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THE ROYAL SOCIETY

The pathophysiology of multiple sclerosis: the mechanisms underlying the production of symptoms and the natural history of the disease

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The pathophysiology of multiple sclerosis is reviewed, with emphasis on the axonal conduction properties underlying the production of symptoms, and the course of the disease. The major cause of the negative symptoms during relapses (e.g. paralysis, blindness and numbness) is conduction block, caused largely by demyelination and inflammation, and possibly by defects in synaptic transmission and putative circulating blocking factors. Recovery from symptoms during remissions is due mainly to the restoration of axonal function, either by remyelination, the resolution of inflammation, or the restoration of conduction to axons which persist in the demyelinated state. Conduction in the latter axons shows a number of deficits, particularly with regard to the conduction of trains of impulses and these contribute to weakness and sensory problems. The mechanisms underlying the sensitivity of symptoms to changes in body temperature (Uhthoff's phenomenon) are discussed. The origin of 'positive' symptoms, such as tingling sensations, are described, including the generation of ectopic trains and bursts of impulses, ephaptic interactions between axons and/or neurons, the triggering of additional, spurious impulses by the transmission of normal impulses, the mechanosensitivity of axons underlying movement-induced sensations (e.g. Lhermitte's phenomenon) and pain. The clinical course of the disease is discussed, together with its relationship to the evolution of lesions as revealed by magnetic resonance imaging and spectroscopy. The earliest detectable event in the development of most new lesions is a breakdown of the blood–brain barrier in association with inflammation. Inflammation resolves after approximately one month, at which time there is an improvement in the symptoms. Demyelination occurs during the inflammatory phase of the lesion. An important mechanism determining persistent neurological deficit is axonal degeneration, although persistent conduction block arising from the failure of repair mechanisms probably also contributes.

Keywords: demyelination; pathophysiology; conduction block; inflammation; ectopic impulses; axonal degeneration

1. INTRODUCTION

This review describes the pathophysiology of multiple sclerosis (MS) first at the membrane level and then at the level revealed by magnetic resonance imaging (MRI). We therefore first describe the electrical events underlying the production of symptoms, both negative (e.g. paralysis and blindness) and positive (e.g. paraesthesiae). Second, we describe the pattern of pathological activity during the course of the disease as

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revealed by MRI and magnetic resonance spectroscopy (MRS) and how this relates to relapse, remission and irrecoverable deficit.

2. PATHOPHYSIOLOGY OF THE AXON

(a) *Negative symptoms: total loss of function*

(i) *Effects of demyelination*

The expression of symptoms such as blindness, paralysis and numbness is primarily due to the loss of conduction in the appropriate pathways, e.g. in the optic nerve in the case of blindness. An important cause of this loss is axonal conduction block and there is abundant experimental evidence that the demyelination which is characteristic of the disease can cause conduction block. Indeed, conduction block was the first electrophysiological consequence of central demyelination to be reliably demonstrated in the laboratory (McDonald & Sears 1969) and it is the dominant feature of many experimental demyelinating lesions in both the central and peripheral nervous systems. The block occurs specifically at the site of demyelination and conduction along the morphologically unaffected portions of the axon on either side of the lesion is usually considered to be unimpaired (McDonald & Sears 1969, 1970).

The likelihood that conduction will be blocked at a site of demyelination is related to both the magnitude of the myelin loss and the time which has elapsed since the demyelination occurred. Certainly, loss of a single whole internode of myelin is more than sufficient to block conduction in a 'typical' axon and, in our experience (primarily in the dorsal column, spinal root and sciatic nerve axons), a period of conduction block is the rule for at least the first few days following segmental demyelination. This period of apparently absolute conduction block is believed to arise from an initial dearth of sodium channels in the newly exposed axolemma. The axolemma beneath the normal myelin sheath has a relatively low sodium channel density ($<25\,\mu m^{-2}$) (see the review by Waxman & Ritchie 1993) and this may be insufficient for the action potential to be actively propagated, as required, across the demyelinated region (Waxman 1989; Waxman & Ritchie 1993; see also Utzschneider *et al.* 1993).

In our experience, conduction is routinely initially blocked in experimentally demyelinated central axons if the demyelination is segmental (i.e. loss of the whole internode) and conduction remains blocked for at least a few days thereafter (McDonald & Sears 1970; Smith *et al.* 1979, 1981). However, it is theoretically possible that conduction could occur in freshly demyelinated axons, particularly if the axons were of small diameter (Bostock 1994) and if the internode preceding the demyelinated region was relatively short (Waxman & Brill 1978; Shrager & Rubinstein 1990; Bostock 1994).

Apart from segmental demyelination, it is also clear that even a modest widening of the nodal gap may cause conduction block. Indeed, experience shows that loss of a given volume of myelin from the paranodes has a much greater effect on conduction than the equivalent loss distributed evenly along the length of the internode and this view is supported by computer models (Koles & Rasminsky 1972; Chiu & Ritchie 1981; Bostock 1993, 1994; Stephanova & Chobanova 1997). This sensitivity of conduction to an increase in the area of the nodal membrane has several causes, but two are particularly noteworthy. First is the fact that nodal widening results in a significant increase in the electrical capacitance of the node. This increase reduces the safety factor (Rushton 1937; Tasaki, 1953; discussed in Smith 1994) for conduction (see §3(b)(ii)) because it increases the current required to depolarize the node to its firing threshold. Second is the fact that the local currents responsible for depolarizing the node during saltatory conduction are no longer focused to the narrow (*ca.* 1 μm) nodal gap, but rather they become dispersed over the wider 'nodal' area. This dissipation of the local current further reduces the safety factor for conduction. These considerations are presented in more detail elsewhere (Waxman & Brill 1978; Waxman & Foster 1980; Bostock 1984; Hille 1992; Smith 1994).

(The safety factor for conduction can be described as the current necessary to depolarize a node to its firing threshold divided by the current necessary to do so (Rushton 1937). In normal axons, the safety factor for saltatory conduction is around three to five, i.e. the local action current flowing from an active node to the next node is three to five times greater than is actually necessary to fire it (Tasaki 1953). In contrast, demyelinated axons have an inherently low safety factor for conduction (discussed in Smith 1994); in fact, the safety factor is typically reduced to near unity. This is a critical level since small improvements in the safety factor, e.g. to 1.1, mean that conduction will be successful, but, conversely, small decreases, e.g. to 0.9, will result in conduction block. In experimental lesions (and presumably in MS) many axons have a safety factor at unity and therefore small changes in the environment of the axons can have pronounced effects on the expression of symptoms. Temperature is one parameter which affects the safety factor and the dramatic effect that body warming and cooling can have on the expression of symptoms in some patients is described in the section on effects of temperature.)

Although conduction can be restored to demyelinated axons over time (see §2(b)(i)), the conduction remains relatively 'insecure' and prone to failure. Factors known to promote a return to conduction block include local inflammation, a rise in body temperature and a recent history of conducting a large number of impulses: these are described below. Other factors which may reasonably be expected to compromise conduction include a large axon diameter (Bostock & Sears 1978; Waxman 1989), the presence of a long internode immediately preceding the demyelinated region (Waxman & Brill 1978), a sub-optimal molecular adaptation of the axolemma to its demyelinated state (e.g. the arrangement and density of sodium channels, ion pumps, etc.) and, possibly, a paucity of astroglial ensheathment. The presence of a long internode impairs the ability of the driving node to fulfil its role of depolarizing the demyelinated axolemma, because it allows more of the action current to become dissipated (through resistive loss and discharging the internodal capacitance) before reaching its target. Computer models have demonstrated the theoretical advantage conferred by a short internode (Waxman & Brill 1978; Shrager &

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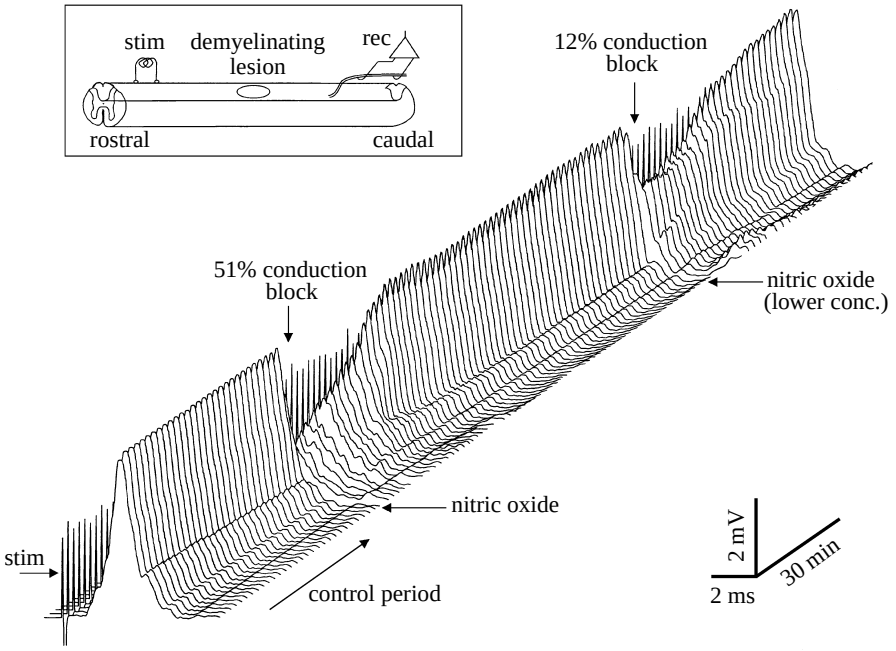

Figure 1. A series of compound action potentials recorded from a dorsal root caudal to a demyelinating lesion induced in the rat dorsal column, as indicated in the diagram. From other recordings it is known that all the axons contributing to the records were affected by the demyelinating lesion. The first record obtained is plotted at the front, and successive records were obtained at 2 min intervals. Following a control period of *ca.* 1 h, an injection of spermine NONOate, a donor of nitric oxide, was made into the demyelinating lesion: the concentration was chosen so that nitric oxide would be released in the concentration anticipated within multiple sclerosis lesions. The injection promptly caused conduction block in approximately half the axons, which recovered during the next *ca.* 30 min as the nitric oxide content of the donor was depleted, and as it dispersed within the spinal tissue. A second injection of a solution partially depleted of its nitric oxide content caused a similar period of conduction block, but restricted to fewer axons. Similar control injections were without effect on conduction. Modified from Redford *et al.* (1997) with permission.

Rubinstein 1990) and this may be able to compensate for other factors which may not be optimal for conduction in a particular axon. Data are limited with regard to the molecular composition of demyelinated axolemma (Waxman & Ritchie 1993), but it is clear that it is remodelled in response to demyelination (e.g. Bostock & Sears 1978; Smith *et al.* 1982) and it is reasonable to anticipate that this will be achieved more effectively in some axons than in others. Remodelling is likely to be a dynamic phenomenon, with the distribution and type of the molecular constituents varying over time (Black & Waxman 1996). One influence which may play a role in the remodelling is the distribution and type of the surrounding cells, such as inflammatory and glial cells. Regional specializations of the demyelinated axolemma have been described, particularly with regard to patches of the axolemma in contact with astroglial processes (Blakemore & Smith 1983; Rosenbluth & Blakemore 1984; Rosenbluth *et al.* 1985; Black *et al.* 1991). It seems likely that the number and diversity of such specializations will become increasingly apparent with advances in the range and specificity of the available probes. Of particular interest will be data revealing the changes in the expression of the different types and subtypes of ion channels, exchangers and pumps (Waxman & Ritchie 1993; Black & Waxman 1996; Rizzo *et al.* 1996).

(ii) *Effects of inflammation*

There is evidence from studies of optic neuritis that inflammation contributes to visual loss (Youl *et al.* 1991; see below). The observations of Moreau *et al.* (1996) suggested that some cytokines may play a role in the conduction block, particularly the pro-inflammatory cytokines tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ). It is clear that cytokines can have indirect effects on neural function (e.g. Brosnan *et al.* 1989) and direct effects on ion channels (e.g. Brinkmeier *et al.* 1992, 1993; Mimura *et al.* 1994; Visentin & Levi 1994), but clear and direct effects on axonal conduction have been difficult to detect (Dugandzija-Novakovic & Shrager 1995; R. Kapoor, E. J. Redford and K. J. Smith, unpublished observations). On the other hand, the cytokines TNF-α and IFN-γ are potent in stimulating the formation of the inducible form of the enzyme nitric oxide synthase (iNOS) (Goodwin *et al.* 1995; Hu *et al.* 1995; Liu *et al.* 1996; Goureau *et al.* 1997) and this enzyme can produce nitric oxide in sustained, high (i.e. low micromolar) concentrations. Indeed, nitric oxide production is raised in MS (Bo *et al.* 1994; Bagasra *et al.* 1995; Johnson *et al.* 1995; De Groot *et al.* 1997; Giovannoni *et al.* 1997; Yamashita *et al.* 1997; Cross *et al.* 1998; Oleszak *et al.* 1998) and so it is interesting that low micromolar concentrations of nitric oxide have recently been demonstrated to mediate conduction block in normal axons (Redford *et al.* 1997; Shrager *et al.* 1998) and, in particular, in axons affected by demyelination (Redford *et al.* 1997) (figure 1). Interestingly, nitric oxide blocks axonal conduction within minutes of exposure and the block is maintained for hours if exposure is maintained. However, conduction is restored within minutes of removal of nitric

oxide. The mechanism of conduction block remains uncertain, but a direct effect on ion channels, particularly sodium channels (Li *et al*. 1998) or on mitochondrial energy production (Bolanos *et al*. 1994, 1997; Brown *et al*. 1995) may be involved. Since, as noted above, there is good evidence that nitric oxide is produced in raised concentrations in MS lesions, it is likely that at least some of the clinical deficit associated with inflammation may be mediated by nitric oxide. If so, measures which reduce nitric oxide production may be effective therapeutically and in this context it is interesting that one action of IFN-β (a recently introduced therapy for MS; Hall *et al*. 1997; Goodkin 1998) is the inhibition of iNOS (Stewart *et al*. 1997; Guthikonda *et al*. 1998; Hua *et al*. 1998).

Inflammation may theoretically also contribute to neurological deficit by modifying the properties of glial cells, particularly astrocytes and microglia (Chao *et al*. 1995; Lee *et al*. 1995; Merrill & Benveniste 1996; Ridet *et al*. 1997). Astrocytes are believed to be important in regulating a wide range of chemical factors in the brain, including the potassium ion concentration and it is easy to imagine that a disturbance in these cells will have consequences on neurological function (see e.g. Largo *et al*. 1996), particularly in demyelinated axons. Apart from their intimate association with neurons, dendrites and synapses, astrocytic processes are also components of the central nodes of Ranvier (Sims *et al*. 1991), where they presumably play an important role in maintaining normal function.

(iii) *A role for synaptic transmission?*

Inflammation in MS is not restricted to the central white matter, but also occurs in regions with a high synaptic density, such as the cerebral cortex (Kidd *et al*. 1999). Several factors associated with inflammation have been shown to disturb synaptic transmission in normal tissue, including interleukin-1 (Martiney *et al*. 1990; Bellinger *et al*. 1993; Miller & Fahey 1994; Yu & Shinnick-Gallagher 1994), interleukin-2 (Park *et al*. 1995), TNF-α (Tancredi *et al*. 1992), IFN (D'Arcangelo *et al*. 1991) and, in particular, nitric oxide (Kilbinger 1996; Holscher 1997; Kara & Friedlander 1998). The possibility that a disturbance in synaptic transmission may contribute to the clinical deficit in MS is rarely discussed, but seems quite likely. If synaptic transmission is depressed in the disease, then it is easy to understand why 4-aminopyridine (4-AP) provides a symptomatic therapy, since the dominant effect of this agent at clinical concentrations may be the potentiation of synaptic transmission (Felts & Smith 1994; Smith *et al*. 2000) (see § (b) (iv); the effects of temperature).

(iv) *Neuroelectric blocking factors*

It is currently uncertain whether the observations regarding cytokines and other inflammatory mediators may help to illuminate the vexed area regarding the existence of 'neuroelectric blocking factors'. The suspicion that such factors may exist originated with a study showing that sera from animals with experimental autoimmune encephalomyelitis or from MS patients during acute exacerbation, blocked reflex activity in cultured central nervous system (CNS) tissue within minutes of exposure and in a complement-dependent manner (Bornstein & Crain 1965). The study was quite small and included few controls, but it was followed by a series of studies which reached similar conclusions (Cerf & Carels 1966; Carels & Cerf 1969; Lumsden *et al*. 1975*a*,*b*; Schauf *et al*. 1976, 1978; Schauf & Davis 1978) and some others which threw doubt on the interpretation of the data (Crain *et al*. 1975; Seil *et al*. 1975, 1976), claiming that the blocking activity was not specific to MS. Where present, the conduction block appears to be contained within the IgG-containing fraction of serum (Crain *et al*. 1975; Schauf & Davis 1978, 1981) and it is diminished by plasma exchange (Schauf & Davis 1981; Stefoski *et al*. 1982). However, if antibodies are involved, they probably act by means other than demyelination, since the blocking activity is both prompt and reversible. The possibility that antibodies may directly interact with ion channels has been discussed (Waxman 1995). Some evidence suggests that factors other than antibodies may play a role (Seil *et al*. 1976), but their identity remains uncertain.

In considering the potential role of neuroelectric blocking factors in MS, two points may be made. First is the fact that neuroelectric blocking factors are assayed for their effects on synaptic transmission and not for their effects on conduction in demyelinated axons. Indeed, sera with potent neuroelectric blocking effects have been found to have no effect on conduction in demyelinated axons (Schauf & Davis 1981). Second, the factors appear to reside in sera and not in the cerebrospinal fluid (Schauf & Davis 1981), even in those patients with potent serum activity. However, these observations do not necessarily rule out a role for neuroelectric blocking factors in MS, since the blood–brain barrier is impaired in the disease and so serum factors could gain access to the damaged tissue and to the synapses near those sites. The potential role of neuroelectric blocking factors has been reviewed in more detail elsewhere (Smith 1994).

There is also evidence for the presence of factors in the cerebrospinal fluid of MS patients which may directly impair sodium channel function (Brinkmeier *et al*. 1993; Koller *et al*. 1996), although the identity of these factors remains uncertain (Brinkmeier *et al*. 1996).

(b) *Recovery of function*

Several factors are likely to contribute to remission, including the restoration of conduction to persistently demyelinated axons and to remyelinated axons, the resolution of inflammation and, perhaps, adaptive changes which compensate for axonal loss and persistent conduction block. The question of adaptive changes is addressed at the end of this review. It seems likely that the relative importance of each mechanism will vary between patients, and in individual patients at different times.

(i) *Restoration of conduction to demyelinated axons*

Clinical observations, including the presence of 'silent' demyelinating lesions, have long suggested that conduction might be restored to some segmentally demyelinated axons (e.g. Namerow 1972; Ghatak *et al*. 1974; Wisniewski *et al*. 1976; Phadke & Best 1983; Ulrich & Groebke-Lorenz 1983; O'Riordan *et al*. 1998), but laboratory proof has been difficult to obtain, despite a number of studies which have examined conduction in axons affected by central demyelinating lesions (e.g. McDonald & Sears

1970; Smith *et al*. 1979, 1981; Pender & Sears 1984; Kaji *et al*. 1988; Pender 1988*a*,*b*, 1989; Chalk *et al*. 1994). However, in a recent study (Felts *et al*. 1997) the conduction properties of single experimentally demyelinated central axons were determined using intra-axonal recording techniques and then these same axons were labelled by the iontophoresis of horseradish peroxidase so that they could later be identified electron microscopically and reconstructed in three-dimensions. It is now clear that, although conduction is seemingly invariably blocked in newly segmentally demyelinated central axons, it can be restored within two or three weeks of demyelination, even when several internodes have been demyelinated and in the proven absence of any repair by remyelination (Felts *et al*. 1997). It is reasonable to believe that the restoration of conduction will tend to reverse the neurological deficit caused by conduction block (Chalk *et al*. 1995).

Presumably, the restoration of conduction involves at least the appearance of sodium channels along the demyelinated axolemma and the adoption of a more continuous (or micro-saltatory) mode of conduction, as occurs in peripheral demyelinated axons (Bostock & Sears 1976, 1978; Smith *et al*. 1982; see also England *et al*. 1990, 1996; Wu *et al*. 1993; Wu & Shrager 1994; Dugandzija-Novakovic *et al*. 1995; Novakovic *et al*. 1998). Indeed, immunohistochemical examination of MS lesions has revealed an increased density of saxitoxin binding within lesions (Moll *et al*. 1991). Saxitoxin is a ligand for sodium channels, but the spatial resolution achieved in this study was not sufficient to distinguish binding to axons from that to glial cells. However, a more recent, immunocytochemical study of central axons demyelinated with ethidium bromide has revealed node-like aggregations of sodium channels at locations along the axolemma (Felts *et al*. 1998). The spacing of the aggregations is not clear and so their relationship to new or, indeed, old nodes remains uncertain. Nevertheless, the aggregations are present at a time when the axons have regained the ability to conduct and so they may have functional importance.

The restoration of conduction is theoretically advantaged by a small axon diameter (Bostock 1994) and a short internode preceding the demyelinated region (Waxman & Brill 1978; Bostock 1994). Both these features occur in axons in the optic nerves and may contribute to the excellent recovery of vision which can occur following optic neuritis. However, experimentally demyelinated central axons as large as 5.5 µm in diameter have been proven to conduct (Felts *et al*. 1997) and so conduction should be possible under ideal conditions in most demyelinated axons in MS, since most human central axons are smaller than 5.5 µm in diameter. Short internodes are a characteristic feature of remyelination, suggesting that remyelination may make an important contribution to the restoration of conduction, even if it only occurs at the edges of a demyelinated lesion.

A role in clinical recovery for the expression of the major histocompatibility expression class I has been implicated in data derived from the Theiler's murine encephalomyelitis virus model of demyelination (Rivera-Quinones *et al*. 1998). Mice deficient in class I were functionally normal despite the presence of significant demyelination. The mechanisms underlying the apparent protection remain uncertain, although the authors proposed an increased expression of sodium channels and the relative preservation of axons.

(ii) *Resolution of inflammation*

Evidence that conduction block is determined in part by nitric oxide production by inflammatory cells in the MS lesion has already been discussed. As explained later, clinical recovery occurs when inflammation (as judged by gadolinium diethylenetriaminepentaacetic acid (DTPA)-enhanced MRI) subsides, suggesting that resolution of inflammation plays an important part in the timing of recovery. This interpretation is consistent with the observation that conduction in axons blocked by nitric oxide is promptly reversed when the agent is removed (Redford *et al*. 1997).

(iii) *The role of glial cells?*

Some earlier ultrastructural studies have revealed a node-like undercoating of the demyelinated axolemma specifically at patches in direct contact with astroglial processes (Blakemore & Smith 1983; Rosenbluth *et al*. 1985). The undercoating has been interpreted as indicating a node-like aggregation of sodium channels and this interpretation is supported by freeze-fracture evidence (Rosenbluth *et al*. 1985). Such observations have encouraged a view that glial ensheathment, even without myelin formation, may be a prerequisite for conduction in demyelinated axons. If so, then axons passing through the 'open' lesions, which are common in MS (Barnes *et al*. 1991), may not be able to conduct. However, the more direct evidence provided by sodium channel immunocytochemistry has now revealed the presence of node-like aggregations at entirely naked regions of axolemma (Felts *et al*. 1998). This information helps in understanding the fact that demyelinated axons have recently been proven to be able to conduct even when at least 88% of their surface area is entirely devoid of glial contacts for several internodes (Felts *et al*. 1997). These observations suggest that conduction may be possible in the sparsely glial-ensheathed axons of open MS lesions. However, it remains true that the simple apposition of glial membranes to demyelinated axons may also favour conduction by affecting the passive cable properties of axons (Shrager & Rubinstein 1990).

(iv) *Characteristics of restored conduction*

Conduction slowing

Although demyelinated axons can regain the ability to conduct, the conduction is neither as fast as normal, nor as secure. The precise velocity along the demyelinated region in central axons has not been determined as accurately as in the peripheral nervous system (Bostock & Sears 1976, 1978; Smith *et al*. 1982), but it is likely to be similar and in the range of 0.5–2.5 m s^{-1}. Although the velocity reduction is restricted only to the demyelinated portion of the axon (McDonald & Sears 1970), the slowing is so marked that it results in a clear increase in the latency of conduction and, thus, a dispersion of the mixed compound action potential. The latency increase, probably coupled with a reduction in the total number of axons conducting, results in diagnostically valuable changes in the latency and form of the visual (Halliday *et al*. 1972, 1973), somatosensory (Small *et al*. 1978) and brainstem auditory (Robinson & Rudge 1977; Hume & Waxman 1988) evoked potentials. However, the latency

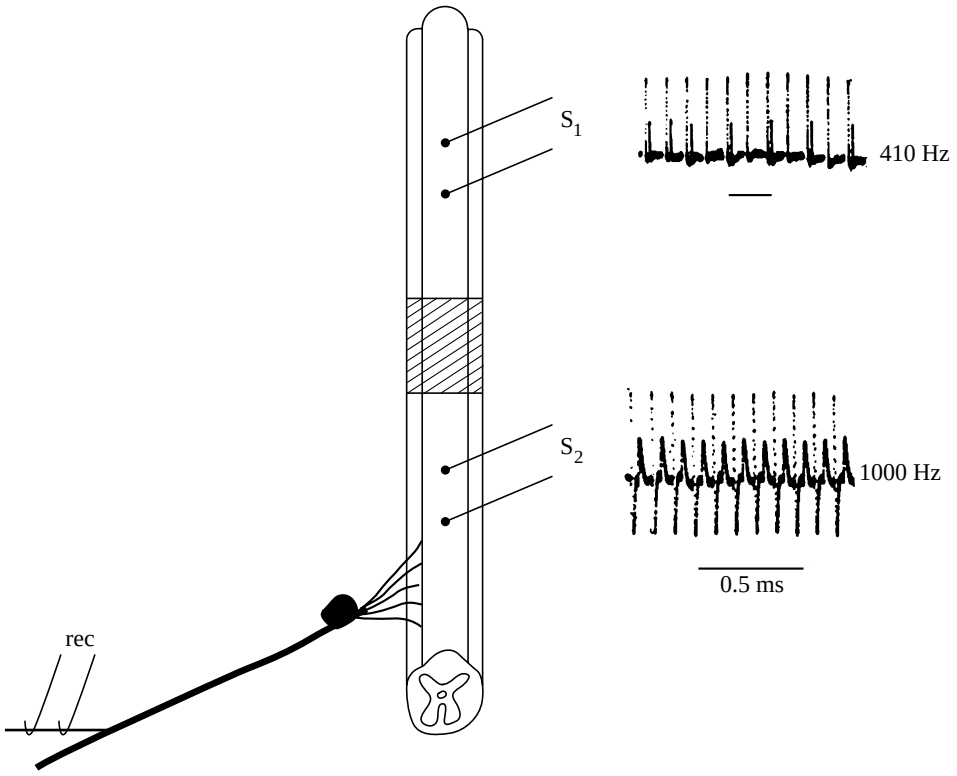

Figure 2. Records of activity in a single unit in a teased dorsal root filament in an intercostal nerve caudal to a demyelinating lesion (hatched region) induced in the dorsal column by the intraspinal injection of diphtheria toxin. The stimulus artefacts appear as dotted lines, and the action potentials as solid lines. Stimulation at S_1 includes the lesion in the conduction pathway, whereas stimulation at S_2 excludes it. Although the axon can conduct faithfully at 1000 Hz excluding the lesion, it only conducts three impulses through the lesion at 410 Hz before alternate impulses are blocked. Reprinted from McDonald & Sears (1970), with permission.

changes appear to be of little consequence to the patient in terms of neurological function. Indeed, apparently normal visual acuity can be preserved in MS patients even when there are gross delays in the visual evoked potential (Halliday *et al*. 1972, 1973; Hume & Waxman 1988).

Under particular circumstances, however, conduction delays can have subtle functional consequences. The Pulfrich phenomenon (i.e. that in healthy people a pendulum appears to swing in the arc of a letter D if a neutral density filter is placed in front of one eye, thereby slowing the visual signal from that eye) can be detected by patients with unilateral optic neuritis; one patient reported that oncoming traffic disturbingly appeared to veer in a curve towards him (K. J. Smith, personal communication). There is evidence that delay interferes with auditory functions dependent upon precisely coordinated information (Levine *et al*. 1994); it is likely that it has a similar effect on vibration sensitivity. Interestingly, Pulfich-like phenomena arising from unilateral optic neuritis can be diminished by placing a neutral density filter over the good eye (A. C. Bird, personal communication).

Conduction of pairs of impulses

Although conduction can be restored to demyelinated axons, it remains rather insecure and prone to conduction block. One measure of the security of conduction is the refractory period of transmission (RPT), a term introduced by McDonald & Sears (1970) to describe the maximum interval between two supramaximal stimuli at which the action potential arising from the second stimulus just fails to be propagated through a lesion. In a healthy axon, the RPT is equal in *value* to the absolute refractory period, but in a focally damaged axon the RPT is prolonged. The magnitude of the prolongation reflects the insecurity of conduction. In axons in the rat dorsal column which were proven to be segmentally demyelinated, the RPT/absolute refractory period along the normal portion of focally demyelinated axons was 0.5–1.4 ms, but, in the same axons, the RPT was prolonged to 1.0–6.0 ms through the lesion (with one axon having a RPT of 27 ms) (Felts *et al*. 1997).

The chronically *amyelinated* axons of the myelin-deficient rat are reported to have refractory properties equivalent to those of normal myelinated axons, at least in the optic nerve (Utzschneider *et al*. 1993). This observation emphasizes that the major cause of prolonged refractoriness in *de*myelinated axons is the low safety factor for conduction at the onset of the demyelinated region, i.e. the low safety factor inherent to the depolarization of an expanse of non-myelinated axolemma by action currents generated remotely, an internodal distance away. The need for remote depolarization does not arise in axons which are not myelinated and so such axons have a high safety factor for conduction, in common with normal unmyelinated axons.

Conduction of impulse trains

It is not possible to calculate the maximum firing frequency from the RPT, i.e. a 1 ms RPT does not imply

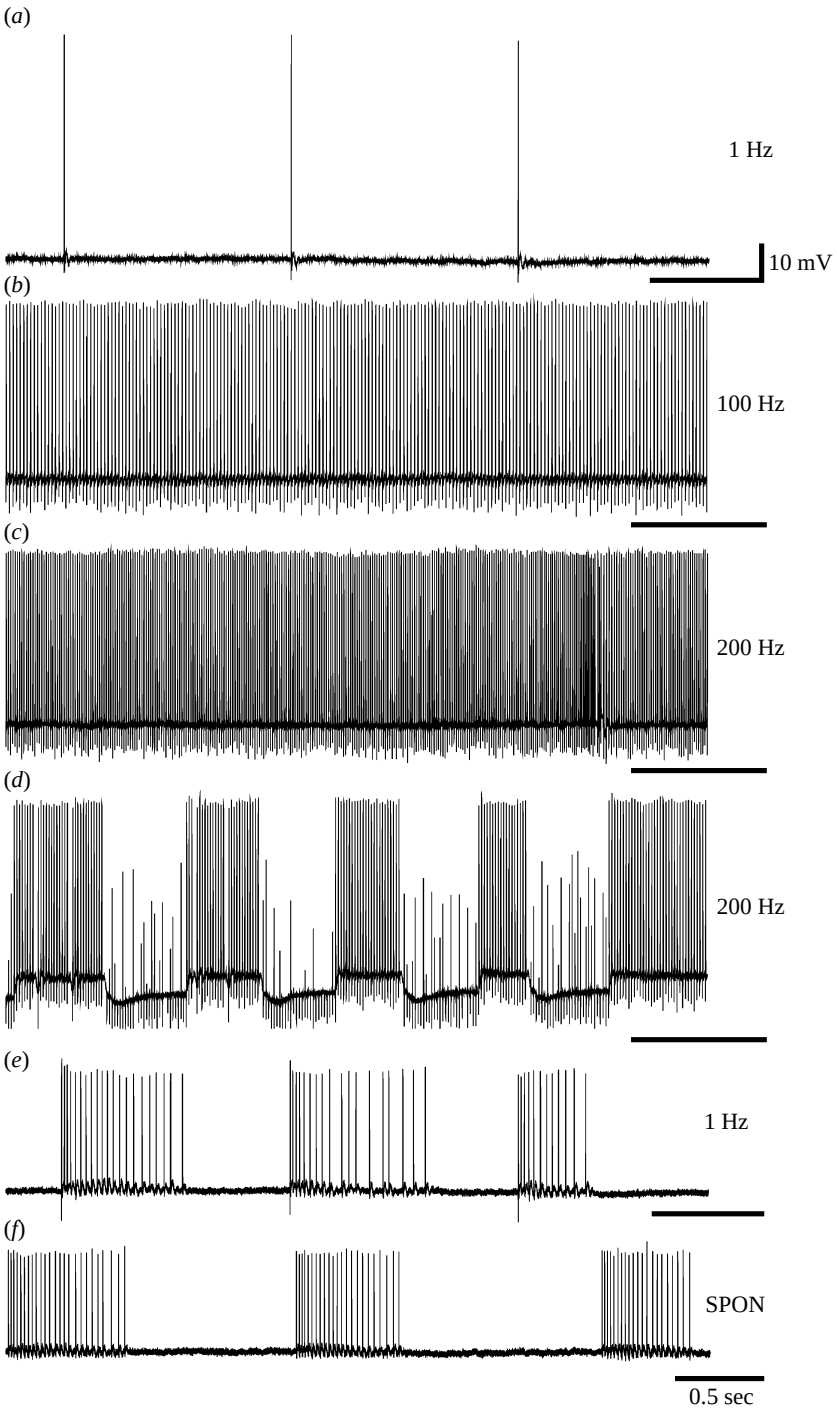

Figure 3. Several portions from a long, intra-axonal record (resting potential −60 mV) obtained from a central axon at or near a site of demyelination induced by the injection of ethidium bromide into the dorsal column 14 days previously. The RPT was prolonged from 0.77 ms in the unaffected portion of the axon to 1.32 ms through the lesion. The record illustrates several electrophysiological properties of demyelinated axons. Initially (*a*) the axon propagated a single action potential through the lesion in response to each supramaximal electrical stimulus presented distal to the lesion at 1 Hz (the stimulus artefacts are not distinguishable). The axon was also able to propagate action potentials faithfully in response to 10 s of stimulation at 100 Hz (*b*) and then to conduct faithfully at 200 Hz (*c*). However, after *ca*. 10 s of such stimulation (*d*) the axon entered intermittent periods of complete conduction block (the irregular spikes during these periods represent stimulus artefacts occasionally captured by the analogue to digital converter). The periods of conduction block were separated by periods when the axon conducted action potentials in response to *ca*. 75% of the stimuli, i.e. the lesioned portion of the axon accumulated refractoriness upon repeated activation, causing it to filter its input of impulses at 200 Hz into an output of only *ca*. 150 Hz. Note that in these intra-axonal records, it is clear that the periods of conduction block coincide exactly with periods of membrane hyperpolarization. This finding is consistent with conduction block mediated by activity of the electrogenic sodium/potassium ATPase (see text). After a total of 30 s of stimulation at 200 Hz, the axon responded to individual stimuli presented at 1 Hz, with bursts of impulses rather than with single impulses, as in (*a*). Furthermore, in the absence of any electrical stimulation (*f*) the axon generated spontaneous bursts of impulses approximately every 2–3 s. Prior to the stimulation at 100 or 200 Hz, this axon was found to generate records similar to those in (*e*) and (*f*), but with briefer bursts, following only 2 s of stimulation at 50 Hz: such an impulse load is well within the normal physiological range (P. A. Felts, R. Kapoor and K. J. Smith, unpublished observations; partially presented in Felts *et al*. (1995)).

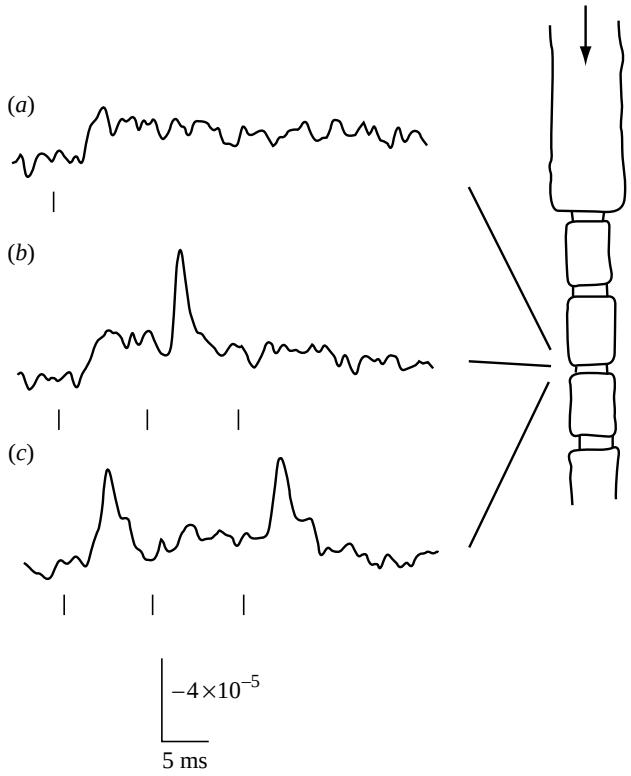

Figure 4. Optical records from a new node of Ranvier (indicated in the sketch) forming on an axon from the sciatic nerve of *Xenopus* 20 days after demyelination was induced with lysolecithin. The records show how the frequency at which impulses are delivered to a damaged region can affect the pattern of transmission (records are averaged, $n = 64$). (*a*) There appears to be total conduction block when impulses are delivered at 10 Hz. (*b*) Only the middle impulse is conducted when a burst of three impulses is delivered at 10 ms intervals. (*c*) Only the first and third impulses are conducted when a burst of three impulses is delivered at 3.3 Hz. From Shrager (1993), with permission.

an ability to fire at 1000 Hz. This restriction arises since the 'RPT' for the third and subsequent impulses is typically much longer than for the second, since the second impulse propagates in the relative refractory period of the first impulse and so propagates more slowly, particularly through the lesion. A further restriction on the ability of demyelinated axons to conduct impulse trains is imposed by their gradual accumulation of refractoriness with repeated activation. This deficit means that the maximum transmissible frequency can become quite dramatically reduced even after a short duration of activity. In the first demonstration of this deficit, McDonald & Sears (1970) described an experimentally demyelinated axon (figure 2) which was initially able to conduct impulses through the lesion at 410 Hz, but, after conducting only three impulses, alternate impulses were blocked at the lesion. The axon was able to conduct at 1000 Hz along its unaffected portion. This lesion acted as a frequency filter, reducing an input frequency of 410 Hz into an output frequency of 205 Hz. The axon illustrated in figure 3*d* also shows frequency filtering, but to a relatively mild extent. However, filtering is a feature common to all demyelinated axons and many filter severely: a maximum output frequency of only 1 Hz has been observed in the peripheral nervous system (R. W. Gilliatt, W. I. McDonald and P. Rudge, unpublished observations). It is easy to appreciate that filtering will distort sensory information and in motor axons it will contribute to weakness, since muscles may not receive a sufficient frequency of motor impulses to achieve maximal contraction.

The increasing severity of frequency filtering may be an important factor contributing to the increasing weakness observed upon sustained muscular exertion in MS patients (McDonald 1975) and possibly to the 'fading' or blurring of vision sometimes described upon fixated gaze (Waxman 1981; McDonald 1998). Frequency filtering is also likely to contribute to the reduced flicker fusion frequency in some MS patients (Titcombe & Willison 1961) and to the reduced ability of visual and somatosensory evoked potentials to follow rapidly presented stimuli (Milner *et al*. 1974; Sclabassi *et al*. 1974; Celesia & Daly 1977).

Although conduction in demyelinated axons typically becomes less likely with repeated activation, sometimes the passage of a preceding impulse can improve the chances of conduction. Some demyelinated peripheral *Xenopus* axons show conduction block when stimulated at low frequency, but successful conduction occurs *after* the initial impulse when a short burst of impulses is studied (Shrager 1993). One possibility is that the initial impulse induces a period of supernormality at the onset of the demyelinated region, and this temporarily increases the safety factor for subsequent impulses. Thus, by varying the frequency at which impulses are delivered to the demyelinated and/or early remyelinated region, different patterns of impulse transmission can be achieved (figure 4). Although such phenomena have not yet been reported in central demyelinated axons, they may well occur and contribute to the disruption of impulse coding.

Progressive weakness and sensory loss will also result from another feature of conduction in demyelinated axons. This is the development during a sustained train of impulses, of intermittent periods of complete conduction block (figure 3*d*). The block can develop after just 1 s of stimulation at 500 Hz (McDonald & Sears 1970) or after longer periods (e.g. 10–30 s) at more physiological frequencies (e.g. 100–200 Hz) (R. Kapoor, P. A. Felts and K. J. Smith, unpublished observations). The intervals of conduction block each persist for periods of *ca*. 0.2–2 s. Demyelinated regions displaying this deficit act like an intermittently operated 'switch', periodically allowing the transmission of information at quite high frequency and then abruptly switching off for a short period. The mechanism responsible is indicated in the changes in the 'baseline' membrane potential of the intra-axonal record in figure 3*d*. The periods of conduction block coincide with membrane hyperpolarization, consistent with the operation of the Na/K ATPase (i.e. the sodium 'pump') (Bostock & Grafe 1985). In elegant experiments in the peripheral nervous system, these authors showed that sustained impulse activity activates the Na/K ATPase and, since this pump is electrogenic, it results in a hyperpolarization of the membrane. Membrane hyperpolarization reduces the safety factor for conduction, in part because it moves the membrane potential away from the firing threshold. Since demyelinated axons already have a reduced safety factor, this additional insult can result in conduction block. As the activity of the pump subsides,

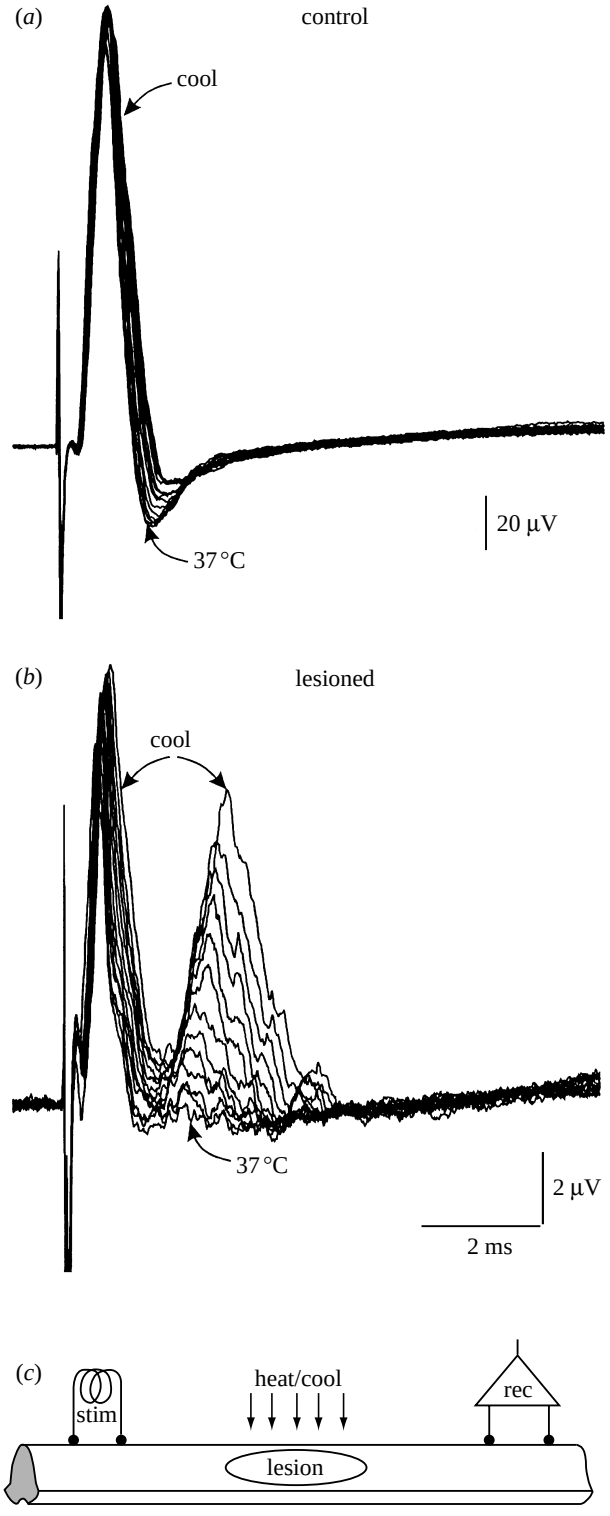

Figure 5. Two families of monophasic compound action potentials recorded *in vitro* from excised dorsal columns from (*a*) a naïve rat ('control') and (*b*) one in which an experimental demyelinating lesion had been induced 21 days previously ('lesioned'). The plots show superimposed, averaged records obtained at 1 °C intervals as the temperature of the lesion was raised from 25 to 37 °C: the temperature of the stimulating and recording sites remained constant (*c*). The temperature changes had little effect on conduction in normal axons, but caused prominent effects in the axons comprising the delayed peak in the lesioned tissue. The delayed peak is composed of axons with a prolonged RPT (data not shown) indicating that the axons are affected by the demyelinating lesion. The delayed axons are markedly temperature sensitive, such that virtually none are able to conduct at normal body temperature.

conditions return to those favouring conduction and the cycle is repeated. Drugs which inhibit the Na/K ATPase have been reported to improve conduction in both central (Kaji & Sumner 1989*a*) and peripheral (Kaji & Sumner 1989*b*; Shrager 1993) demyelinated axons. Benefit has also been observed in three out of seven patients with probable or definite MS (Kaji *et al*. 1990), although the therapy has not been widely adopted. Other mechanisms which may contribute to the development of conduction block during trains of impulses include the accumulation of intra-axonal sodium ions (Rasminsky & Sears 1972) and extracellular potassium ions (Brismar 1981).

Effects of temperature

Many MS patients notice that at least some of their symptoms can be markedly affected by small changes in body temperature. The effects can be dramatic and improvements in vision upon drinking a glass of cold water have been described (Hopper *et al*. 1972; McDonald 1986) and, conversely, of deterioration during a hot shower (Waxman & Geschwind 1983), sunbathing (Berger & Sheremata 1985) or the use of a hair dryer (Brickner 1950). The circadian change in body temperature also affects some patients (Namerow 1968; Davis *et al*. 1973). The deleterious effects of warming occur promptly, such that a patient may require support to prevent drowning in a hot bath (Guthrie 1951). Indeed, a fatal case of scalding has been described (Waxman & Geschwind 1983), together with deaths from hyperthermia during sunbathing: the patients were believed to have been prevented from moving into the shade by weakness induced by body warming (Harbison *et al*. 1989; Avis & Pryse-Phillips 1995). Such patients are described by neurologists as illustrating Uhthoff's phenomenon (Uhthoff 1890; Selhorst & Saul 1995), although Uhthoff (1890) himself ascribed such effects as being due, in his patients, to exercise, even though one of his patients complained that she felt the same exacerbation of symptoms upon standing next to a hot stove. Certainly, exercise does provoke Uhthoff's phenomenon and one of the authors (K.J.S.) has encountered a patient later diagnosed with MS who was presented with unexplained loss of vision after a few minutes of aerobics. Uhthoff's phenomenon was in the past sometimes used diagnostically in the form of the 'hot bath test' (Malhotra & Goren 1981). The effects of temperature are typically fully reversible, but exceptions have been reported (Berger & Sheremata 1983, 1985; Davis 1985).

The clinical observations suggest that warming will promote conduction block, with the opposite effect upon cooling, and these consequences have often been demonstrated in peripheral demyelinated axons (Davis & Jacobson 1971; Rasminsky 1973; Davis *et al*. 1975; Sears *et al*. 1978; Pencek *et al*. 1980; Sears & Bostock 1981; Pender & Sears 1984). While examining central demyelinated axons, one of the authors (K.J.S.) was impressed by how few axons are able to conduct at normal body temperature in some lesions, despite there being a large number of axons present which are capable of conducting when

cooled (figure 5). Such observations encourage a belief that a useful symptomatic therapy for MS may derive from the use of a cooling jacket, or drugs which, at normal body temperature, may mimic the effects of body cooling (Davis & Schauf 1981; Sears & Bostock 1981; Waxman *et al*. 1994). This belief has now resulted in a partially effective symptomatic therapy for MS.

To understand the approach underlying this therapy, it is first necessary to understand the mechanism responsible for the beneficial effects of body cooling. The germane effect of cooling is a resultant small prolongation in action potential duration (Schoepfle & Erlanger 1941; Paintal 1966), since the temperature coefficient for sodium inactivation is larger than that for sodium activation (Schauf & Davis 1974; Davis & Schauf 1981). The prolongation in duration means that the flow of local action currents generated by the node prior to the demyelinated region is also prolonged, and this favours successful conduction since it increases the likelihood that the demyelinated axolemma will be depolarized to its firing threshold. To mimic the effects of cooling the goal has been to prolong the action potential pharmacologically, either by delaying the inactivation of sodium channels or by blocking potassium channels (such as those exposed at demyelinated paranodes). Both approaches have proven effective in the laboratory (Bostock *et al*. 1978, 1981; Sherratt *et al*. 1980; Targ & Kocsis 1985; Bowe *et al*. 1987). Interestingly, therapy based on the inactivation of sodium channels may inadvertently have been tested, apparently with some favourable consequences, when patients have been stung by scorpions (Breland & Currier 1983): scorpion venom markedly prolongs sodium currents (Bostock *et al*. 1978). Based on related experimental observations, the potassium channel blocking agent 4-AP has been tested for efficacy in MS and a number of clinical trials have now proven its value (reviewed in Bever 1994; see also Schwid *et al*. 1997). The therapy has not, however, come into widespread use, largely because patients find that its overall effect on disability is modest and there is also a danger of convulsions (Blight *et al*. 1991; Bever *et al*. 1994).

Despite the foregoing rationale, the common assumption that 4-AP acts primarily to restore conduction to demyelinated axons has been questioned on the basis of data suggesting a more dominant action on synaptic transmission (Smith *et al*. 2000). Doubt also remains as to whether the dominant effect of 4-AP is on the driving node or the demyelinated axolemma at the demyelinated site.

(v) *Restoration of conduction by remyelination*

It is now clear that repair by remyelination is common in MS (Prineas & Connell 1979; Prineas *et al*. 1987, 1993; Lassmann, this issue). However, the new internodes are shorter (and thinner) than normal and so the new nodes are formed at sites previously covered by myelin (Gledhill & McDonald 1977). The nodes are therefore formed at sites normally lacking a high density of sodium channels, raising the possibility that the new nodes may not be excitable. However, studies in which conduction has been serially followed in the same group of axons as they sequentially undergo demyelination and remyelination have established that remyelination is effective in restoring conduction, probably to all of the axons affected (Smith *et al*. 1979, 1981). Remyelination is effective in restoring conduction irrespective of whether it is primarily achieved by oligodendrocytes (Smith *et al*. 1979, 1981), Schwann cells (Felts & Smith 1992; Honmou *et al*. 1996) or transplanted olfactory ensheathing cells (Imaizumi *et al*. 1998; see also Utzschneider *et al*. 1994). It is probably safe to assume that each new node is excited in turn, as occurs in peripheral remyelinated axons (Smith *et al*. 1982). Indeed, peripheral axons show aggregations of sodium channel immunoreactivity at remyelinated nodes of Ranvier (Dugandzija-Novakovic *et al*. 1995; Novakovic *et al*. 1996) and such aggregations have now also been observed in the CNS (Felts *et al*. 1998). Importantly, the security of conduction is restored (i.e. the RPT becomes as short as in normal axons; Smith *et al*. 1979, 1981) and so remyelinated central axons may be expected to function well at physiological frequencies (Jeffery & Blakemore 1997). Where it occurs, it is reasonable to believe that remyelination will contribute to the restoration of function during remission. The promotion of repair by remyelination therefore offers some prospect as a symptomatic therapy in MS and may theoretically have an additional advantage of helping to protect axons from the harmful effects of recurrent inflammation. Since repair by Schwann cells is as effective as oligodendrocytes in this respect, and since Schwann cells are not normally affected by the disease, these peripheral myelinating cells have been a target of interest in transplantation studies (Franklin & Blakemore 1993; Bunge 1994; Baron-Van Evercooren *et al*. 1997). There is currently growing interest in the possibility of promoting remyelination by central glial cells (Scolding, this issue).

(c) *Positive symptoms*

MS patients can experience a wide range of positive phenomena and recordings in experimentally demyelinated axons suggest that many of them can be explained by the electrophysiological properties of axons rendered hyperexcitable by their response to demyelination. This interpretation is supported by the fact that at least some positive sensations in patients (e.g. tingling in the trunk and limbs induced by neck flexion, i.e. Lhermitte's phenomenon) are associated with impulse activity in primary sensory axons, and this activity correlates in intensity and time with the sensation (Nordin *et al*. 1984) (figure 6*b*). The role of axonal hyperexcitability in the generation of positive symptoms in demyelinating disease has recently been reviewed in detail (Smith *et al*. 1997).

(i) *Persistent paraesthesiae*

Experimentally demyelinated central (Smith & McDonald 1980, 1982) and peripheral (Burchiel 1980; Calvin *et al*. 1982; Bowe *et al*. 1987; Baker & Bostock 1992) axons (and amyelinated axons) (Huizar *et al*. 1975; Rasminsky 1978, 1987) can acquire the property of spontaneously generating trains of spurious impulses which arise at the demyelinated site and propagate away from it in both directions (Smith & McDonald 1980, 1982; Baker & Bostock 1992). In our experience, newly experimentally demyelinated axons are not spontaneously active, but they may acquire this property after one week or more has elapsed.

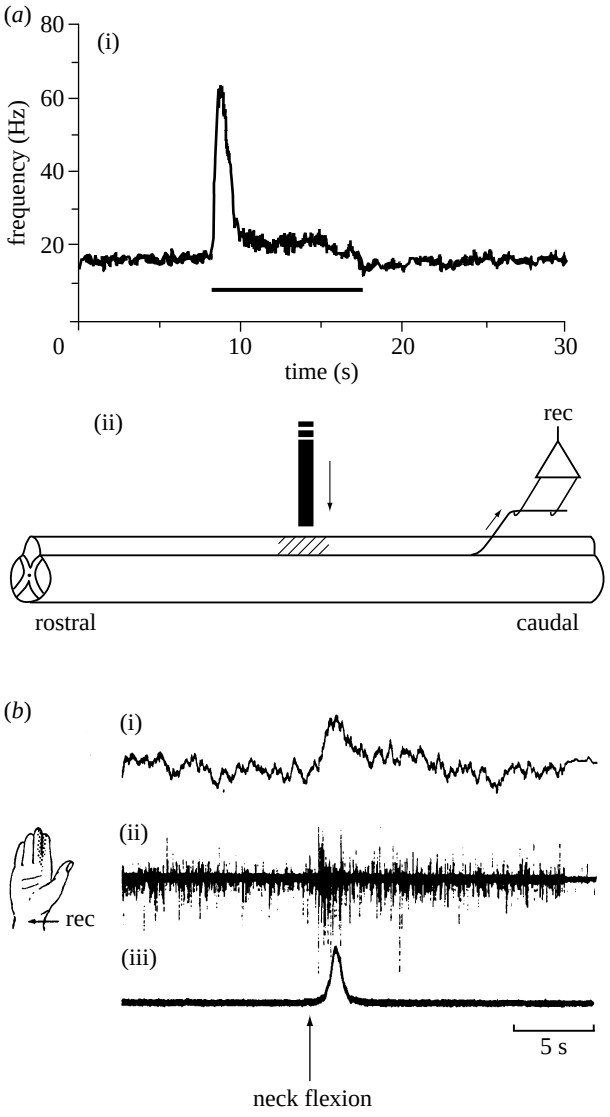

Figure 6. (*a*) Graph showing the firing frequency of a single unit isolated in a teased dorsal root filament caudal to a demyelinating lesion (cross-hatched) induced in the spinal dorsal columns by the injection of lysolecithin. The unit is spontaneously active, with impulses arising at the lesion at *ca*. 18 Hz. The unit also shows mechanosensitivity and it responds to a sustained 10 s, 0.75 mm deformation of the lesion by a phasic burst of activity, followed by a slower, more tonic discharge. The activity was unaffected by similar deformations applied to the dorsal columns at sites remote from the lesion. Redrawn from Smith & McDonald (1980), with permission. (*b*) Recording from a skin fascicle in the right median nerve (see diagram) of a patient with MS and a six-month history of Lhermitte's phenomenon. When she flexed her neck (vertical arrow) she experienced a non-painful paraesthesia (an 'electric feeling') in all fingers of her hands, indicated by the peak in the force record (iii). (ii) The evoked multiunit burst of activity, which coincides with the paraesthesiae, and (i) the integrated neurogram (time constant 0.5 s). From Nordin *et al*. (1984), with permission.

Two patterns of activity have been described, namely trains of evenly spaced impulses at frequencies of 10–50 Hz, and bursts of impulses (figure 7). The individual bursts are of very variable duration, but often persist in an individual axon for periods of 0.1–5 s, separated by silent intervals of 0.1–100 s. Both the continuous and bursting patterns of activity can be generated continuously for hours in the absence of any identifiable stimulus. The authors have suggested that, when such trains of impulses arrive at the brain, generated concurrently in hundreds of different sensory axons, the brain may interpret the information as a tingling sensation referred to the body parts normally innervated by those axons (Smith & McDonald 1980, 1982; see also Rasminsky 1981). This view is supported by evidence from microneurography in patients, as noted above (Nordin *et al*. 1984) (figure 6*b*).

The combination of intra-axonal recording methods and pharmacological manipulation of ion channels has allowed the study of the potential mechanisms generating the ectopic impulses (figure 7). The regular discharges have been related to a slow inward sodium current which can appear at sites of demyelination (Rizzo *et al*. 1996; Kapoor *et al*. 1997; see also Stys *et al*. 1993; Honmou *et al*. 1994; Cummins & Waxman 1997), whereas the bursting discharges may be generated by a prolonged, inward potassium current resulting from an accumulation of potassium ions in a compartment surrounding the axon (Kapoor *et al*. 1993; Felts *et al*. 1995; see also Young *et al*. 1989; Burke 1993). However, it is clear that it is not possible to link a particular mechanism to a particular pattern of activity rigidly, since both single and bursting discharges have been observed in different preparations due to currents believed to be carried by sodium and potassium ions (P. A. Felts, R. Kapoor and K. J. Smith, unpublished observations). A clear role for sodium currents in the generation of at least some paraesthesiae is supported by the efficacy of therapeutic agents such as carbamazepine, which act to block these channels (Schwarz & Grigat 1989). However, carbamazepine may provide unconvincing relief from paroxysmal pain in MS, other than trigeminal neuralgia (Matthews 1998). The role played by potassium currents in patients remains less clear, but, in view of the role of astrocytes in potassium homeostasis and the fact that astroglial cells are sparse in some MS lesions (Barnes *et al*. 1991), dysregulation of the potassium ion concentration may sometimes be expected.

'Spontaneous' bursting activity can also sometimes be provoked by stimulation of axons at physiological frequencies. For example, the axon illustrated in figure 3 generated 'spontaneous' bursts following only 2 s of stimulation at 50 Hz. It is possible that this period of activity results in the loading of an extra-axonal compartment with potassium ions.

Positive sensory phenomena are much more common in MS patients than their motor counterparts, although manifestations such as facial myokymia and myoclonus can occur (Andermann *et al*. 1961; Hjorth & Willison 1973; Kapoor *et al*. 1992; Jacobs *et al*. 1994).

Positive phenomena are often enhanced by hyperventilation (Davis *et al*. 1970) and this can be explained by an increase in axonal excitability (Burchiel 1981) in response to a change in membrane surface charge due to a reduction in the concentration of extracellular free calcium (Frankenhaeuser & Hodgkin 1957; Hille 1992; Burke 1993).

(ii) *Ephaptic transmission between axons*

Tingling sensations can be attributed to ectopic activity occurring asynchronously in demyelinated axons, but some patients exhibit symptoms that imply relatively

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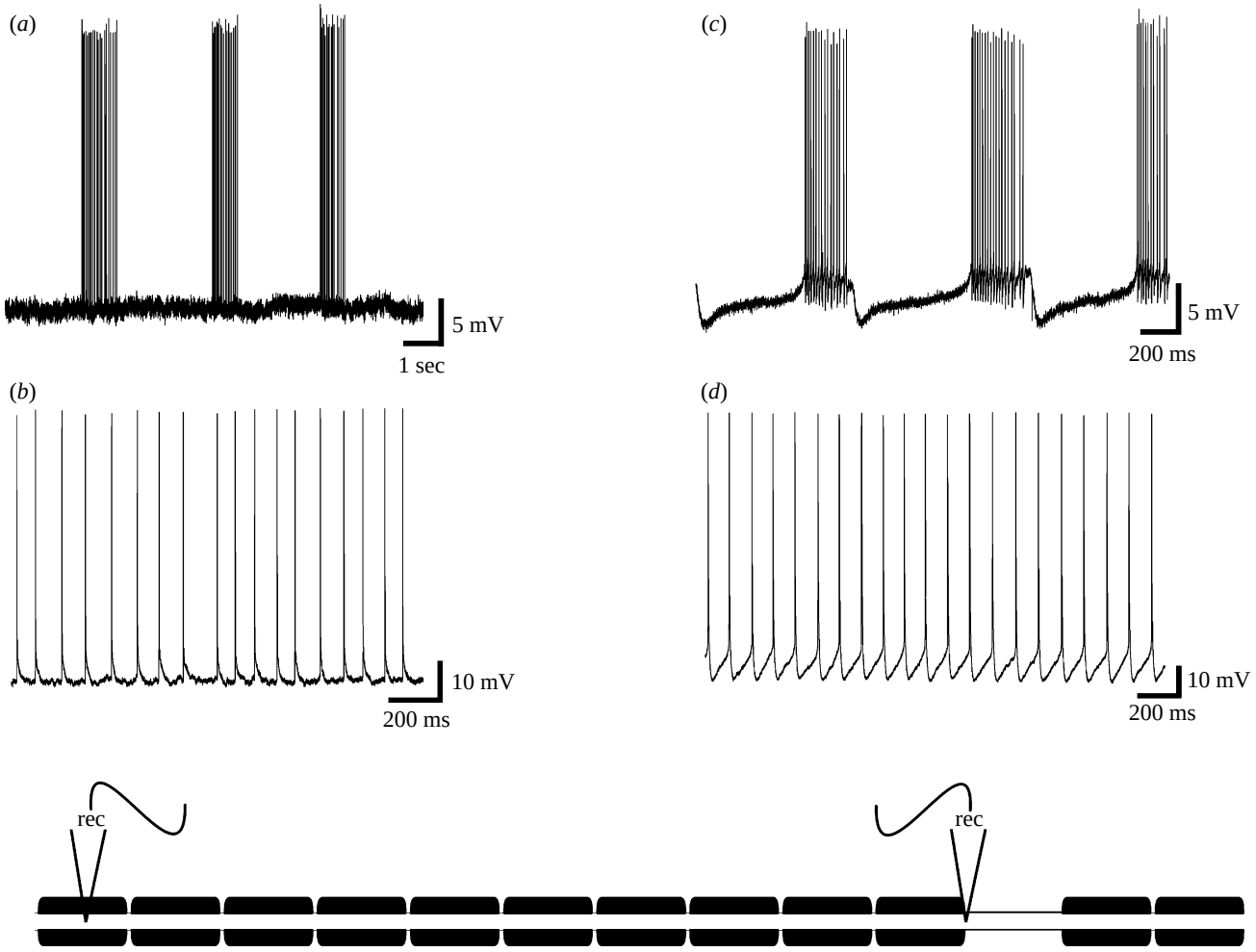

Figure 7. A series of four intra-axonal records obtained from four different demyelinated central axons. (*a*,*b*) Records were obtained with the recording micropipette at a site remote from the site of demyelination, while (*c*,*d*) are records obtained at or near the demyelination. The records show the two types of continuous discharge evoked by such axons in the absence of any deliberate stimulation, namely a bursting discharge (*a*,*c*) or a more evenly spaced discharge (*b*,*d*). The records obtained at or near the site of demyelination reveal the changes in membrane potential associated with the generation of the impulses. (*b*,*d*) were obtained in the presence of 4-AP. Redrawn from Felts *et al*. (1995) and Kapoor *et al*. (1997), with permission.

synchronous discharges in many axons (Matthews 1975, 1998). Furthermore, some of the more complicated paroxysmal phenomena (e.g. Kapoor *et al*. 1992) are most easily explained by postulating the lateral spread of excitability across different but anatomically adjacent spinal tracts. Such transmission between physically adjacent axons is described as 'ephaptic', to distinguish it from true synaptic transmission.

Ephaptic transmission is frequently invoked in the clinical literature to explain a host of obscure phenomena and it may well occur in patients, but there is relatively little direct experimental evidence supporting it. Although the phenomenon has been clearly demonstrated between single amyelinated and normal axons in the 'dystrophic' mutant mouse (Rasminsky 1978, 1980), it has yet to be proven involving demyelinated central axons. However, since some demyelinated axons in MS appear to be sufficiently hyperexcitable that they are spontaneously active, it seems safe to believe that others will sometimes be poised very near their firing threshold. If so, then impulses may well be triggered ephaptically by the effects of action currents generated by activity in neighbouring axons.

In this context, it may be of interest that mass synchronous firing arising from the spinal cord has very occasionally been observed in animals with experimental demyelinating lesions in the dorsal columns. The activity takes the form of the spontaneous generation of a series of compound action potentials propagating anti-dromically along the dorsal roots (figure 8) (Smith *et al*. 1997). The phenomenon presumably arises from the self cross-excitation of a large population of neurons via an ephaptic interaction, but whether this interaction occurs within the central demyelinating lesion or within the dorsal horn is currently uncertain. If it occurs in the grey matter, it may indicate mechanisms similar to those involved in epilepsy, which is reported to occur more commonly in MS than in the general population (an incidence of 2.3% in definite MS; Ghezzi *et al*. 1990).

(iii) *Triggered sensations*

On clinical evidence, it seems that some positive phenomena can be triggered by the propagation of normal impulse traffic through a site of demyelination (e.g. when light touch evokes pain referred to the same

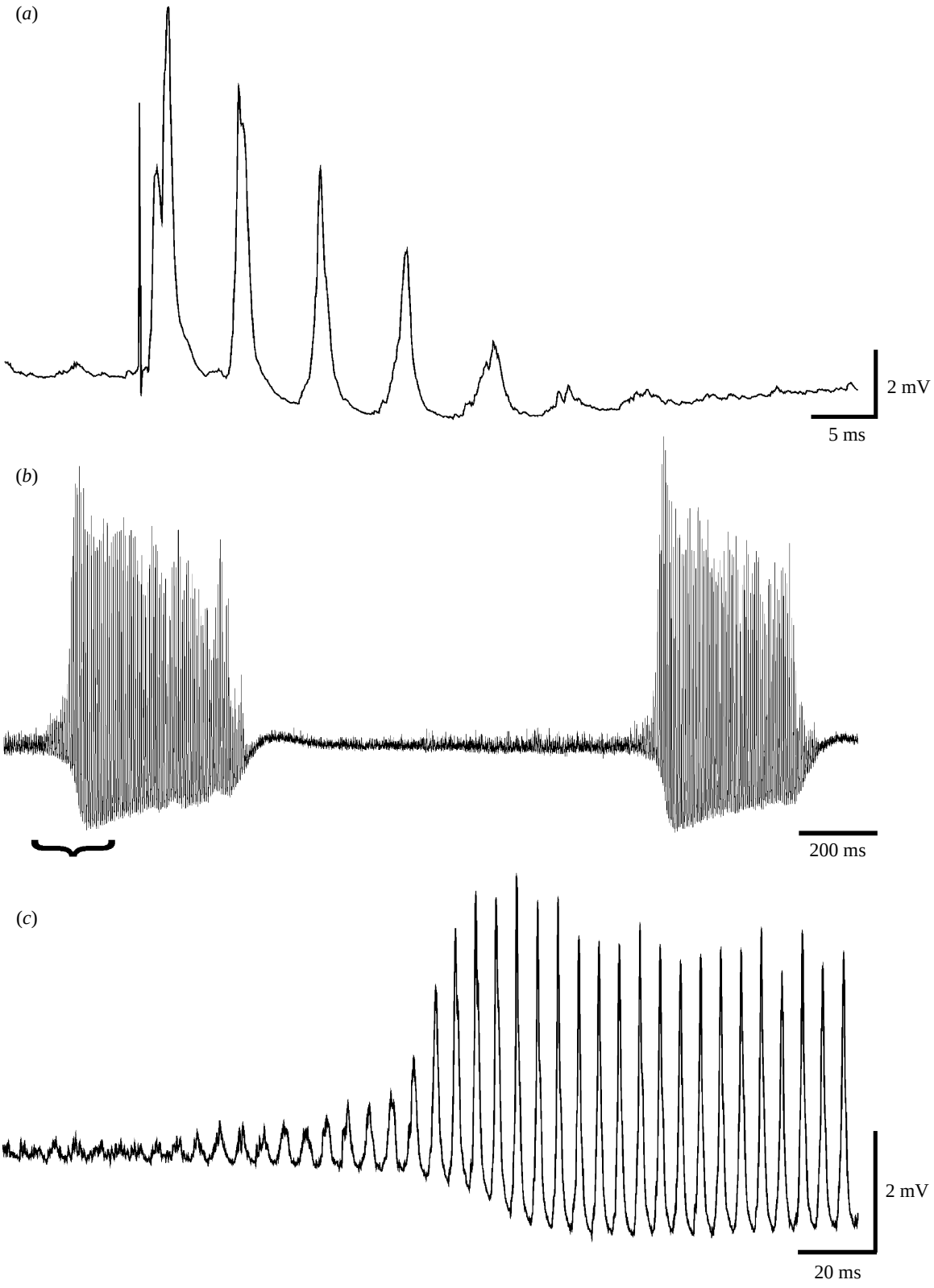

Figure 8. Records of activity obtained under general anaesthesia (halothane) from a dorsal root caudal to a central demyelinating lesion induced 43 days previously in rat dorsal columns by the intraspinal injection of ethidium bromide. (*a*) A series of compound action potentials evoked in response to a single supramaximal electrical stimulus applied to the dorsal columns rostral to the lesion. The first peak following the stimulus artefact denotes the synchronous conduction of impulses evoked by the stimulus itself, while the later peaks show synchronous activity initiated by the axons themselves. (*b*) Two sustained bursts of synchronous, compound action potentials which arose 'spontaneously', i.e. in the absence of any deliberate stimulation. The onset of the burst (see bracket) is shown on an expanded time-base in (*c*), indicating that the bursts arose spontaneously by the progressive recruitment of additional axons to what may have been a rhythmic cycle of ongoing activity in only one or a few axons. The downward drift of the baseline during bursts is due to the AC coupling of the amplifiers. (M. Davies, R. Kapoor, V. Samtani and K. J. Smith, unpublished observations.)

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receptive field). Correspondingly, in some experimentally demyelinated axons prolonged bursts of ectopic impulses can be triggered at the demyelinated region by the passage of single impulses propagating through the demyelinated site (Huizar *et al*. 1975; Burchiel 1980; Calvin *et al*. 1982; Bowe *et al*. 1987; Felts *et al*. 1995). In central demyelinated axons, this behaviour can sometimes be induced by prior stimulation of the axon at high frequency (figure 3*e*). The period of stimulation may be expected to increase the extracellular potassium ion concentration, favouring a role for this ion in the underlying mechanism (Kapoor *et al*. 1993; Felts *et al*. 1995), but other mechanisms are also possible.

In some demyelinated axons, impulses can be 'reflected' from sites of demyelination (Howe *et al*. 1976; Calvin *et al*. 1977, 1982; Bostock 1994), i.e. an impulse propagating through a demyelinated site can result in the formation of a second impulse which travels back along the same axon in the opposite direction. Reflection is possible because of the prolonged duration of the action potential at the demyelinated membrane, which means that the action currents arising from it can re-excite the preceding normal node once it has emerged from its refractory period. A pair of reflecting sites could result in action potentials shuttling back and forth, generating a train of sister impulses at each site of reflection. It is easy to see how this mechanism could also generate a burst of impulses in response to the propagation of a single impulse, if the reflection periodically failed.

Paroxysmal itching has been described in MS (Osterman & Westerberg 1975; Osterman 1976) and it may conceivably be explained by the induction of activity, such as that described above, in itch modality axons.

(iv) *Movement-induced sensations*

MS patients often report the presence of sensations evoked upon body movements expected to distort portions of the nervous system containing demyelinated axons. For example, in patients with demyelinating lesions affecting the sensory axons in the cervical posterior columns, flexing the neck can provoke 'electric shock' or tingling sensations which radiate down the limbs and body (Lhermitte's phenomenon; Lhermitte *et al*. 1924; Kanchandani & Howe 1982). Similarly, patients with demyelinating lesions of the optic nerve may experience the perception of flashes of light, or other phosphenes, upon eye movements (Davis *et al*. 1976). Such observations suggest that demyelinated axons may become mechanosensitive and this view has been supported by microneurography in patients with Lhermitte's phenomenon (Nordin *et al*. 1984) (figure 6*b*) and demonstrated by recordings from experimentally demyelinated central axons (Smith & McDonald 1980, 1982) (figure 6*a*). The pattern of ectopic discharge elicited by distortion of demyelinated axons is similar to that produced by distortion of certain mechanoreceptors, suggesting that demyelinated axolemma may acquire ion channel characteristics which resemble those of unmyelinated receptors. Some demyelinated axons only display a tonic response to deformation (Smith & McDonald 1982), in contrast to the phasic and tonic response shown in figure 6*a*. Whether demyelinated membranes contain specific stretch-sensitive ion channels (such as the TRAAK channel; Maingret *et al*. 1999) remains to be determined.

(v) *Pain*

Pain is a common complaint in MS patients (Shibasaki *et al*. 1981) and it appears to have several origins. Some of these are beyond the scope of this review since they may not involve demyelinated axons directly. However, it is reasonable to predict that painful sensations will result from ectopic impulses generated in pain fibres, although the authors are not aware of such reports. It may be relevant that systemic injections of lignocaine are typically effective in treating pain in MS patients (Petersen *et al*. 1986), consistent with an underlying mechanism involving sodium channels (see above).

Since the concentration of TNF-α is raised in MS lesions (Brosnan *et al*. 1995; Hartung *et al*. 1995; Navikas & Link 1996; Chandler *et al*. 1997; Carrieri *et al*. 1998), it may be relevant that the application of 'low' concentrations of TNF-α to normal peripheral axons elicits increased impulse activity in the Aδ and C (unmyelinated) fibres which mediate pain (Sorkin *et al*. 1997). Even low firing rates in such nociceptive axons may produce 'wind-up' in dorsal horn neurons, exacerbating or eliciting painful sensations. The intrathecal administration of either TNF-α or interleukin-1α have, in contrast, been reported to promote analgesia (Bianchi *et al*. 1992), but dose may be a complicating factor in the interpretation of such data, since, in the former study, higher concentrations of TNF were found to reduce firing in nociceptors (Sorkin *et al*. 1997). TNF-α may exert some of its effects via the induction of interleukin-1β and nerve growth factor (Woolf *et al*. 1997).

3. THE COURSE OF MULTIPLE SCLEROSIS

One of the surprising results yielded by serial MRI of groups of patients with MS has been the evidence that new pathological activity (as judged by the appearance of new lesions on T2-weighted scans or by gadolinium-DTPA enhancement of T1-weighted scans) is often ten times more frequent than clinical relapse (Isaac *et al*. 1988; Willoughby *et al*. 1989; Thompson *et al*. 1991). An extreme example is illustrated in figure 9 in which areas of enhancement (red) have been superimposed on co-registered unenhanced scans. In this 24-year-old woman who had recently entered the secondary progressive phase of MS there were 97 separate areas of gadolinium-DTPA enhancement, but only three clinical relapses over a period of nine months.

Why are so many lesions clinically silent? Two important factors are the location of the lesion and the functional state of the nerve fibres which are affected. Standard MRI gives invaluable evidence about location and from it we know that lesions commonly occur in the periventricular white matter. Here, acute lesions would not be expected to produce symptoms unless the lesions were very large. A second aspect of location relates to the proportion of nerve fibres subserving a given function which is affected by the lesion, and the length of the damage sustained by these fibres. Repair of demyelinated regions appears to advance from the edges towards the middle and, thus, repair of axons with long regions of demyelination tends to be delayed. Furthermore, the longer the lesion, the greater the probability of the inclusion of a region favouring conduction block. Since lesions are orientated around venules, which do not necessarily

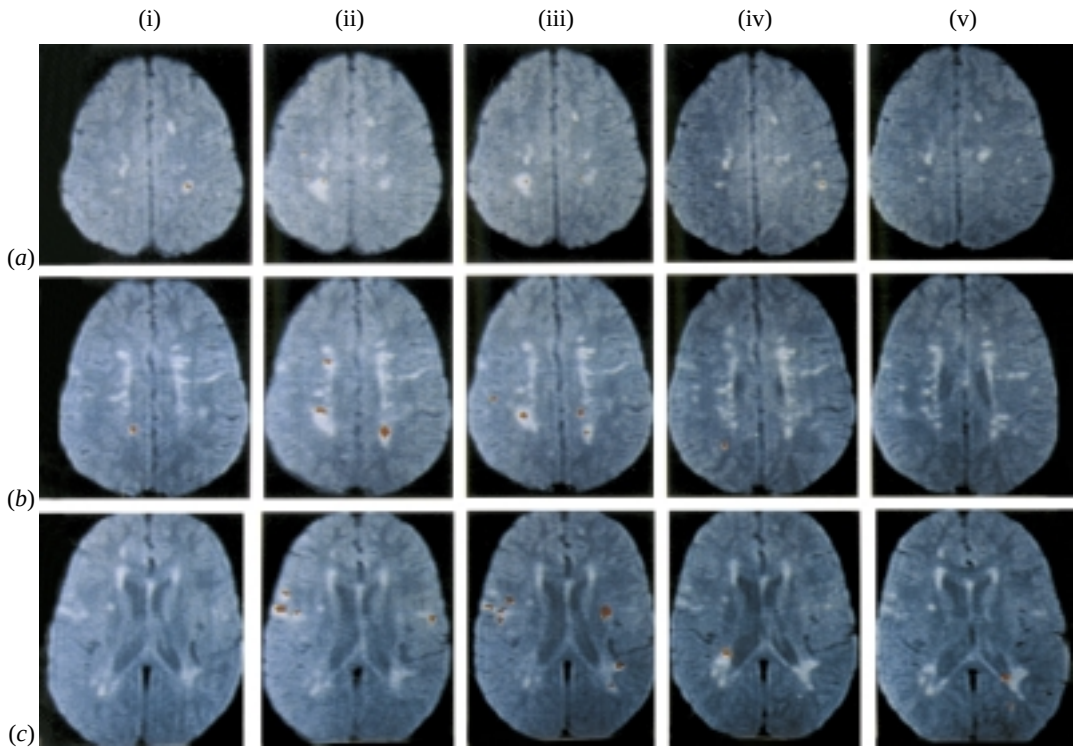

Figure 9. Superimposed serial T2-weighted and gadolinium-DTPA-enhanced T1-weighted MRI scans at monthly intervals in a 24-year-old patient with secondary progressive multiple sclerosis. The areas of enhancement are shown in red (from Miller *et al*. 1997).

follow the course of nerve fibres, a lesion of a given volume may affect many or only a few internodes of a particular axon. The second factor, the functional state of the nerve fibres in the lesion, depends not only on the extent of demyelination, but also on the extent of axonal degeneration, whether there has been time for the compensatory mechanisms (described above) to develop and their effectiveness. A further important factor is the presence or absence of inflammation.

In summary, putting aside the complexity of detail, the overall picture is clear; when a new acute lesion affects the majority of nerve fibres subserving a particular function, as in optic neuritis, that function is impaired or lost. Early in the course of MS the compensatory mechanisms are so efficient that virtually complete clinical recovery is usual, although an abnormal MRI may persist: the MRI depends not upon the functional state of the axons, but on changes in the amount and distribution of water in the intra- and extracellular spaces in the affected area (see Barkhof & Van Walderveen, this issue).

(a) *Mechanisms of relapse and remission*

Before describing the natural history of the new acute lesion in MS it is useful to remind the reader that the MRI abnormalities seen with standard techniques correspond to the plaques seen at post-mortem (Stewart *et al*. 1984; Ormerod *et al*. 1987) and that areas of enhancement following the intravenous injection of gadolinium-DTPA represent focal regions of breakdown of the blood–brain barrier in areas showing marked inflammation (Katz *et al*. 1993; Bruck *et al*. 1997).

Comparison of MRI and post-mortem findings and the exploitation of other nuclear magnetic resonance techniques, together with evoked potentials and precise clinical observation, have shed light on the mechanism of relapse and remission in MS. In the course of serial studies of patients at intervals of one week to one month, it was possible to chart the evolution of the new lesion in MS. The earliest detectable event in the great majority of new lesions in relapsing–remitting and secondary progressive MS is a breakdown of the blood–brain barrier, signalling in this context the presence of inflammation (Kermode *et al*. 1990). The lesion increases in size over the course of approximately one month (Willoughby *et al*. 1989). Enhancement then ceases (Miller *et al*. 1988) and the lesion decreases in size as a result of the resolution of oedema (Larsson *et al*. 1988) to leave a smaller residual area of abnormality. Serial proton MRS reveals that demyelination occurs during the active inflammatory phase (Davie *et al*. 1994).

Acute optic neuritis provides a good opportunity for exploring the relationship between conduction, inflammation and the expression of symptoms. Figure 10 shows that, in addition to a delay, there is a decrease in the amplitude of the visual evoked potential from the affected eye (largely attributable to conduction block) during the acute phase when vision is poor. One month later, the amplitude of the evoked potential has returned towards normal, indicating that conduction block has partially resolved: vision has also returned to normal. These changes correlate with the cessation of enhancement, presumably signalling a decline in the intensity of inflammation. Demyelination probably persisted, given that there was a persistent delay in the evoked potential. These observations are consistent with a contribution of the inflammatory process itself in conduction block

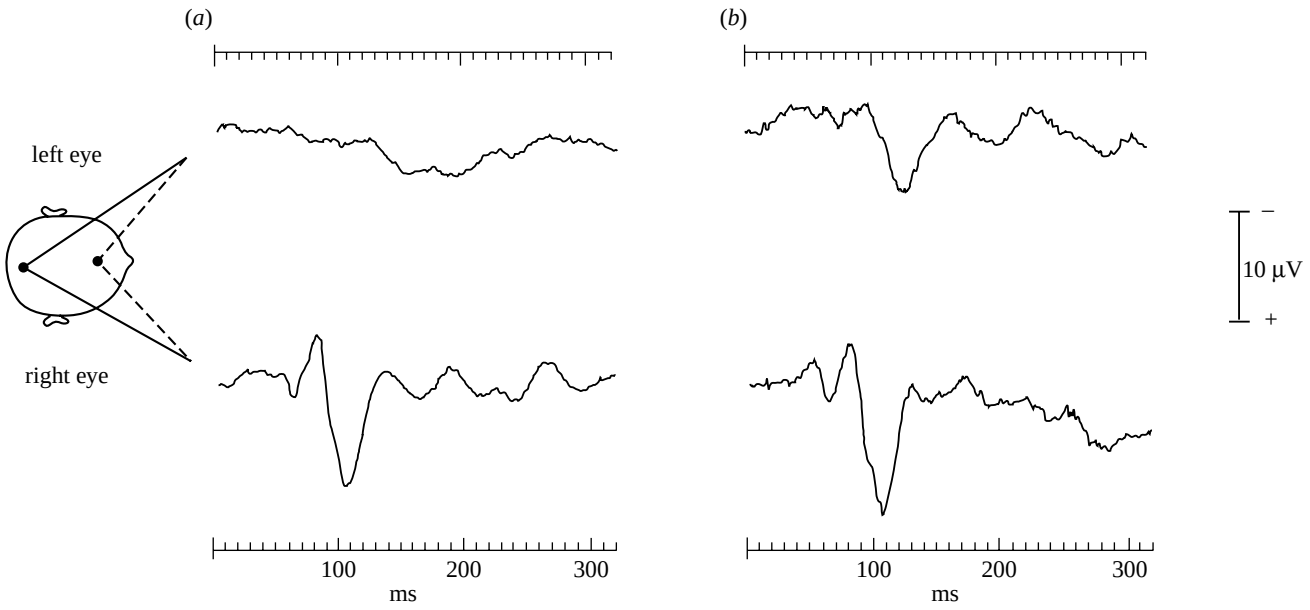

Figure 10. Visual evoked potentials from a 29-year-old female patient with acute left optic neuritis recorded (on 20 February 1990) (*a*) within two weeks of onset of symptoms and (*b*) 31 days later. There is a small, delayed response from the left eye at the first recording which has recovered in amplitude at the second. Redrawn from Youl *et al.* (1991), with permission.

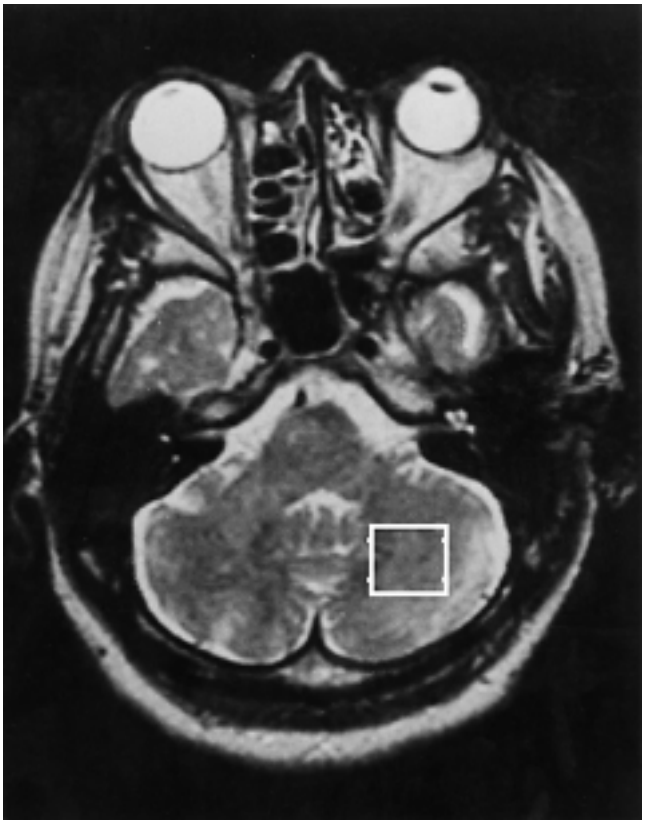

Figure 11. Proton-density weighted scan showing the volume of interest (figure 12) localized in the cerebellar white matter (box).

(see §2(a)(ii)), and with the capacity of persistently demyelinated axons to conduct (see §2(b)(i)) (Youl *et al.* 1991). Thus, an important mechanism of relapse is an acute episode of inflammatory demyelination, and an important mechanism of remission is the resolution of inflammation together with the acquisition of the ability to conduct by demyelinated axons. Remyelination may play little part in the early stages of recovery, given the usual persistence of delay in evoked potentials.

The above account covers the great majority of new lesions. However, some new lesions do not show evidence of enhancement despite the use of special techniques to detect subtle changes (Silver *et al.* 1997) and, in primary progressive MS, gadolinium-DTPA enhancement is exceptional, perhaps because the lesions are less inflammatory in nature (Thompson *et al.* 1991; Revesz *et al.* 1994). These observations provide further evidence for the heterogeneity in the pathogenesis of the MS lesions discussed by Lassmann (this issue).

(b) *Mechanism of irrecoverable deficit*

If remission is so complete in the early stages of MS, why does irrecoverable disability develop later in almost all patients? In principle, two mechanisms might operate. The first is axonal degeneration. Second, where there is failure of repair by remyelination, there may also be failure in the reparative mechanisms which can restore conduction in persistently demyelinated axons, perhaps as a result of repeated inflammatory damage.

(i) *Axonal loss*

It is well established (although often forgotten) that axonal loss is a prominent feature in the MS lesion (see Lassmann, this issue; Perry & Anthony, this issue). From the neurologist's perspective, the interesting question is whether axonal loss contributes to irrecoverable disability. Several lines of evidence suggest that it does. Losseff *et al.* (1996) showed a graded relationship between spinal cord atrophy and disability as measured on the Kurtzke (1983) extended disability status scale. Since loss of myelin alone will contribute to atrophy, a more specific index of axonal loss is required. This index can be provided by proton MRS. The normal proton spectrum from the brain is

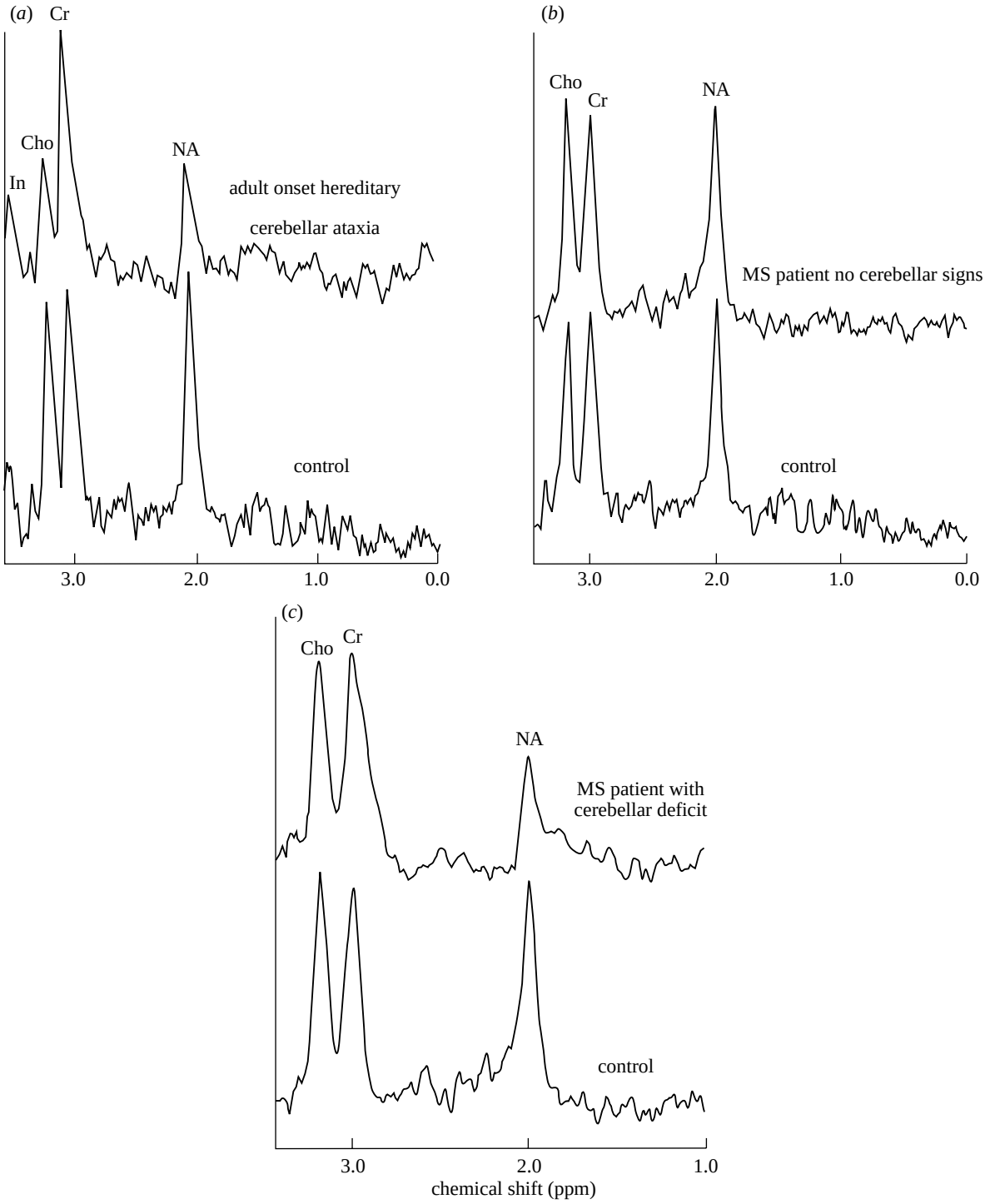

Figure 12. Proton MRS from (*a*) a patient with autosomal dominant cerebellar ataxia and a healthy control, (*b*) a patient with MS but no cerebellar deficit, and a healthy control, and (*c*) a patient with a severe cerebellar deficit due to MS, and a healthy control. Cho, choline; Cr, creatine; In, inositol; NA, *N*-acetyl aspartate. From Davie *et al.* (1995), with permission.

dominated by a peak attributable to *N*-acetyl aspartate, which in the adult brain is virtually confined to neurons and their processes. It is therefore to be predicted that, if neurons (including axons) are lost, there would be a sustained reduction in the concentration of *N*-acetyl aspartate. This has been demonstrated in stroke (Gideon *et al*. 1992) and (of more interest in the present context) in dominantly inherited cerebellar degeneration, in which axonal loss is known to be the pathological basis for atrophy (Davie *et al*. 1995). Against this background, Davie *et al*. (1995) investigated two groups of patients with MS, one without ataxia, in which the level of *N*-acetyl aspartate proved to be normal and one with severe intractible ataxia, in which it was reduced in association with a measured reduction in cerebellar volume (figures 11 and 12). These qualitative results were confirmed quantitatively.

Further evidence for a contribution to disability from axonal loss comes from the work of Barkhof and his colleagues, who demonstrated a correlation between the

progression of disability and an increase in T1 hypointensity in T1-weighted images, and that the latter in turn correlates at post-mortem with axonal loss in the brain (Truyen *et al*. 1996; Van Walderveen *et al*. 1998) as it does in the spinal cord (Mottershead *et al*. 1999).

Taken together, these observations provide strong evidence that axonal loss makes an important contribution to the irrecoverable deficit so common in the later stages of MS.

The factors leading to axonal degeneration are currently of considerable interest. Recent studies have shown that, in patients treated with the humanized anti-CD-52 monoclonal antibody Campath-1H, the risk that disability will continue to progress after treatment is directly related to the amount of inflammation present before treatment, as judged by the volume of gadolinium-DTPA enhancement (Coles *et al*. 1999). The patients in whom disability continued to advance showed progression of cerebral atrophy which, in turn, correlated with a reduction in the concentration of *N*-acetyl aspartate in affected white matter. The latter observation provides further evidence that advancing disability corresponds with progressive axonal loss.

The experimental evidence that inflammation can lead to axonal degeneration was reviewed by Perry & Anthony (this issue). Little is known about the elements of the inflammatory process which might damage axons. Nitric oxide is a candidate. The production of nitric oxide is raised in MS (Bo *et al*. 1994; Bagasra *et al*. 1995; Johnson *et al*. 1995; De Groot *et al*. 1997; Giovannoni *et al*. 1997; Yamashita *et al*. 1997) and so it is interesting that exposure of axons to nitric oxide, at the concentrations anticipated within MS lesions, can result in persistent conduction block and evidence of morphological damage to the fibres concerned (Kapoor *et al*. 1998). These consequences arise if the exposure to nitric oxide occurs in conjunction with sustained impulse activity at physiological frequencies. The potential mechanisms involved remain uncertain, but since inflammatory concentrations of nitric oxide inhibit mitochondrial energy production (Bolanos *et al*. 1994, 1997; Brown *et al*. 1995) and since sustained impulse activity increases the requirement for energy, it is possible that demand exceeds supply resulting in a loss of ionic homeostasis and, thereby, axonal damage.

(ii) *Persistent conduction block*

Persistent conduction block is known to occur in chronic demyelinating peripheral neuropathy (Lewis *et al*. 1982). Although there is no direct evidence that it occurs in central demyelinating disease, it seems likely that it does, for a number of reasons. For example, in §2(a)(i) we noted that the safety factor for conduction in demyelinated axons is often near unity and, thus, it is easy to imagine that, in some axons, the safety factor may be persistently below unity, resulting in persistent conduction block. One structural feature which may theoretically impose this condition is a long internode preceding the demyelinated region, as previously discussed. It is also easy to imagine that the conditions in some lesions may predispose to a safety factor below unity if, for example, there is failure of potassium homeostasis due to a paucity of glial cells, or a tendency to anoxia due to a local paucity of capillaries. Indeed, figure 5 shows records obtained from a lesion in which nearly all the demyelinated axons exhibited conduction block at normal body temperature, and these axons would be expected to show persistent conduction block during normal life since the temperature of the CNS is closely maintained. Finally, it is also possible that some patients may be genetically predisposed to a poor repair of lesions, perhaps due to a deficiency in glial progenitor cells. However, while all these conditions (and many others) are easily imagined, they remain speculative at present.

4. THE CEREBRAL CORTEX IN MULTIPLE SCLEROSIS

Little attention has so far been paid to the cerebral cortex in MS, either structurally or functionally, perhaps because of the rarity of clinical features (e.g. epilepsy or aphasia) deriving from cortical damage. However, it has long been known that cortical plaques do occur and it has recently been shown that they are common (Kidd *et al*. 1999).

It is also possible that there are secondary effects on cortical organization deriving from neuronal loss consequent upon axonal degeneration. This is perhaps particularly likely in the visual cortex, given the high frequency of involvement of the visual pathways in MS and the propensity for transsynaptic degeneration in the visual system. By the same token, changes might also be expected in the retina. These possibilities have yet to be investigated.

The importance of axonal degeneration in MS raises the question of whether adaptive changes might help to contribute to functional recovery. That they do so in stroke and are associated with changes in the pattern of cortical activation has been demonstrated by functional imaging (Weiller *et al*. 1993). Few data are available regarding MS, although recent studies in the visual system (Werring *et al*. 1999) suggest that comparable changes may occur. Whether and to what extent they contribute to remission from relapse is currently being investigated, including whether they may retard the accumulation of neurological deficit in the continuously progressive phase of the disease.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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