Motor axon excitability during Wallerian degeneration

Mihai Moldovan,1 Susana Alvarez1 and Christian Krarup 1,2

1 Division of Neurophysiology, Institute of Neuroscience and Pharmacology, Panum Institute, University of Copenhagen, Copenhagen, Denmark
2 Department of Clinical Neurophysiology, Neuroscience Center, Rigshospitalet, Copenhagen, Denmark

Correspondence to: Prof. C. Krarup, MD DMSc FRCP, Department of Clinical Neurophysiology NF3063, Neuroscience Center, Rigshospitalet, 9 Blegdamsvej, 2100 Copenhagen, Denmark
E-mail: ckrarup@rh.dk

Axonal loss and degeneration are major factors in determining long-term outcome in patients with peripheral nerve disorders or injury. Following loss of axonal continuity, the isolated nerve stump distal to the lesion undergoes Wallerian degeneration in several phases. In the initial ‘latent’ phase, action potential propagation and structural integrity of the distal segment are maintained. The aim of this study was to investigate in vivo the changes in membrane function of motor axons during the ‘latent’ phase of Wallerian degeneration. Multiple indices of axonal excitability of the tibial nerve at ankle distal to axotomy were monitored by ‘threshold-tracking’. The plantar compound muscle action potentials (CMAPs) were recorded under anesthesia in three animal models: 8-week-old wild-type mice, 8-week-old slow Wallerian degeneration mutant mice and 3-year-old cats. We found that the progressive decrease in CMAP following crush injury was associated with slowing of conduction and marked abnormalities in excitability: increased peak threshold deviations during both depolarizing and hyperpolarizing threshold electrotonus, enhanced superexcitability during the recovery cycle and increased rheobase. In the context of decreased current-threshold slope and increased chronaxie, these deviations in excitability were consistent with a decrease in voltage-dependent Na⁺ and K⁺ conductances. Our data suggest that during the ‘latent phase’ of Wallerian degeneration there is a gradual disruption in ion-channel function leading to abnormalities in excitability that precede conduction failure and axonal disintegration. These findings may have clinical relevance and should be taken into consideration in interpretation of the specificity of abnormalities in excitability measures in disorders characterized by axonal degeneration.

Keywords: excitability; internode; ion channels; node of Ranvier; nerve activity; regeneration

Abbreviations: ALS = amyotrophic lateral sclerosis; CMAP = compound muscle action potential; RRP = relative refractory period; TEd = peak threshold reduction during depolarizing electrotonus; TEh = peak threshold increase during hyperpolarizing electrotonus

Introduction

The interruption of a peripheral nerve continuity sets in motion a complex sequence of electrical, chemical and morphological events referred to as ‘Wallerian degeneration’ (Waller, 1850). These events include: (i) disintegration of the axonal cytoskeleton; (ii) fragmentation of myelin sheets; (iii) degradation of myelin and axonal debris by activated macrophage; and (iv) remodelling of the distal stump by proliferating Schwann cells. In the distal severed stump, an initial ‘latent phase’ of varying intervals of several hours–days in different species is characterized by persistent evoked electrical activity and morphological integrity (Lubinska, 1977; Beirowski et al., 2005). Over time distal segment electrical conductivity fails first before morphological changes become apparent, followed ultimately by axonal disintegration. With the introduction of the CS7Bl/Ola mouse mutant with
delayed Wallerian degeneration (Lunn et al., 1989), now formally named the Wld<sup>S</sup> mouse (Lyon et al., 1993), it has become clear that axonal disruption marks the initiation of an active ‘axonal self-destruction program’, in many aspects similar to (Sievers et al., 2003) but not identical to apoptosis (Ikegami and Koike, 2003).

During the ‘latent phase’ of Wallerian degeneration, electrical stimulation of the distal nerve stump is able to evoke normal compound motor action potentials (CMAPs) for extended periods of time until progressive failure of neuromuscular transmission occurs (Okamoto and Riker, 1969; Miledi and Slater, 1970; Gilliatt and Hjorth, 1972). Persistence of conduction in the distal stump itself could be studied in vitro on excised nerve (Lunn et al., 1989) and following sciatic nerve lesion. Axons in the distal stump were able to conduct action potentials ~1 day in wild-type mice and 1–2 weeks in Wld<sup>S</sup> mice (Lunn et al., 1993), 1989) and following sciatic nerve lesion. Axons in the distal stump were able to conduct action potentials <50% of axons appeared morphologically intact, suggesting that an impairment of axonal membrane function precedes cytoskeletal dissolution (Tsao et al., 1994).

Membrane function of motor axons can be monitored in vivo using the clinically available technique of ‘threshold-tracking’, which allows the strength of a sub-maximal stimulus (threshold) to be continuously adjusted by computer to achieve a constant fraction of ~40% of the maximal CMAP (Bostock et al., 1998). By comparing relative changes in threshold under different test conditions, several measures of axonal excitability can be derived to explore both nodal and internodal biophysical properties of myelinated axons at the site of stimulation (Kiernan et al., 2000). Such excitability studies revealed functional abnormalities in a wide spectrum of neuropathies with variable degree of ongoing axonal degeneration, including amyotrophic lateral sclerosis (ALS) (Bostock et al., 1995; Kanal et al., 2006; Tamura et al., 2006; Vucic and Kiernan, 2006). Nevertheless, in the clinical setting it was not possible to distinguish to which extent the observed abnormalities reflected specific changes associated with the disease process in axons or the impairment of excitability in axons committed to degenerate.

The aim of this study was to investigate the changes in excitability of motor axons during experimental Wallerian degeneration. We tested both mouse and cat animal models of tibial nerve lesions and found concordant changes in excitability indices. Brief preliminary reports on some of these changes were previously published (Moldovan and Krarup, 2005; Moldovan et al., 2006).

**Material and Methods**

**Animals and experimental design**

The electrical properties of the distal stump of the tibial nerve motor axons following crush injury were monitored in vivo by serial conduction and excitability studies under anaesthesia. Three different animal models were used: (i) wild-type mice (n = 20, adult female, 18–22 g, C57BL/6J HSD, Harlan); (ii) Wld<sup>S</sup> mutant mice (n = 15, adult female, 18–22 g, C57BL/6J WLDS, Harlan); and (iii) normal cats (n = 4 nerves from two female adult cats, 3- to 4-years old, Iffa-Credo, France). Three additional wild-type mice were used for control experiments.

In mice, repeated electrophysiological investigations were carried out 10, 14 and 17 h following axotomy and then continued daily during the following week. In cats, investigations were carried out daily in the week following axotomy. At the completion of experiments, the mice were killed by cervical dislocation and the cats were killed by an overdosage of 250 mg pentobarbital (i.v.). Experimental procedures were approved by the Danish National Animal Experiment Committee.

**Anaesthesia, nerve lesions and experimental setup in mice**

Surgical procedures and electrophysiological investigations were carried out under anaesthetic and analgesic mixture containing Fentanyl (0.45 mg/kg), Droperidol (30 mg/kg) and Midazolam (3 mg/kg). The mixture was injected subcutaneously 0.4 ml every 30 min. The relatively large volume ensured that the animals were adequately hydrated during the investigations. The ECG (Sirecust 341, Siemens) was monitored.

After shaving the right hind-limb, surgical procedures were carried out with the aid of a stereomicroscope (MZ6, Leica, Inc.). Through a minimal incision, the right sciatic nerve was crushed at mid-thigh level (~1 cm above the knee) by maximally clamping the nerve with a smooth-jawed micro-forceps for 30 s. After the crushing procedure the injured nerve remained translucent and in continuity. The surgical wounds were closed with three stitches. No weight-loss was observed during the daily measurements after the surgery.

For the serial electrophysiological investigations the mouse was fixed in a stereotaxic frame (dual manipulator with mouse adaptor 51624, Stoelting) on a temperature controlled pad (HB 101/2, LSI Letica) set to 37°C. The right leg was placed on a piece of hydrophobic cotton to reduce the stimulus artefact and fixed (but not stretched) with a clamp from the distal toes.

Tibial nerve was stimulated with the cathode placed at ankle and the anode placed ~1 cm proximally on the leg. Two types of stimulation electrodes were used: (i) surface electrodes (Ag/AgCl cup EEG electrode filled with 10–20 EEG paste and folded around the ankle) (n = 5); and (ii) platinum needle electrodes (n = 15).

The evoked CMAP was recorded from plantar muscles using custom-made platinum needle electrodes inserted into the foot ~0.5 cm apart. A ground electrode was inserted subcutaneously in the left thigh.

**Anaesthesia, nerve lesions and electrophysiological setup in cats**

Surgical procedures and electrophysiological investigations were carried out under anaesthesia by a mixture of 0.7 ml Ketamine (10 mg/kg) and 0.4 ml Xylazine (2 mg/kg) i.m., maintained by subcutaneous injection as needed, and monitored by the suppression of the corneal reflex elicited by air puff.

Nerve lesions were carried out under aseptic conditions. The hindlimbs were first shaved and skin disinfected by iodine. Tibial nerves were transected ~3 cm proximal to the ankle through a small incision. The surgical wounds were closed in layers. After full recovery from anaesthesia the cats were returned to a communal cage. No signs of infection, gait alterations or other behavioural disturbances were observed as a result of the lesion. All cats stayed healthy during the observation period.
For the electrophysiological investigations, the hind limbs were shaved and skin was wiped with ether to ensure good electrode contact. Nerve stimulation was performed via non-polarizable surface electrodes (Blue sensor disposable electrodes N-10-A, Ambu, Denmark). The cathode was placed over the tibial nerve at ankle between the bones and the Achilles’ tendon about 5 cm distal to the nerve lesion. The anode was placed over muscle 5 cm further proximally. Recording was carried out from plantar muscles through a subcutaneous needle electrode referenced to the first toe. A ground electrode was placed around the foot between stimulation and recording. Temperature was maintained at 37°C by placing the animal on a thermostatically heated rubber pad and covering it with hydrophobic cotton. An additional feed-back operated heating lamp was used to maintain the skin temperature over the tibial nerve above 35°C.

Multiple tests of nerve excitability

Threshold tracking

Stimulation was carried out with a custom made, photo-isolated, linear, battery-powered, constant current stimulator (maximal output 100 mA). The test pulse duration was 1 ms except for surface stimulation in mice where it was reduced to 0.4 ms to reduce the stimulus artefact. The evoked CMAP (10Hz–6kHz, 10C02, Dantec) was digitized by computer (PC Pentium) with an analog-to-digital (A/D) board (DT2812, Data Translation, Marlboro, MA, USA), using a sampling rate of 10kHz. CMAP amplitudes were measured peak-to-peak. Latencies of the fastest conducting axons were measured to the first onset from baseline.

The target ‘threshold’ CMAP was set to 40% of maximum peak-to-peak CMAP amplitude. Stimulation and recording were controlled by standard threshold tracking software (QTRAC, copyright Institute of Neurology, London).

Excitability protocol

Investigations were performed using a protocol designed to measure excitability of human nerves (TRONDHM, Kiernan et al., 2000). The full sequence of tests included: (i) stimulus-response curves for 0.2 and 0.4 or 1 ms test currents; (ii) threshold electrotonus induced by 100 ms polarizing currents set to +40% (depolarizing) and –40% (hyperpolarizing) of the control threshold; (iii) current-threshold (I/V) relationship tested with 1 ms pulses at the end of 200 ms polarizing currents altered in a ramp fashion from 50% (depolarizing) to –100% (hyperpolarizing) of the control threshold and (iv) recovery of excitability from 2 to 200 ms after a supramaximal conditioning stimulus.

Off-line threshold estimation

During ‘on-line’ measurement, an acceptable threshold current was considered after evoking three consecutive CMAP responses within 15% of target, or after evoking alternate responses each side of the target. All stimulus-response pairs were however saved to allow further analysis. Our method for ‘off-line’ threshold estimation was previously described in detail (Moldovan and Krarup, 2004b). Briefly, we estimated the sigmoid that fitted the stimulus-response relationship ‘at rest’ and then we calculated its average displacement on the stimulus axis for each group of stimulus-response pairs saved in a particular test condition.

Excitability indices

Values of several excitability indices (Burke et al., 1999) were derived from excitability tests for numerical analysis. The naming and calculations were largely similar to those described for multiple excitability tests (Kiernan et al., 2001).

Rheobase and chronaxie

Chronaxie (ms) and rheobase (mA) were estimated at threshold (40% responses) from the sigmoid fits through the 0.2 and 1.0 ms stimulus-response relationships as previously described in detail (Moldovan and Krarup, 2004b). The term ‘chronaxie’ is preferred in this study to the ‘strength-duration time constant’ (Bostock, 1983) or $r SD$ (Mogyoros et al., 1996) to indicate that a slightly different method of calculation was used.

Maximal threshold changes during sub-threshold depolarization and hyperpolarization

The accommodation to prolonged polarization induces complex non-linear changes in threshold with a time-course that differed slightly between the animal models tested. For simplicity, we limited our quantification to the peak percentage threshold reduction during 40% depolarization (TD0), and the peak threshold increase during 40% hyperpolarization (TEh).

Resting I/V slope

As a measure of ‘resting’ input conductance, we calculated the ‘resting I/V slope’ as the dimensionless slope of the current-threshold relationship calculated for polarizing currents between –10% and +10% of resting threshold.

Relative refractory period and superexcitability

During the recovery cycle the relative refractory period (RRP, ms) was calculated as the time to the first intercept with the delay-axis (computed by linear interpolation of the two nearest points). Superexcitability (%) was the peak threshold reduction following RRP.

Data analysis and statistics

All computations for ‘off-line’ threshold estimation, data analysis and data presentation used in this study were performed using a custom-made toolbox implemented in MATLAB (version 2006a, MathWorks, Inc.). Both recorded and calculated parameters were stored in an SQL database to maintain consistency and facilitate Mann–Whitney statistical comparisons using SPSS (version 13, SPSS, Inc.). Results in numbers are given as mean ± SEM.

Results

Conduction studies

An overview of conduction studies in the three animal models is presented in Fig. 1. Prior to the lesion, WldS mice were electrophysiologically indistinguishable from wild-type controls. Stimulation of the tibial nerve at ankle evoked CMAPs with an amplitude of $7.5 ± 1$ mV and a latency of $1.2 ± 0.1$ ms (Fig. 1A). In cats, stimulation of the tibial nerve at ankle evoked CMAPs with an amplitude of $21.1 ± 1$ mV and a latency of $2.2 ± 0.2$ ms (Fig. 1C).

The observed time-course of CMAP amplitude decay was markedly different in the three animal models investigated: (i) in wild-type mice, CMAP was unchanged at 10 h and then dropped rapidly within the next 8 h (Fig. 1D); (ii) in WldS...
mutant mice the decay was much slower and CMAPs could still be evoked after 1 week (Fig. 1E); and (iii) in cats CMAPs remained unchanged for at least a day after the lesion and then progressively declined in amplitude within the next 4 days (Fig. 1F). In all animal models, the decay in amplitude was associated with a progressive increase in latency up to ~25% (Fig. 1D–F). As a consequence of CMAP decay reliable threshold estimates could be obtained from most nerves up to ~14 h in control mice, ~3 days in WldS mice and ~4 days in cats (marked with grey areas in Fig. 1).

---

**Fig. 1** Conduction studies during Wallerian degeneration. CMAP recordings are presented for a wild-type mouse (A), WldS mouse (B) and cat (C). Latencies of the fastest motor axons are marked. Traces in A are obtained after stimulation with needle electrodes and in B after stimulation with surface electrodes; note the larger stimulus artefact in the latter. The corresponding changes in amplitude (filled symbols) and latency (open symbols) are presented for wild-type mice (D), WldS mice (E) and cats (F). In mice, means include measurements from investigations with both surface and needle electrodes. Error bars represent SEM. Grey areas indicate the time-points where reliable threshold estimates for excitability testing could still be obtained from most nerves.
Excitability studies with surface and needle stimulation in mice

To identify the most suitable method for excitability testing in mice, we compared excitability measures in six tibial nerves from three wild-type control mice stimulated via (i) a large Ag/AgCl non-polarizable surface electrode; (ii) a thin non-polarizable Ag/AgCl wire inserted percutaneously at ankle; and (iii) platinum needles inserted at ankle. These data are illustrated in Fig. 2.

As compared with surface stimulation, stimulation using needle electrodes required a much lower current reflected by a 4× reduced rheobase (Fig. 2B). The evoked CMAPs had similar latency and ~5% smaller amplitudes. The more focused stimulation delivered via needle electrodes induced a much smaller volume conducted stimulus artefact (Fig. 1A) than surface stimulation (Fig. 1B).

The recovery cycle (Fig. 2F) and chronaxie (Fig. 2B) that did not require the use of sustained DC currents did not differ between...
the stimulation techniques. Nevertheless, with platinum needle electrodes deviations during threshold electrotonus and current-threshold relationship were smaller than with surface electrodes (Fig. 2A, C and E). The measures with platinum electrodes were relatively similar to the measures with the Ag/AgCl wire, suggesting that the differences were more likely related to electrode geometry than to an artefact reflecting polarization of the platinum electrode. We, therefore, decided to investigate excitability in mice both using surface and needle electrodes (the Ag/AgCl wire was not suitable for repeated investigations).

Multiple measures of motor axon excitability in wild-type mice

No changes in excitability could be detected at 10 h after the lesion (data not shown). At 14 h excitability measures were markedly abnormal.

The largest abnormalities were seen in response to polarizing currents (Fig. 3). Peak threshold deviations during threshold electrotonus were enhanced (Fig. 3A and C). In response to depolarization, there was a larger than normal decrease in threshold (TED was enhanced from 50.7 ± 3.4% to 73.3 ± 0.6%, P < 0.05) that seemed to accommodate (in the sense of returning to 40%) within 100 ms (Fig. 3A). In response to hyperpolarization there was a larger than normal threshold increase (TEh was enhanced from −97.3 ± 5.1% to −120.8 ± 5.3%, P < 0.05), that did not accommodate (in the sense of returning to −40%) within 100 ms (Fig. 3C). Accommodation to hyperpolarization did occur within 200 ms as indicated by the current-threshold relationship (Fig. 3F). The current-threshold relationship was, however, abnormal at low polarization currents, as indicated by a decreased resting I/V slope from 1.2 ± 0.2 to 0.7 ± 0.1 (P < 0.05). The recovery cycle did not show statistical significant abnormalities (Fig. 4A). Nevertheless, the strength-duration properties were also abnormal, revealing an increased chronaxie (Fig. 6C).

By comparison with surface stimulation, using needle stimulation revealed deviations in threshold electrotonus (Fig. 4A and C) and current-threshold relationship (Fig. 4F) in the same direction albeit of a lower magnitude. As in the wild-type mice, the changes in recovery cycle were more pronounced when investigated using needle electrodes (Fig. 5D). The superexcitability was markedly enhanced from −12.4 ± 1.3% to −28.3 ± 2.5% (P < 0.05). Furthermore, the late subexcitability was also enhanced from 17.2 ± 1.1% to 22.1 ± 2.4% (P < 0.05). Both rheobase (Fig. 6B) and chronaxie (Fig. 6D) were increased.

Multiple measures of motor axon excitability in cats

Prior to lesion, excitability measures were similar to our previous report on the same model (Moldovan and Krarup, 2004b) and markedly deviated (P < 0.05) from their corresponding measures in mouse: cat nerves showed larger rheobase and chronaxie, larger TEh, smaller I/V resting slope and longer RRP (Fig. 7).

At Day 4 after the lesion, there were marked changes in excitability that were similar to the observed changes in mice: there were increased peak threshold deviations during both depolarizing (Fig. 7A) and hyperpolarizing (Fig. 7C) threshold electrotonus accompanied by an increase in both rheobase (Fig. 7D) and chronaxie (Fig. 7B). The changes in current-threshold relationship were much more pronounced in the cat (Fig. 7E) than in the mice showing a marked decrease in resting I/V slope from 0.8 ± 0.03 to 0.4 ± 0.03 (P < 0.05). Changes in recovery cycle were, however, less pronounced showing only a slight reduction in RRP from 3.7 ± 0.3 m to 2.6 ± 0.2 ms (P = 0.05, Fig. 7F).

Discussion

We investigated in vivo changes in motor axon excitability during experimental Wallerian degeneration of the tibial nerves of cat and mouse. Following axotomy, there was a progressive decrease in CMAP and slowing of conduction that occurred before structural disintegration of axons took place. These changes in conduction were associated with marked abnormalities in excitability that could reflect disruption of ion-channel function. Our findings may have clinical relevance and should be taken into consideration in interpretation of the specificity of abnormalities in excitability measures in disorders characterized by axonal degeneration.
Technical issues regarding excitability studies in different animal models

During excitability testing, long-lasting DC current pulses are used to produce a non-propagating change in membrane potential thereby altering the activity of voltage-dependent conductances. In humans, this can be achieved by stimulation via non-polarizable, Ag/AgCl surface electrodes. This is technically challenging in small experimental animals, in particular because stimulation via large surface electrodes of the mouse tibial nerve (Sawai et al., 2008) may recruit other nerves in the leg confounding ‘threshold-tracking’ responses. A more focal and precise stimulation requiring less current can be achieved with subcutaneous needles (Moldovan and Krarup, 2006). However, polarization of platinum needles is a potential complication. To address these methodological concerns, we investigated stimulation with both

**Fig. 3** Accommodation to polarization in mice applied with surface electrodes. Depolarizing threshold electrotonus (A and B), hyperpolarizing threshold electrotonus (C and D) and current-threshold relationship (E and F) are presented for wild-type and Wld^S^ mutant mice, respectively. Mean traces are presented after 14 h (filled symbols) and 3 days (open symbols). In one, wild-type mouse recordings could be carried out after 17 h (stippled lines) and the corresponding mean at 17 h is illustrated for Wld^S^ mice. Error bars represent SEM. Grey areas represent control mean ± SEM.
surface and needle electrodes in the mouse. Furthermore, we investigated excitability changes in a cat model of Wallerian degeneration where human-type surface electrodes could be used (Moldovan and Krarup, 2004b).

Though the time-course of Wallerian degeneration following axotomy differed, and different stimulation methods were employed, we found that abnormalities in excitability were remarkably consistent in degenerating tibial nerves of the wild-type mice, WldS mutant mice and cats with both surface and needle electrodes. It is therefore unlikely that our findings reflected technical issues of the experimental model.

**Progressive abnormalities in excitability of degenerating axons**

We found that in degenerating motor axons increasingly more current was required to evoke propagated action potentials, as reflected by the increase in rheobase. Conduction of action
potentials in myelinated axons is primarily dependent on the nodal Na⁺ channels and passive membrane properties (Chiu et al., 1979). Thus at a time when the myelin sheath is morphologically intact and the axonal cytoskeleton is largely preserved (Lubinska, 1977; Tsao et al., 1994; Beirowski et al., 2005; Alvarez et al., 2008), the increased rheobase could be explained by a reduction in Na⁺ channel function (Mogyoros et al., 1998, 1999). Furthermore, such a decrease in voltage-dependent Na⁺ conductance could also account for the observed conduction slowing (Yokota et al., 1994).

The effects of specific reduction of Na⁺ conductance has been studied in puffer fish poisoning (Kiernan et al., 2005). The threshold increase during hyperpolarizing electrotonus in our study was consistent with Na⁺ channel reduction but it was inconsistent with the increased reduction in threshold during electrotonus depolarization and with the increase in refractoriness and in supernormality. The ‘fanning-out’ (Kaji, 1997) of both hyperpolarizing and depolarizing threshold electrotonus suggests an additional reduction in K⁺ conductances. Since the deviations of excitability in depolarizing threshold electrotonus appeared to accommodate

---

**Fig. 5** Recovery cycle in wild-type mice (A and C) and Wldś mice (B and D) were obtained after stimulation with surface electrodes (top) and needle electrodes (bottom), respectively. Mean traces are presented after 14 h (filled symbols) and 3 days (open symbols). Error bars represent SEM. Grey areas represent control mean ± SEM.
within 100 ms, and there was a large late subexcitability during the recovery cycle (Fig. 5D) it was likely that slow K⁺ conductances were less affected than fast K⁺ conductances (Schwarz et al., 2006). Furthermore, a reduction in the paranodal fast K⁺ channel conductance was also consistent with the enhanced superexcitability during the recovery cycle (Bostock et al., 1981; Barrett and Barrett, 1982; Kiernan and Bostock, 2000).

The strength duration time constant is voltage-dependent due to the persistent fraction of the Na⁺ channels open at rest (Bostock and Rothwell, 1997). In the context of increased rheobase, chronaxie was unlikely to reflect an increase in the conductance of persistent Na⁺ channels. In vivo observations of peripheral axons suggested that a widening of the nodal gap due to paranodal myelin retraction could occur very early during Wallerian degeneration (Williams and Hall, 1971). It therefore possible that the higher resistance of the internodal membrane exposed by myelin retraction could account for the observed increased chronaxie (Brismar, 1981).

Taken together with the general fall in membrane conductance reflected by decreased resting I/V slope (Kiernan and Bostock,
2000; Kiernan et al., 2000) the observed abnormalities in excitability during Wallerian degeneration may suggest a progressive reduction in both voltage-dependent Na\(^+\) and K\(^+\) conductances.

**Mechanisms of decreased voltage-dependent conductances**

Degenerating motor axons showed increased rheobase, ‘fanning-out’ of threshold electrotonus, decreased resting I/V slope and increased superexcitability. All these deviations in excitability measures could be induced during experimental membrane hyperpolarization of intact nerves of humans (Kiernan and Bostock, 2000), cats (Moldovan and Krarup, 2004a) and mice (Moldovan and Krarup, 2006). Hyperpolarization alters excitability by reducing the fraction of K\(^+\) and Na\(^+\) channels normally opened at resting membrane potential in peripheral axons (Chiu and Ritchie, 1984; Bostock et al., 1991; Baker, 2000). Hyperpolarization also reduces chronaxie by decreasing the fraction of the persistent Na\(^+\) channels opened at rest (Mogyoros et al., 1998, 1999). In apparent

---

**Fig. 7** Multiple excitability measures during degeneration after axotomy in four tibial nerves from cat: depolarizing (A) and hyperpolarizing (C) threshold electrotonus; chronaxie (B) and rheobase (D); Current-threshold relationship (E) and recovery cycle after a single impulse (F). Grey areas represent control mean ± SEM. Recordings were obtained 4 days after the lesion and are presented individually as thin lines.
discrepancy with the deviations of the other excitability measures, chronaxie was increased and not decreased in degenerating nerves. Nevertheless, chronaxie was not found to be particularly voltage-dependent (Kiernan and Bostock, 2000). It is therefore possible that the net deviations in chronaxie were dictated by other factors, such as changes in passive membrane properties, and did not accurately reflect membrane hyperpolarization.

What then could be the mechanisms of a hypothetical membrane hyperpolarization? One of the first ionic balances found in degenerating axons was an increase in intracellular Na⁺ (LoPachin et al., 1990). It is possible that a Na⁺ leak would drive the Na⁺/K⁺ pumps (Skou, 1965) to restore the ionic equilibrium leading to net membrane hyperpolarization (Ritchie and Straub, 1957; Kaji and Sumner, 1989). Threshold tracking techniques were previously found to be particularly sensitive to detect hyperactivity of the Na⁺/K⁺, induced by repetitive conduction (Bostock and Bergmans, 1994; Kiernan et al., 1996, 2004; Moldovan and Krakup, 2006) and during reperfusion after ischemia (Weigl et al., 1989; Bostock et al., 1991, 1994; Kiernan and Bostock, 2000). With time, the Na⁺/K⁺ pumping and hyperpolarization would be overcome by the Na⁺ leak giving way to ‘Na+‐mediated axonal degeneration’ (Bechtold and Smith, 2005). Nevertheless, such events would occur after conduction failure and are not accessible by excitability studies.

Whether a transient increase in Na⁺ leading to hyperactivity of Na⁺/K⁺ pump and hyperpolarization does occur in degenerating axons prior to energy failure and depolarizing block (Manjo and Karnaowsky, 1958; Bachelard and Silva, 1966) remains, however, speculative. A more direct explanation for the decreased voltage‐dependent Na⁺ and K⁺ channel function observed in degenerating motor axons remains a progressive reduction of the density of voltage‐dependent channels mimicking the effect of K⁺ and Na⁺ channel closure by membrane hyperpolarization. Due to the limited half‐life of voltage‐dependent ion channels, such an ‘acquired channelopathy’ would not be unexpected in axon membrane no longer maintained by axonal transport and fits with the prediction of mathematical modeling (Kanai et al., 2006).

Comparison of excitability changes in experimental Wallerian degeneration and ALS

Early nerve excitability tests in ALS patients revealed an increased chronaxie (Mogyoros et al., 1998) and abnormalities in excitability consistent with either decreased accommodation (Type 1 abnormality) or an unstable membrane with markedly increased accommodation (Type 2 abnormality) to sub‐threshold depolarization (Bostock et al., 1995). Later excitability studies confirmed the increased chronaxie, whereas, other abnormalities in excitability appeared less pronounced and primarily of Type 1 (‘fanning‐out’ of threshold electrotonus and increased superexcitability) (Horn et al., 1996; Kanai et al., 2006; Vucic and Kiernan, 2006). Paradoxically, it was found that with the disease progression there is a ‘pseudo‐normalization’ of excitability abnormalities in ALS that was attributed to either loss of ‘the sickest’ axons (Vucic and Kiernan, 2006) or an increase ‘leakiness’ of axonal membrane (Kanai et al., 2006).

We report here that motor axons undergoing Wallerian degeneration show an increase in chronaxie and changes in excitability largely similar to Type 1 abnormalities found during the time‐course of ALS (Kanai et al., 2006; Vucic and Kiernan, 2006). Our data suggest that a gradual decrease in functional ion‐channels precedes conduction failure and axonal disintegration during Wallerian degeneration. It is therefore possible that a similar ‘acute channelopathy’ is reflected in excitability measures during axonal degeneration in ALS. This view is supported by the fact that Type 1 abnormalities in ALS appear maximal during disease stages when the largest number of axons degenerate as also observed in ALS (Winhammar et al., 2005; Vucic and Kiernan, 2006). Thus the contribution of degenerating but still conducting axons to excitability measures should be taken in account when interpreting excitability measures in disorders characterized by axonal degeneration.

Acknowledgements

We are grateful to Steven H. Horowitz, M.D. for comments on the manuscript.

Funding

Lundbeck Foundation; the Novo Nordisk Foundation; the Danish Medical Research Council; the Ludvig and Sarah Elsass Foundation; the Foundation for Research in Neurology.

References