Multiple Sclerosis and other demyelinating disorders

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Multiple Sclerosis and Optic Neuritis

MS is primarily a disease of nerve tissue and especially of the fibres which connect different parts of the nervous system. Many of these fibres are covered with myelin, that facilitates saltatory conduction along the nerve. In MS myelin is destroyed in discrete areas of the brain or spinal cord (the plaques of demyelination) leaving the nerve fibres or axons mostly intact. Loss of myelin is the critical factor in MS, with severe loss of myelin or demyelination completely blocking nerve conduction. Hence, the symptoms observed in an individual patient will depend on the location and degree of the myelin loss. The sites in the nervous system particularly susceptible to the initial development of the disease are the optic nerves, the upper region of the spinal cord, the brain stem and the cerebellum. Earlier categorisation of the disease included the classes of possible, probable or definite MS (Poser et al. 1983), more recent guidelines suggest a redefinition in MS, not MS (McDonald et al. 2001)

MS appears with different clinical patterns consisting of acute attacks followed by complete or partial recovery, further attacks involving different areas of the Central Nervous System (CNS), months or years after the first attack, the so called relapsing remitting course, or with an insidious onset and continuous progression of disability, the primary progressive MS. In the early phases of the relapsing-remitting course, attacks are usually followed by a full or almost full recovery; as the disease evolves sequelae from the attacks start to accumulate and about 90% of the patients enter a progressive course with a continuous deterioration of neurological functions. It has been estimated that 50% of the patients have some disability after 10 years of disease and 70% need assisted deambulation after et al. 20 years. The intraindividual variability of disease evolution and recovery from attacks is very high, however as soon as the patients enter the secondary progressive phase of the disease the variability decreases and a prolonged disease stability become exceptional (O’Connor et al. 2002).

Acute optic neuritis, isolated or associated to other symptoms, is the presenting symptom of MS in about a quarter of patients (Paty et al. 1997). Brain MRI shows multiple white matter lesions in about 50-70% of the cases and oligoclonal bands are electively present in cerebrospinal fluid in about 60% of the patients. The risk of development of MS after isolated instances of ON increases with the length of the follow-up. In one series (Rodriguez et al. 1995), the 10-year risk of clinically definite multiple sclerosis was 39%, the et al. 20-year risk was 49%, the 30-year risk was 54%, and the 40-year risk was 60% (Rodriguez et al. 1995). The most important risk factor is the presence of white matter lesions at brain MRI evaluation (Beck et al. 1993). After 10 years the risk of developing MS is 60% in patients with positive MRI and close to zero in patients with negative MRI (Ghezzi et al. 2000). The risk is
increased in women (Rizzo and Lessel 1988, Sandberg-Wollheim et al. 1990), in patients with retinal vascular abnormalities (Lightman et al. 1987), HLA-DR2 positive (Sandberg-Wollheim et al. 1990, Hauser et al. 2000) and with oligoclonal bands in the cerebrospinal fluid (Sandberg-Wollheim et al. 1990, Rizzo and Lessel 1988). The risk of development of MS after bilateral simultaneous ON of childhood is much lower (Kriss et al. 1988, Parkin et al. 1984). The percentages quoted above, resulting from several multicenter studies (Rodriguez et al. 1995, Rizzo and Lessel 1988, Sandberg-Wollheim et al. 1990, Lightman et al. 1987), underline that ON is a focal demyelinating process that may or may not evolve in MS with an appreciable 50% of patients who will be safe from invalidating outcomes. ON with its evolution, time course and complex of symptoms is considered as a model for the study of demyelinating diseases.

ON is an acute inflammatory optic neuropathy. Patients experience a unilateral, or more rarely a bilateral, impairment of vision of acute onset. Visual acuity is depressed but this can vary from a mild reduction in acuity to severe cases in which no light perception is present. In the acute phase of ON, the visual loss appears to its full extent in a matter of days, and current treatments, based on corticosteroid boluses, are only indicated in the first days after the appearance of symptoms. Within 2-3 weeks, visual symptoms start aborting, and in 3-4 months commonly the recovery phase is completed (McDonald 1998, Onofrj et al. 1996a), but residual clinical abnormalities can be noticed, depending on the extent of action of the different mechanisms leading to recovery.

After this recovery period there may be fluctuations in visual acuity during the day, with visual acuity deteriorating during daytime and improving at night. Improvements of visual acuity at night may be related to the secretion of melatonin which lowers body temperature (Sandyk 1995). A characteristic feature of ON is the presence of "Uthoff's symptom", i.e. the visual loss is more severe after exercise (Selhurst and Saul 1995), or a hot meal or a hot bath. It is usually not encountered in the acute stage of optic neuritis, though it can rarely be the presenting feature of MS. This variability in vision is associated with a reduction in amplitude of the visual evoked potentials (Persson et al. 1981), and can be partly accounted for the extreme sensitivity of conduction in partially demyelinated fibres to small changes in temperature (Ramsminski et al. 1973).

Other transient phenomena, such as phosphenes (flashes of light), often precipitated by eye movement are experienced by approximately one third of patients (Lightman et al. 1987, Davis et al. 1976) during the acute phase of optic neuritis.
A sense of disorientation in moving traffic is experienced by some patients and is probably due to the “Pulfrich effect”, a phenomenon attributable to unequal conduction between the two optic nerves. This effect can be experienced in normal individuals by placing a neutral density filter over one eye: a pendulum swinging in one plane then appears to be describing an ellipse (Rushton 1975, Ell and Gresty 1982).

In patients with ON, the colour vision is often impaired after the recovery phase more seriously than visual acuity (Porciatti and Sartucci 1996). However, the deficits in colour vision are often complex, with the ability to detect a colour change being less affected than the ability to detect an object using chromatic stimuli (Cole 1995). In addition, contrast sensitivity using chromatic gratings (Porciatti and Sartucci 1996) and colour visual field analysis may be more sensitive indicators of the presence of MS than simple luminance stimuli (Accornero et al. 1998). The colour vision problem observed in MS patients suggests that it may be the smaller diameter fibres of the optic nerve which are more susceptible to demyelination. This hypothesis is supported by post-mortem studies of the optic nerve revealing atrophic areas including large portions of the papillo-macular bundle (Gartner 1953).

Visual field defects are common in patients with ON. The classical field loss is a central scotoma, but it is important to appreciate that a range of defects may be found, to some extent depending on the stage at which the patient is examined. Occasionally, a defect with a horizontal margin may evolve over a week or two in central scotoma. During the recovery phase, atypical central scotoma may become patchy and later leave an enlarged blind spot with an arcuate scotoma; fragments of this defect may persist indefinitely. However, the North American Optic Neuritis Treatment Trial (ONTT, Beck 1998) noted a wide variety of visual loss (48% of patients), altitudinal defects (15%), quadrant loss (6%), while central and centro-caecal scotomas accounts for less than 5% of the patient population (Keltner et al. 1993). More rarely, cases of MS may begin with homonymous hemianopias (Gundry et al. 1998) or bitemporal visual field defects (Demaerel et al. 1995).

Fig 1 shows some visual field defects observed in ON in the acute phase and after recovery.

**Differential diagnosis**

Since the diagnosis of demyelinating optic neuropathy is usually made on clinical grounds, ancillary tests may be of limited value in typical cases. However, atypical cases are not so rare and the differential diagnosis requires a meticulous clinical history and an extensive instrumental evaluation.
Acute loss of central vision, besides demyelinating ON, can also be due to other ocular diseases that involve the eye media or the retina. Moreover, functional visual loss that occurs typically in children and young adults presents with a clinical picture that may be a difficult diagnostic challenge.

Optic neuropathies that may present with acute loss of central vision include: ischemic, infectious, traumatic, toxic, genetic and radiation induced optic neuropathy. Anterior segment and retinal diseases should be included in the differential diagnosis of central visual loss. Visual loss in optic neuritis is commonly characterized by painful eye movement. Ocular pain may be due to corneal abrasion but it is uncommonly associated with retinal diseases. A few anterior segment diseases can mimic optic neuritis and they can easily be detected by slit lamp examination. Inflammatory or traumatic diseases involving the central portion of the cornea, anterior chamber reaction with cells and acute angle closure glaucoma can be responsible for acute loss of vision, in some cases associated with eye pain. Anterior segment involvement commonly occurs with intense hyperemic conjunctival reaction, which is not observed in optic neuritis or other posterior intraocular diseases.

More challenging is the differential diagnosis with diseases involving the retina, since symptoms due to central retinal involvement can be very similar to those of optic neuritis. Furthermore, although most retinal diseases are bilateral from the onset, they can mimic optic neuritis when strictly unilateral or when visual dysfunction is highly asymmetric. They may cause central visual impairment when macular function is damaged. Unlike retinal disease, optic neuritis is commonly associated with a relative afferent pupillary defect, a sensitive sign of unilateral optic nerve dysfunction. A relative afferent pupillary defect is rarely due to retinal disease, and it occurs only in severe cases such as retinal detachment or central artery occlusion.

Optic neuritis can also be mimicked by functional, nonorganic visual loss which frequently occurs in young subjects. Feigned visual loss includes both malingers and hysterics and the distinction between the two categories is difficult for ophthalmologists. The diagnosis of functional visual loss can be helped by observing the patient’s behaviour and the inconsistency of results on subjective visual function tests. Optic nerve tests can be used as an additional objective demonstration of normal anterior visual pathway function.

Nonorganic visual loss is commonly unilateral, its onset can be abrupt, and it is usually associated with other neurological symptoms such as pain, headache or dizziness. On neuro-ophthalmological examination, a normally reactive pupil and the absence of a relative afferent pupillary defect on the involved side strongly suggest the diagnosis of feigned visual loss. Moreover, when visual acuity is
severely reduced on subjective tests, the 4 prism dioptries test and the presence of a normal optokinetic nystagmus indicate a functional disease. Finally, the diagnosis of functional visual loss can be confirmed with laboratory tests, such as flash visual evoked potentials (VEP).

**VEPs and optic neuritis**

Already in 1963 Halliday and Wakelfield (1963) showed that somatosensory evoked potentials to electric stimuli could be delayed in patients with MS when plaques involved the dorsal column and medial lemniscal pathways. Later studies (Namerow et al. 1968, Desmedt and Noel 1973, Matthews et al. 1974, Celesia 1978) confirmed that conduction in the central nervous system (CNS) could be delayed in presence of demyelinating diseases, similarly to what happens in diseases of the peripheral nervous system (PNS).

When pattern visual stimulators, instead of flash stimulators, were introduced in clinical practice, allowing a reduction of the statistical variability of Evoked Responses in controls, Halliday et al. (1972) showed that latency of visual evoked potentials (VEPs) was delayed in as much as 94% of patients affected by ON. Several other researchers (Namerow et al. 1968, Lowitzch et al. 1976, Collins et al. 1978, Shahrohki et al. 1978, Chiappa 1980, Kjaer 1980, Celesia et al. 1986) confirmed the finding, even though the prevalence of VEPs delays in ON and MS was identified in a range of 70-85%, as reported in table I, smaller than the prevalence reported by Halliday et al. (1972), but yet the highest prevalence of abnormalities ever identified with evoked potentials (EP), to the point that VEPs are still considered as the gold standard measure for the evaluation of ON, in comparison with other available techniques including MRI (O'Connor et al. 1998, Gronseth et al. 2000). Fig. 2 shows examples of delayed VEPs.

In demyelinating diseases of PNS, like diphteria or the Guillan-Barre' syndrome, delays of action potentials were found, due to slowed conduction of nerve potentials. Comparably EP delays have been interpreted as being due to a shift "en masse" by several milliseconds of an EP virtually conserving its waveform characteristics (Halliday et al. 1972, Lowitzch et al. 1976, Collins et al. 1978, Shahrohki et al. 1978, Chiappa 1980, Kjaer 1980).

As late as in et al. 2001 this shift "en masse" or delayed VEP with well preserved waveform is considered a typical of MS by International Guidelines (McDonald et al. 2001).
Whether this simplification, derived from the need of preserving a role for neurophysiological studies in the evaluation of MS, is correct or not, will be part of the discussion of the present chapter.

The interpretation of EP abnormalities in MS and related disorders revolves around two basic concepts deriving from knowledge of nervous conduction abnormalities in PNS demyelinating disease: conduction block, with disappearance of EP components, and conduction slowing, leading to delays. In the first attempt to analyse the mechanisms of EP delays, McDonald (1977) tried to relate the central conduction slowing to the length of plaques in MS. However not all the EP latency increments could be interpreted on the basis of the conduction slowing in a plaque and further debates (Halliday et al. 1977a,b) wondered whether conduction could be preserved, and to which extent, in large demyelinated CNS plaques.

In several attempts to correlate VEP alterations with magnetic resonance imaging (MRI) findings, the mechanisms of EP delays remained controversial and the conclusion was that "the origin of the delays seen in demyelinating disease is still not fully understood" (Ormerod et al. 1986a,b). Thorpe et al. (1995) showed that in optic neuritis the length of the lesion was positively correlated with both the visual deficit and the reduced amplitude of the VEP, but not with the VEP latency increase.

Ulrich and Groebke-Lorenz (1983), in a pathological study, found a correlation between the extent of demyelination in the optic nerve and the last antemortem visual acuity. No studies have correlated the extent of axonal loss in optic nerve to the visual function and VEP abnormalities, but examination of the retinal nerve fibre layer at the optic disc revealed that more than 50% of neural tissue in a given area must be lost before a visual defect becomes clinically evident (Quigley and Addicks et al. 1982).

These observations may explain some clinical/neurophysiological and neurophysiological/MRI discrepancies:

1) patients who fully recover from optic neuritis have usually persistent VEP delays and VEP may be abnormal in asymptomatic eye (Celesia 1992)

2) asymptomatic MRI lesions can be detected in an optic nerve which also display a normal VEP (Thorpe et al. et al. 1995)

We have to consider that due to inter-subject variability on VEP amplitude and latency, actual abnormalities may not be evident on an individual-based analysis. In fact, in a study evaluating MS patients with normal visual acuity and normal VEPs (Diem et al. 2003), group comparison revealed significantly decreased VEP amplitude compared with normal subjects, suggesting that pathological
changes in optic nerve fibres may go undetected before a visual defect becomes evident both clinically and at VEPs.

Many studies have demonstrated a good correlation between symptoms and signs secondary to the damage of a given nervous pathway and abnormalities of the corresponding EP (Celesia 1992; Ingram et al. 1988; Comi et al. 1989). It is rare (<5% in the acute phase of the optic neuritis) to find a normal VEP in the symptomatic eye. In acute optic neuritis the involvement of a small proportion of fibres may not modify the latency of the VEP, but may determine morphological abnormalities of the response which can be easily demonstrated by multielectrode recordings (Onofrj 1990).

In patients with isolated syndromes suggestive of MS the frequency of abnormal EPs is low, with the exclusion of optic neuritis patients (Filippini et al. 1994; Lee et al. 1991); the frequency of abnormal responses increases progressively with the disease duration (Comi et al. 1989).

As a general rule a negative EP does not exclude an active lesion in the explored pathway, but a significant increase of latency or a morphological modification of EPs is confirmatory that a new lesion has injured the explored pathway. Because most of the active lesions demonstrated by MRI do not affect sensory-motor pathways it is clear that MRI is much more sensitive than EPs in demonstrating disease activity in the brain.

Given the fact that VEP alterations in MS are nowadays still granted a role in the identification of objective abnormalities in MS, this chapter will review the experimental models of VEP alterations, the clinical aspects of Demyelinating Optic Neuritis and the different VEP findings during the course of ON.

The experimental evidence of conduction slowing in demyelination induced by toxic agents

The early studies on pathophysiology of conduction were performed on the spinal cord of cats. (McDonald 1977). The demyelinating agent used was the diphteria toxin (DTX). Immediately after the application of DTX the conduction in demyelinated fibres was blocked and no inward sodium current was observed along the demyelinated segment. Four or five days after DTX new points of inward current were observed along the demyelinated fibre (Bostok and Sears 1978) and conduction reappeared: impulse conduction in single fibres demyelinated by DTX was similar to microsaltatory conduction in amyelinic fibres, ranging inside the 1-2 m/s. Changes in capacitance or in the safety
factor of the nerve fibre were thought to be dependent on the appearance of new Na+ channels on the axonal membrane (Schauf and Davis 1974).

McDonald (1977) showed that the ability to conduct train of impulses was reduced in demyelinated nerve fibres, and described failures even at 1 Hz.

By considering the studies (McDonald and Sears 1970, Rasminsky and Sears 1972) showing that maximum internodal conduction time in demyelinated fibres was more than 25 times the mean normal value and considering a probable conduction velocity of 10m/s in the CNS, McDonald and Sears suggested that a demyelinated area of 1 cm could delay the conduction of 25 ms; yet in their paper they raised some open questions: first that conduction is likely to fail altogether when there is a succession of internodes with very long internodal conduction time (and therefore he suggested that the addition of successive delays might justify more appropriately the delays measured in clinical application of VEPs) and second he considered cortical or retinal contribution to the mechanism of VEP delays. Their conclusion was that "evidence" was "however, inadequate to justify the conclusion that slowing of conduction is the major source of delay in demyelinating disease".

Other experimental evidences were obtained (Smith et al. 1982) using the neurotoxin lysophosphatidyl choline (LPC), deterging the glicolipids of the myelin sheath but preserving the nerve axons. Following LPC application on single nerve fibres, conduction is blocked and there is lack of any inward Na+ current in the internodes, just as it happened in the DTX model.

Remyelination begins 7-8 days after the direct application of LPC but already on the 5th day conduction returns on demyelinated tract, where new foci of inward Na+ current, called “φ” nodes, appear: these foci are uniformly distributed on the demyelinated tract with an average distance of 100µm one from each other, and therefore shorter than the known internodal distance of et al. 200µm (Schauf and Davis 1974, Smith et al. 1982). On the 5th day following LPC application a microsaltatory conduction is recorded between the “φ” nodes, but this conduction is likely to fail (block) when the extent of the demyelination exceeds few millimetres because of inadequate capacitance and high impedance. When remyelination begins (approximately on the 7th day) the appearance of small foci of remyelination increases the distance between the “φ” nodes. The distance between foci of Na+ inward current will be however shorter in remyelinated fibres than prior to LPC application. Nerve conduction velocity will increase proportionately to the distance between “φ” nodes. During remyelination the amplitude of action potentials and the conduction velocity increase.
Impulse conduction in fibres with “ø” nodes appears to have a low safety factor, and to be highly temperature sensitive (Schauf and Davis 1974, Smith et al. 1982), corresponding to the Uthoff phenomenon previously described.

Waxman (1977) and Sears et al. (1978) pointed out that at the junction between normally myelinated and demyelinated axons the inadequate current density may constitute a physiological barrier blocking conduction. The interposition of short internodes due to remyelinated regions (Waxman and Brill 1978) might resolve the impedance mismatching and restore conduction. Fig.3 exemplifies the mechanism of conduction block and conduction recovery.

Waxman and Wood (1984) showed that an increment of sodium conductance or a decrement of potassium conductance due to minor changes in the axon membrane properties has significant effects on impulse invasion properties. According to this report a minor degree of membrane reorganisation, involving redistribution of channels via lateral diffusion within the membrane, can restore conduction in the demyelinated area by sustaining continuous conduction.

**Experimental allergic encephalomyelitis (EAE)**

EAE is a demyelinating disease of the CNS obtained in rats (Creel et al. 1974), monkeys (Hayreh et al. 1981), guinea pigs (Lidski et al. 1980, Onofrj and Gambi 1986) by systemic administration of myelinated components of the mammalian CNS. Owing to the resemblance of histopathologic and immunologic findings in this disease and in MS, EAE is a “commonly used model for the study of human demyelinating disorder” (Lassman 1983).

The EAE can be obtained in different forms: acute fatal, chronic relapsing and chronic progressive.

VEPs were studied in acute EAE by several authors (Hayreh et al. 1981, Lidski et al. 1980, Onofrj and Gambi 1986, Bilbool et al. 1983). All the studies agreed in showing VEP delays and amplitude reductions early in the course of the disease, even before the appearance of motor symptoms of EAE. The latency delay of VEP components, ranged from 3 to 14 ms in guinea pigs and rats (Creel et al. 1974, Lidski et al. 1980, Onofrj and Gambi 1986, Bilbool et al. 1983) (where N1 and P1 have mean latencies of 28 and 46 ms) and above et al. 20 ms in monkeys, where P1 VEP component has mean latency of 90 ms (Hayrey et al. 1981).

In the animals sacrificed after the detection of VEP delays, demyelinating plaques were not observed in histopathologic specimens. Scattered mononuclear infiltration (Onofrj and Gambi 1986), maximal
inflammatory reaction with no demyelination (Lidski et al. 1980), perivasculitis, periventriculitis and parenchymatous cellular infiltration in only one half of the animals showing abnormal VEPs (Bilbool et al. 1983) ad minimal cellular infiltration without any demyelination or loss of nerve fibres (Onofrj and Gambi 1986) were reported by the different authors.

The electron microscopic alterations (Raine et al. 1974) of optic nerves in acute EAE consisted of "macrophages surrounding the myelinated axons, disrupting the myelin lamellae stripping away the entire myelin sheath" in patchy areas that did not show the confluent characteristics of a plaque.

In the chronic relapsing form of EAE, a progressive increment of EP latency was observed already in the first 2 months following the onset of the disease, accompanied or not by further amplitude decrements. The latency delays range from 8 to 24 ms for P1 component.

Transient latency reduction by 30-79% of a previously delayed VEP were also observed.

The histophatologic specimens obtained 50 and 75 days after the onset of the chronic relapsing form of EAE when clinical relapses and recoveries, VEP delays and recoveries of delayed VEPs had been observed showed perivenous sleeves of demyelination, together with perivenous inflammatory infiltrations with hystomonocytic and plasma cells. Histopathologic evidence of remyelination in chronic relapsing EAE was reported by Lassman (1983) and Lassmann and Wisniewski et al. (1979) not earlier than on the 80th day after the disease onset, and at least after the formation of the confluent plaques.

In his electron microscopic study of chronic relapsing EAE Rao (1981) showed thin strips of myelin sheath and oligodendroglial cells around demyelinated axons "suggesting" the beginning of remyelination 55-80 days after the disease onset.

In EAE therefore modest myelin alterations, only evident at the electron microscopy, consisting of myelin vesciculation (vescicular disruption) or stripping by macrophages, seem sufficient to induce EP delays.

Together with the early attack to myelin, conduction could be blocked by the myelin alterations leading to a physiological barrier to impulse conduction dependent on inadequate current density between the contiguous areas of normal and abnormal myelin, as suggested by the toxic models of demyelination.

Minor, rapid reorganisation of the axon membrane, with redistribution of sodium channel via lateral diffusion could increment sodium conductance (or decrement potassium conductance), overcome the
physiological barrier and sustain slow continuous conduction. If oligodendrocytes are still intact, "remyelinative" nodes could progressively normalise conduction velocity.

The studies on dissected nerve fibre groups (Bostok et al. 1978, Schauf and Davis 1974, Smith et al. 1982, Waxman 1977, Waxman and Brill 1978, Waxman and Wood 1984, Sears et al. 1978) show that conduction appears on totally demyelinated nerves when axon membranes rapidly reorganise to form foci of inward sodium current (nodes) and that more “ø” nodes, supporting a new saltatory conduction, are evident in areas where early remyelinating activities appear.

The “ø node” model of myelin alteration is compatible with early amplitude alteration of EPs, or with EPs waveform changes, with the early EP delays, with sensitivity of VEPs delays to changes of temperature or electrolytic environment (Regan et al. 1977), and with recovery of EP latencies and amplitudes.

According to this model, though, conduction should fail across an extended plaque and further EP changes should be expected if astrocyte activation appears around the area of demyelination, further altering conduction and leaving to axon membrane reorganisation less chance to restore conduction (Waxman 1988).

In EAE the origin of delayed EPs can not be identified in plaques, since plaques are absent when EP delays are obvious.

The mechanism subserving the delay appears subtler than a conduction alteration across the plaque, and related to the complex pathological alteration of the myelin sheath and early remodelling of axon membrane properties.

In a review on pathophysiology in the clinical course of MS, Waxman (1988) supported the concept that prolonged EP latencies can be interpreted as reflecting, in human subjects, abnormal conduction along demyelinated axons. Waxman (1988) underlined the evidence that EP studies provide demonstration of "focal" alterations, and focused his attention on the mechanism through which demyelination alters conduction. While the basic mechanism might consist of autoantibodal reaction against proteins located within the myelin sheath or protein located in the paranodal part of axon membrane, eliciting the immune response as exposed via loss of the myelin sheath, Waxman insisted that EP and clinical alterations in MS can occur on the basis of the biophysics of demyelination.

Waxman (1988) stressed the findings coming from several of the previously quoted studies, that pathological mechanisms as well as plasticity at several levels contribute to modifications in the
function of demyelinated axons. These mechanisms included cell pathology and plasticity, with
demyelination and/or remyelination and gliosis; molecular pathology and plasticity, with reorganisation
of the axon membrane in demyelinated fibres leading to the appearance of ø nodes; functional
alterations, which affect the excitability of the axon.

He suggested that the participation of multiple pathophysiological mechanism at several organisational
levels (cellular, molecular, functional) might contribute to the complexity of clinical remissions and
explain the difficulty in understanding its physiology.

The myelin stripping effected by macrophages induces a widening of nodal and paranodal regions,
where myelin debris can be found detached from myelin sheaths. Myelin alteration lead to a
physiological barrier to impulse conduction, dependent on inadequate current density between the
contiguous areas of normal and abnormal myelin. The inflammatory process itself might contribute to
the block of conduction: recent experiments on the rat spinal cord have provided evidence that nitric
oxide (which is produced in abundance by macrophages, which are a prominent part of acute lesions in
MS) has a concentration related conduction blocking effect on demyelinated and normal fibres
(Redford et al. 1997).

The experimental evidences suggest therefore that several mechanisms linked to the inflammatory
process and membrane reorganisation in axons stripped of their myelin totally or in prenodal region
might explain the different aspects of EP delays and of the eventual recovery of the delay.

One final argument must be addressed on the conduction properties of different sensory pathways of
CNS. As reported in the initial part of the paragraph, conduction velocity in the CNS is, generically,
considered to be around 10 m/s. Several studies show instead that conduction velocity is different in the
different pathways examined by EP techniques.

In monkeys the conduction velocities of optic nerve fibres range from 1.3 to et al. 20 m/s.

In the visual pathway of primates the myelinated nerve fibres have in the majority a fibre diameter
(axon plus myelin) of 2 microns or less (Potts et al. 1972). These nerve fibres conduct at a maximum
rate of approximately 6 m/s (velocity in m/s=3.2 x diameter in microns) (Ogden et al. 1966). A second
large group of fibres conduct at 8 m/s (Ogden et al. 1966).

The optic nerve fibres serving the central part of the visual field are generally of small diameter and
occupy the lower end of the optic nerve fibres size spectrum (Potts et al. 1972) and conduct at 5m/s or
less. The intraretinal optic nerve fibres conduct much more slowly at 0.5-1 m/s (Ogden et al. 1966).
If the calculations proposed by Bostock and Sears et al. (1978) are accepted suggesting that, if conduction block does not occur, demyelination should slow conduction to approximately 1/200th to 1/50 of the normal conduction velocity, demyelination of a 1 cm segment could increase conduction from 2 ms at 5 m/s to 40 ms at 0.0125 m/s.

For SEP components recorded at cervical level Desmedt and Cheron (1981) recorded a normal conduction velocity of 58 m/s. Since the large axons in CNS have an internode of about 500 µm, conduction velocity in the somatosensory pathway is quite higher than in the visual pathway, and a demyelination extending for 1 cm would induce (according to calculations by Bostock and Sears (1978) and McDonald (1976)) a slowing of less than 10 ms.

The conclusions of this note is that demyelinated areas of same extent could induce different conduction delays: the delay can be at least 4 times longer in VEPs than in SEPs.

Histopathology in MS, plaques vs demyelination and the role of MRI

Acute inflammatory lesions are prominent aspects of early MS lesions, with perivenous infiltrates of lymphocytes, monocytes, plasmacells and macrophages (Guseo and Yellinger 1975, Prineas 1978).

All the histopathological reports in the different studies confirmed the findings of neuropathologists in acute EAE describing absence of demyelinated plaques and a perivascular infiltrate of lymphocytes, monocytes, plasmacells and macrophages (Prineas 1978, Seitelberger 1973). The evolution of acute inflammatory lesions towards the areas of demyelination is admitted on the basis of the evolution towards MS of the acute disseminated leukoencephalomyelitis (Seitelberger 1973, Krucke et al. 1973).

Relatively recent lesions show a partial or complete destruction and loss of myelin throughout a zone formed by the confluence of many small, predominantly perivenous, foci, with sparing of axons and a variable but modest degeneration of oligodendroglia, a neuroglial reaction and perivascular and paraventricular infiltration with mononuclear cells and lymphocytes.

The initial myelin alteration in MS are similar to ones found in EAE (Lassman et al. 1983): myelin stripping due to macrophages (cellular immunity) and vascular disruption due to an antibody mediated reaction (humoral immunity). The myelin stripping effected by macrophages induces a widening of nodal and paranodal regions, where myelin debris can be found detached from myelin sheaths.

Remyelination by oligodendrocytes is described in MS (Lassman et al. 1983, Lumsden 1970) mostly at the border of newly formed plaques, the “remyelinated shadow plaque”. Later, a large number of microglial phagocites infiltrate the lesions and astrocytes appear in and around them. Long-standing
lesions show thickly matted, relatively acellular fibroglial tissue, with only occasional perivascular lymphocytes and macrophages, the “demyelinated sclerotic plaque”.

Oligodendrocytes are spared in early lesions, but there are oligodendrocyte loss and secondary axonal degeneration in old plaque.

**MRI in MS**

The diagnosis of MS has been revolutionised by the introduction of MRI. The characteristic changes are best demonstrated by T2 weighted images. Areas of high signal are seen in the periventricular regions of 98% of patients. The abnormalities are either discrete and focal or confluent T1 weighted images, and Magnetisation Transfer images have properties for identifying parenchimal disruption and tissue disorganisation. The fluid attenuated inversion recovery (FLAIR) has the advantage of greater sensitivity than T2 images but only in the cerebral hemispheres.

MRI lesions are seen to wax and wane in size over a matter of weeks, or residual abnormal signal are seen in the long standing, probably gliotic plaques.

Recent lesions in MS are thought to be identified by MRI because of the larger proportion of tissue occupied by water molecules secondary to oedema and inflammation and accompanying the loss of myelin, which is instead made of lipids.

Recent lesions evidenced by MRI in MS are described as “very similar” to lesions of EAE (Bottomley et al. 1984, Ormerod et al. 1986a,b).

In chronic lesions of MS the MRI imaging is thought to depend on the increase in water content per unit volume (Ormerod et al. 1987) supported by gliosis (since astrocytic processes are cytoplasm rich). Studies of post mortem brains provided covering evidence that MRI abnormalities in MS correspond with plaques detected at post mortem examination, and that the source of abnormal MRI signals in acute lesions is oedema and in chronic lesions is gliosis (Ormerod et al. 1986a,b, Newcombe et al. 1991).

Yet it should be pointed out that the early plaque-like lesions evidenced, in the acute phases of the disease, by alterations of relaxations times or by gadolinium injection (Grossman et al. 1988, Miller et al. 1988a)- should be considered as a very early phenomenon, that is not easily identifiable by the anatomical study.
The disseminated alterations observed by MRI in MS patients should appropriately be considered as an atemporal rendering of the amount of demyelinating lesions, where early oedema or late gliosis should necessarily be differentiated by appropriate techniques. The early abnormal signals due to oedema might undergo different natural evolution, becoming a shadow plaque or a vanishing lesion when the early cause of inflammation and oedema are overcome, or being doomed to become the patognomic demyelinated sclerotic plaque when microglial and astrocytic activation disrupt definitively the chance of recovering functions and induce axonal pathology.

Already in et al. 1996 it was suggested that the term of plaque when interpreting MRI should be confined only to lesions indicating the long standing, probably gliotic areas (Onofrj et al. 1996b).

With different words, but reinforcing the same concept, McDonald (1998) wrote "Standard MRI does not reveal either normal or pathological myelin and the common practice of referring to regions of abnormal signal as areas of demyelination, is wrong, may be misleading in diagnosis and is to be deplored".

Based on the studies quoted above it was likely that, with the introduction of MRI, attempts would have been made to correlate VEP abnormalities in ON with the extent of lesions observed with MRI. The task resulted undoubtedly more difficult than was thought at first approach, because of inherent difficulties in the imaging of optic nerve.

The anatomy of the optic nerve explains some of the difficulties encountered. Each optic nerve consists of approximately 1 million myelinated nerve fibres; the optic nerve-sheath complex measures 4-6 mm in diameter and its length from the globe to the optic chiasm varies between 45 and 55 mm. Successful imaging of the optic nerve is difficult as it is a small, often tortuous and mobile structure surrounded by fat within the orbit, by bone within the optic canal, and by cerebrospinal fluid (CSF). Intracranially, it lies very close to the carotid arteries (Gass and Moseley 2000).

The optic nerve usually can be differentiated in the anterior orbit from surrounding CSF within its dural sheath. The width of the optic nerve sheath narrows progressively from the globe towards the orbital apex with no CSF signal seen in the optic canal.

In ON lesions may involve the partial cross-section of the nerve, can involve the full thickness, while perineural involvement is not common. The lesion of ON typically involves segments of the optic nerve, rather than its full length (Simon and McDonald 2000). Lesions of the intracanicular segment
have been considered a greater risk factor for poor outcome, as swelling may result in bony compression and vascular compromise (Miller et al. 1988b).

The CT study provides only a composite picture of optic nerve combined with CSF and investing tissues (pia-arachnoid and dura), and virtually no information about the intrinsic abnormalities of the optic nerve, unless there is considerable enhancing tissue or expansion. MRI has become the method of choice for evaluating the optic nerve in optic neuritis and MS.

Despite the high sensitivity of MRI in detecting brain lesions in patients with MS, the initial attempts to detect ON were disappointing (Atlas et al. 1988, Guy et al. 1992, Larsson et al. 1988), because of the chemical shift artifact between optic nerve and orbital fat observed with T1-T2 weighted sequences. The high proton density and short T1 relaxation time of orbital fat results in a high signal intensity concealing intraorbital structures and contributing to partial volume averaging artifacts. These problems were partly overcome by the introduction of the short tau inversion recovery (STIR) sequence with conventional short time echo (STE-STIR) (Daniels et al. 1986, Simon et al. 1988).

Early MRI studies of the optic nerve in patients with a clinical diagnosis of optic neuritis were unrewarding (Sobel et al. 1985), until et al. 1986 when Miller et al. (1986) drew attention to the fact that high signal lesions could be detected in about 85% of individuals with a clinical diagnosis of acute optic neuritis using STIR sequence. Patients who recovered vision slowly or incompletely had longer lesions than those who made a rapid and/or good recovery; poor recovery was also associated with lesions in the posterior, intracanicular segment of the nerve.

STE-STIR sequence evidenced increased signal intensity of optic nerves in 70% of acute ON, and in et al. 20-50% of optic nerves studied when acute ON had recovered (Miller et al. 1988b, Youl et al. 1991a).

STE-STIR sequence, with inversion time (T1) at the “null point” of fatty tissue, results in a signal void of the orbit fat: the normal optic nerve however, appears relatively hyperintense and clear separation between optic nerve and perioptic cerebrospinal fluid (CSF) is lost on STE-STIR images, because in sequences obtained with short T1 (<150 ms), image contrast is dependent on T1 and T2 (Hendrick and Roff 1992).

Although the image contrast in STE-STIR domain is due to the additive effects of correlated T1, T2 and N(H) inherent contrast (Hendrick and Roff 1992), when a TE reaching 80 ms is used it is possible to obtain a contrast differentiation between oedema/gliosis and CNS structures. Based on this
theoretical issue, Finn et al. (1991) introduced a double time echo STIR sequence (STE:22 ms and LTE:80ms) and obtained an increment of the signal to noise ratio in comparison with the spin echo T2-weighted images of white matter lesions.

The STE-STIR images are characterised by signal intensities very similar for optic nerve (nerve fibres and perineural space), cerebral cortex, CSF and white matter lesions, so that the presence of oedema, demyelination of proliferative glial events is not always identifiable. A further technical problem lies in the non-homogeneous detection of the signal by the head coil, that render STE-STIR more sensitive to magnetic field dishomogeneities, something resulting in a left-right difference of signal intensity of intracranial and intraorbital structures (Atlas et al. 1988). This may led to misinterpretation of MRI images, particularly when comparing signals of the two optic nerves, or of each nerve with other parts of the brain. The LTE-STIR images of normal optic nerves by approximating a T2 contrast (Finn et al. 1991, Hendrick and Roff 1992) can allow a distinction between meningeal sheath and nerve fibres, while cerebral cortex, CSF and white matter have different signals intensity and are homogeneously represented in the two sides of the head.

Onofrj et al. (1996b) compared the sensitivity of STIR sequences with an STE of 22 ms and an LTE of 80 ms in detecting lesions of optic nerves in 26 patients with acute or previous ON assessed by clinical, neuro-ophtalmological examinations and visual evoked potentials recordings. They showed that LTE-STIR sequence is more effective than the STE-STIR sequence in showing acute or previous lesions of the optic nerve in patients affected by ON. The LTE-STIR sequence gives an improved contrast resolution of ON increasing by 14% the detection of abnormality in acute ON and by 35% the detection of previous ON, where the altered signal is probably due to glial reorganisation. Furthermore, altered signals are consistently detected in more slices with LTE-STIR than with conventional STE-STIR sequence. Precise measurement of the optic nerve is however considered barely reliable because of oblique position of the nerve that lies 5-10% off a true perpendicular axis. Despite the difficulties, some attempts were made to correlate the size of MRI observed lesions with EP alterations: as expected a lack of correlation between MRI evidenced plaques and EP alterations was described, and this even though MRI is able to identify lesions of 5 mm or less width (Ormerod et al. 1986a,b); in this study the authors conclued that “the mechanism of EP delays is still obscure”. The theoretical delay of 25 ms per 1 cm of demyelination was not confirmed.

VEP delays of 15 ms or more were recorded (Onofrj et al. 1996a, Youl et al. 1991) when no lesions were evidenced with MRI. The amount of delay was not strictly dependent on the size of the newly
formed or old plaque since MRI observed lesions, often of more that 1 cm extent, were inducing small EP delays and severe delays were found in sensory pathways that did not appear attained by lesions evidenced by MRI.

All in all the comparison of focal MRI lesions in MS and EP delays shows an incongruence between the extent of lesions and the amount of delays: acute as well as chronic lesions can delay conduction in MS (Onofrj et al. 1996a).

A better estimation of the type of damage affecting the optic nerve is coming from the application of some new MRI techniques, such as magnetization transfer ratio (MTR). In a recent study the optic nerve affected by demyelinating neuritis had a significantly lower mean MTR compared to the value observed in the optic nerve with a full recovery (Inglese et al. 2002). Interestingly enough, optic nerves affected by a degenerative disease, Leber Hereditary Optic Neuropathy, had also very low values of MTR, indicating that this measure provide information on axonal degeneration.

In order to understand the relationship between VEP delays and MRI alterations one further argument, concerning the interpretation of VEPs, should also be considered.

**Delayed and pseudodelayed VEPs in ON**

Current interpretation of the EP delay mechanism (Chiappa and Perez-Arroyo 1992, Halliday 1982) is still based on the classic discussions on pathophysiology of ON by McDonald (1977) and Halliday et al. (1977a,b). These authors described a loss of the VEP in the acute phase of ON. During follow up, when vision improves a delayed VEP of small amplitude is recorded. The VEP amplitude progressively recovers, in parallel with the recovery of visual acuity. According to Halliday et al. (1977a,b) the increased latency of delayed VEPs does not return to normal, and they conclude, therefore, that "once established, the latency for a particular patient appears to be a stable phenomenon, unless a further attack of ON occurs, which may increase the delay". Their findings were used to hypothesise that "the amount of delay depends on some fairly stable anatomical feature, such as the length of the demyelinated fibres in the plaque, while the amplitude recovery may reflect the restoration of conduction in initially blocked nerve fibres".

The Queen Square group (Youl et al. 1991) furtherly attempted to correlate VEP abnormalities with magnetic resonance imaging (MRI) findings and, contrary to their previous descriptions, they reported that the latency of VEPs recovered by 10-33 ms, in at least 50% of their patients, in the et al. 20-32 days following acute ON and returned to normality, in the same time, in 3 of 9 patients.
Although in one of his books Halliday et al. (1982) wrote "the return of a delayed PR-VEP to normal latency is more common than was formerly believed", the discussion of the paper by Youl et al. (1991) kept the initial interpretation on the pathophysiology of VEP delay: "in the recovery phase the amplitude recovers (though not completely) but the delay persists". Other authors have instead described "early" delays in ON and "early" delay reductions (Asselman et al. 1975, Matthews and Small 1979, Becker et al. 1984, Papakostopoulos et al. 1989). Fig. 4 shows further examples of VEP findings in MS with follow up.

While the hypothesis of the Queen Square group (Halliday et al. 1977a,b, 1992, McDonald 1977, Youl et al. 1991) focused the interpretation of EP abnormalities on the formation of the "classic" demyelinated plaque, other authors, as reported in previous paragraphs (Waxman 1988, Takigawa et al. 1995), suggested that some EP abnormalities are dependent on alterations at the "molecular" level (e.g. Na+ channels may be the target of antibodies).


These authors (Halliday 1982, Blumhardt et al. 1982, 1984, 1985) pointed out that examination of the published illustrations of delayed VEPs in MS patients revealed obvious wave-form differences between the responses from the affected and asymptomatic eyes of same patient. The interpretation of VEP latency changes is a difficult task as VEPs are compound responses resulting from the summation of several definable “major” components. A lesion of the optic nerve or retina, blocking the afference that generates a “major” component (e.g. P1 or P100), will induce disappearance of this major component while other components, which were concealed by this component, will become prominent on scalp derivations.

These other components might be erroneously interpreted as “delayed” VEP components, but they are the result of a partial block of the visual pathway, rather than of a “delayed” conduction shifting normal components: in case of partial block, the delay is therefore only a “pseudodelay”.

Using an array of electrodes, Blumhardt et al. (1982, 1984, 1985) showed that the activity evoked by a full-field (FF) stimulus presented centrally is a compound response resulting from the sum of a number of definable major components, including N75, P100 (major positive) and N140 components ipsilateral
to the stimulating half-field (HF), and of P70, N105 and P135 controlateral to the half-field. Onofrj et al (1987, 1990) described the same components, but called them N1-P1-N2 and iP1-iN2-iP2 or c (controlateral) cP1-cN1-cP2, as the latency of the different is changed by the use of stimuli of different spatial frequencies. Fig 5 shows examples of the different components.

The conclusion drawn in the different papers by Blumhardt et al. (1982, 1984, 1985) is that any of the different VEP components may be prominent in different situations, and that the controlateral P135 (iP2) component is often erroneously interpreted as a “delayed” P100 (P1) component (Blumhardt et al. 1982, 1984, 1985). The redefinition of abnormal VEPs as “delayed” and “pseudodelayed” or “apparently delayed” VEPs was therefore suggested (Blumhardt et al. 1982, 1984, 1985). Fig. 6 shows examples of the pseudodelayed VEPs.

Furthermore, Blumhardt (1986) also suggested that VEP studies could be divided into two main categories: “first generation studies”, describing the VEPs recorded using only one derivation, and “second generation studies”, in which VEPs are recorded using multiple electrode array. The latter makes it possible to verify whether an apparently delayed VEP is in reality the result of the disappearance of one of the components and the “dominance” on the midline of other components which are not evident in normal recordings.

Onofrj et al. (1996a) evaluated VEP findings in acute and previous ON by comparing results with LTE STIR MRI of optic nerves and with visual field tests.

20 patients affected by ON underwent serial VEP recordings, performed with multiple electrode arrays, and with stimuli of 1 and 3 cycles per degree (cpd) for 1 year. Many studies (Celesia 1984, Onofrj 1990, Novak et al. 1988, Dawson and Maida 1984) have shown that different pattern element sizes can help to identify VEP components in abnormal VEP waveshapes. Distinct alterations corresponding to delay or pseudodelay could be found in the same patient with 1 and 3 cpd, the first corresponding to the contrast sensitivity peak of paracentral-eccentric retinal projections, the second corresponding to contrast sensitivity of central retinal projection, 2-6° radius from the anatomical fovea (Dawson and Maida 1984, Bodis-Wollner et al. 1987a). This evidence confirmed the hypothesis that parallel spatial frequencies (SF) pathways can be differentially affected in ON (Novak et al. 1988, Dawson and Maida 1984, Bodis-Wollner et al. 1987a,b, Plant et al. 1992).
During follow-up several VEP changes were observed: VEPs at onset were present in 28.5% (1cpd) and 14.3% (3cpd), but delayed or pseudodelayed VEPs were recorded in all patients 3 weeks after the onset of ON.

The latency of delayed VEPs to 1 or 3 cpd stimuli could decrease in the first weeks or months after acute ON, and the amplitude could increase.

In the pseudodelayed VEPs, either normal or delayed components appeared in the first 4 months following acute ON or pseudodelay remained as a persistent findings, with minor amplitude or latency changes.

The VEP findings were tentatively categorised into major groups. In the first group, pseudodelayed VEPs to 1 or 3 cpd stimulation were recorded 3 weeks after acute and were persistent findings 4 months after ON; in these patients persistent pseudodelays were observed at 1 year, with prominent central visual field abnormalities, especially in those patients with pseudodelayed 3 cpd VEPs.

In the second group, delayed or normal VEPs to 3 cpd were recorded at least 4 months after acute ON. In this case, only paracentral visual field abnormalities were recorded 1 year after ON. VEP amplitudes (and latencies) for 3 cpd stimulation improved progressively; VEPs to 1 cpd stimuli could be delayed or pseudodelayed.

MRI showed lesions in 95.2% of acute ON and in 66.6% of the 1 year follow up. The longest lesions were observed in patients with lowest visual acuity, most severe field defects, and smallest VEP amplitudes. These correlation were found in the acute as in the follow-up evaluations.

Both in the acute and in the follow-up studies, lesion length never correlated with the latencies of VEP components; a correlation with lesion lengths was instead observed in patients with pseudodelayed VEPs.

The conclusion from this study was that when delayed or normal VEP components appear in the first 4 months they are accompanied by functional recovery. Otherwise, field defects persist and are paralleled by VEP pseudodelays. This 4 months time limit corresponds to the known break-point in predicting functional recovery, as described in previous reviews (McDonald and Barnes 1992). This correspondence between functional recovery and delays and between earliest delays and best prognosis shows that delays can be considered as the effect of conduction reorganisation in the visual pathway.

The correlation between pseudodelays and visual field defects might suggest that mechanisms other than demyelination are involved in the generation of pseudodelays: either the oedematous nerve fibres
might suffer from compression and ischemia, because the fibrous septa of the optic nerve and the bony optic canal limit expansion, or, as suggested by previous studies (Holder 1991), retrograde degeneration might induce retinal involvement and contribute to the type of VEP abnormality.

Early delays could be reasonably be interpreted as evidence that mechanisms other than the cellular events that lead to plaque formation or remyelination are acting shortly after the acute myelin lesions of ON or MS relapses.

The paradoxical conclusion for this study was that, as VEP delays in ON appear when vision is recovering, real delays should be interpreted as a favourable sign, indicating that the mechanisms involved in the restoration of conduction are successfully overcoming the demyelination lesions. Pseudodelays consists of inverted polarity components (mostly at N105 and a P135) with a controlateral scalp distribution to HF stimuli, contrary to normal N1 (N79) or P1 (P100), which have the normal paradoxical ipsilateral distribution to HF stimuli or quadrant stimuli. They are found when central scotomata are present and can be interpreted as evidence of conduction block: unless a breakthrough of normal or delayed components appears during the first 4 months after acute ON (if scotomata improve), the presence of pseudodelays indicate irreversible persistence of central scotomata and represent the evidence of unfavorable prognosis in demyelinating disease.

**VEPs and PERGs changes during ON**

An alternative way of reaching prognostic evaluation in ON is based on simultaneous recording of pattern electroretinogram (PERGs), and VEPs. PERG consists of two main components P50, at approx. 52 ms, and N95, at approx 93 ms (Holder 1991). When the optic nerve axons are severed by a traumatic or inflammatory disease, PERG can disappear because of retrograde degeneration (Celesia et al. 1986, 1987), while flash ERGs and part of VEPs will still be recorded (Maffei and Fiorentini 1981). Therefore the disappearance or amplitude reduction in PERGs will indicate unfavourable prognosis for ON.

In ON however the most relevant finding was an amplitude reduction of N95 (Kaufman et al. 1988, Tobimatsu et al. 1989). Froelich and Kaufman (1994) showed that N95 PERG abnormalities are evidenced if the central 8° visual field radius are persistently involved by the visual loss.

PERGs abnormalities appeared furthermore later than the 4-months breakpoint for pseudodelays and in less than 12% of patients after the first ON. Moreover, in patients with repeated ON attacks the kind of
VEP abnormality which accompanied relapses gave earlier information than PERGs. The PERG recordings, therefore, can only track the course of a minority of patients affected by ON, as already suggested in other PERG studies.

**Recording of VEPs to different stimuli in MS patients.**

Diagnostic value of VEPs is dependent on the characteristics of stimulus, such as type and dimension of pattern elements, orientation, mean luminance, contrast chromaticity and stimulated field dimension (Porciatti and Sartucci 1996, Camisa et al. 1981, Bodis-Wollner et al. 1986, 1987a,b, Bobak et al. 1987, Celesia 1982)

Camisa et al. (1981) noticed an orientation dependent delay in VEPs in 50% of MS patients that matches with results of a recent study (Logi et al. 2001).

Onofrj et al. (1987) recorded VEPs to different spatial frequencies (1, 2, 4, cycles per degree, corresponding to 30, 15 and 7.5 minutes of visual angle) and different stimulus configurations (vertical, horizontal gratings and checkerboard) in 47 MS patients and in controls subjects. Changing the stimulus configuration (vertical to horizontal bar or checkerboards) and spatial frequency raised the diagnostic yield by 2-3% and 12% respectively.

The medium spatial frequencies, that is 4 cpd, which gives the highest diagnostic yield, elicit consistent and reliable VEPs only when gratings (or bars) patterns are used. Checkerboard patterns of 7.5’ or arc size (corresponding to the width of 4 cpd gratings) give smaller VEPs than bars of the same sample size. Half-field stimulation might throw light on some diagnostic controversies dependent on the finding of “W” shaped P1 components, but, all in all, the use of half-field stimuli was not helpful in increasing the VEP diagnostic yield in MS (Celesia 1978). Moreover, whereas coarse pattern (1 cpd or less) may elicit “W” shaped VEPs, fine patterns (4cpd) elicit always VEPs with a regularly shaped major positive component. For these reasons, Onofrj et al. (1996a) suggested that 2-4 cpd (near to the contrast sensitivity function) grating patterns might be the best for the diagnosis of MS and ON.

To study the effects of stimulus spatial orientation (Onofrj et al. 1987) on VEP it is necessary to use a grating rather a checkerboard pattern, since checkerboards stimulate simultaneously many different spatial frequency (SF) with several orientations, especially oblique (Bodis-Wollner and Camisa 1980, 1986), while gratings, vertical or horizontal, work on a single SF and orientation (Camisa et al. 1981). Such stimulation is the best to assess retinal response, being the monodimensional sinusoidal stimuli
the most elementary for the retina, while any other pattern is to be considered as sum of several single sinusoidal components (Andrews and Pollen 1979, Parker et al. 1982).

Contrast sensitivity (CS) is defined as the reciprocal of minimal contrast necessary to perceive a given SF and allows the analysis of visual sensitivity to several stimuli and dimension (Bodis-Wollner et al. 1980, 1986). The human CSF has a “dome” shape with a peak at 4/6 cycle/degree (c/d) and attenuation for high and low SF (Bodis-Wollner et al. 1986, Sartucci and Domenici 1993).

CS is affected by refraction errors (Campbell and Green 1965, Mitchell et al. 1974, Maffei and Fiorentini 1976), luminance reduction, binocular vision which diminish CS threshold, and pattern orientation (Bodis-Wollner et al. 1986, Sartucci and Domenici 1993). Campbell and Maffei (1970) found out that CS is higher for vertical bars as respects to other orientations. CS and pattern VEPs are strictly related because there is a direct logarithmic relation between amplitude of pattern VEPs waves and contrast used (Bobak et al. 1987).

Kupersmith et al. (1984) recorded grating VEPs in MS patients with 3 SF and 4 pattern orientation, finding out an orientation-specific loss, both monocular and binocular, without any prevalence of one orientation respect to others. To explain these results the authors suggested a cortical involvement. Since typical MS lesions are placed at the edge of white and grey matter, there should be a functional loss of cortical architecture, in order to explain this feature.

Logi et al. (2001) have recently demonstrated that the use of vertical grating in clinical routine is more reliable both for VEPs and contrast sensitivity (CS) luminance testing. In fact the rate of abnormalities resulted higher for P100 wave, followed by N70 and P60 and for all vertical respect to horizontal grating. In MS patients with a history of ON or even without clinical visual dysfunction, CS analysis reveals deficits for some SF and orientations (Camisa et al. 1981, Regan et al. 1980, Coupland and Kirkham 1982, Kupersmith et al. 1984, Leys et al. 1991) providing more information than routine pattern VEPs (Nordmann et al. 1987, Neima and Regan et al. 1984). In addition CS can be abnormal even with normal VEPs; this could mean an early impairment of CS and provide useful indications about a subclinical involvement of visual cortex. Even if for clinical routine analysis VEPs represent the more suitable tool to investigate visual pathways in MS, CSF could represents the most sensitive technique.

*Chromatic VEPs*
VEPs to patterned stimuli with pure chromatic contrast (red-green) have been reported to represent a useful tool to evaluate visual involvement in MS (Porciatti and Sartucci 1996). To compare the relative involvement of the two colour opponent subsystems of the visual pathway (red-green: R/G, subserved by the P stream, and blue-yellow, subserved by the koniocellular stream), Porciatti and Sartucci (1999) developed a set of stimuli parameters yielding robust and reliable onset VEPs, specific for either pathway.

They studied 30 patients with MS, 17 of them had active symptoms or had a past history of ON, compared to controls. Chromatic visual stimuli were R/G or B/Y equiluminant horizontal sinusoidal gratings of 2 cpd and different Michelson contrast (90 and 25%). For both types of stimuli and contrast levels, the main response component was a negative-positive complex at stimulus onset, which represents the chromatic VEP response. The rate of latency abnormalities depended on the degree of disease, being much higher in definite MS than in probable MS. The study showed that equiluminant R-G and B-Y VEPs are altered in MS to the same extent. These data were in contrast to the reported anatomical and functional differences in fibre size and number (Porciatti and Sartucci 1999, Livingstone and Hubel 1988, Lennie et al. 1997, Dacey 1994, Rabin et al. 1994) present in the optic pathways and also in contrast with the findings that the blue-cone pathway is particularly vulnerable to many retinal and post-retinal disease (Korth et al. 1994).

Moreover, chromatic VEPs in this patient group were more abnormal than standard transient VEPs to reversing checkerboards, even if these differences were not statistically significant. While equiluminant stimuli are adequate to activate selectively the colour-opponent pathways, achromatic stimuli are not selective for the achromatic pathway. The high rate of abnormalities found by Porciatti and Sartucci (1996) with chromatic VEPs confirmed the high vulnerability of the visual subsystem involved in colour processing, probably due to smaller diameter and less myelinated sheath of their axons compared with those of the M stream (Porciatti and Sartucci 1996).

Pragmatically, the chromatic stimulus does not appear to increase substantially the sensitivity of standard VEPs in clinical testing of patients with MS. However, the combined assessment of chromatic and achromatic VEPs to selected stimuli provides a means for a more comprehensive evaluation of the visual pathway and a better understanding of the pathophysiology of MS.
VEPs in MS monitoring

The demonstrated stability of evoked responses is an important requisite for their utilisation in follow up studies. All the studies on the reproducibility of early components of EPs revealed a quite low variability of morphology and latency, which never exceeded 10% (Hammond et al. 1987; Shaw and Synék 1987; Andersson and Persson et al. 1990; Romani et al. 1992, Bergamaschi et al. 1992). However test-retest variability is usually larger in MS population, because of both technical and physiopathological reasons (Aminoff et al. 1984; Anderson et al. 1987; Ingram et al. 1988).

Different methods have been utilised to evaluate the longitudinal EP changes: latency and amplitude measures, non parametric measures (improved, stable, deteriorated), conventional scores taking into consideration both latency and morphology of the responses, indices expressed as Z-scores (Sorensen et al. 1996; Aminoff et al. 1984; Bednarik and Kadanka et al. 1992; Iragui et al. 1986). The modifications of amplitude or morphology of the responses or the absence of an expected wave can be problematic to interpret because such findings may occur for technical problems or poor co-operation of the patient. To be accepted as an abnormality they have to be consistently reproduced in repeated exams. There is also uncertainty about the magnitude of latency change which can be significant of improvement or deterioration in conduction. It has been proposed to consider as significant only the latency changes exceeding the mean value + 2 SD of the test/retest variability observed in the control population or the maximum interside difference observed in normal subjects (Aminoff et al. 1984; Anderson and Persson 1990). A significant increase of latency or deterioration of the morphology of the evoked response at two consecutive examinations indicates that the damage in the explored pathway has progressed. The EP changes may be reversible, indicating the efficiency of the reparative mechanisms, but if they persist for more than 6 months a successive improvement of the responses should be considered highly unlikely. Even if a latency prolongation does not "per se" implicate a deterioration of the corresponding neurological function, it indicates that the "safety margin" of that specific pathways is reduced.

No or equivocal correlation between clinical and EP changes has been found in some studies (Matthews and Small 1979; Aminoff et al. 1984; Davis et al. 1985b); while a good correlation has been found in others (De Weerd and Jonkman 1982; De Weerd 1987; Walsh et al. 1982; Ghezzi et al. 1986; Nuwer et al. 1987; Andersen and Siden 1991). Some authors (Anderson et al. 1987) criticised the use of EPs in longitudinal studies because clinical and EP factors often change independently, as well as different EP modalities. We believe that this is exactly what we can expect from the nature of the
disease, with a complex interplay of demyelination and remyelination, quite variable from time to time and from site to site in the CNS.

A study on a small sample of MS patients (Hickman et al. 2002) evaluated serial MRI of the optic nerve using fat-saturated short-echo fast fluid-attenuated inversion recovery (sTE fFLAIR) sequence, with basal evaluation performed after a median time of 19.5 months after ON and the second 1 year later. The mean area of the affected optic nerve significantly decreased at follow up and atrophy was associated with poor visual acuity and decreased VEP amplitude.

Fuhr et al. (2001) combined the serial evaluation of VEP and motor evoked potentials (MEPs) in 30 patients with relapsing-remitting MS. The latencies of VEPs and MEPs over time were correlated with EDSS changes; moreover, VEPs and MEPs at baseline correlated with the EDSS after 2 years, suggesting a possible predicting value of electrophysiological assessment with respect to the clinical course. In 11 patients with chronic progressive MS, serial VEP were performed together with BAEP and MRI scans (Sater et al. 1999). Despite no detectable changes in the EDSS, in the ambulation index and in the MRI T2 plaque burden and BAEP parameters did not show significant changes over a 1.5 year period, significant progression of VEP P100 was observed. In a 2-year follow-up study using VEPs and psychophysical methods 31 patients after ON (Brusa et al. 2001), improvement of latency occurred in the affected eye over the first and, to a lesser extent, over the second year, while contrast sensitivity improved during the first 9 months, but successively tended to deteriorate. The authors suggested that even though improvement of VEP latency was not strictly related to functional improvement, it could be due to ion channel reorganization or to remyelination occurring even after two years after ON and which may have an important role in protecting the axons from degeneration. Similar findings have been reported by the same group in a 6 months and 3 years follow-up (Brusa et al. 1999), with concomitant significant latency prolongation in the fellow unaffected eye, the latter findings suggesting a latent pathological process occurring also in the unaffected eye.

In 90 untreated patients at the onset of ON and after 2, 4, 12 and 52 weeks (Frederiksen and Petrera 1999). After one year, of the 69 patients (77% of total) with initially abnormal VEPs, 19% showed VEP normalization. Latencies, amplitudes and combined VEP scores in the eyes with acute ON were significantly associated with the time after onset. Latencies were shorter in patients with isolated ON than in patients with ON as part of clinically defined MS, possibly as a correlate of disease duration and insidious demyelination prior to ON. In another study, multimodal EPs showed minor changes
after one year in 70 patients with acute ON (Frederiksen et al. 1996) except VEP in eyes with acute ON.

VEPs combined with other EPs have been used as secondary end points in some clinical trials, they did not provide useful information in most of the studies (Smith et al. 1986; Weiner and Dawson 1980; Compston et al. 1987; Dweer et al. 1987; Dau et al. 1980; Knobler et al. 1984). Nuwer et al. (1987) utilized multimodal EPs in a 3 year controlled study on the effects of Azathioprine, with and without steroids, in chronic progressive MS. A significant difference in VEP and SEP in favour of active treatment group was observed one year before corresponding differences were seen clinically.

VEPs have been also utilised to monitor the effects of drugs modifying the nervous conduction in partially demyelinated fibres. An improvement of BAEP and VEP has have been observed in a group of MS patients treated with Verapamil, a calcium antagonist (Gilmore et al. 1985) and more recently a significant improvement of amplitude and latency of VEP was observed in patients treated with 4-aminopyridine, an inhibitor of the potassium channels (Davis et al. 1990; van Diemen et al. 1993).

Conclusions

Visual evoked potentials are very easy techniques currently routinely utilised in most of the laboratories of clinical neurophysiology. The tests produce only minor discomfort to the patients, and do not produce damages; EP can be performed in a few minutes with very low costs, particularly if compared to MRI exams. The major VEP components are quite reproducible and the estimation of the signal to noise ratio and of the residual noise for the averaged evoked responses allows to carefully define the technical quality of the exam. The use of VEPs, and of EPs in general, in multiple sclerosis has been essentially limited to the diagnostic phase, but there are accumulating evidences that EPs are useful also in the monitoring of the disease evolution. Even if MRI is by large superior to EPs in revealing the disease activity, EPs are more strictly related than MRI to the impairment and disability affecting the patient. The changes of evoked responses describe the loss of function consequent to the accumulation of lesions along the sensory and motor pathways. Small lesions invisible to MRI and microscopic changes of the white matter affect the evoked responses. In patients who complain of vague and indefinite disturbances they allow to objectivate the nervous dysfunction, moreover they can reveal the subclinical involvement of the sensory-motor pathways.
The use of EPs as paraclinical measures in clinical trials have been limited, mostly because of difficulties in the standardization of the procedures among labs. This criticism is not justified, as recently indicated by Brigell et al. (1994) who demonstrated the feasibility of the utilization of VEP in a multicentre trial. As discussed it has been already proved that evoked responses can be useful in evaluating new treatments to modify the disease course in MS and symptomatic treatments. For such an utilisation the two main limitations are the more advanced phases of the disease, because most of the evoked responses can be absent, and the early phases of the disease because most of the responses are normal.

The phase II clinical trials are performed in order to select drugs potentially effective on the disease course; with this type of trial it is important to demonstrate that the tested treatment is able to modify the disease activity. MRI, for its high sensitivity to detect the disease activity is an excellent surrogate clinical endpoint, while evoked responses are not useful because their sensitivity to the disease activity is quite poor. On the contrary in phase III clinical trials it is important to demonstrate that the tested treatment is able to reduce the progression of disability and the increase of the nervous damage. Evoked responses are an objective and direct measure of the function of the nervous pathways and therefore may support the clinical measures in the assessment of the effects of the tested treatment.

Evoked potentials may also play an important role in the evaluation of treatments promoting the remyelination or improving the nervous conduction. For this type of therapeutic interventions evoked responses should be used both in phase II clinical trials to select potentially effective treatments and in phase III clinical trials to objectively confirm the clinical effects.
References


**Figure Legends**

Figure 1. Visual field abnormalities detected by Humphrey Field Analyzer, evaluating the central 10° of visual field, in 20 patients with ON. Levels of gray indicate statistical significance in comparison with 1000 age matched controls (P<0.5, P<0.1, P<0.005, P<0.01, P<0.005).

Figure 2. Transient VEP of a patient with Definite MS without ocular disturbances. The upper row is the right eye. The CII latencies are bilaterally delayed by about 30 ms both to 30’ and 15’ checks. Note the marked amplitude asymmetry which causes some difficulty in finding the CII peak with 15’ checks stimulation.

Figure 3. (a) Computed action potentials for a fiber focally demyelinated in the region between D1 and D4, carried out under the assumption that the demyelinated axon membrane had developed a high density of sodium channels (similar to that of normal nodal membrane). Despite the assumption of excitability in the demyelinated zone, there is failure of invasion of the action potential from the normal region into the demyelinated region. This conduction block is a result of inadequate current density at the transition region between myelinated and demyelinated zones, primarily because of capacitative current loss.

(b) Computed action potentials for a demyelinated fiber similar to that shown in (a) to show the effect of interposing several short internodes (f nodes) proximal to the demyelinated zone. (f=f nodes).

Figure 4a. VEPs in a patient affected by ON (4 cpd reversal stimuli). Notice latency modifications (reported beside each set of traces) appearing shortly after the onset of ON (5/3/83). Notice also further latency increments in serial follow-up after ON.

Fig 4b. Visual evoked potentials to pattern reversal recorded from a girl 13 years at presentation, 3 days, 9 days and 12 years after onset of an attack of acute bilateral ON. (Kindly provided by Drs M Halliday and A Kriss).
Figure 5. Organization of VEP generators as suggested by Blumhardt et al., 1987. NPN components are ipsilateral to the stimulated hemifield and generated in the central retinal projection of primary visual areas. The cP-cN-cP components (labelled PNP) are generated in the peripheral retinal projection of primary visual areas.

Fig 6a. Delayed VEPs to 3 cps, pseudo-delayed VEPs to 1 cpd (with bifid waveshape) with abnormal distribution. The abnormal distribution of 1 cpd VEPs is explained by the prominent field defect involving the lower paracentral field. The VEPs to the left hemifield therefore consist of iP1-iN1-ip2 components, widespread on the scalp. The components from the left and right half-fields together generate the pseudo-delayed VEPs to 1 cpd. The 3 cpd VEPs are really (symmetrically) delayed, and this delay is further reduced after 1 year. The real delay, and normal distribution of 3 cpd VEPs corresponds to central vision recovery.

Fig 6b. Pseudo-delayed VEPs to 3 cpd persisting 1 year after bilateral ON. Notice that the visual fields are also abnormal 1 year after ON and, although the visual acuity of the patient recovered because one half of the central fields were intact, the abnormal fields incapacitate his working life. The distribution of VEPs to left eye stimuli corresponds to the lower field defect, as shown by the polarity inversion in the inion leads: modestly delayed iP1-iN1-ip2 components are recorded from all scalp leads. The distribution of VEPs to right eye stimuli shows a modest delay (P1=137 ms) and right central hemianopsia.

Fig 6c. Spuriously delayed responses from a 47 year old patient with a small absolute central scotoma due to “clinically definite” MS. The major positivity of the wide-field response appears markedly “delayed” at 145 msec, but half-field recordings show that it is composed predominantly, if not entirely, of contralateral P135 waves projecting into the midline (arrows). Note that in each half-field response the ipsilateral channels contain two small positive waves; the first of these are within normal latency limits for P100 components. They can also be seen in the widefield responses. The latency of the second of these two small ipsilateral subcomponents coincides approximately with that of the 135 wave, which appears to project across the midline.