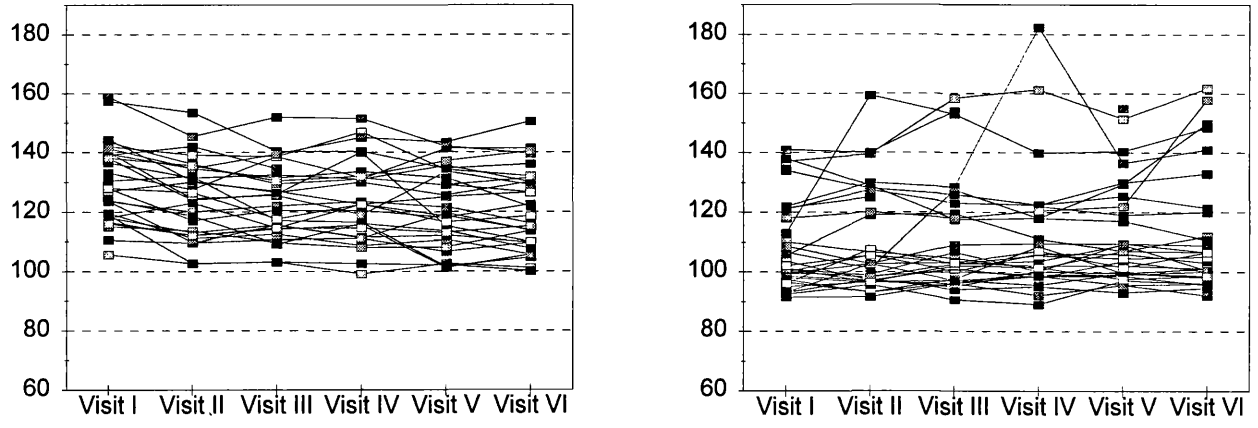
Fig. 3. VEP latency (msec) changes for individual patients during 2 year follow-up (one case with latency >200 msec was excluded).

Affected eye Fellow eye

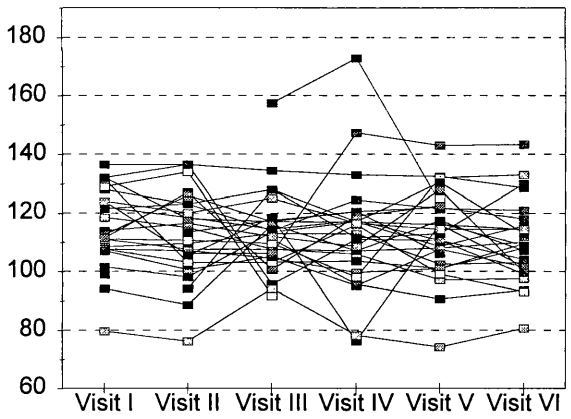
Central field

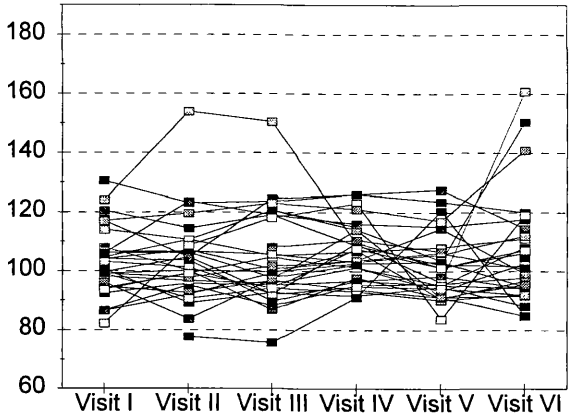


Whole field

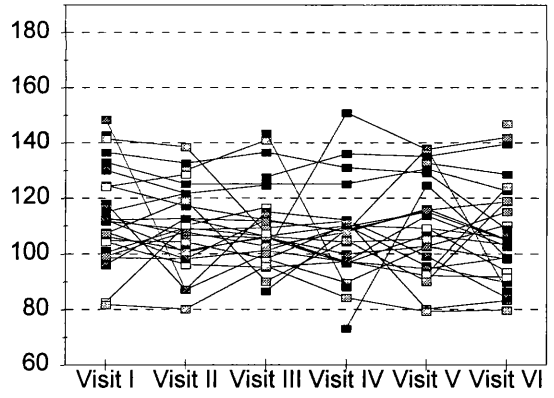


Right hemisurround field





Left hemisurround field



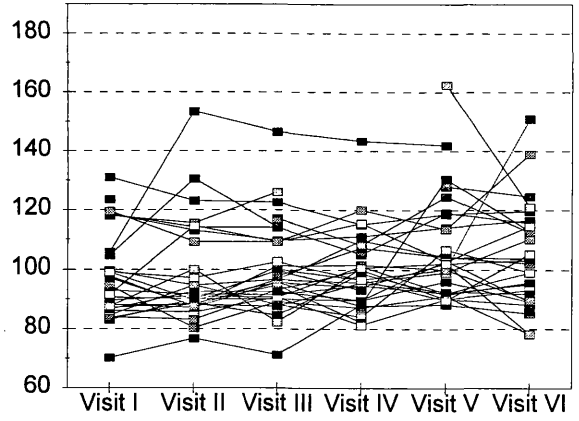


Fig. 4. Visual field mean threshold in the central 10° radius (as dB of attenuation): changes for individual patients during 2 year follow-u

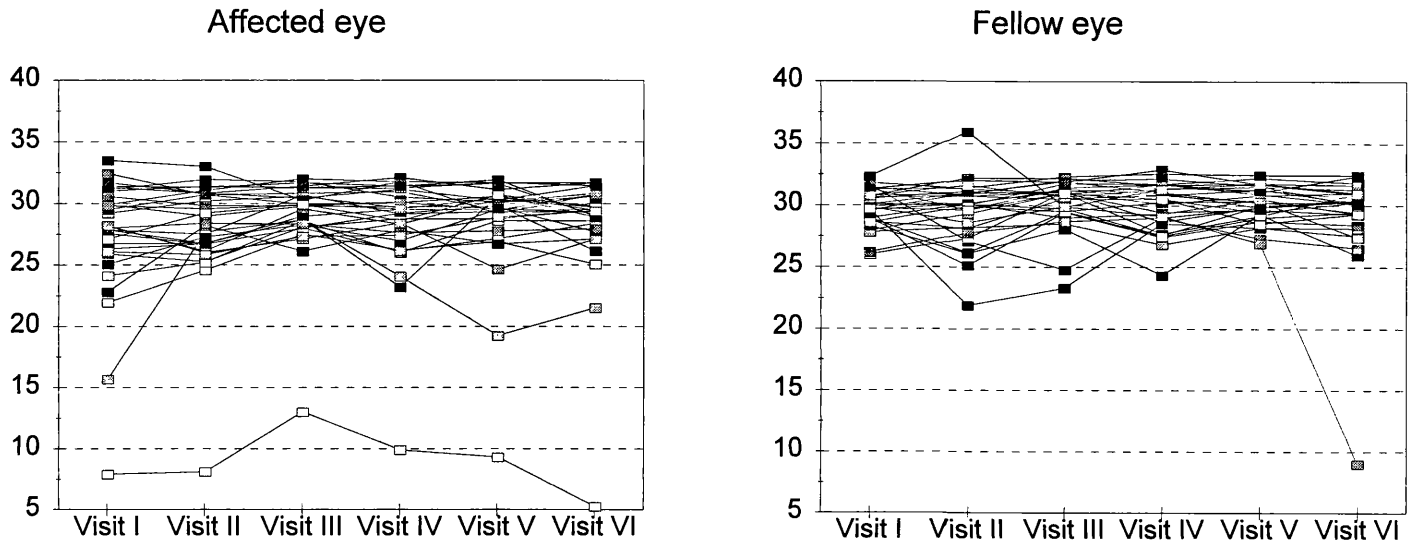
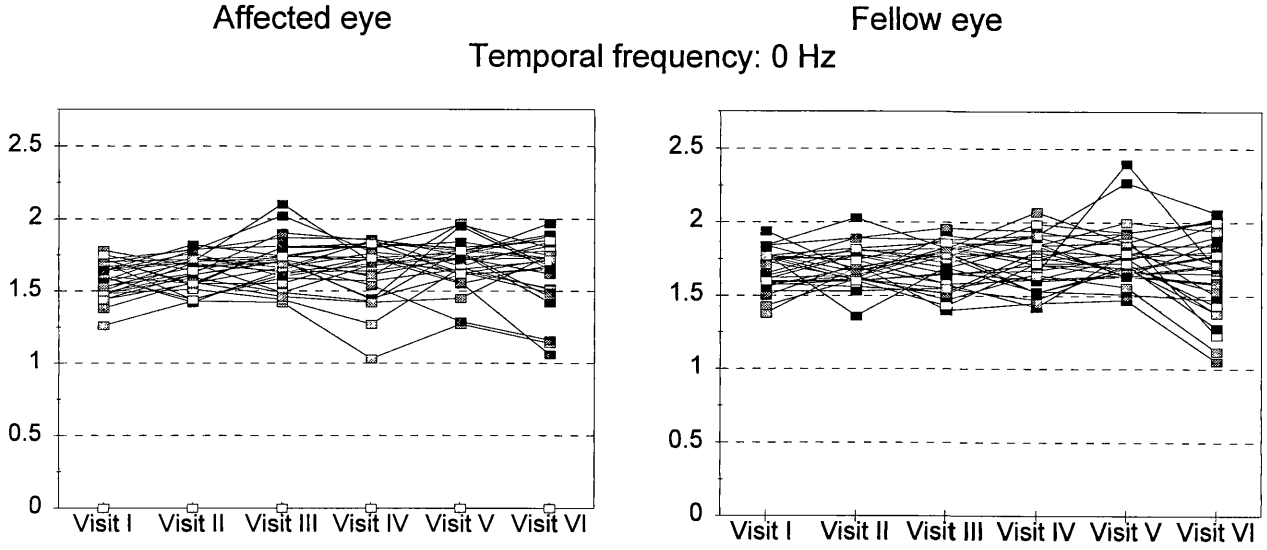
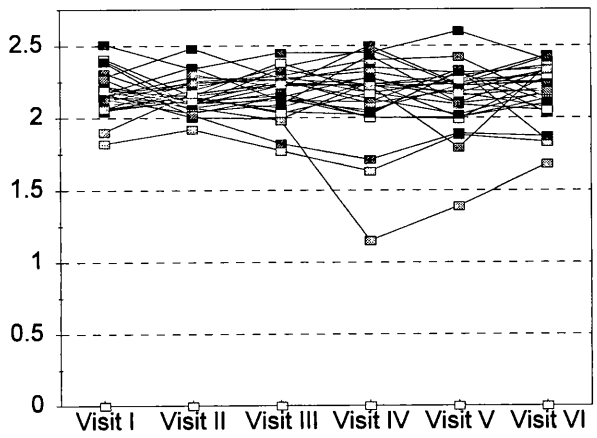


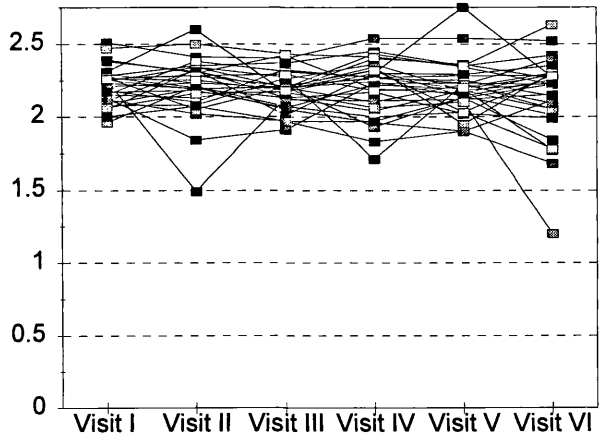
Fig. 5. Contrast sensitivity data (log) changes for individual patients during 2 year follow up.

Spatial frequency: 0.5 c/deg

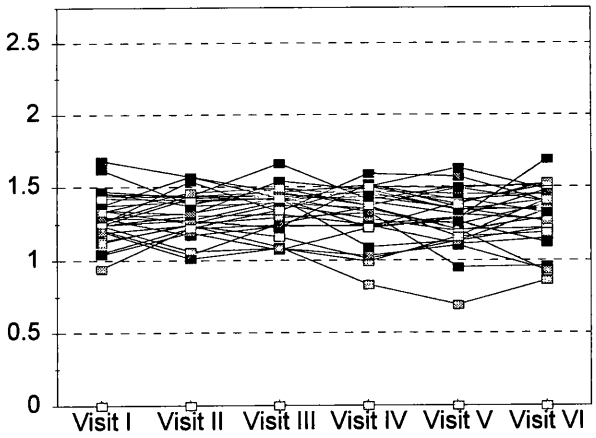


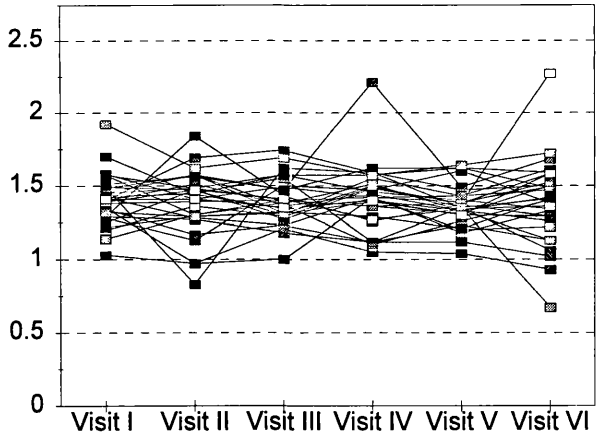
Temporal frequency: 8 Hz





Temporal frequency: 32 Hz



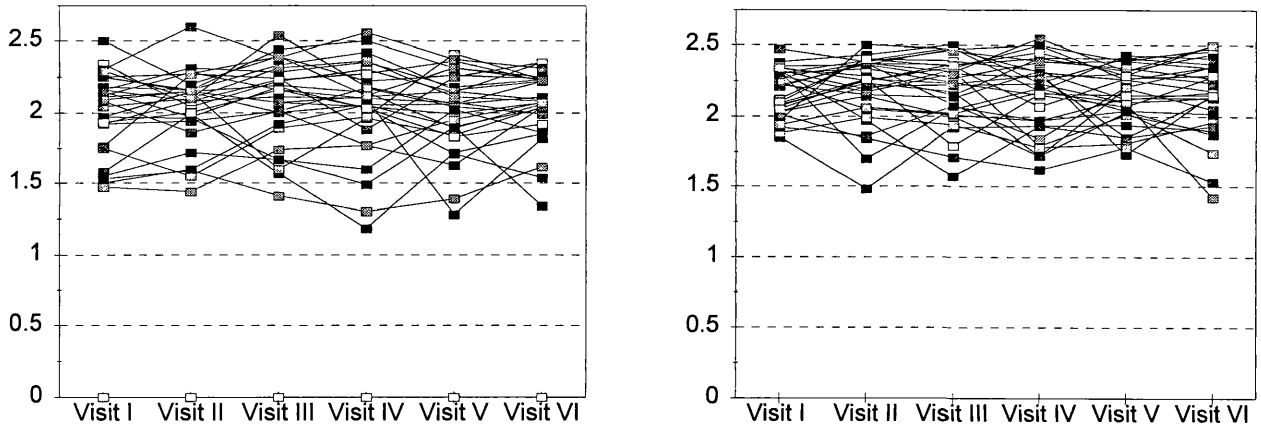


Spatial frequency: 4 c/deg

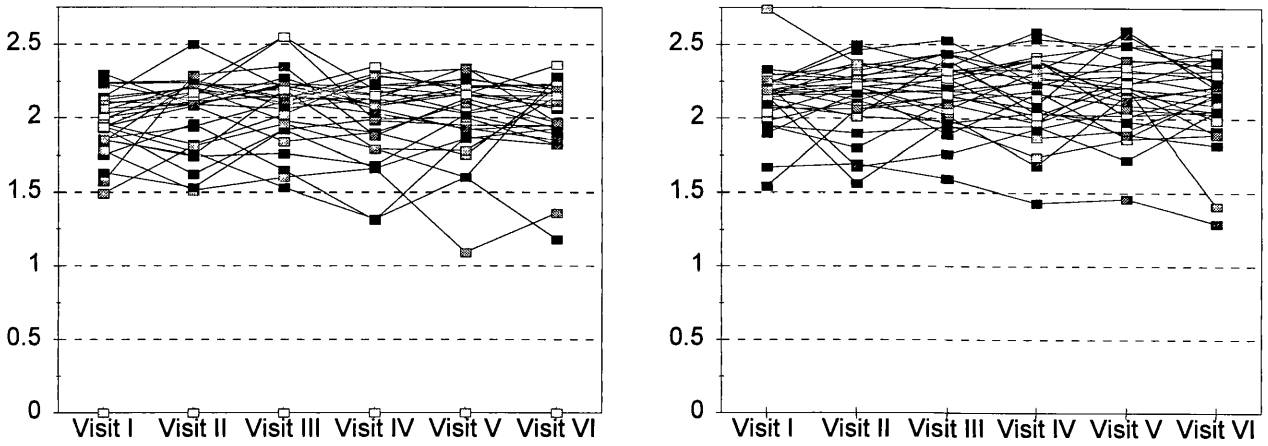
Affected eye

Fellow eye

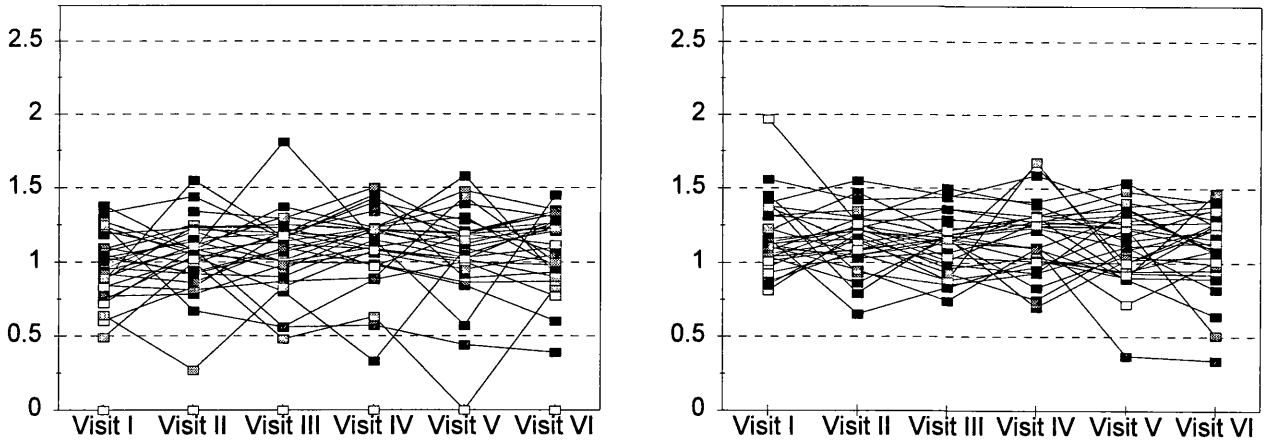
Temporal frequency: 0 Hz



Temporal frequency: 8 Hz



Temporal frequency: 32 Hz



4.2.4 Repeated measure analysis

VEP, Visual field and Contrast sensitivity data were analysed for equally spaced intervals of 3 months (Visits I - IV) or 6 months (Visits II, IV, V, VI) for groups A (all patients) and B (excluding those patients who had an additional episode of ON in the affected or fellow eyes prior to recruitment or during follow-up). The VEP data (amplitude and latency) did not show any difference between ON patients and MS patients as classified at presentation and at 2 year follow-up and there were no interactions (MS and ON patients did not show a different behaviour in time).

The contrast sensitivity data showed a marginal difference between ON and MS patients as classified at presentation or at 2 year follow-up only when Group A was analysed (the ON patients had a higher sensitivity than the MS patients, more evident for the affected eye) but not Group B. However, there was no significant interaction ON vs MS x time.

The visual field data showed a statistically significant difference between ON and MS patients as classified at presentation (the ON patients had a mean threshold in dB of attenuation (28.8±2.9) higher than MS patients (25.3±4.9) in the affected eye). A significant interaction eye x time x ON vs MS patients was present when patients with symptomatic ON in the fellow eye were excluded. The findings will be therefore discussed later in greater detail. There was also a statistically significant difference between ON patients and MS patients

as classified at 2 year follow-up when Visits I-IV were analysed but not Visits II, IV, V and VI. No interaction was found in this case.

There was no statistically significant difference between those patients who showed changes between MRI at presentation and at the end of the study and those who did not for VEP, Visual field and Contrast sensitivity data and no MRI changes x time interaction. However, these results may be due to the fewer patients who did not show a change (n=5) as compared to those who did (n=18).

Further analysis was performed excluding the within subject factors except for the Visual field data (ON patients vs MS patients at presentation).

4.2.4.1 VEP amplitude

The amplitude analysis (Table 4) comprised 3 variables. The variable "eye" had 2 levels, affected/fellow (eye 2), the variable "field" had also 2 levels, centre/whole (field 2) and the variable "time" had 4 levels, Visits I-IV in the first analysis and Visits II, IV, V and VI in the second (time 4).

As expected, a significant difference between the affected eye and the fellow eye (affected eye of lower amplitude than fellow eye) as well as a significant difference between the central and the whole field responses (central field responses of lower amplitude as compared to whole field responses) was seen in all situations. However, there were no differences among visits and no trend in time was seen from the affected eye or the fellow eye. The behaviour in time for the two eyes (affected/fellow) and two fields (centre/whole) did not show any difference, although the starting point was different (as shown by the significant difference between affected and unaffected eyes or central and whole fields).

Table 4. Mean VEP amplitude (μV) ±SD and p-values

Eye	*Field*	3 mths*	6 mths*°	9 mths*	12 mths*°	18 mths°	24 mths°
Group A							
Affected	*Centre*	4.42±3.4	4.20±2.5	4.04±2.4	4.63±2.9	4.23±2.6	4.51±2.4
	Whole	5.79±3.6	6.54±2.6	6.82±3.5	6.99±3.5	6.19±3.6	6.91±3.5
Fellow	*Centre*	5.75±3.7	5.57±3.1	5.57±3.0	5.79±3.7	5.12±3.2	4.96±3.1
	Whole	9.19±4.7	8.80±4.6	9.56±4.6	9.78±4.8	8.28±4.2	8.17±4.0
Group B							
Affected	*Centre*	4.15±3.5	4.18±2.5	3.99±2.4	4.55±2.7	4.18±2.5	4.51 ±2.2
	Whole	5.51±3.6	6.88±2.8	6.80±3.7	6.77±3.1	6.13±3.9	6.47±3.7
Fellow	*Centre*	5.76±3.3	5.99±2.6	5.88±2.8	6.23±3.3	5.63±3.0	5.43±3.0
	Whole	9.91±4.9	10.27±3.8	10.32±4.6	10.56±4.3	8.54±4.0	8.95±4.4

* analysed together for trend; ° analysed together for trend

Group		A	B	A	B
		Visits I - IV		**Visit II - VI**	
Affected/fellow eye	*centre*	p=0.001	p=0.007	p=0.009	p=0.011
(eye 2, time 4)	*whole*	p=0.000	p=0.000	p=0.000	p=0.000
Central/whole field	*affected*	p=0.000	p=0.000	p=0.000	p=0.000
(field 2, time 4)	*fellow*	p=0.000	p=0.000	p=0.000	p=0.000
Time	*affected*	NS	NS	NS	NS
(field 2, time 4)	*fellow*	NS	NS	NS	NS
Eye x time		NS	NS	NS	NS
Field x time		NS	NS	NS	NS
(eye 2, field 2, time 4)					

4.2.4.2 VEP latency

The latency variables were as described for amplitude (eye 2, field 2 and time 4).

There was a significant difference between the affected eye and the fellow eye (affected eye of longer latency as compared to the fellow eye). There was no significant difference between central and whole field responses for either eye.

There was a statistically significant time effect (latency becoming shorter with increasing time) for the affected eye for Visits I - IV and Visits II, IV, V and VI, but not for the fellow eye. The trend was linear in nature and its pattern did not show ant difference for central and whole field (no field x time interaction). Simple contrast analysis showed a significant difference between Visit I and Visits II, III and IV in the analysis of 3-month intervals, and a significant difference between Visit II and Visits V and VI, but not Visit IV in the 6-month interval analysis. The repeated contrast did not show any significant difference between adjacent visits, apart from Visits I and II as described previously.

For the fellow eye there was no time effect and therefore no trend could be shown, which explains the finding of a different behaviour in time (trend) for the affected eye and the fellow eye (eye x time interaction) (Table 5).

Table 5. Mean VEP latency (msec) ±SD and p-values

Eye	Field	3 mths*	6 mths*°	9 mths*	12 mths*°	18 mths°	24 mths°
Group A							
Affected	*Centre*	129.8±10.1	122.7±11.2	123.6±9.4	122.4±12.1	119.0±10.9	118.4±11.0
	Whole	129.8±13.5	123.5±13.1	122.4±12.1	122.7±13.4	120.4±13.0	119.7±12.8
Fellow	*Centre*	106.9±11.9	108.9±15.9	109.2±13.1	109.2±15.8	108.4±12.8	109.9±18.0
	Whole	104.9±12.5	106.7±16.0	107.1±14.5	108.8±18.9	108.8±13.7	110.3±19.2
Group B							
Affected	*Centre*	129.3±8.9	121.6±11.1	122.7±10.6	120.2±10.6	117.3±10.1	115.7±9.8
	Whole	127.5±13.2	120.6±12.4	119.8±12.6	120.5±13.9	116.2±12.8	114.5±11.7
Fellow	*Centre*	103.0±10.9	102.9±7.8	103.9±8.0	102.6±6.8	102.7±8.7	101.1±7.3
	Whole	100.1±10.0	99.8±8.5	100.2±8.1	101.4±7.9	101.1±7.9	100.3±6.9

* analysed together for trend; ° analysed together for trend

Group		A	B	A	B
		Visits I - IV		**Visits II - VI**	
Affected/fellow eye	*centre*	p=0.000	p=0.000	p=0.000	p=0.000
(eye 2*)	*whole*	p=0.000	p=0.000	p=0.000	p=0.000
Central/whole field (eye2, field 2, time 4)		NS	NS	NS	NS
Time	*affected*	p=0.000	p=0.002	p=0.03	p=0.016
(field 2, time 4)	*fellow*	NS	NS	NS	NS
Eye x time		p=0.002	p=0.013	p=0.029	p=0.011
Field x time (eye 2, field 2, time 4)		NS	NS	NS	NS

* general factorial

4.2.4.3 Visual fields

The mean threshold in dB of attenuation of the central 10° (radius) was used in the statistical analysis. For the visual fields there were two variables: eye and time. The former had two levels, affected/fellow eyes (eye 2) and the latter 4 levels, Visits I-IV and Visits II, IV, V and VI (time 4).

The repeated measure analysis comprising the within subject factor ON vs MS patients at presentation showed for Visits I to IV an interaction between ON vs MS x eye x time and therefore the affected and the fellow eye were analysed separately. There was a significant difference in the sense of a higher sensitivity for the ON patients as compared with the MS patients in the affected eye, and a similar difference was just significant for the fellow eye.

For the affected eye there was also an interaction between ON vs MS x time for Group A so that the trend was different between the ON and the MS patients. Therefore the two subgroups were analysed separately, although there were in total only 9 MS patients. The affected and the fellow eyes were different for the ON patients in Groups A and B whereas there was no significant difference in the MS group when all patients were analyzed together (there were too few patients in Group B to perform a statistical analysis). For the MS patients there was a significant improvement over time (the largest improvement occurred between 6 and 9 months) and but no time effect was seen for the ON patients (group A). There was a significant

improvement over time in Group B for the affected eye (the largest improvement occurred between 6 and 9 months but a statistically significant difference was also seen between 9 and 12 months). There was no interaction eye x time so that there was no difference in the beahviour of the two eyes in time in the ON and MS subgroups.

For the fellow eye there was a marginal difference between ON and MS patients in Group A, but no statistical analysis was performed for Group B. There was no interaction (ON vs MS x time) and no time effect was seen in either group.

When Visits II, IV, V and VI were analysed, there was no significant ON vs MS x eye x time interaction in all groups. However, there was a significant difference between ON and MS patients in Group A and the affected and the fellow eyes were significantly different in both groups. There was no time effect in either group. There was no interaction eye x time or ON vs MS x time so that the trend in time was in the same direction for the affected eye and the fellow eye or ON and MS patients, although they started at different levels (Table 6).

Table 6. Mean threshold (in dB of attenuation) ±SD and p-values

Eye	3 mths	6 mths	9 mths	12 mths
Group A*				
Affected	27.3±5.1	27.9±4.4	28.9±3.4	28.2±4.1
Fellow	29.8±1.6	29.5±2.7	30.1±2.0	29.8±2.0
Group B				
Affected	27.0±5.8	27.2±5.5	28.5±4.2	27.7±4.9
Fellow	29.9±1.4	30.4 ±2.1	30.7±0.9	30.4±1.4

* 30 subjects

Eye	6 mths	12 mths	18 mths	24 mths
Group A*				
Affected	28.0±4.3	28.3±4.1	28.5±4.3	28.3±4.8
Fellow	29.5±2.6	29.9±2.0	30.1±1.4	29.2±4.1
Group B				
Affected	27.4±5.4	27.9±4.9	28.6±5.0	28.1±5.9
Fellow	30.3±2.1	30.5±1.4	30.7±1.1	30.5±1.3

* 31 subjects

Group		A	B
		Visits I - IV	
ONvsMS (wsf*)	*affected*	p=0.000	-
(time 4, wsf*)	*fellow*	p=0.053	-
Affected/fellow eye	*ON*	p=0.014	p=0.038
(eye 2, time 4)	*MS*	NS	-
Time	*affected ON*	NS	p=0.031
	MS	p=0.008	(time 4)
(time 4 + wsf*)	*fellow*	NS	NS
Eye x time	*ON*	NS	NS
(eye 2, time 4)	*MS*	NS	-
ONvsMS x time	*affected*	p=0.02	NS
(time 4 + wsf*)	*fellow*	NS	NS
		Visits II - VI	
ONvsMS (wsf*)		p=0.005	NS
(eye 2, time 4, wsf*)			
Affected/fellow eye		p=0.043	p=0.009
(eye 2, time 4 + wsf*)			
Time		NS	NS
(eye 2, time 4 + wsf*)			
Eye x time		NS	NS
(eye 2, time 4 + wsf*)			
ONvsMS x time		NS	NS
(eye 2, time 4 + wsf*)			

* within subject factor

The Visual field data were also analyzed with a specific software fir Windows, PROGRESSOR, where each patient was analysed separately across 6 visits. In addition, each point tested in the field (74 in total) was analyzed separately. In Group A there were 12/31 affected eyes which showed a deterioration (increase in number of points where the threshold in dB of attenuation from maximal decreased by 1 dB at a significance level <0.05) of which 9 were affected by isolated or recurrent ON and 3 by MS (clinical data); 19/31 did not show any deterioration, of which 13 were affected by ON and 6 by MS (clinical data). For the fellow eye 17 patients showed a deterioration (15 were affected by ON and 2 by MS) and 14 did not show any progression (7 patients in each group). A Chi-square test did not show any significant difference for the affected eye, but showed that a significantly higher number of patients classified at presentation as affected by ON rather than MS deteriorated in the fellow eye (also when those patients who had a new episode of ON during follow-up were excluded).

4.2.4.4 Contrast sensitivity

There were 4 variables for Contrast sensitivity: "eye" as previously described (eye 2), "spatial frequency" (two levels, spfr 2), "temporal frequency" (three levels, tefr 3) and time as previously described.

There was a significant difference between the affected eye and the fellow eye for the high SF (4 c/deg) (affected eye lower sensitivity than fellow eye), but only a marginal difference for the low SF (0.5 c/deg) for Visits I-IV and II, IV, V and VI. The 3 temporal frequencies (0, 8, and 32 Hz) were significantly different for high SF and low SF for Visits I-IV and II, IV, V and VI for either eye. There was a significant effect of time for the affected eye for Group A (Visits I-IV) but no effect between Visits II, IV, V and VI or for the fellow eye. There was a statistically significant difference between Visit VI and Visit I at simple contrast but not between adjacent visits in both groups. A marked spatial frequency effect was present for the affected eye. A similarly marked effect was also present for the fellow eye, except for the data at 8 Hz for Group B (I-IV) and Group A and B (II, IV, V and VI) where the differences did not reach statistical significance. The two eyes did not differ in their behaviour over time (no eye x time interaction) and the behaviour in time was also independent of the spatial and temporal frequencies used (no spfr x time and tefr x time interactions) (Table 7).

Table 7. Mean contrast sensitivity (log) ±SD and p-values

Eye		3 mths	6 mths	9 mths	12 mths
Group A*	*Temporal frequency*		*Spatial frequency 0.5 c/deg*		
Affected	*0 Hz*	1.53±0.3	1.58±0.3	1.63±0.3	1.59±0.4
	8 Hz	2.10±0.4	2.08±0.4	2.10±0.4	2.07±0.5
	32 Hz	1.25±0.3	1.27±0.3	1.30±0.3	1.27±0.3
Fellow	*0 Hz*	1.67±0.1	1.69±0.1	1.70±0.1	1.72±0.2
	8 Hz	2.21±0.1	2.21±0.2	2.19±0.1	2.22±0.2
	32 Hz	1.41±0.2	1.38±0.2	1.39±0.2	1.39±0.2
			Spatial frequency 4 c/deg		
Affected	*0 Hz*	1.94±0.5	1.97±0.4	2.01±0.5	1.97±0.5
	8 Hz	1.91±0.4	1.96±0.4	2.01±0.4	1.93±0.4
	32 Hz	0.94±0.3	1.00±0.3	1.02±0.3	1.05±0.3
Fellow	*0 Hz*	2.17±0.2	2.19±0.2	2.18±0.2	2.14±0.3
	8 Hz	2.14±0.2	2.14±0.2	2.17±0.2	2.15±0.3
	32 Hz	1.18±0.2	1.15±0.2	1.12±0.2	1.18±0.2
Group B	*Temporal frequency*		*Spatial frequency 0.5 c/deg*		
Affected	*0 Hz*	1.51±0.4	1.54±0.4	1.62±0.4	1.58±0.4
	8 Hz	2.08±0.6	2.05±0.5	2.05±0.5	2.02±0.5
	32 Hz	1.22±0.4	1.25±0.4	1.26±0.4	1.26±0.4
Fellow	*0 Hz*	1.68±0.1	1.72±0.1	1.73±0.2	1.78±0.1
	8 Hz	2.25±0.1	2.29±0.2	2.20±0.1	2.27±0.2
	32 Hz	1.43±0.2	1.46±0.2	1.42±0.2	1.40±0.2
			Spatial frequency 4 c/deg		
Affected	*0 Hz*	1.92±0.5	1.93±0.6	1.98±0.6	1.99±0.6
	8 Hz	1.89±0.5	1.92±0.5	1.95±0.5	1.90±0.5
	32 Hz	0.91±0.3	0.96±0.4	1.01±0.4	1.04±0.3
Fellow	*0 Hz*	2.22±0.1	2.25±0.2	2.23±0.2	2.21±0.3
	8 Hz	2.20±0.2	2.21±0.2	2.22±0.2	2.21±0.3
	32 Hz	1.17±0.2	1.23±0.2	1.14±0.2	1.20±0.2

* 31 subjects

Eye		6 mths	12 mths	18 mths	24 mths
Group A*	*Temporal frequency*		*Spatial frequency 0.5 c/deg*		
Affected	*0 Hz*	1.58±0.3	1.60±0.4	1.64±0.3	1.60±0.4
	8 Hz	2.09±0.4	2.08±0.5	2.08±0.4	2.11±0.4
	32 Hz	1.28±0.3	1.28±0.3	1.24±0.3	1.27±0.3
Fellow	*0 Hz*	1.69±0.1	1.73±0.2	1.78±0.2	1.66±0.3
	8 Hz	2.21±0.2	2.20±0.2	2.20±0.2	2.15±0.3
	32 Hz	1.40±0.2	1.42±0.2	1.36±0.1	1.39±0.3
			Spatial frequency 4 c/deg		
Affected	*0 Hz*	1.98±0.4	1.98±0.5	1.95±0.4	1.99±0.4
	8 Hz	1.97±0.4	1.94±0.5	1.96±0.5	1.97±0.4
	32 Hz	1.01±0.3	1.07±0.3	1.03±0.4	1.05±0.3
Fellow	*0 Hz*	2.19±0.2	2.15±0.3	2.15±0.3	2.15±0.3
	8 Hz	2.15±0.2	2.15±0.3	2.16±0.3	2.12±0.3
	32 Hz	1.15±0.2	1.18±0.2	1.12±0.3	1.11±0.3
Group B	*Temporal frequency*		*Spatial frequency 0.5 c/deg*		
Affected	*0 Hz*	1.55±0.4	1.60±0.4	1.61±0.4	1.55±0.5
	8 Hz	2.06±0.5	2.05±0.5	2.07±0.5	2.07±0.5
	32 Hz	1.26±0.4	1.27±0.4	1.24±0.3	1.26±0.4
Fellow	*0 Hz*	1.73±0.1	1.78±0.2	1.83±0.2	1.73±0.3
	8 Hz	2.29±0.2	2.24±0.2	2.22±0.2	2.21±0.2
	32 Hz	1.47±0.2	1.45±0.3	1.39±0.2	1.46±0.3
			Spatial frequency 4 c/deg		
Affected	*0 Hz*	1.96±0.6	2.01±0.6	1.96±0.6	1.98±0.6
	8 Hz	1.94±0.5	1.92±0.5	1.94±0.5	1.93±0.5
	32 Hz	0.98±0.4	1.07±0.3	1.06±0.4	1.00±0.3
Fellow	*0 Hz*	2.26±0.2	2.23±0.3	2.20±0.2	2.22±0.3
	8 Hz	2.22±0.2	2.21±0.3	2.21±0.3	2.16±0.3
	32 Hz	1.23±0.2	1.21±0.3	1.13±0.3	1.18±0.3

* 30 subjects

Group			A	B	A	B
			Visits I - IV		Visit II - VI	
Affected/fellow eye	*high SF*		p=0.01	p=0.029	p=0.024	p=0.044
(eye 2, tefr* 3, time 4)	*low SF*		p=0.06	p=0.084	p=0.078	p=0.068
0 / 8 / 32 Hz	*high SF*		p=0.000	p=0.000	p=0.000	p=0.000
(eye 2, tefr* 3, time 4)	*low SF*		p=0.000	p=0.000	p=0.000	p=0.000
Time	*affected*		p=0.035	NS	NS	NS
(spfr° 2, tefr* 2, time 4)	*fellow*		NS	NS	NS	NS
High SF/low SF	*affected*	0 Hz	p=0.000	p=0.000	p=0.000	p=0.000
(spfr° 2, time 4)		8 Hz	p=0.000	p=0.000	p=0.000	p=0.000
		32 Hz	p=0.000	p=0.000	p=0.000	p=0.000
	fellow	0 Hz	p=0.000	p=0.000	p=0.000	p=0.000
		8 Hz	p=0.015	NS	p=0.057	NS
		32 Hz	p=0.000	p=0.000	p=0.000	p=0.000
Eye x time			NS	NS	NS	NS
(eye 2, spfr° 2, tefr* 3, time 4)						
Spfr x time	*affected*		NS	NS	NS	NS
(spfr° 2, tefr* 3, time 4)	*fellow*		NS	NS	NS	NS
Tefr x time	*affected*		NS	NS	NS	NS
(spfr° 2, tefr* 3, time 4)	*fellow*		NS	NS	NS	NS

* temporal frequencies; ° spatial frequencies

4.2.5 VEP, Visual field and Contrast sensitivity data comparing Visit VI to Visit I

A paired T-test for all parameters between Visit I and Visit VI was also performed for Groups A and B (Table 8).

When the VEP amplitude was assessed the affected eye responses on average increased, whereas the fellow eye responses decreased. The decrement for the fellow eye was close to significance in Group A for the central field.

For VEP latency there was a significant difference in both groups for the affected eye (whole and central field responses), the latency being shorter at Visit VI than at Visit I. For the fellow eye there was no statistically significant difference.

The visual field thresholds in dB of attenuation were on average better at Visit VI (affected eyes: both groups; fellow eyes: Group A) but the difference did not reach statistical significance (the affected eye difference was just not significant in Group A).

All contrast sensitivity data were on average better at Visit VI than at Visit I for the affected eye (apart from low SF temporally modulated at 8 Hz in Group B). In the fellow eye the pattern was more variable with some conditions of higher, lower or stable sensitivity. The difference was close to significance in Group A for the fellow eye high SF at 8 Hz (Visit VI worse than Visit I) and for the affected eye low SF at 32 Hz (Visit VI better than Visit I).

Table 8. VEP, Visual field and Contrast sensitivity data comparing Visit VI to Visit I

Mean VEP amplitude (μV) and SD

Eye	*Field*	VISIT I	VISIT VI	RATIO	p-value
Group A					
Affected	*Centre*	4.42±3.4	4.51±2.4	1.02	NS
	Whole	5.79±3.6	6.91±3.5	1.19	NS
Fellow	*Centre*	5.75±3.7	4.96±3.1	0.86	p=0.037
	Whole	9.19±4.7	8.17±4.0	0.89	NS
Group B					
Affected	*Centre*	4.15±3.5	4.51 ±2.2	1.09	NS
	Whole	5.51±3.6	6.47±3.7	1.17	NS
Fellow	*Centre*	5.76±3.3	5.43±3.0	0.94	NS
	Whole	9.91±4.9	8.95±4.4	0.90	NS

Mean VEP latency (msec) and SD

Eye	Field	VISIT I	VISIT VI	Difference	p-value
Group A*					
Affected	*Centre*	129.2±10.4	118.3±10.8	-10.9	0.000
	Whole	129.4±13.5	119.4±12.6	-10.0	0.000
Fellow	*Centre*	107.0±11.7	110.0±17.7	3.0	NS
	Whole	105.6±12.7	110.7±18.9	4.9	NS
Group B*					
Affected	*Centre*	129.3±8.9	115.7±9.8	-13.6	0.000
	Whole	127.5±13.2	114.5±11.7	-13.0	0.000
Fellow	*Centre*	103.3±10.6	101.3±7.2	-2.0	NS
	Whole	100.2±9.7	100.6±6.8	0.4	NS

* All patients who had a response at Visit I and Visit VI were analysed

Mean threshold (in dB of attenuation) and SD

Eye	VISIT I	VISIT VI	RATIO	p-value
Group A				
Affected	27.3±5.1	28.1±4.8	1.03	p=0.052
Fellow	29.8±1.6	29.1±4.1	0.98	NS
Group B				
Affected	27.0±5.8	28.0±6.0	1.04	NS
Fellow	29.9±1.4	30.3±1.3	1.01	NS

Mean contrast sensitivity (log) and SD

Eye		VISIT I	VISIT VI	Difference	p-value
Group A	*Temporal frequency*		*Spatial frequency 0.5 c/deg*		
Affected	*0 Hz*	1.53±0.3	1.59±0.4	0.06	NS
	8 Hz	2.10±0.4	2.11±0.4	0.01	NS
	32 Hz	1.25±0.3	1.27±0.3	0.02	NS
Fellow	*0 Hz*	1.67±0.1	1.65±0.3	-0.02	NS
	8 Hz	2.21±0.1	2.14±0.3	-0.07	0.074
	32 Hz	1.41±0.2	1.38±0.3	-0.03	NS
			Spatial frequency 4 c/deg		
Affected	*0 Hz*	1.94±0.5	1.98±0.4	0.04	NS
	8 Hz	1.91±0.4	1.96±0.4	0.05	NS
	32 Hz	0.94±0.3	1.04±0.3	0.10	0.057
Fellow	*0 Hz*	2.17±0.2	2.14±0.3	-0.03	NS
	8 Hz	2.14±0.2	2.11±0.3	-0.03	NS
	32 Hz	1.18±0.2	1.10±0.3	-0.08	NS
Group B	*Temporal frequency*		*Spatial frequency 0.5 c/deg*		
Affected	*0 Hz*	1.51±0.4	1.54±0.5	0.03	NS
	8 Hz	2.08±0.6	2.06±0.6	-0.02	NS
	32 Hz	1.22±0.4	1.25±0.4	0.04	NS
Fellow	*0 Hz*	1.68±0.1	1.71±0.3	0.03	NS
	8 Hz	2.25±0.1	2.20±0.2	-0.05	NS
	32 Hz	1.43±0.2	1.45±0.3	0.02	NS
			Spatial frequency 4 c/deg		
Affected	*0 Hz*	1.92±0.5	1.96±0.6	0.04	NS
	8 Hz	1.89±0.5	1.91±0.5	0.02	NS
	32 Hz	0.91±0.3	0.99±0.4	0.08	NS
Fellow	*0 Hz*	2.22±0.1	2.25±0.2	-0.02	NS
	8 Hz	2.20±0.3	2.14±0.3	-0.05	NS
	32 Hz	1.17±0.2	1.17±0.3	0.00	NS

4.2.6 Regression analysis

Because of the high number of variables compared some tests may reach "significance" just by chance. To avoid this problem only p-values ≤ 0.01 were considered as significant.

All analysis has been performed on group A, although a patient whose VEP latency delay was well above the range of all the other patients was excluded.

4.2.6.1 VEP amplitude

Central and whole field amplitudes at Visit I were linearly correlated with the equivalent amplitudes at Visit VI ($p<0.001$ for the central field at Visit I and $p=0.002$ for the whole field at Visit I) (Fig. 6 and 7). Whole field amplitudes (Visit I) were inversely correlated with the degree of amplitude changes between Visit I and Visit VI for the whole field ($p=0.005$) and central field ($p=0.007$) (fig. 7), and central field amplitudes at Visit I correlated inversely with the degree of change in central field amplitude between Visit I and Visit VI ($p=0.001$) (Fig. 6).

Whole and central field amplitudes were not correlated with latencies (whole and central field at Visits I and VI) or changes in latency between Visit I and Visit VI.

For the contrast sensitivity data there was a significant linear correlation between the whole field amplitude at Visit VI and CS high

SF at 8 Hz (p=0.01) and the central field amplitude at Visit VI was also linearly correlated with CS high SF at 8 Hz (p=0.007).

Whole and central field response amplitudes at Visit VI were not linearly correlated with visual fields data (Visits I and VI).

Whole and central field response amplitudes were not correlated with VA (Visits I and VI).

4.2.6.2 VEP latency

Central and whole field latencies (Visit I and VI) were not correlated with whole and central field amplitudes (Visit I and VI).

The central field response latencies (Visit I) were positively correlated with central field response latencies at Visit VI (p=0.005) (Fig. 8). Central field latency at Visit I was also significantly correlated with the latency difference (Visit I and Visit VI) for both the central (p=0.007) and whole (p=0.003) field responses, indicating that responses which were initially the most delayed showed the greatest degree of recovery. The whole field response latencies (Visit I) were correlated linearly with central (p=0.000) and whole field (p=0.000) latencies at Visit VI (Fig. 9).

Central and whole field response latencies (Visits I and VI) were not correlated with the contrast sensitivity, the visual field data or the VA (Visit I and Visit VI).

4.2.6.3 Age and VEP latency and amplitude

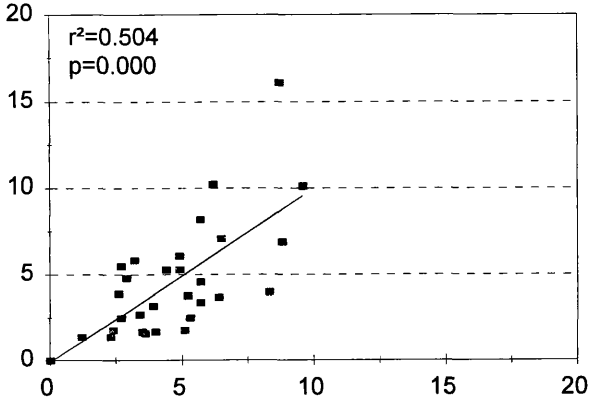
A regression analysis between age of the subjects and actual delay in the affected eye, amount of latency recovery in the affected eye or amount of latency delay in the fellow eye (Visit I and Visit VI), amplitude or amplitude difference (Visit I and Visit VI) was not significant. The analysis was performed with the same results for whole and central field responses.

4.2.6.4 MRI and VEP amplitude and latency

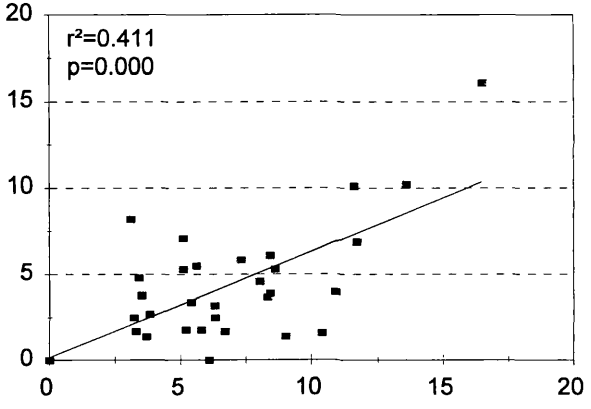
A regression analysis for a linear model between MRI lesions (difference in number of lesions in the MRI performed at presentation and that performed at the end of the study) and amplitude and latency change (Visit I and Visit VI) was not significant.

Fig. 6. Regression analysis for central field amplitude at Visit I (y-axis).

Central field amplitude at Visit VI (x-axis).



Whole field amplitude at Visit VI (x-axis).



Central field amplitude difference: Visit VI - Visit I (x axis).

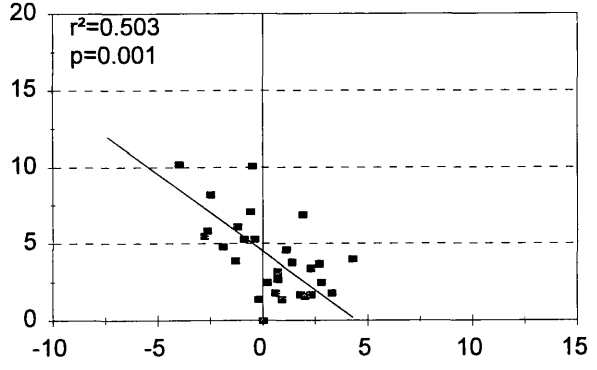
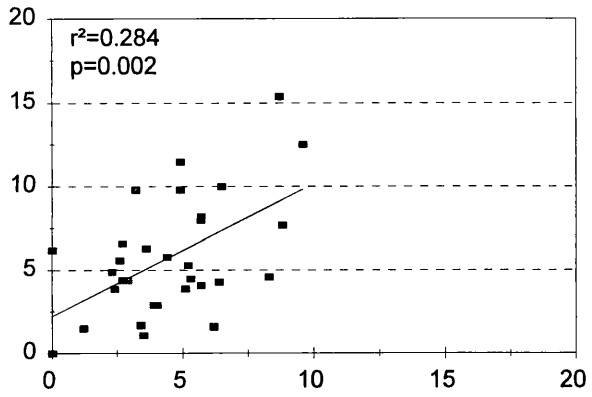
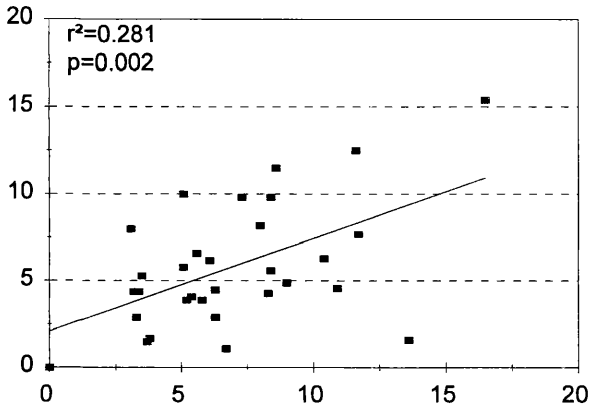


Fig. 7. Regression analysis for whole field amplitude at Visit I (y-axis).

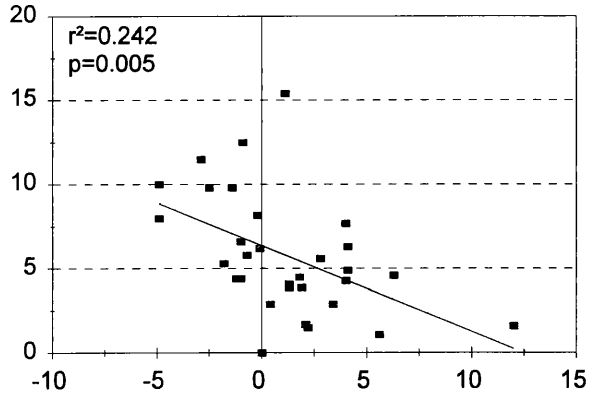
Central field amplitude at Visit VI (x-axis)



Whole field amplitude at Visit VI (x-axis)



Whole field amplitude difference: Visit VI - Visit I (x axis)



Central field amplitude difference: Visit VI - Visit I (x axis)

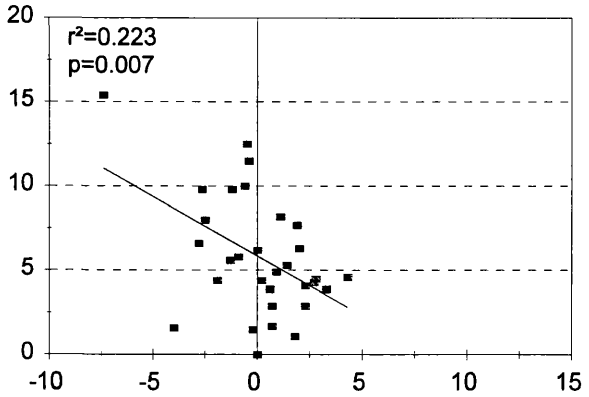
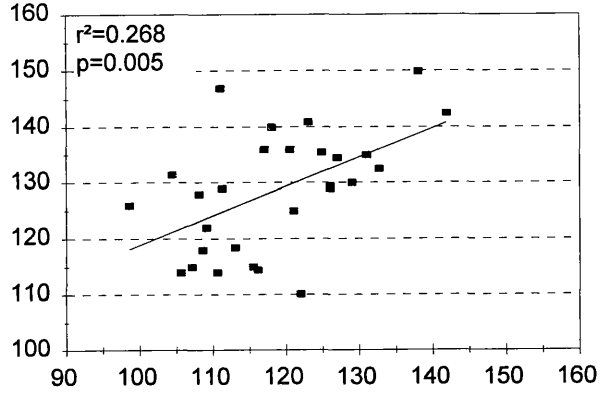
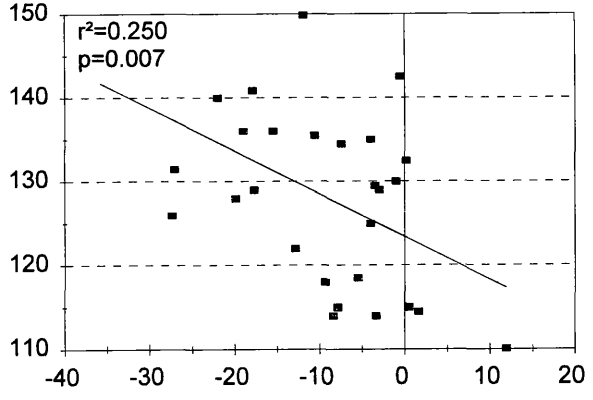


Fig. 8. Regression analysis for central field latency at Visit I (y-axis).

Central field latency at Visit VI (x-axis).



Central field latency difference: Visit VI - I (x-axis).



Whole field latency difference: Visit VI - I (x-axis).

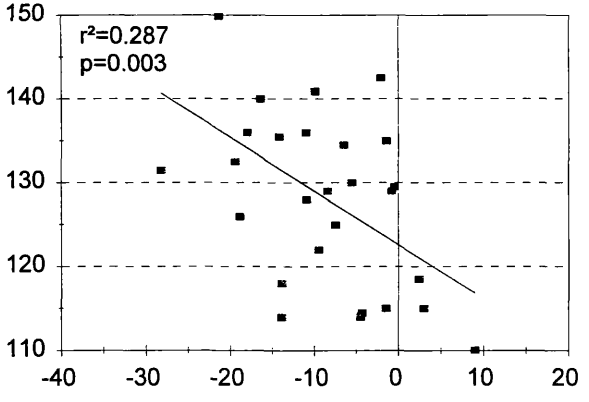
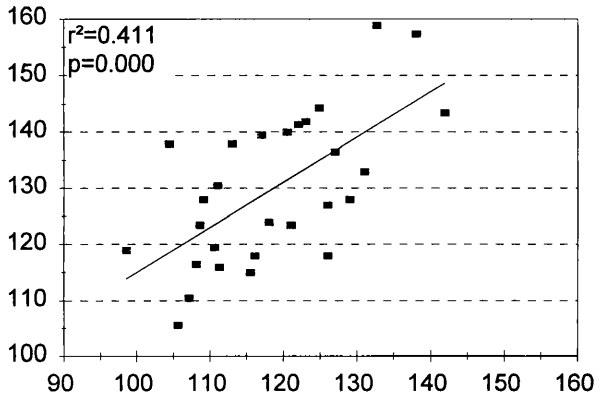
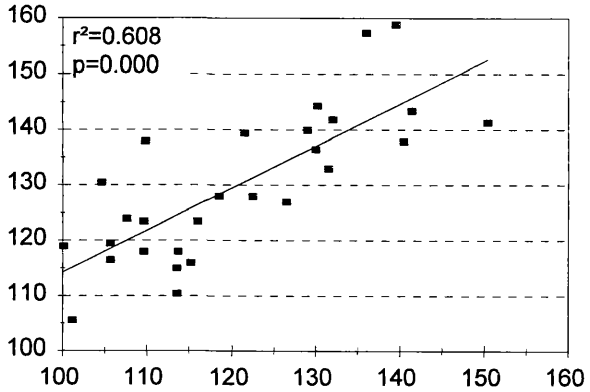


Fig. 9. Regression analysis for whole field latency at Visit I (y-axis).

Central field latency at Visit VI (x-axis).



Whole field latency at Visit VI (x-axis).



4.2.7 Summary of the results

For the affected eye VEP latency shortened throughout the follow-up period to the greatest extent in the first year and in particular in the interval between 3 and 6 months after the acute episode of ON. During the first year, VEP amplitude, although on average increasing, did not show any significant trend over time, whereas Contrast sensitivity and Visual field data improved significantly, to the greatest extent between 6 and 9 months. The VEP latency recovery was also significant during the second year when VEP amplitude, Contrast sensitivity and Visual fields, although on average still improving, did not show any statistically significant trend over time.

As far as the fellow eye is concerned there was no evidence of a trend over time in VEP latency or amplitude, Contrast sensitivity or Visual field data over 2 year follow-up and the direct comparison between 3 months and 2 years also did not show any statistically significant difference.

Patients affected by ON and patients with MS (as diagnosed at presentation and at two year follow-up) did not show any significant difference in their behaviour over time. One exception was the Visual field data: in the 3 month interval analysis the MS patients improved significantly over time while those with isolated ON failed to show improvement. Patients who showed changes and patients who

did not show changes in the MRI between presentation and 2 year follow-up did not differ in their behaviour over time. In addition no correlation was found between number of new lesions and extent of VEP recovery (latency or amplitude).

There was no significant correlation between patients' age at presentation and the VEP latency and amplitude in the affected eye .

Central and whole field VEP amplitudes and latencies at 3 months were positively correlated with the equivalent VEP amplitudes and latencies at 2 years. Whole and central field amplitudes at 3 months were also inversely correlated with the degree of amplitude change between 3 months and 2 years, whereas only the central field latencies at 3 months were inversely correlated with the degree of latency change in the whole and central field between 3 months and 2 years.

4.2.8 Examples of VEP responses

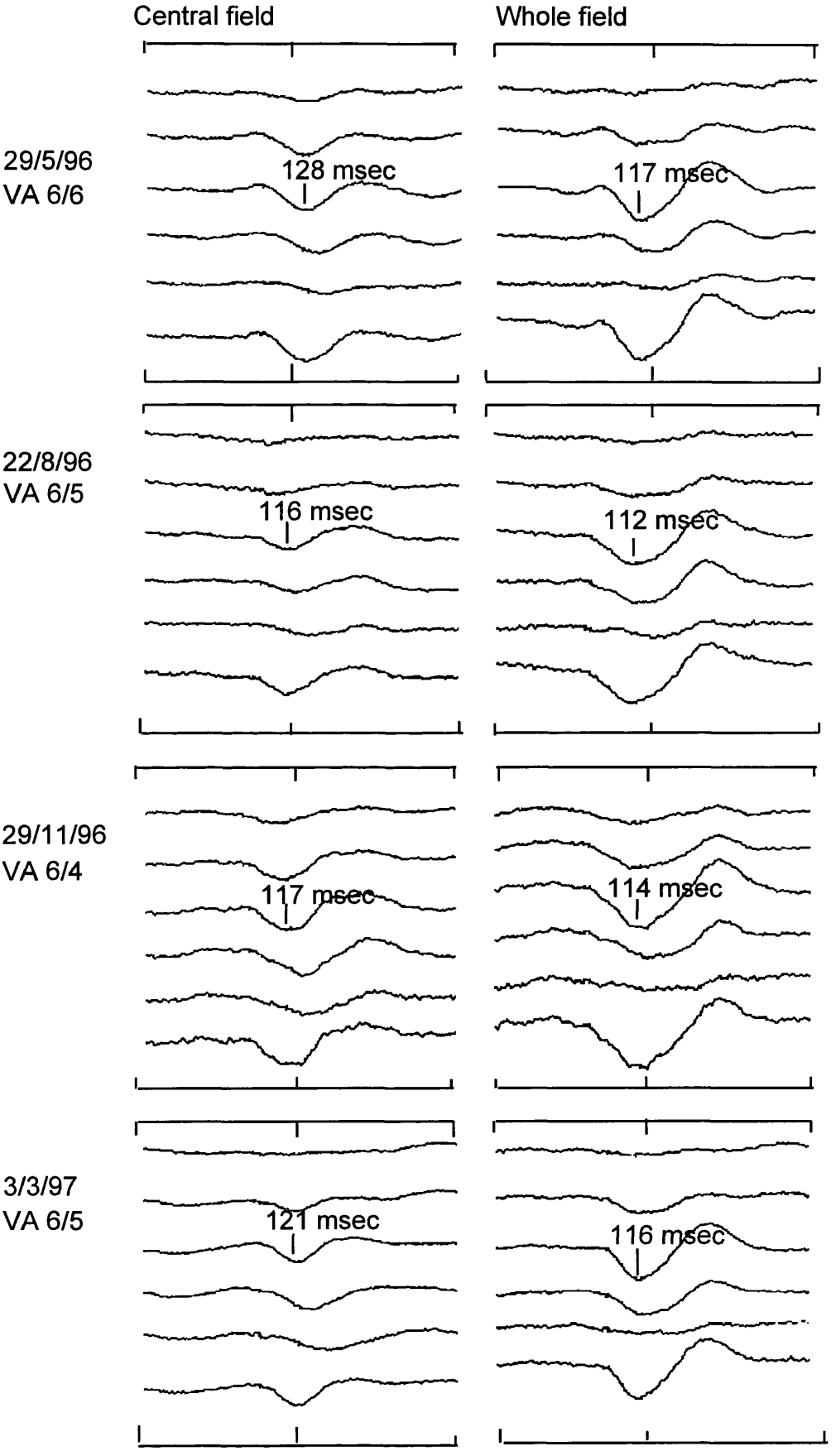
All responses shown in the following pages are taken from single patients (affected eye and/or fellow eye) on six occasions in 2 year follow-up. They are the average of 2 or 3 runs of 100 sweeps for the whole and the central field responses, respectively.

The first 5 channels correspond to the recording from a chain of electrodes fixed 5 cm above the inion (right to left) and number 6 to the recording from an electrode placed 2.5 cm above the inion.

The latency of the central and the whole field responses from the affected eye might recover to normal values (Fig. 10) or recover to a limited extent without reaching the limit of normal (Fig. 11) or until a new episode of symptomatic optic neuritis occured (Fig. 12). It could also be that the response would become bifid (Fig. 13; in this case the whole field has become bifid with the earlier peak of normal latency and the second peak still delayed. The central field did not show any latency recovery. The fatc that neither peak in the whole field response corresponded in latency to the single positivity from the central field suggests that the latter was probably a truly delayed P100 while the later peak in the whole field response was probably a P135). The fellow eye responses could be: delayed but asymptomatic at presentation (Fig. 14; latency recovered as if the pathological process had occurred in recent times); or delayed because of a previous episode of ON (Fig. 15; latencies remained

stable until a new symptomatic episode of ON occurred in that eye. VA nevertheless recovered fully); or normal throughout the study (Fig. 16; latencies, however, increased slightly between Visits I and VI).

Fig. 10. VEP responses from the affected eye (case 21).



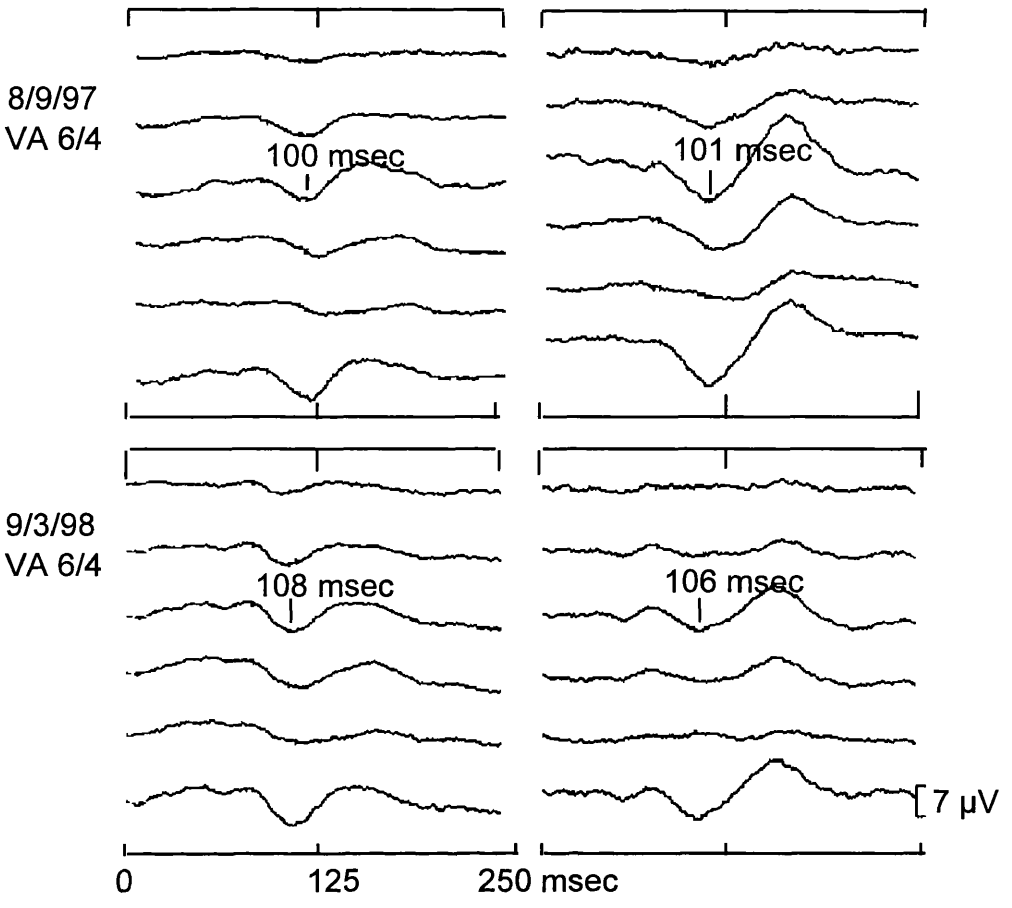
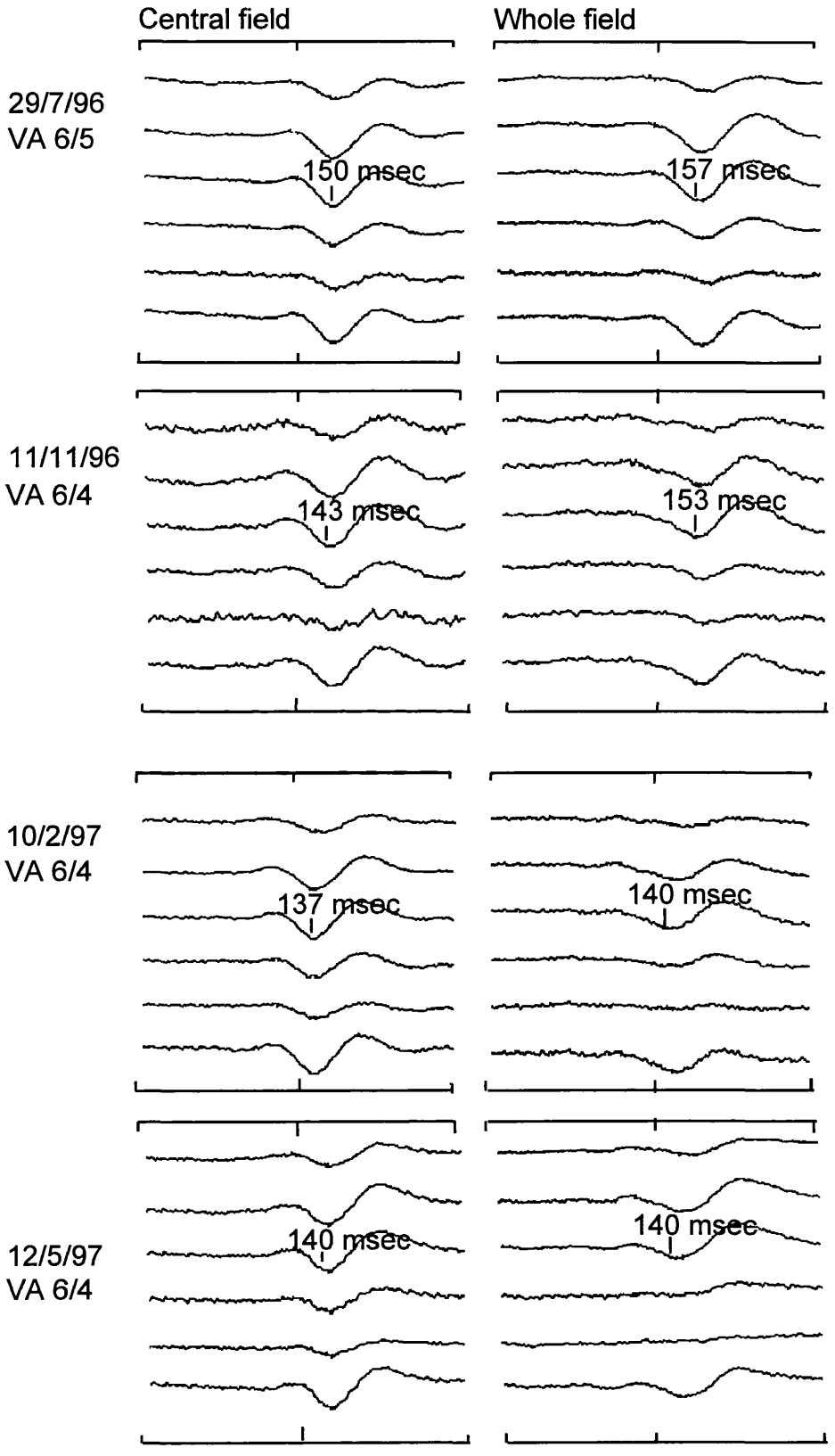

8/9/97
VA 6/4
100 msec
101 msec
9/3/98
VA 6/4
108 msec
106 msec
7 μV
0
125
250 msec

Fig. 11. VEP responses from the affected eye (case 24).



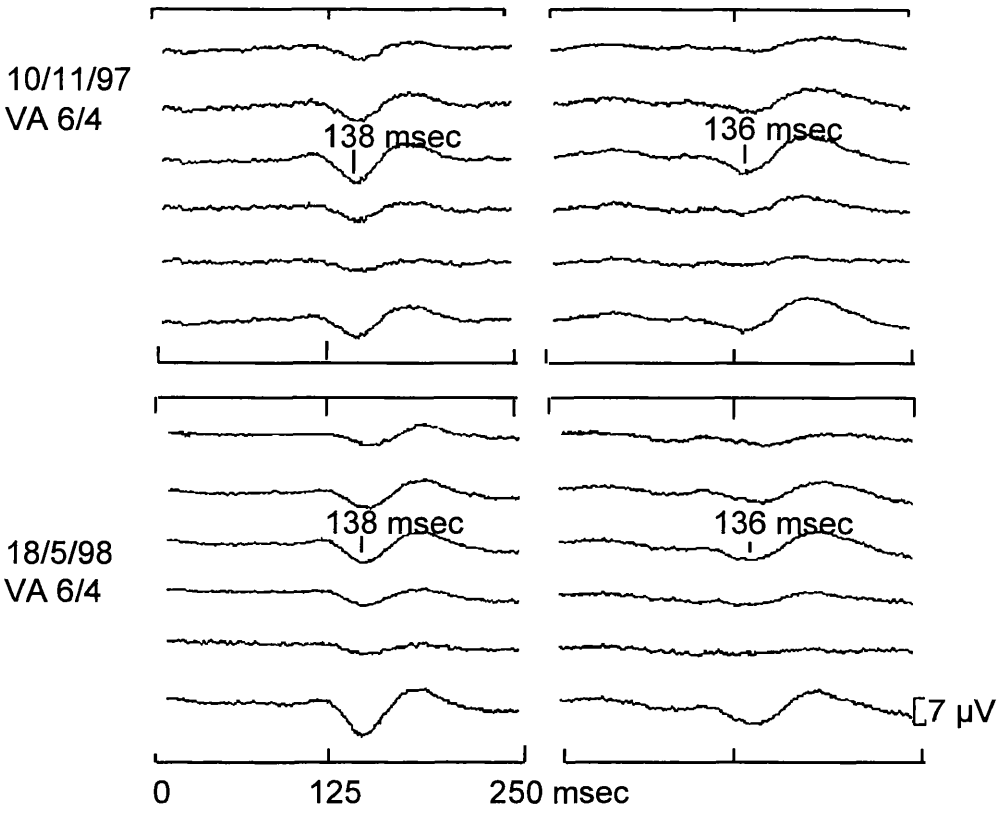
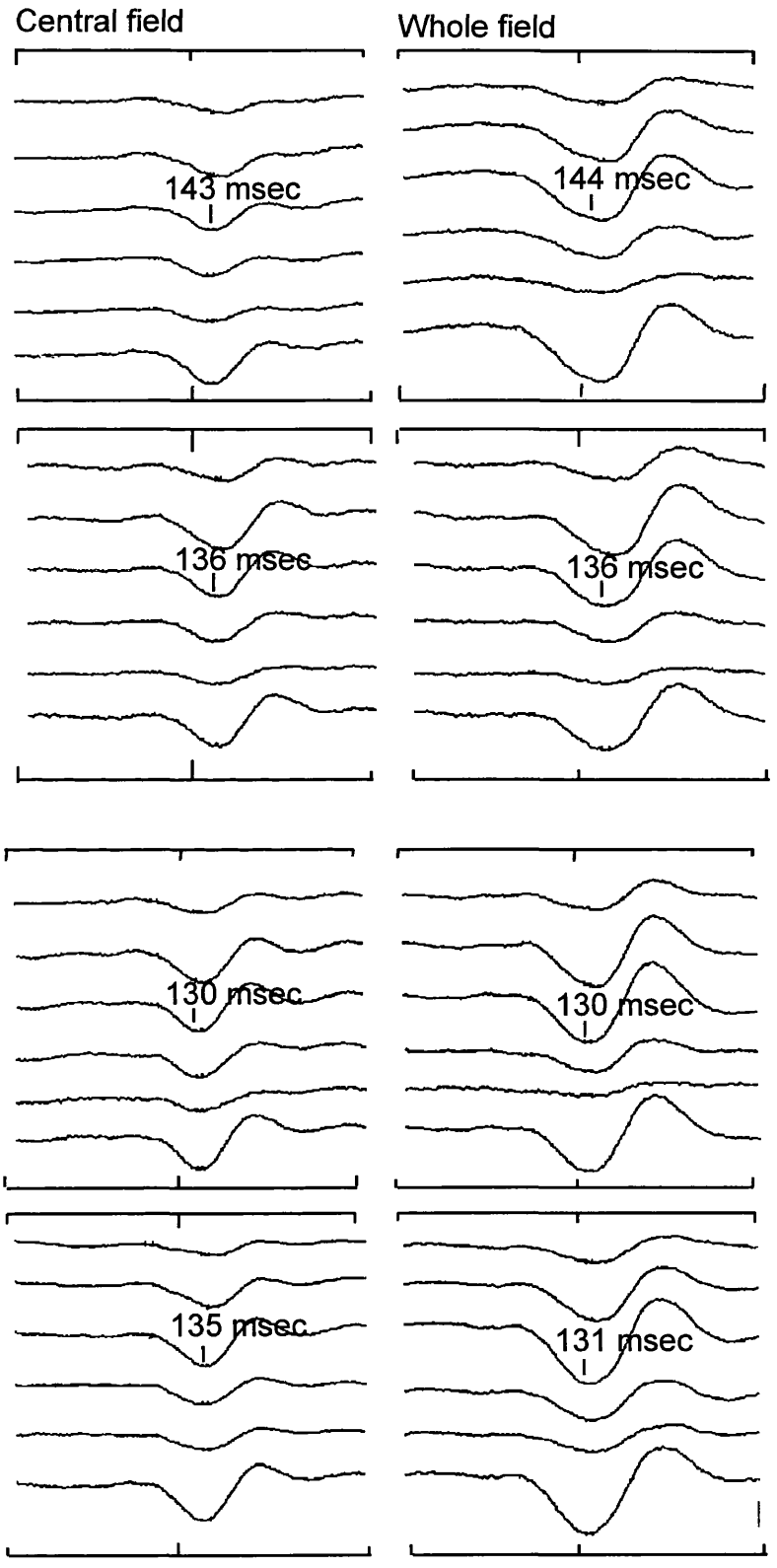

10/11/97
VA 6/4
138 msec
136 msec
18/5/98
VA 6/4
138 msec
136 msec
7 µV
0
125
250 msec

Fig. 12. VEP responses from the affected eye (case 25).



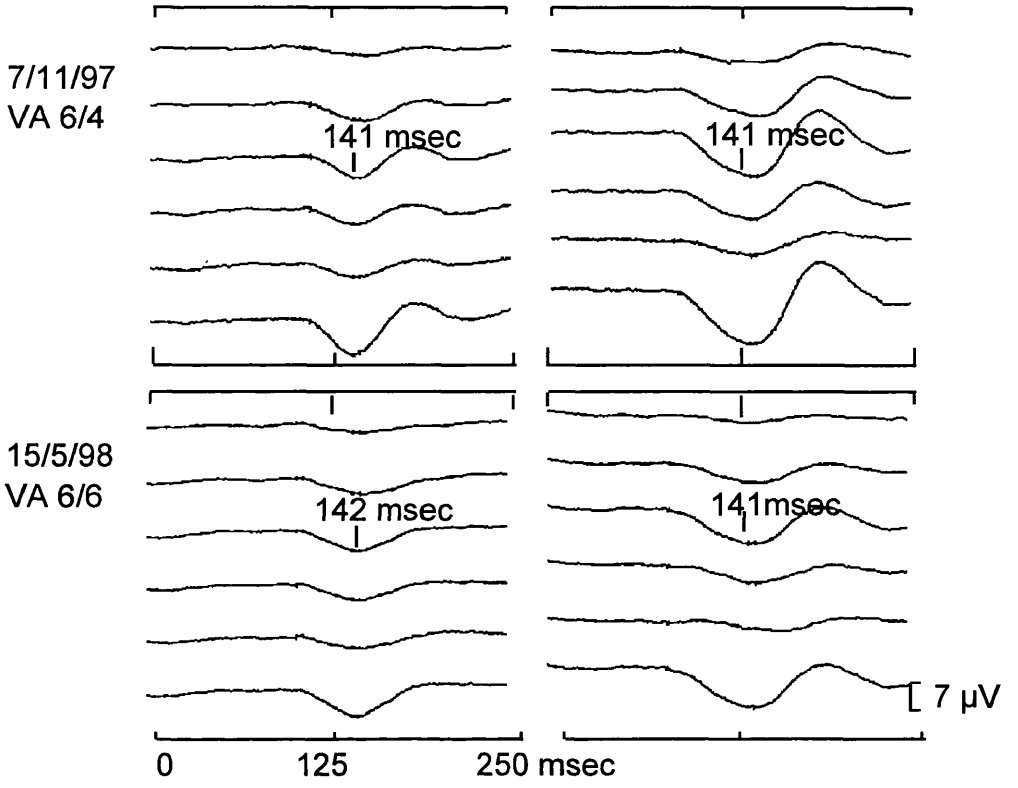
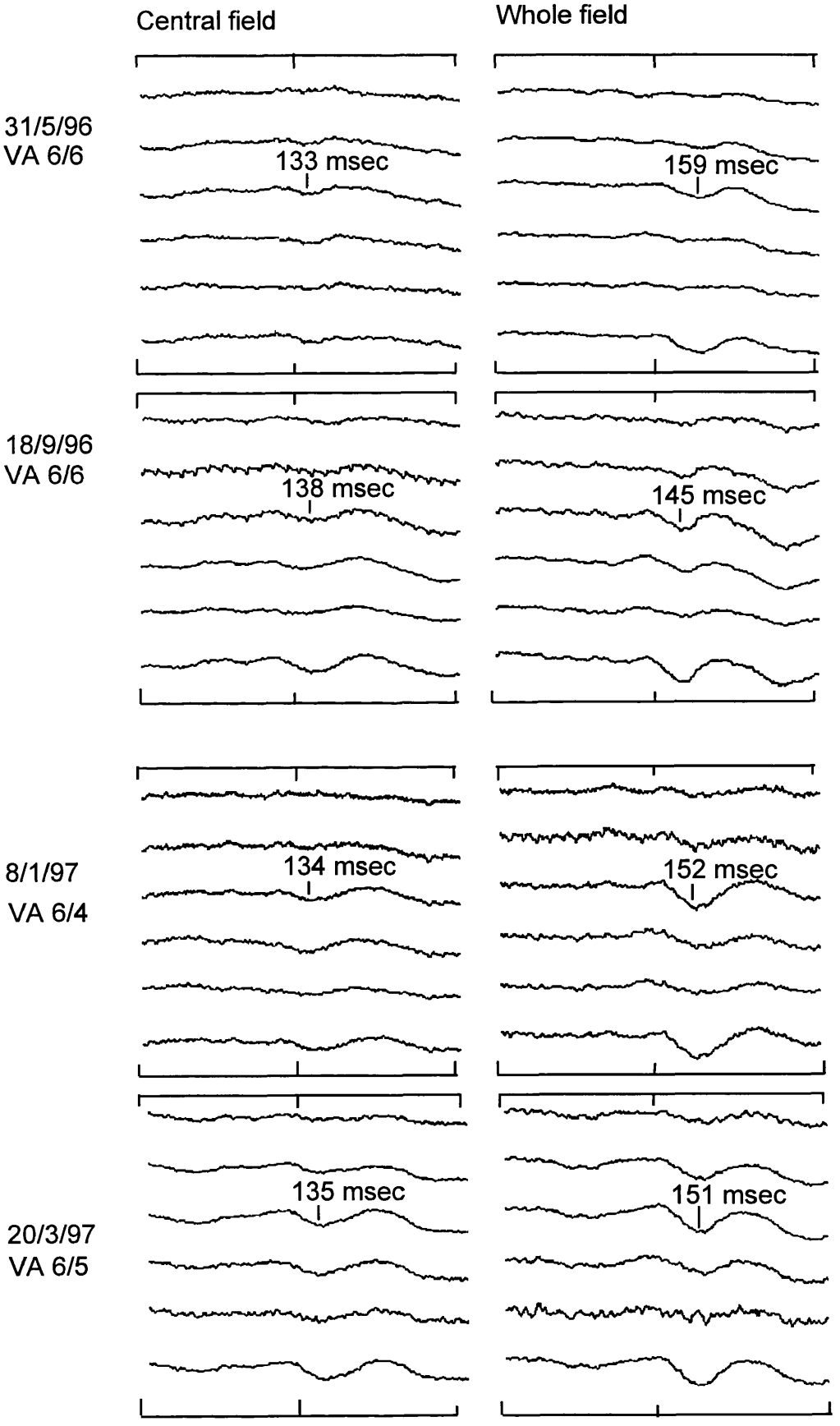

7/11/97
VA 6/4
141 msec
141 msec
15/5/98
VA 6/6
142 msec
141msec
7 µV
0
125
250 msec

Fig. 13. VEP responses from the affected eye (case 23).



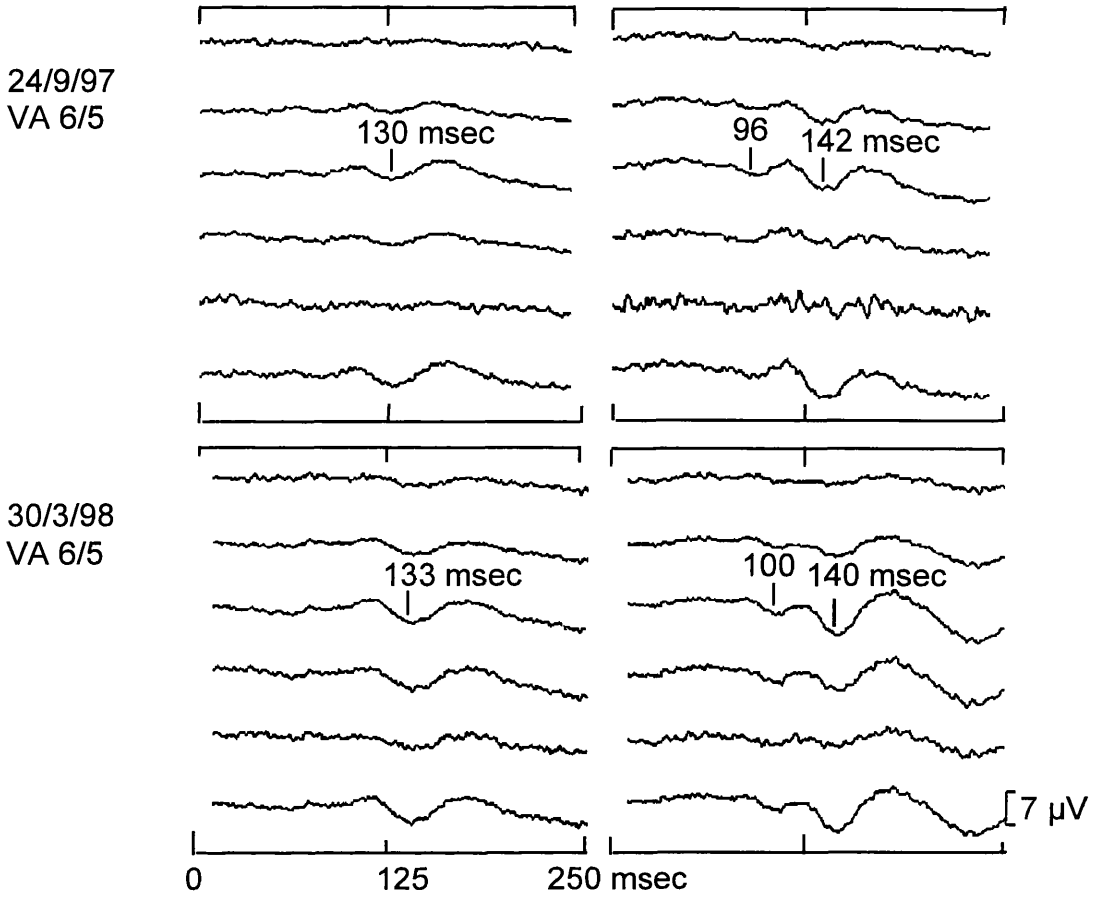
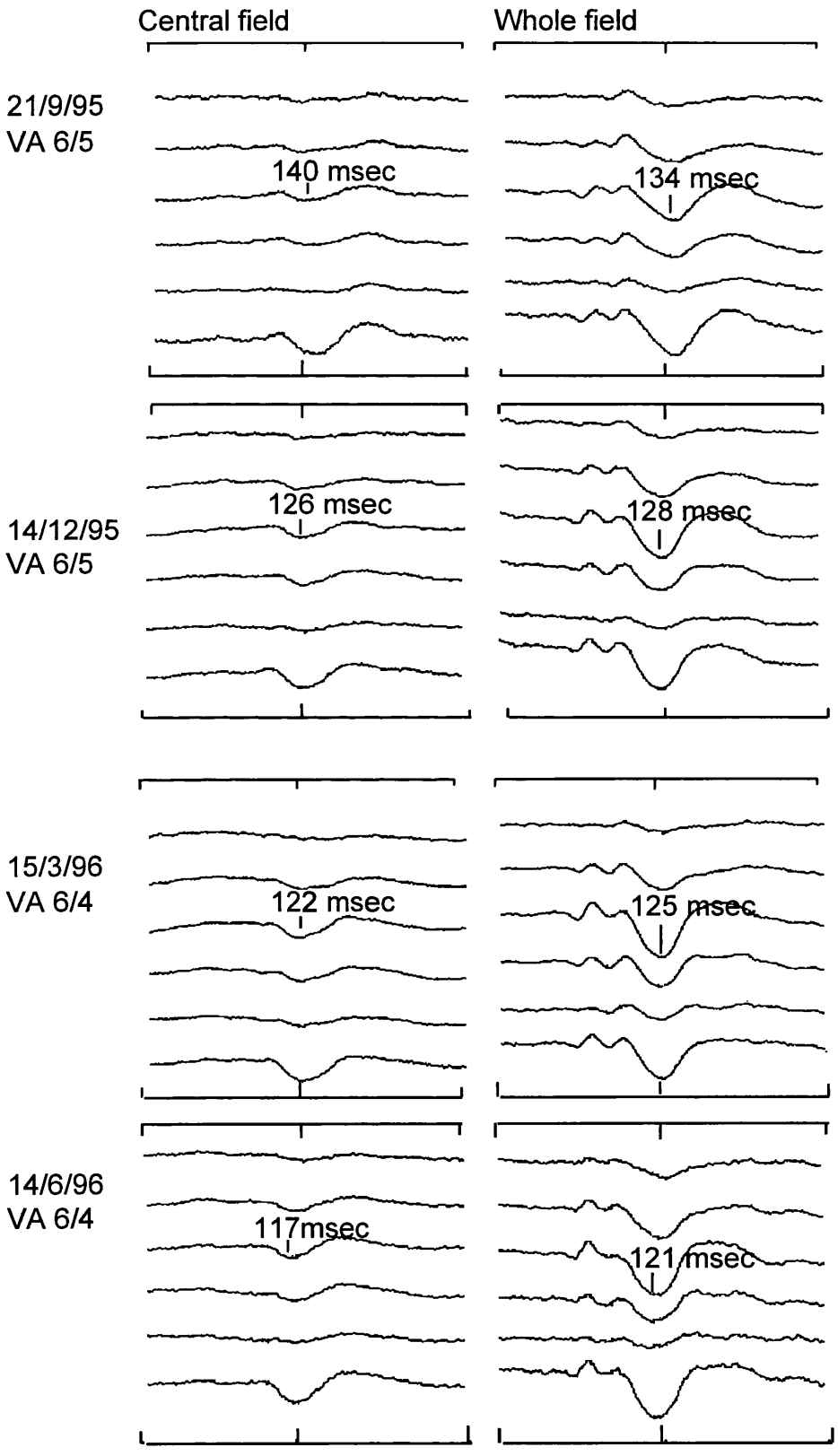

24/9/97
VA 6/5
130 msec
96
142 msec
30/3/98
VA 6/5
133 msec
100
140 msec
7 µV
0
125
250 msec

Fig. 14. VEP responses from the fellow eye (case 6).



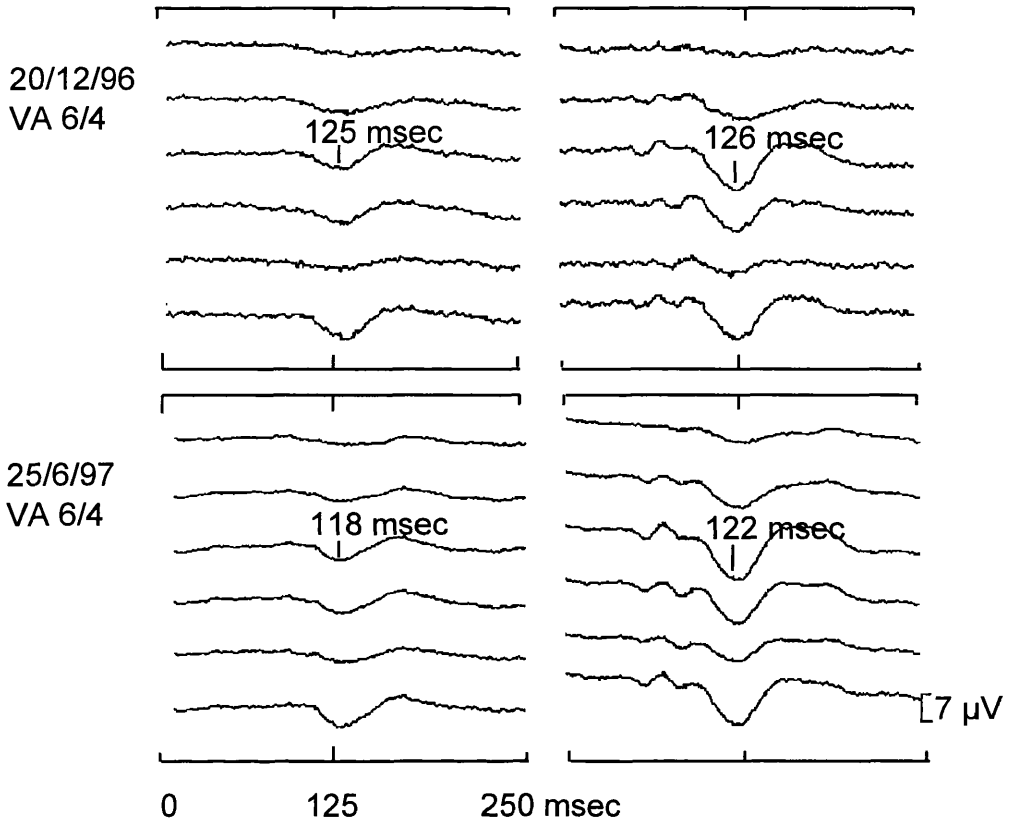
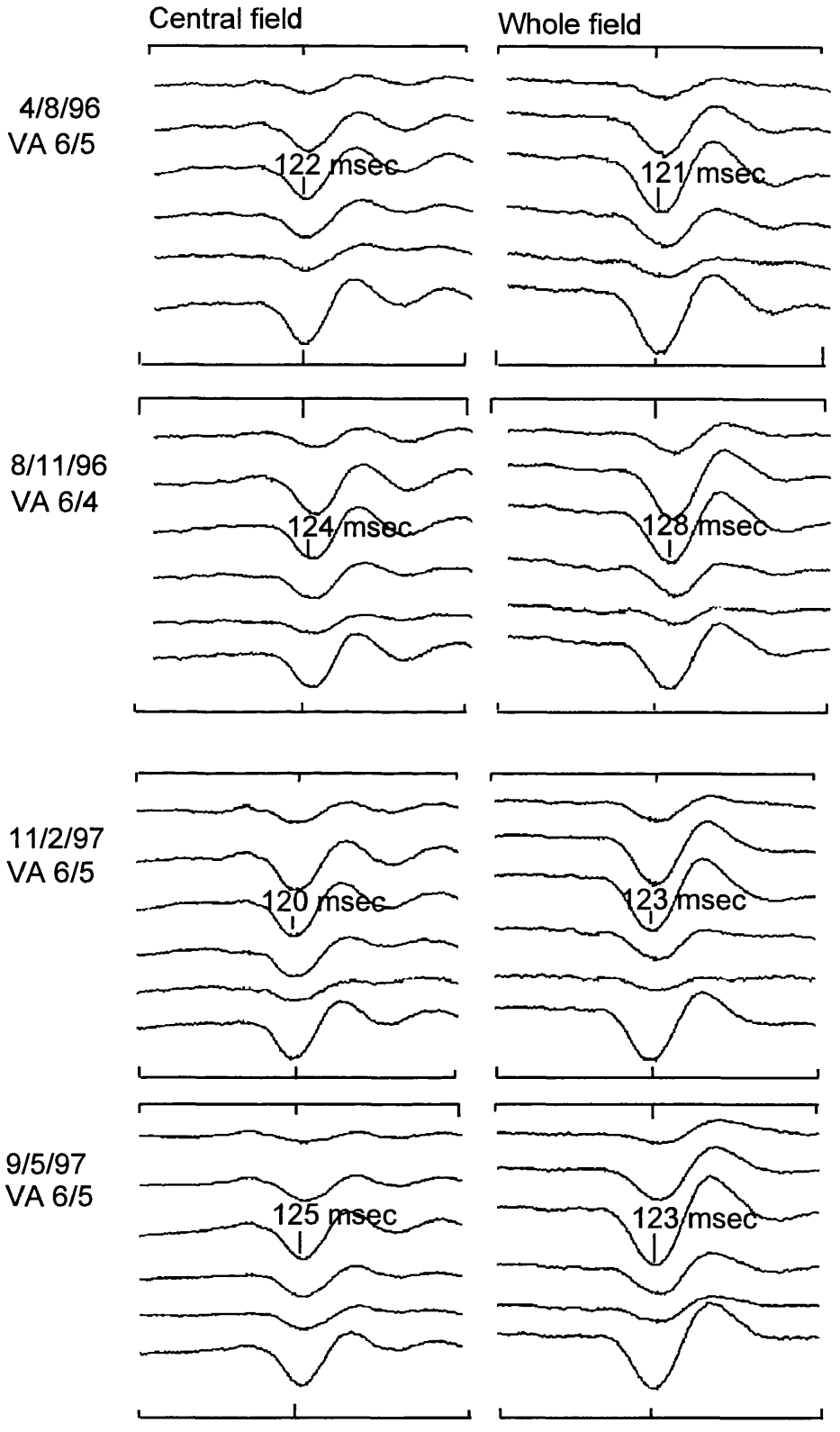

20/12/96
VA 6/4
125 msec
126 msec
25/6/97
VA 6/4
118 msec
122 msec
7 μV
0
125
250 msec

Fig. 15. VEP responses from the fellow eye (case 25).



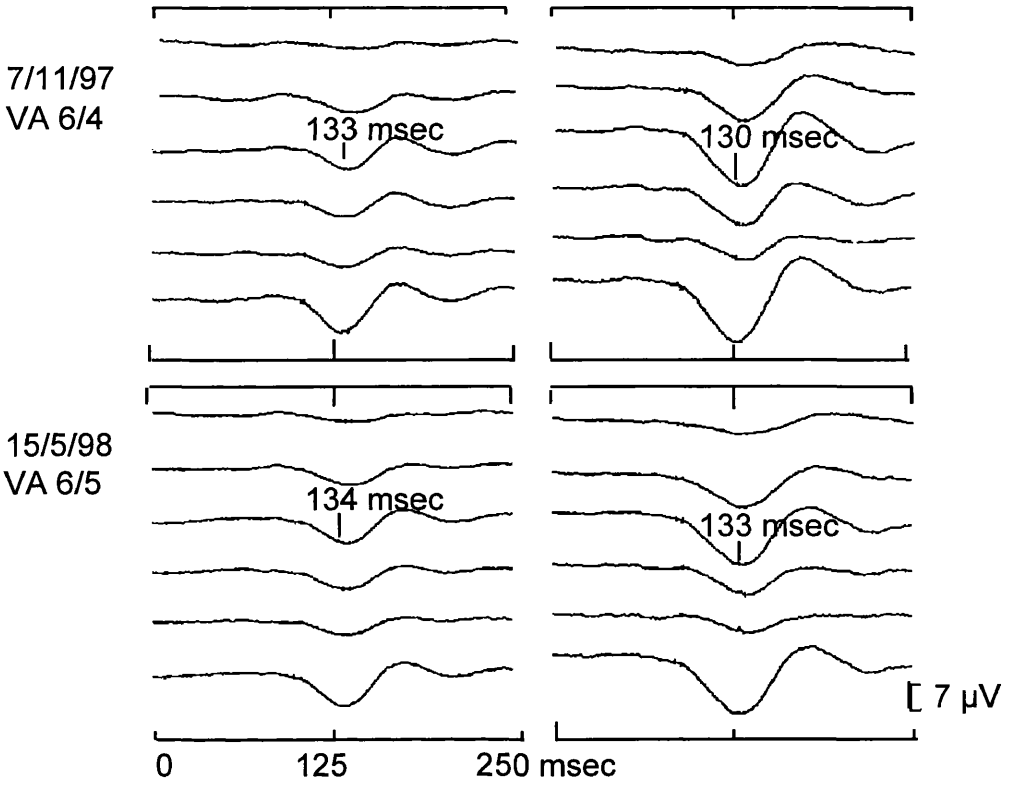
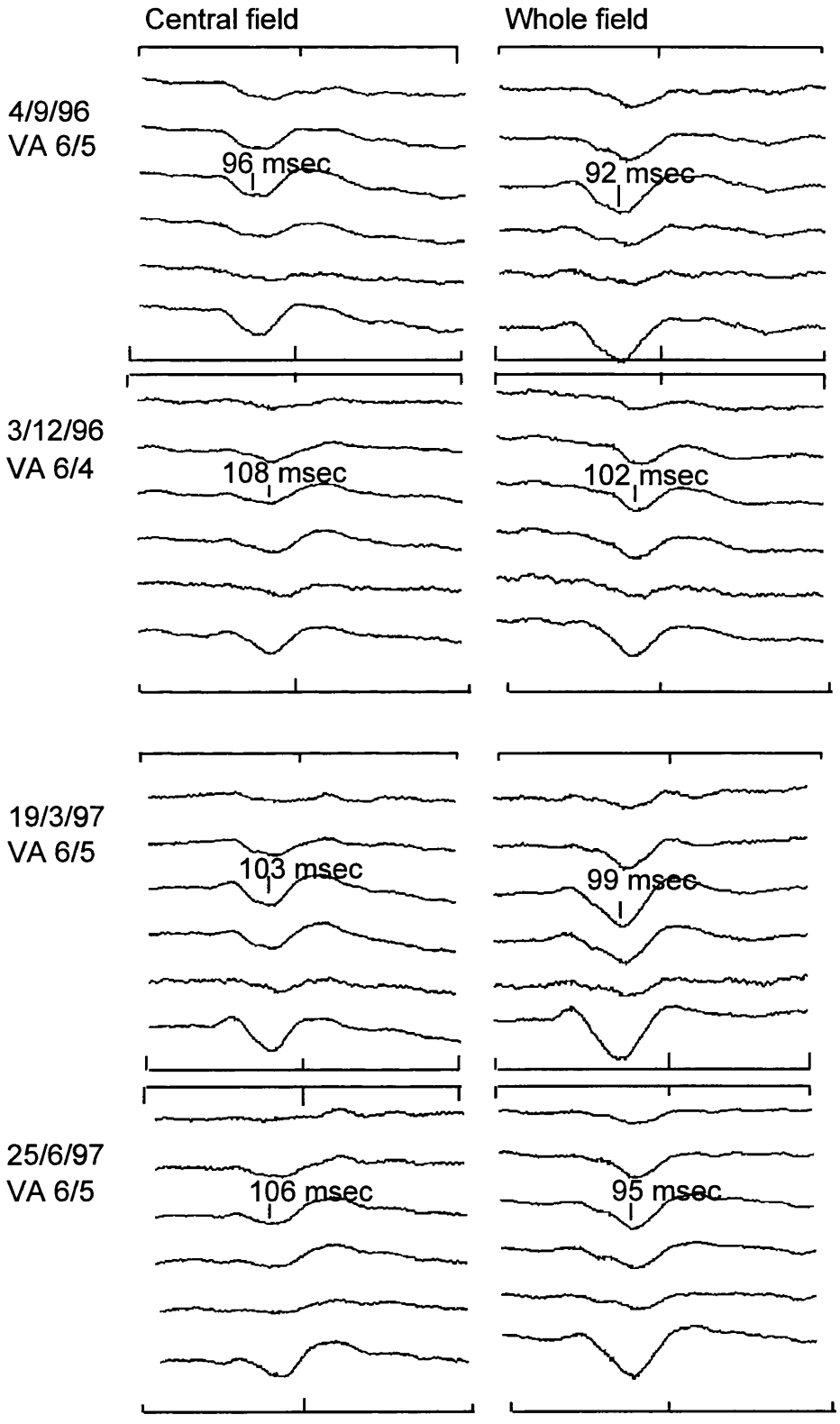
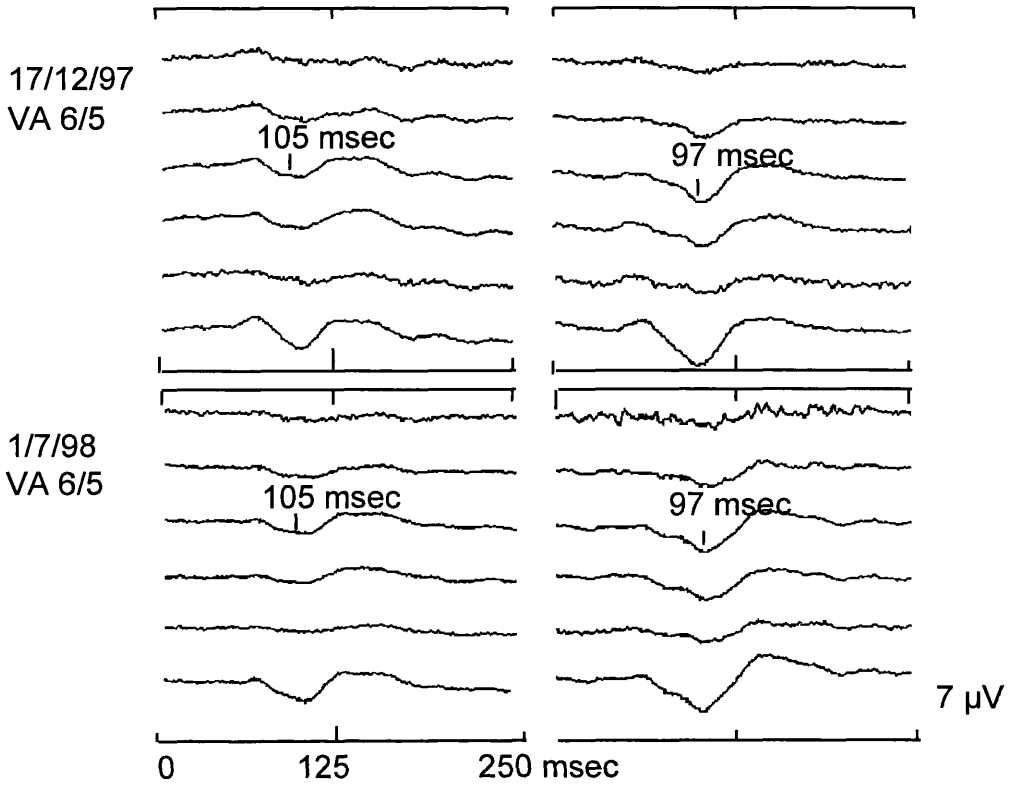

7/11/97
VA 6/4
133 msec
130 msec
15/5/98
VA 6/5
134 msec
133 msec
7 μV
0
125
250 msec

Fig. 16. VEP responses from the fellow eye (case 29).

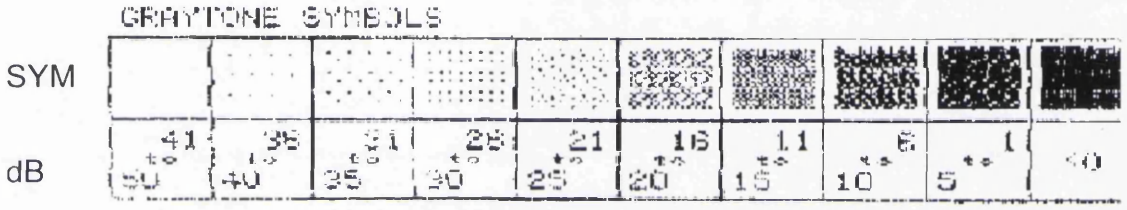



17/12/97
VA 6/5
105 msec
97 msec
1/7/98
VA 6/5
105 msec
97 msec
7 μV
0
125
250 msec

4.2.9 Examples of visual field printouts

Visual field printouts for single patients (affected eye and/or fellow eye) recorded on six occasions are shown in the following pages. The greytone scale (see below for the conversion scale in dB) is reported on the left of the figure, the "total deviation" in the centre and the "pattern deviation" on the right.

Fig. 17. Greytone symbols.



The date of recordings, the VA at the time of recording and the outcome of the test are also reported.

At presentation the affected eye responses could be normal (Fig. 18) or abnormal. When they were abnormal various situations could occur: some responses became normal during follow-up (Fig. 19), some improved but were still abnormal at the end of the study (Fig. 20 some improved until a new episode of ON occurred (Fig. 21) and some did not show any improvement (Fig. 22). The fellow eye responses also could be normal (Fig. 23) or abnormal (Fig. 24) at presentation. Most frequently they did not show any significant change (Fig. 24) unless a new episode of ON occurred (Fig. 23).

Fig. 18. Affected eye (case 23).

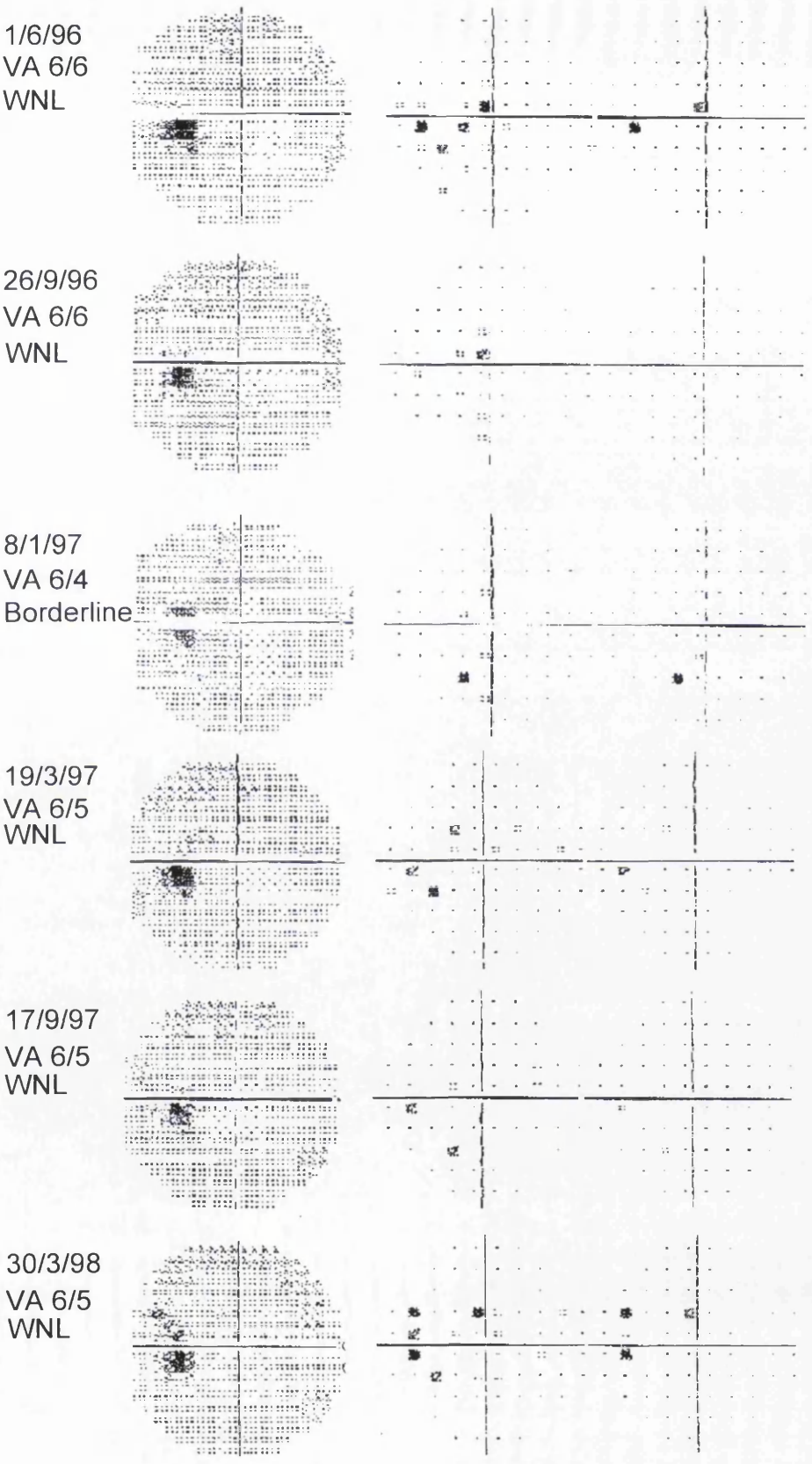


Fig. 19. Affected eye (case 26).

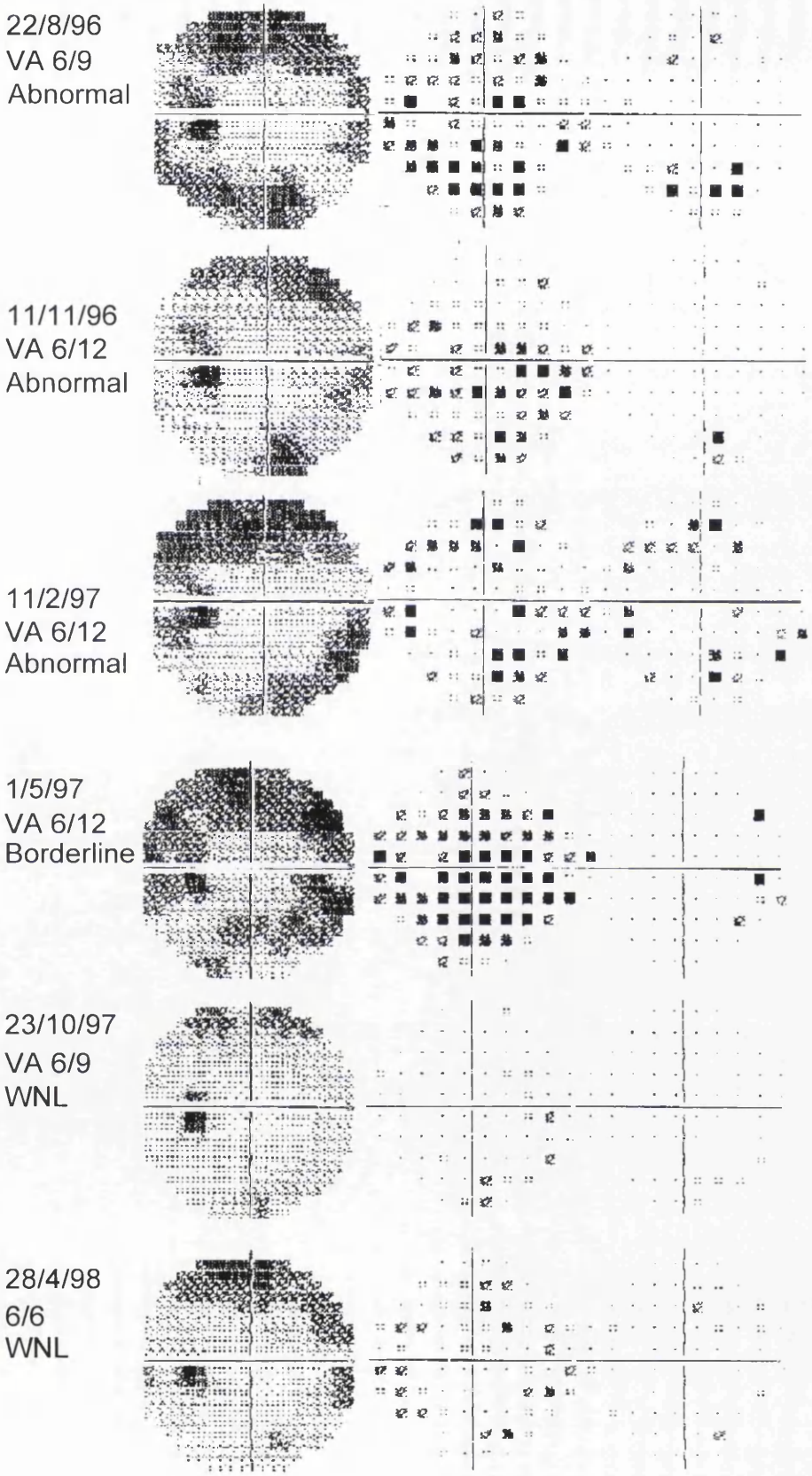
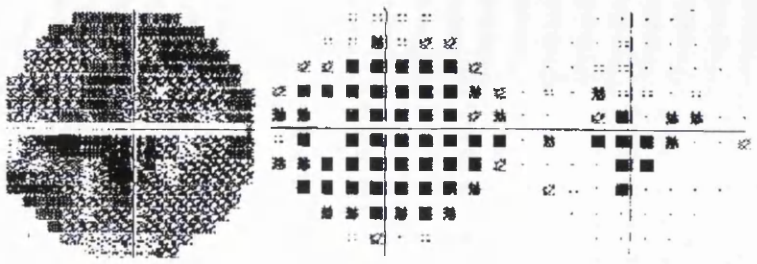
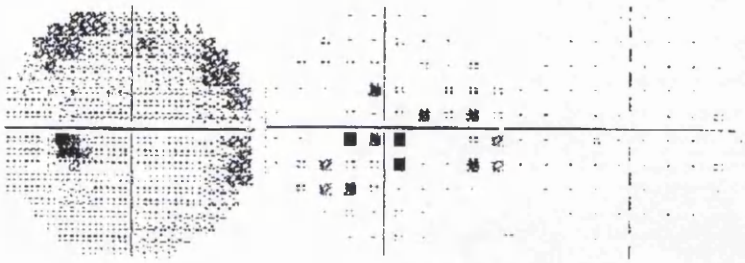


Fig. 20. Affected eye (case 23).

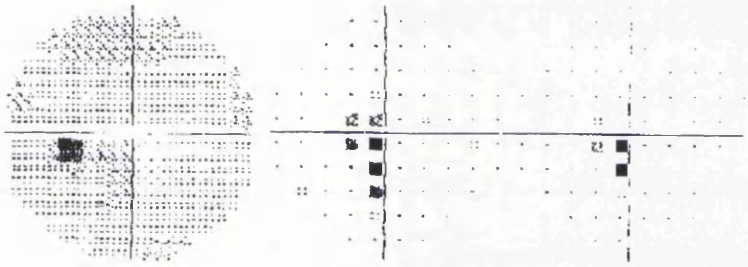
2/2/96
VA 6/9
Abnormal



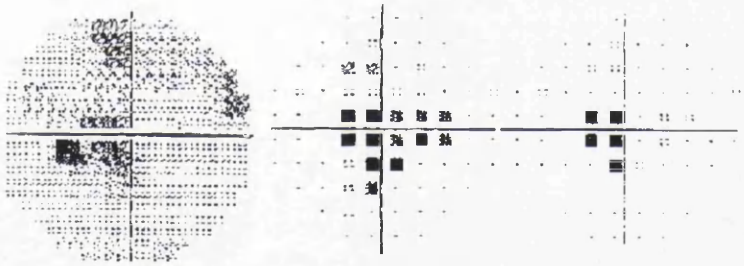
4/5/96
VA 6/6
WNL



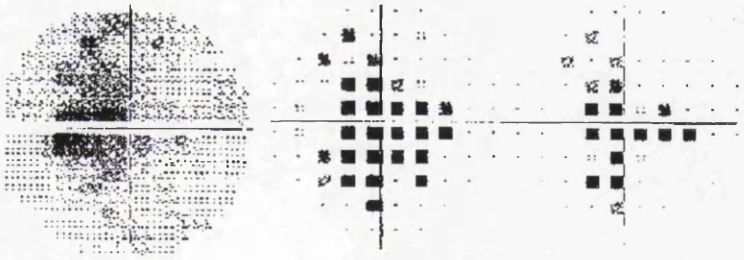
3/8/96
VA 6/9
Abnormal



8/11/96
VA 6/9
Abnormal



21/5/97
VA 6/18
Abnormal



14/11/97
VA 6/9
Abnormal

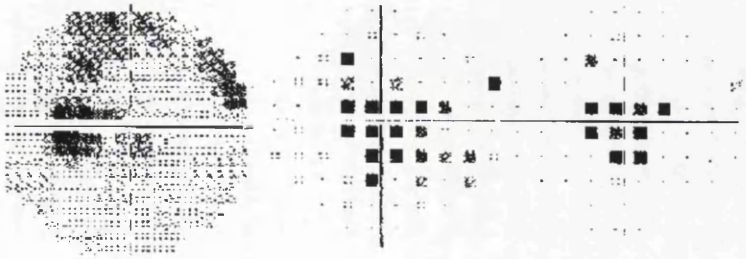
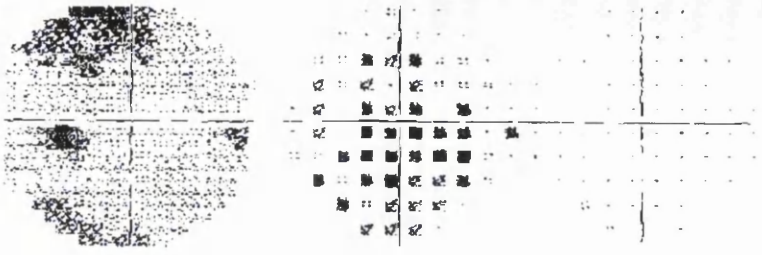
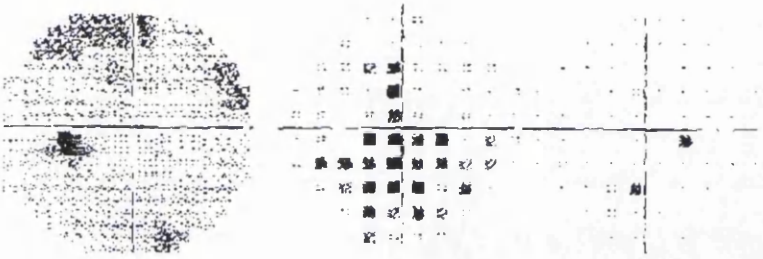


Fig. 21. Affected eye (case 20).

15/5/96
VA 6/5
Abnormal

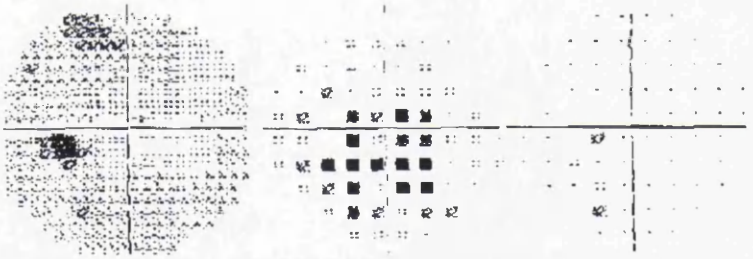


6/8/96
VA 6/6
Borderline

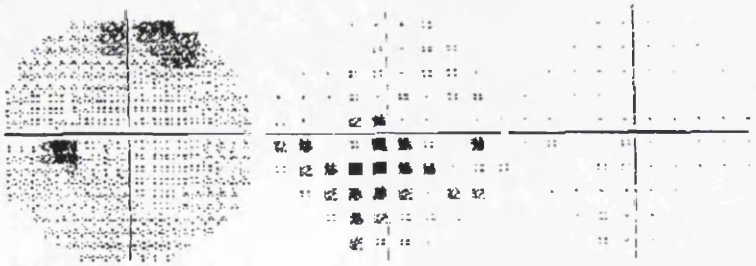


5/11/96
VA 6/4
WNL

3/2/97
VA 6/4
Abnormal



28/7/97
VA 6/5
Abnormal



26/1/98
VA 6/6
Abnormal

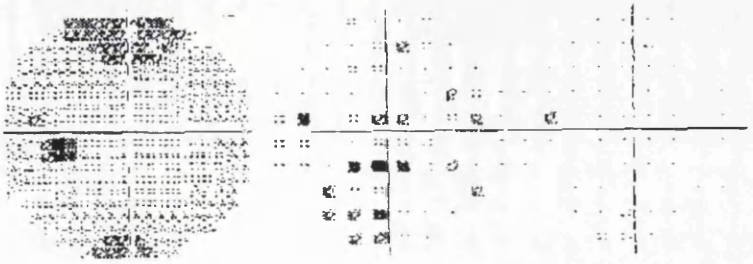


Fig. 22. Affected eye (case 31).

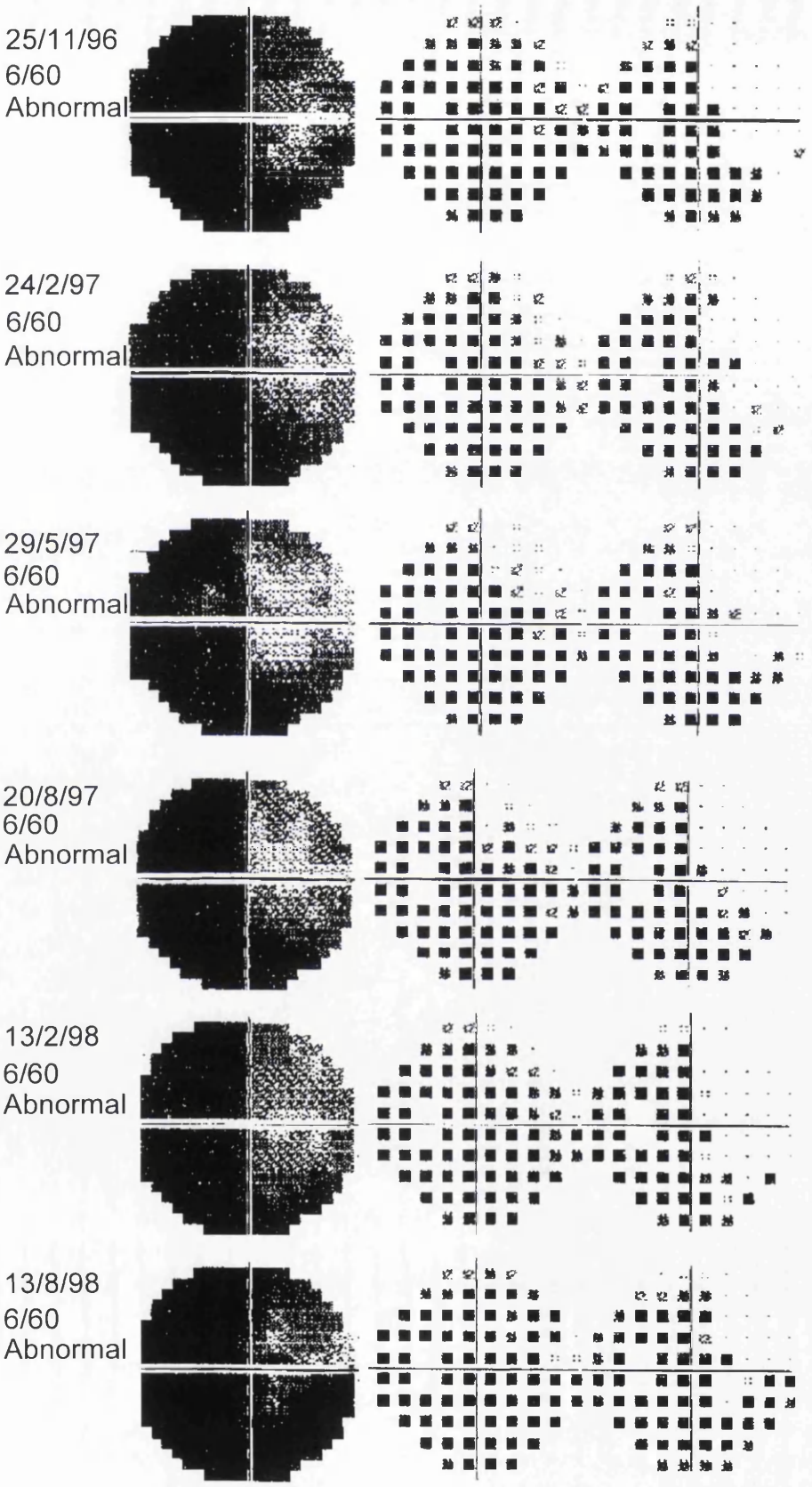
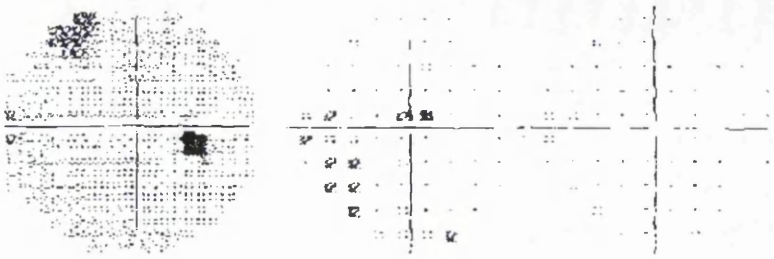


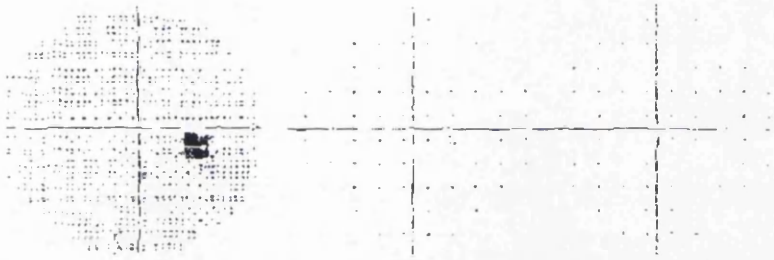
Fig. 23. Fellow eye (case 20).

15/5/96
VA 6/5
WNL

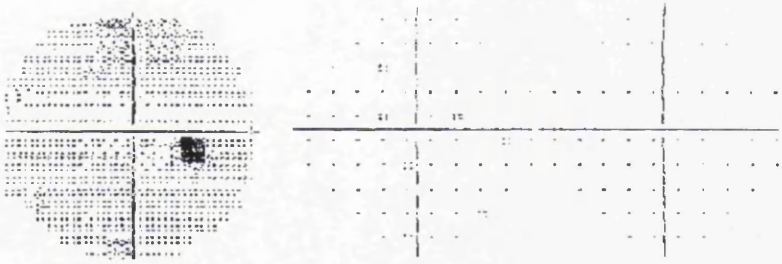


6/8/96
VA 6/5
WNL

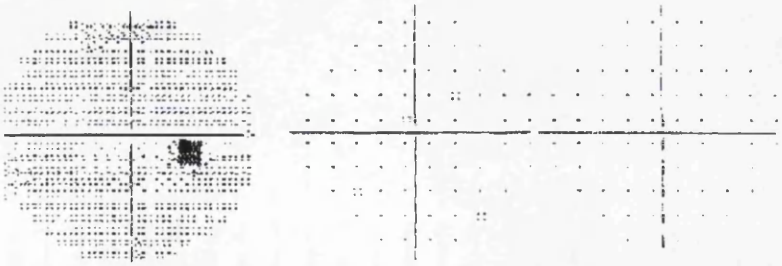
5/11/96
VA 6/5
WNL



3/2/97
VA 6/4
WNL



28/7/97
VA 6/4
WNL



26/1/98
VA 6/4
Abnormal

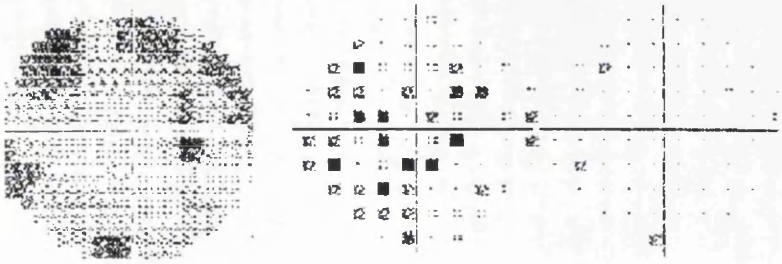
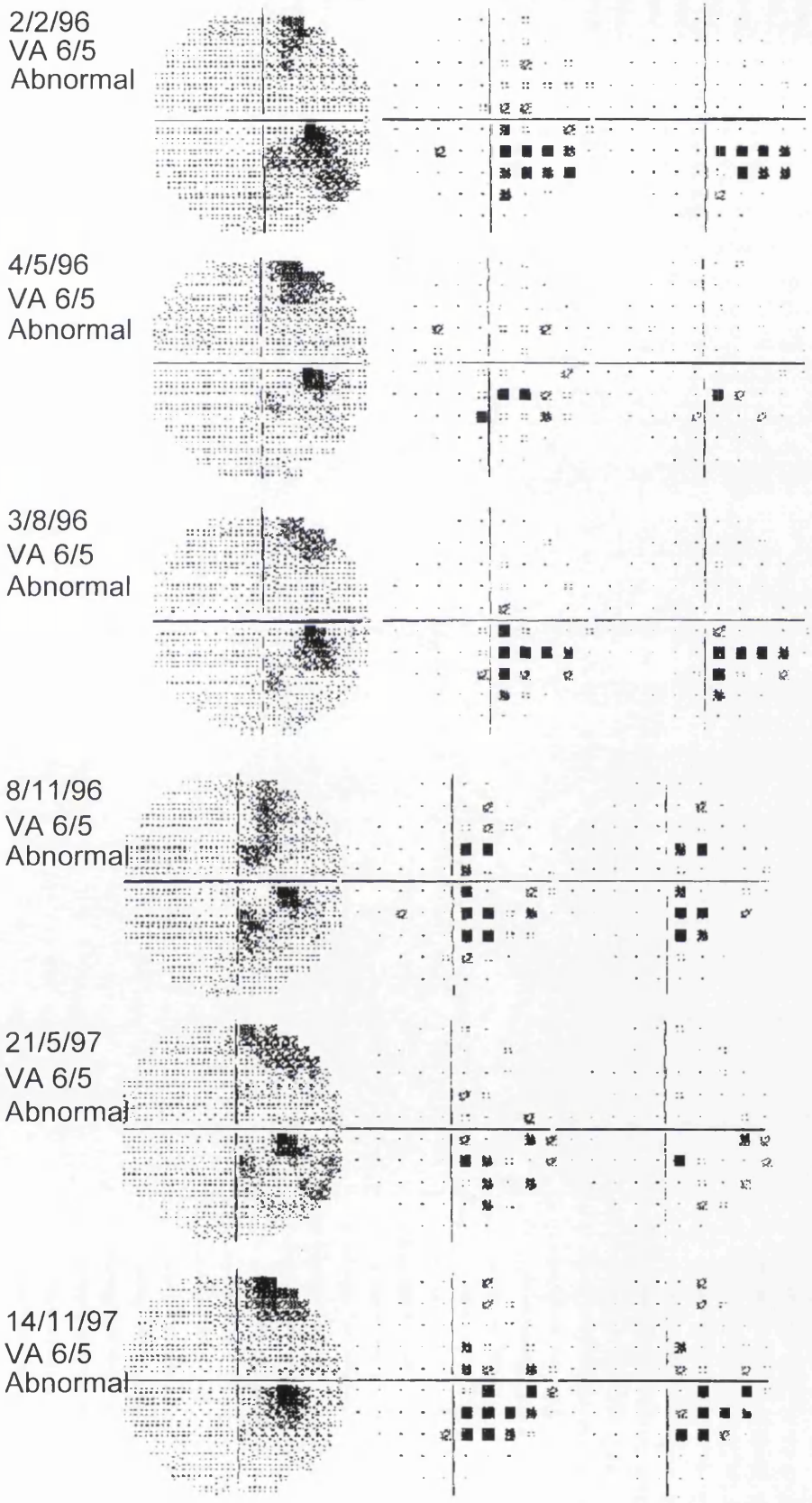


Fig. 24. Fellow eye (case 14).



5. DISCUSSION

5.1 ELECTROPHYSIOLOGICAL FINDINGS AND THEIR IMPLICATIONS

For the affected eye the VEP latency showed a significant decrease between 3 months and 1 year (3 month interval ANOVA) and between 6 months and 2 years (6 month interval ANOVA). In the analysis of contrasts the largest latency recovery occurred between 3 and 6 months (7-8 msec difference for whole and central field responses when all patients with a symptomatic ON were excluded. The latter group will be referred to from here onwards unless otherwise stated) but it was less marked after that time and it was necessary to wait for a further year before a statistically significant difference could be noted (6 months as compared to 18 months: 4 msec difference for either field). In the comparison between 6 months and 2 years, however, the latency difference was about 6 msec for central and whole field responses. In a previous study (Jones, 1993) the mean P100 latency was reported to be shorter on average by 6 msec in a group of patients tested 6-24 months after the onset of symptoms, as compared with an independent group tested between 3 and 6 months. In the ON treatment trial the latency reduction between 6 months and 3 years was slightly greater: 11 msec for the central field and 8 msec for the whole field. If the data from the two studies can be considered comparable (somewhat different VEP recording techniques were employed), this suggests that VEP latency recovery may proceed for

longer than 2 years. Combining the evidence of the two studies the best estimate of the mean latency reduction is circa 8 msec in the first year, mostly occurring before 6 months, circa 5 msec in the second year and 2-5 msec in the third year.

The repeated measure analysis for the fellow eye did not show any trend in time and whole and central field responses showed a similar invariance over 3 or 6 month intervals. In the comparison between 3 months and 2 years whole and central field responses were on average both longer in latency at 2 years when all patients were analysed together but the central field responses were on average 2 msec shorter at 2 years than at 3 months when all patients with symptomatic ON in the fellow eye were excluded (the difference did not reach statistical significance in either case). These findings differ from the ON treatment trial where the latency increased 3 msec on average for both the whole field (statistically significant difference) and the central field responses (the difference did not reach statistical significance). In the follow-up study, therefore, there was no evidence for insidious demyelination as suggested in the ON treatment trial. One possible explanation for the different findings would be that the effects of insidious demyelination (increase in VEP latency) become evident only when responses are compared after more than 2 years have elapsed. It is possible that only after that time might the delay exceed the random variability and become measurable.

The affected eye responses did not recover on average to absolutely normal latency values but almost 20% of whole field

responses and almost 30% of central field responses were within the limits of the normal range at 2 years as compared with 3% (whole and central field) at 3 months. The increase in the number of "normal" responses for the affected eye was significant, whereas there was no significant change for the fellow eye.

For the VEP amplitude no significant trend in time was found in either eye, although the affected eye responses were on average larger in amplitude at 2 years than at 3 months and the fellow eye responses on average lower. The ON treatment trial also failed to show any significant difference in either eye but in that case the responses from both eyes were on average lower in amplitude at 3 years than at 6 months. Although no definite conclusions are possible regarding long-term VEP amplitude changes, what was observed for the affected eye suggested an initial improvement followed by a subsequent deterioration. The findings from the fellow eye, conversely, suggested only a deterioration. Therefore it is possible that the findings from the affected eye in the present studies are due to a combination of 2 opposing processes, remyelination and insidious demyelination, whereas for the fellow eye the findings reflect only insidious demyelination.

5.2 PSYCHOPHYSICAL AND CLINICAL FINDINGS AND THEIR IMPLICATIONS

For the visual field data it appeared that at 3 month intervals the effect of the factor "time" on the affected eye depended on the clinical classification of the patients (MS or isolated ON) when all patients were analysed together. There was a significant improvement of the central sensitivity for the affected eye in the MS subgroup between 3 months and 1 year but no effect for the ON subgroup or the fellow eye. When those patients who had a new episode of ON were excluded the trend in time was similar for ON or MS patients and there was a significant improvement over the first year. No trend was seen for the analysis at 6 month intervals in the affected or fellow eyes. The comparison between 3 months and 2 years showed on average an improvement in central sensitivity for either eye when patients who had a new episode of ON were excluded. In the ON treatment trial study there was no significant difference between the two visits but the affected eye on average improved and the fellow eye deteriorated. Central sensitivity does tend to improve for up to 2 years, but this may be obscured by further episodes of ON or in the longer term by insidious demyelination (contrary to the most natural assumption, further deterioration occurred more commonly in the ON than the MS patients of this study).

The number of normal/abnormal responses did not change

significantly for either eye during follow-up. It is interesting to note that the percentage of abnormal visual field data was very similar at 3 months for the two eyes and that the results improved in 2 years in the same percentage for the two eyes. At presentation the percentage of abnormal visual fields from the fellow eye (40%) was higher than the percentage of abnormal VEPs from the same eye (about 30%). An analysis of progression (deterioration) over 2 years was performed on single patients after splitting the group into ON and MS subgroups. For the affected eyes a roughly equal number of subjects showed a deterioration in the ON and the MS subgroups, but for the fellow eyes a deterioration was seen more frequently in patients with ON (also when patients with symptomatic ON affecting the fellow eyes were excluded). There appears to be no obvious explanation for these findings.

For the contrast sensitivity data there was a significant improvement for the affected eye when visits up to a year were analysed and the major changes occurred between 6 and 9 months. No time effect was seen for the fellow eye or when data at 6 month intervals up to 2 years were analysed. This finding suggests that for the affected eye the influence of the recovery process on Contrast sensitivity is seen only for the first year. The comparison of the 3 month and 2 year visits for the affected eye showed an improvement of the CS on average but the difference did not reach statistical significance.

The non-significant repeated measure findings for CS in the

fellow eyes were reflected by the comparison between 3 months and 2 years when although the responses on average deteriorated for almost all conditions tested there was no significant difference. These findings differ from the ON treatment trial because on that occasion CS on average deteriorated, reaching significance for some conditions. The two studies could be reconciled if again we can assume that the influence of a subclinical demyelinating process is mild when measured over a period of two years, but then because of an accumulation of its pathological effects becomes evident at 3 years. The CS findings for the fellow eyes, therefore, tend to support the VEP findings suggesting a slow process of deterioration.

For the affected eye, the contrast sensitivity data were mostly abnormal when the stimulus was temporally modulated at 32 Hz, most markedly so for the high SF. The most striking improvement was seen for the affected eye low and high SF temporally modulated at 32 Hz (the percentage of normal responses doubled at 2 year follow-up as compared to presentation and the difference was just significant in a Chi-square test). For the fellow eye over a 2 year period the increase in the percentage of abnormal responses to low spatial frequency was significant at 0 Hz and close to significant at 8 Hz. It does appear surprising that those temporal frequencies (low SF at 0 and 8 Hz) which were less affected at presentation after the episode of ON in the affected eye were then more frequently affected at 2 year follow-up in the fellow eye. One possibility might be that as a consequence of symptomatic ON CS at high SF is mostly affected, but insidious

demyelination mainly affects the low SF. In a similar way insidious demyelination might account for abnormal visual fields in the fellow eyes which were a more frequent finding at presentation than abnormal VEPs.

As far as the clinical data are concerned there was no significant difference in the number of eyes with normal visual acuity (6/6 or better) or normal discs between 3 months and 2 years for either eye. The number of affected eyes with absent APD and normal colour vision increased significantly between 3 months and 2 years but there was no difference for the fellow eye. The findings from the affected eye indicate an improvement between 3 months and 2 years for some of the indices. The VA was normal in more than 70% of subjects at presentation and therefore the lack of a significant increment at 2 years was not unexpected. For the fellow eye no evidence of VA deterioration was found even including the cases who had symptomatic ON during the follow-up period, presumably because patients were mostly tested after the acute phase and had made a good recovery (4 patients had a VA 6/12 or worse at different times during follow-up following an episode of ON in the fellow eye).

5.3 CORRELATION OF ELECTROPHYSIOLOGICAL, CLINICAL AND PSYCHOPHYSICAL DATA

The regression analysis showed that the whole field VEP latency at 3 months significantly predicted the latency at 2 years (whole and central field), indicating that greater delays tend to remain so, whereas with the central field latency at 3 months it was possible to predict only the central field response latency at 2 years. Central field latency at 3 months, however, predicted the degree of latency recovery at 2 years, the greater delays showing the greater recovery. From the VEP amplitude at 3 months it was possible to predict the VEP amplitude at 2 years. No correlation between latency and CS was found, whereas as far as amplitude is concerned there was a correlation at 2 years with CS at high SF temporally modulated at 8 Hz. In a previous study the pattern VEP latency was found to be correlated with the contrast sensitivity for temporally modulated gratings, in particular at the high temporal frequencies(Wright et al., 1987); conversely, the VEP amplitude was correlated with CS at the high spatial frequencies. Other studies showed a correlation between spatial contrast sensitivity and VEP amplitude (Plant, 1983) but not latency (Bodis-Wollner et al., 1979).

No correlation was found between age at presentation and VEP (amplitude or latency at 2 years) and the degree of VEP latency

recovery between presentation and 2 years so there was no evidence this factor affected the recovery process or the fellow eye deterioration in this patient group. Jones (1993) found a weak correlation between age at presentation and VEP latency for those patients who were studied more than 2 years after the onset of symptoms, but no correlation with VEP amplitude. The failure to replicate this effect in the present study may be due to the longer mean duration of the follow-up in the previous study (Jones, 1993) where the patients showing significant correlation were tested between 2 and 19 years after the episode of ON.

In the present study it was attempted to correlate the activity of the disease as measured on MRI by the number of new lesions with the extent of VEP latency recovery. The data did not confirm a previous observation (Jones, 1993) of a more rapid recovery both in terms of amplitude during the first 3 months and latency over 2 years for those patients who showed disease activity, as defined by additional symptoms of CNS involvement outside the visual pathway, before or after the episode of ON. A possible explanation for the difference between the two studies might be that the recovery is only accelerated in patients with symptomatic MS, rather than asymptomatic MRI deterioration. It is still debated what is the best index to measure disease activity on MRI (number of new lesions, lesion load in total or in particular locations, etc.). However, for the purpose of this study to reach a general idea of the phenomenon the number of new lesions on MRI at least 2 years apart was considered to

be adequate and the most practical index.

5.4 PATHOPHYSIOLOGICAL IMPLICATIONS

It is possible to summarize the findings from the follow-up study and the ON treatment trial with the hypothesis that during 3 years two processes are ongoing: a pathological and a recovery process. The recovery process appears to prevail in the first 2 years, but afterwards the cumulative effects of the pathological process become more apparent. The latency recovery process which plausibly represents remyelination seems to be accompanied by visual function improvement for the first year approximately. During the second year there was no evidence of visual function improvement although VEP latency continued to recover, at a slower rate. In the third year there was some suggestion of a further recovery in VEP latency, but also a suggestion that VEP amplitude may be declining and function deteriorating. In the fellow eye there was no clear evidence for VEP latency prolongation for the first two years, and there was no significant deterioration of VEP amplitude or the visual function, whereas at 3 years there was evidence for latency delay accompanied by visual function deterioration (contrast sensitivity), although no significant changes in VEP amplitude or visual field data were found.

Although remyelination is a plausible explanation for the findings of the present study, it is necessary to consider other mechanisms which are known to be able to restore conduction in a demyelinated

axon:

1) manipulation of the currents generated by demyelinated axons,

2) changes in the ion channel distribution.

As far as the first point is concerned, it is well known that conduction block can sometimes be overcome by a small reduction in temperature, with increment of the duration of the nerve action potential (Bostock, Sherratt and Sears, 1978). A different method is the use of 4-aminopyridine (4-AP), a K+-channel blocking agent which causes an increment of the net inward current (Waxman, 1988). Neither of these are likely explanations for the present results, because the changes are temporally restricted to the period of manipulation.

As regards reorganization of the axon membrane, studies of experimental demyelination with diphtheria toxin and lysolecithin showed contrasting results, the former causing paranodal and segmental demyelination, the latter mainly segmental demyelination. Bostock and Sears (1978) showed continuous conduction at greatly reduced velocity a few days after demyelination following exposure to diphtheria toxin. This would suggest a redistribution and an increment in number of the Na++-channels along the length of the demyelinated axon membrane to sustain the conduction. Conversely, when lysolecithin was used to cause demyelination, conduction 4 days after injection was saltatory between the so called phi-nodes. This would suggest that sodium channels are aggregated at these sites (Smith, Bostock and Hall, 1982) prior to the formation of new myelin and that they are separated by inexcitable membrane. Moll et al. (1991)

identified by means of light microscopic autoradiography a 4 fold increase of the number of sites for Na++channels in multiple sclerosis lesions, confirming the former findings also in humans. These processes may possibly contribute to the rapid recovery of the symptoms after an episode of demyelination, because they restore conduction along the demyelinated nerve which was previously unable to conduct (Rasminsky, 1984; Moll et al., 1991). However, although they restore conduction within a few weeks it is difficult to comprehend how they might be responsible for any decrement of the P100 latency in the longer term.

It is also necessary to consider the possibility of cortical reorganization, a phenomenon known to occur in different species and in different systems (somatosensory, motor, visual and auditory) following lesions of sensory surfaces, peripheral nerves or more central structures (Kaas et al., 1990; Kaas, 1991; Darian-Smith and Gilbert, 1995). In particular in the visual system it has been shown that following lesions of the retina on one side (and eliminating any sort of input from the other eye) in the cat the retinotopic cortical maps are altered: after 2 to 6 months the cortical area associated with the damaged retinal region acquired new receptive field from regions surrounding the lesion. The authors suggested that the sensory perceptual system maintains the capacity to change to adjust to environmental changes or to damages (Kaas et al., 1990). In an experiment on cats and monkeys (Darian-Smith and Gilbert, 1995) stimulation of an area within or outside the boundaries of focal and

homologous binocular retinal lesions was found to elicit no responses at cortical level soon after the lesion was produced. However, a substantial cortical reorganization occurred between 2 and 12 months so that the previously silenced cortex was now activated by stimulation of the areas immediately surrounding the retinal lesion. The importance of this study resides in the observation that cortical reorganization cannot reflect a reorganization at the lateral geniculate nucleus because at this level at 2 and 12 months a silent zone corresponding to the lesioned area in the retina was still present. In the present study latency recovery was largely dissociated from functional recovery, therefore it is possible that, if cortical reorganization occurs, although not responsible for latency recovery (it would be unlikely that 10 msec decrement in VEP latency could be due to cortical reorganization) it might be partially responsible for functional recovery in the first year after acute ON. Recently, by means of fMRI, data have been obtained which suggest that cortical reorganization may occur in patients affected by isolated ON and who have made a good functional recovery. In particular, the authors found activation of brain regions (insula-claustrum, lateral temporal cortex and thalamus) which are not normally activated following photic stimulation and were not activated following stimulation of the "unaffected" eye. It is suggested that this recruitment may be due to functional compensation of persistent abnormality of the visual input. However, it is at present very difficult to be certain of the interpretation of these results and more data are awaited (Werring et al., unpublished data).

5.5 PATHOLOGICAL IMPLICATIONS

The findings from the present studies give electrophysiological support to pathological studies which showed that a reparative process occurs after demyelination in multiple sclerosis. From these studies it appears that a lesion would naturally tend to remyelinate, unless a further demyelinative event occurs (Prineas et al., 1993). According to authors such as Prineas et al. (1987) and Lucchinetti et al. (1997) oligodendrocytes and myelin are initially destroyed, but within weeks or months the surviving oligodendrocytes or those differentiated from migratory progenitors start to proliferate and new myelin is formed. Raine (1997) suggested that myelin is the primary target in MS, but due to progression of the inflammatory event oligodendrocytes are eventually destroyed, probably via a cytolytic pathway. These studies lead to the conclusion that remyelination and active demyelination may proceed at the same or different times within the same lesion, and the balance between these processes will determine the evolution of the plaque (Prineas et al, 1984; Prineas et al., 1993). At present it is thought that remyelination may be the normal consequence of demyelination, which is prevented from proceeding to completion by inhibitory factors, or alternatively stimulatory factors may be present to differing degrees in the demyelinating lesions and determine the degree of oligodendrocyte proliferation and differentiation (Lucchinetti et al., 1997). The findings of the present studies support the concept of MS lesions, not as areas of irreversible myelin loss but as areas

where demyelination and remyelination are in a dynamic equilibrium.

In a pathological study on acute, early and late chronic MS Lassman et al. (1994) found evidence for different mechanisms of plaque formation depending on the patients and the stage of the disease, so that for example oligodendrocytes are less affected by the first attack of the disease than in those attacks arising after years of disease duration when some new factor intervenes which causes a selective destruction of the oligodendrocytes as well as the myelin. The authors concluded that the pathogenesis of lesions in the acute, early and late chronic disease stages is different. If it is true that MS and as a consequence optic nerve lesions can have a different pathogenesis in different patients and in different phases of the disease it is easier to understand why the recovery process may not be present in all patients and why the rate of recovery may be different.

In the evolution of a demyelinating lesion, it is also necessary to take into account the occurrence of axonal degeneration, once believed to occur only late in the course of the disease and to be responsible for the late progressive deficit (Ghatak et al., 1973; Ludwin, 1981; McDonald, Miller and Barnes, 1992). There is recent evidence, however, that this phenomenon may be an early event and not only present in chronic lesions (Ferguson et al., 1997; Trapp et al., 1998). Therefore at any stage of the disease the clinical deficit may be attributable to axonal degeneration rather than to demyelination per se. Electrophysiological studies have shown that when remyelination occurs, although the characteristics of the myelin sheaths differ from

the normal myelin, conduction is relatively secure along the axons (Waxman, 1981). Demyelination when not accompanied by inflammation may produce no clinical symptoms as shown in experimental demyelination in the absence of inflammation as occurs after administration of lysolecithin (van Engelen et al., 1994). However, if the visual deficit is mainly due to axonal degeneration remyelination will have little or no functional benefit. Ferguson et al. (1997) observed a high number of damaged axons close to the areas with inflammation, active demyelination and macrophages which suggests that axonal damage is associated with inflammation. In addition, it has been shown that demyelinated axons are more vulnerable to toxic agents than myelinated ones (Ferguson et al., 1997; Trapp et al., 1998). Therefore, successful remyelination might have a different function, such as to protect demyelinated axons from subsequent degeneration (as speculated by van Engelen et al. in 1994) and as a consequence limit the extent of persistent disability (van Engelen et al., 1994; Waxman, 1998).

5.6 FUNCTIONAL IMPLICATIONS

One important conclusion from the follow-up study is that, contrary to what was previously suggested by the ON treatment trial, there is a long-term recovery process which has the effect of improving visual function. This could not be seen in the ON treatment trial, possibly because the effects of insidious demyelination counteract those of remyelination over longer periods. After 3 months, although inflammation is thought to have subsided and simple measures of VA had recovered to normal, in this patient group we cannot completely exclude a contribution of inflammation resolution in the later recovery process. The hypothesis of a visual deficit due partially to demyelination itself (part of the deficit which can recover following remyelination) and partly to axonal degeneration (this part of the deficit cannot recover) is strongly suggested on the basis of these findings. It has been previously argued that conduction block is more important than delayed conduction in causing clinical deficits (Waxman, 1981). In particular the occurrence of Uhthoff's phenomenon supports this view: clinical symptoms in patients with documented VEP delay can sometimes be worsened by increasing body temperature but there is no further increase in VEP latency (MacCana et al., 1983). In this case symptoms are characteristically transient and full recovery is seen after cooling (Waxman, 1981). It may be argued that conduction block is present in some demyelinated axons even at normal temperature and therefore cause visual impairment. An alternative possibility is that of

temporal dispersion which may also be the cause of visual deficit but in particular of impairment of CS at high TF.

If we assume that there is an initial recovery of visual function over approximately 9 months which is lost later, it is possible to reconcile the results of the present studies with the findings of Jeffery and Blakemore (1997). These authors showed that experimental demyelination of the spinal cord by means of gliotoxin ethidium bromide caused locomotor deficits which disappeared following spontaneous remyelination (the time of recovery was about 5 weeks after the injection).

The evidence for a functional deterioration in the fellow eye probably due to insidious demyelination might suggest that once the pathological process has become symptomatic (or possibly even independently from a symptomatic demyelinating episode) not only is the patient more likely to be affected by a new overt episode of demyelination but also the disease becomes insidiously progressive. From the data of the present study it seems that insidious demyelination has functional consequences which are different from those of overt demyelination: the CS to low SF and visual field sensitivity seem to be more frequently affected in the former situation even in the absence of a clear cut delay in VEP latency which is conversely seen in almost all cases of symptomatic ON. However, on the basis of VEP data, it appears that the progression (insidious demyelination or axonal loss) may be counteracted or prevented by the recovery process for up to 3 years and the balance between these two

opposing processes will probably determine the final outcome of the disease.

5.7 DIAGNOSTIC IMPLICATIONS

The occurrence of a VEP delay in the absence of ON symptoms has been shown in the past by authors such as Feinsod et al. (1973) on the basis of abnormal VEP to flash stimulation. Chiappa (1989) in a summary of various studies on MS patients without clinical evidence for optic nerve involvement found VEP abnormalities in about 50% of cases (the percentage, though, differed greatly amongst the various studies). In different MS studies, as reported by Halliday (1993), the percentage of VEP abnormalities in patients without ON but affected by MS (definite, probable and possible subgroups) varied between 36 and 93% but narrowed to between 57 and 95% when only the definite MS cases were included. In the present study, the occurrence of insidious delay over a period of 2 and a half years accompanied by a mild deterioration of the visual function is quantified apparently for the first time. By means of the present data it is not possible to determine whether insidious demyelination could start even before an overt episode of demyelination (for example at the occurrence of silent demyelinating brain lesions) and it is also not known whether a slowly progressive demyelinating process rather than a more acute (albeit asymptomatic) episode could account for the delay seen accidentally in patients without ON. The observation of a delay at a time when the patient does not experience any visual symptoms could be in favour of a progressive process which, once having reached a certain level, becomes manifested in a reduced CS.

In the follow-up study the VEP latency from the affected eye was normal at presentation in 3% of cases (whole and central field responses) and at 2 years in 19% of whole field responses and 29% of central field responses. Jones (1993) found normal whole field responses in 11% of cases between 1 and 4 weeks, in 22% of cases between 27 and 104 weeks and in 28.5% of cases more than 2 years after an episode of ON. Hely et al. (1986) observed that 95% of symptomatic eyes had abnormal VEPs (55' check size; 32° field size) at presentation which reduced to 81% at follow-up (on average 46 months later). Celesia (1990) found that none of 19 patients affected by ON had normal VEPs (15' check size; 22° field size) at presentation; at 12 month follow-up 95% of the responses were abnormal. Therefore, VEPs are still useful more than 2 years after ON to detect abnormalities, although a certain proportion of cases can be expected to have recovered to within normal limits.

The data from the Contrast sensitivity testing appear to differ from those of Hess and Plant (1983) who showed a higher involvement of low SF as compared to high SF on increasing the temporal frequency. For both SF in the present study the highest number of abnormal responses was detected at 32 Hz (2 or 3 times the number found at 0 and 8 Hz) and the extent of the involvement did not appear to be greater for the low SF as compared to the high SF. It is possibly true that the pattern of abnormalities can be patchy and the involvement can either be selective to particular spatial and temporal frequencies or more diffuse (Hess and Plant, 1986). In either study it appears that the

high TF is more sensitive in detecting CS defects in demyelinating disease. In addition in the present study it seems that the CS to high SF is more useful when assessing the sensitivity following an overt episode of demyelination, whereas the low SF appears to be more useful when insidious demyelination is suspected to have occurred.

The status of the fellow eye after optic neuritis was serially evaluated by Beck et al. (1993): an overall improvement of the psychophysical tests performed at 6 month follow-up as compared to presentation was found. The authors concluded that the abnormalities detected at presentation may not be attributable in most cases to long-standing lesions, but to acute inflammation. In the 3 year ON treatment trial follow-up some of the VEP abnormalities in the fellow eye were presumably due to episodes of optic neuritis prior to recruitment, but there was also evidence of new lesion formation. However, in the 2-year follow-up study there was only very marginal evidence for deterioration and the high incidence of abnormal Visual fields (although VEPs were normal) may suggest to some extent an involvement of the fellow eye prior to recruitment. The hypothesis that contrast sensitivity deterioration observed in the 3-year ON treatment trial study was due to a longer follow-up as compared to the American ONTT is supported by the Follow-up study where patients followed-up for 2 years did not show statistically significant deterioration.

In the follow-up study approximately 1/3 of the patients had normal-appearing optic discs in the affected eye as reported previously (McDonald and Barnes, 1992). Most of the patients had normal VA at

3 months (74%) and the number increased up to 1 year (84%), confirming reports that a slow recovery can occur up to 12 months after the acute episode (Celesia et al., 1990) and supported by the contrast sensitivity findings. The same applies to colour vision which was already normal at 3 months in 50% of affected eyes and in 84% at 2 years (abnormal colour vision is reported in the literature to vary between 50 and 100% of cases (Perkin and Rose, 1979)). APD was present in about 50% of affected eyes at presentation and in about 25% at 2 year follow-up. This finding is also in agreement with the literature where it is reported that APD most frequently disappears but can also become chronic or worsen, particularly when VA remains poor (Slamovits et al., 1991).

For the analysis of the data the patients were split into two subgroups firstly according to the MRI data and secondly according to the clinical data. In particular, as far as the MRI data are concerned patients were split into those who showed changes in the scan between presentation and 2 year follow-up and those who did not. Regarding the clinical data, patients were split into one subgroup if they were affected by isolated or recurrent ON and into a second subgroup if they were affected by MS (firstly for data at presentation and secondly for data at 2 year follow-up). The VEP and psychophysical data were analysed in order to determine whether the changes of these data over time could be different in the various subgroups (only the visual field data showed some different behaviour between the patients classified as ON or MS at presentation). From

this analysis no evidence was found for differential effect on VEPs or visual function according to the degree of disseminated disease activity. However, the lack of significant differences might be due to some extent to the fewer patients affected by MS than ON at presentation and the fewer patients who did not show changes on MRI over time than those who did.

5.8 THERAPEUTIC IMPLICATIONS

In the therapeutical view, the observation that oligodendrocytes survive after an episode of demyelination and are also capable of producing new myelin (according to Raine (1997)) should suggest that a therapeutic intervention might be possible. Recently attempts have been made to reduce inflammation and demyelination, to promote remyelination or to limit axonal loss in order to reduce the clinical effects of "demyelination" (Scolding, 1997). Two studies have been performed recently on patients affected by ON to whom corticosteroids were administered: the American ONTT and the ON treatment trial performed in London. The former (Beck et al., 1992) included 457 patients who were randomly assigned to receive oral prednisone (1 mg/Kg/day), ivMP (1 g/day for 3 days) followed by oral prednisone (1 mg/Kg/day) or placebo. The authors found a faster recovery in those patients who received ivMP as compared to patients who received oral prednisone or placebo in a 6 month follow-up (patients were seen on 7 occasions over that period of time). Although the difference between groups decreased with time, visual fields, contrast sensitivity and colour vision were still slightly better at 6 months in the group that received ivMP than in the remaining groups. Moreover, patients who received oral placebo showed a higher risk of developing a new episode of ON than the remaining two groups. The ON treatment trial (Kapoor et al., 1998) was undertaken after the observation that visual recovery tended to be poorer when the intracanalicular portion of the

optic nerve was affected or the lesion as seen on MRI (performed, however, at various times after the development of optic neuritis) was relatively long. The hypothesis was that reduction of inflammation (as may be produced by corticosteroid treatment) may help to reduce the incidence of poor recovery particularly in these types of lesions. Therefore ivMP (1g/day for 3 days) or iv saline were administered in a double blind fashion and the 66 patients were also split into subgroups according to short or long lesion length on MRI of the optic nerve. This was also the case when the patients were split into short and long lesion subgroups according to MRI findings in the optic nerve. VEP amplitude was on average larger (whole and central field) at 2 week follow-up in the treated group (borderline significant for all patients and the short lesion subgroups) but no significant differences in latency were found between the two groups. At 6 month follow-up there were no significant effect of iv methylprednisolone treatment on VEP amplitude or latency, clinical examination, psychophysical tests or MRI (Kapoor et al., 1998). The findings suggested that ivMP treatment caused a more rapid recovery from conduction block associated with inflammation but had no effect in the long term (6 months) on visual function, MRI or VEP data in either subgroup.

The administration of iv immunoglobulin (ivIg) has also been studied by authors such as Achiron et al. (1992) and van Engelen et al. (1992 and 1994). In the study of Achiron et al. (1992) ivIg was administered to patients affected by relapsing/remitting MS. The authors showed a significant reduction in exacerbation rate in a 1-year

follow-up of the treated group. The data were compared firstly with those of a 2 year pre-treatment period and secondly with those obtained from an untreated group of patients (relapsing/remitting MS) matched to the treated group for age, disease duration and number of attacks. Van Engelen et al. (1992) measured VA, colour vision and light discrimination and performed VEP and MRI in patients affected by MS but with stable ON (unchanged visual impairment in the previous 6 months) before and 3 months after the administration of ivIg. The authors found an improvement of different indices in different patients which appeared to be time related with treatment. It was not clear, however, whether the improvement was due to a regulation of immune activity and therefore to reduction of inflammation/demyelination or to promotion of remyelination. Following experimental observations after administration of ivIg in Theiler's virus-induced demyelination in mice, Van Engelen et al. (1994) reached the conclusion that the main effect of this therapy is that of promoting remyelination, as a consequence limiting axonal degeneration.

Another possible therapy would be the use of growth factors which have been shown to promote the synthesis of the myelin at least in vitro (PDGF, FGF and IGF) but more data are awaited in vivo (McMorris et al., 1990; McMorris and McKinnon, 1996).

In recent years the transplantation of glial cells such as oligodendrocytes, Schwann cells, astrocytes or stem cells (highly proliferative and pluripotential cells) has been attempted in order to promote remyelination (Blakemore and Franklin, 1991; Duncan, 1996;

Scolding, 1997). This type of study is at present experimental, but the feasibility of the technique has been shown in mice and rats (Blakemore and Franklin, 1991). Many problems can arise following cell-transplantation: the immune reaction against the graft may be responsible for new antioligodendrocyte activity which would determine a deterioration in the patients. In addition there are possible adverse effects on the host directly due to the implanted cells such as for example the occurrence of tumours after the Schwann cell transplantation. There are also ethical problems regarding the production of the cells to transplant: in fact as far as the oligodendrocytes are concerned the main source is abortion-derived human CNS tissue whereas the Schwann cell may be cultured from adult peripheral nerve biopsies (autologous transplantation), a more ethically acceptable way. Of course, once the cells are transplanted, they also need to maintain the ability to proliferate and migrate to the area of the lesion (which is why it would be better to transplant immature oligodendrocyte precursors rather than mature cells) and it is necessary to prove that remyelination following transplantation is a stable phenomenon before the technique could be applied to humans (Scolding, 1997). Some authors doubts that transplantation will ever be used in clinical studies on a large scale (Scolding, 1997) but others do think it is a possible approach although more details about the characteristics of the cells to transplant (safety for the recipient, availability, ability to migrate, divide and produce stable remyelination) are necessary (Duncan, 1996).

Although most therapies to promote remyelination are still at experimental stage it is plausible that in the next few years an easy and effective way to promote remyelination may be found. The findings of the present study appear to suggest that remyelination is most likely responsible for the visual function recovery over a period of up to one year (although VEP latency recovers for at least 2 years) after the acute episode of demyelination, and in addition may have a protective role in limiting the degeneration of demyelinated axons. Of course in the present study no treatment was used and the recovery observed in this group of patients was a spontaneous process: it is possible then that the therapeutical promotion of remyelination would produce an even more marked recovery, encouraging research in this direction. It is also possible that the best approach may involve the combination of various therapies directed to promotion of remyelination, to reduction of inflammation and demyelination and to reduction of exacerbation rate. The therapy with β-interferon (Hall et al., 1997; Yong et al., 1998), for example, is believed to cause a reduction of the exacerbation rate in relapsing-remitting MS. If it is correct as suggested by Lassman et al. (1994) that the mechanism of plaque formation is different according to the stage of the disease it is also plausible to think that the best therapy for the single case may differ at different stages of the disease.

5.9 CONCLUSION

The present studies suggest that two opposing processes are present over a 3 year follow-up: remyelination and insidious demyelination. Remyelination causes shortening of VEP latencies for at least 2 years after an acute episode of ON and this is accompanied by visual function recovery for the first year. A partial explanation for the dissociation between VEP latency and visual function recovery might be that visual symptoms can also be caused by axonal loss and remyelination would not have any influence on this part of the symptoms. However, remyelination after the first year may still have an indirect effect on visual function, by protecting demyelinated axons against further inflammatory episodes which might result in permanent degeneration. Insidious demyelination, whose effect on VEP latency and contrast sensitivity is possibly counteracted by the remyelinating process in the affected nerve, has effects which are more clearly seen in the "unaffected" nerve. These effects become detectable only over a follow-up period of more than 2 years, although it is most likely that in some cases insidious demyelination starts even before the acute episode of ON.

The balance between remyelination and demyelination will probably determine the evolution of CNS plaques and therefore the course of the functional deterioration. It seems plausible to think that when insidious demyelination and/or axonal loss prevail over remyelination a progressive phase of functional deterioration starts.

However, given that the findings of the present studies are due to spontaneous remyelination, research on therapies directed to promote remyelination should be encouraged.

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