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Decompaction of CNS myelin leads to a reduction of the conduction velocity of action potentials in optic nerve

Rafael Gutiérrez*[,1], Detlev Boison[2], Uwe Heinemann[1], Wilhelm Stoffel

Institut für Biochemie and Institut für Neurophysiologie, Universität zu Köln, Josef Steltzmann Strasse 9, 50931 Köln, Germany

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Abstract

The conduction velocity of action potentials in nerve fibres is proportional to the degree of myelination. Here we studied the influence of myelin ultrastructure on the compound action potential conduction velocity in optic nerves of the proteolipid protein (PLP)-deficient mouse model, which displays loose myelination in central fibres. We show that a myelin decompaction leads to a suboptimal conduction velocity. The significance of myelin ultrastructure for conduction of action potential in the optic nerve is discussed.

Keywords: Conduction velocity; Optic nerve; Myelin decompaction; Gene targeting; Proteolipid protein

It is well documented that myelinated fibres have a higher conduction velocity than unmyelinated fibres. Myelinated and unmyelinated central axons have been studied in the optic nerve of naturally occurring mouse mutants [1,9]. Estimations of optic nerve conduction velocity for unmyelinated and myelinated axons range between 0.4 and 2.4 m/s respectively [4,5]. These investigations do not give any clues to the precise influence of myelin ultrastructure on nerve conduction properties. Here we used the proteolipid protein (PLP)-deficient mouse model [3] exemplifying structurally altered decompacted CNS myelin sheaths lacking the PLP-mediated adhesion of the outer membrane surfaces of oligodendrocyte processes, to assess the importance of myelin compaction on the conduction velocity of action potentials. For these investigations the optic nerve is a well suited part of the CNS consisting exclusively of astro- and oligodendroglia and parallel bundles of axons without cell bodies. We compared the action potential velocities of optic nerves of homozygous and heterozygous PLP-deficient mice with those of wild type mice. The heterozygous mice show a cellular mosaicism due to a random inactivation of the X-linked PLP-gene during Barr body formation leading to a mosaic-like packing of their myelin sheaths. As a control, to exclude any effects other than myelin ultrastructure, we compared conduction velocities in juvenile animals, where myelination is still incomplete.

Optic nerves were obtained from mice of two different age groups: 8–10 weeks old (adults) and 16 days old (juveniles). The mice were grouped as wild type (WM), homozygous PLP-deficient (TM), and heterozygous PLP-deficient (HM). Optic nerves were prepared after decapitation under deep CO_2 anesthesia. They were dissected from the beginning of the optic chiasm to the eyeball and placed in a recording chamber over a nylon net. They were constantly perfused with prewarmed (35°C) and oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 124; KCl, 5; NaH_2PO_4, 1.25; $MgSO_4$, 2; $CaCl_2$, 2; $NaHCO_3$, 26; glucose, 10 (pH 7.35). For recording of compound action potentials a glass microelectrode (10 MΩ) filled with 150 mM NaCl, was inserted into the optic nerve. The action potentials were recorded against a reference electrode consisting of an Ag–AgCl pellet with direct contact to the

* Corresponding author.

[1] Present address: Abteilung Neurophysiologie, Institut für Physiologie der Charité, Humboldt Universität zu Berlin, Tucholskystrasse. 2, 10117 Berlin, Germany.

[2] Present address: Institute of Pharmacology, University of Zurich, Winterthurerstrasse 190, CH-8057, Zurich, Switzerland.

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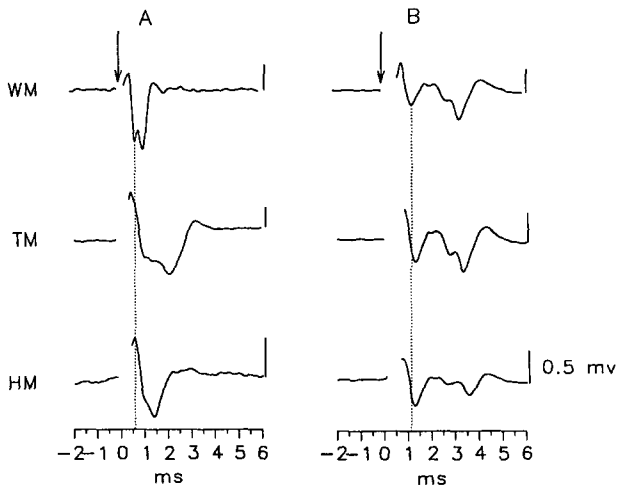

Fig. 1. Typical recordings of averaged ($n = 20$) compound action potentials of adult (A) and juvenile (B) optic nerves from WM, TM and HM. Arrow, onset of the stimulus. The distance (d) between recording and stimulating electrodes (in mm) and the conduction velocities (in m/s) for each component ($c_{1,2,3}$) were as follows. (A) WM, $d = 1.69$, $c_1 = 1.97$, $c_2 = 1.32$; TM, $d = 1.37$, $c_1 = 1.19$, $c_2 = 0.59$; HM, $d = 1.15$, $c_1 = 1.007$, $c_2 = 0.73$. (B) WM, $d = 2$, $c_1 = 2.34$, $c_2 = 1.28$, $c_3 = 0.56$; TM, $d = 1.24$, $c_1 = 1.08$, $c_2 = 0.57$, $c_3 = 0.43$; HM, $d = 2$, $c_1 = 1.56$, $c_2 = 0.78$, $c_3 = 0.56$.

bath. Bipolar platinum wire electrodes (diameter 50 μm) were used for stimulation. The stimulating electrode was placed at the surface of the nerve 1 mm away from the cut end. Stimulation consisted of square pulses with a duration of 10–30 μs and a stimulus intensity of 10–20 V. Stimulus intensity was initially set to get a maximal response which usually consisted of three components. However, in such conditions the first component of the compound action potential was contaminated by the stimulus artifact. The stimulus intensity was subsequently lowered to a level where the first component in the compound action potential was readily visible and at a level which permitted recording of at least two components. This was well above the threshold for the first action potential component. The distance between the recording and stimulating electrodes was read from a calibrated micromanipulator. The latency from the beginning of the stimulation artifact to the first and subsequent negative peaks was measured after averaging 20 compound evoked potentials (SIGAVG PC-compatible program, Cambridge Electronic Design Ltd.) and apparent conduction velocities were calculated. Mean values and standard deviation (SD) were determined and the data were statistically compared using Student's t-test.

Typical responses from adult nerves obtained from 8–10 week old animals are shown in Fig. 1A. The compound action potential in nerves from WM consisted of a first small positive deflection and two negative components, with a larger positive component and a broader negativity with two peaks in the TM and HM (Fig 1A). In normal animals often a third small negative component was seen. The presence of different negative peaks is consistent with different conduction velocities. Nerves of WM had an apparent mean conduction velocity of 2.12 ± 0.14 m/s (mV ± SD, $n = 8$) for the first component and 1.18 ± 0.2 m/s for the second component. For TM, the apparent conduction velocities were 1.05 ± 0.25 m/s for the first component and 0.7 ± 0.13 m/s for the second component ($n = 8$). HM presented a conduction pattern similar to that of TM (Fig. 1A). The conduction velocity was 1 ± 0.01 m/s and 0.77 ± 0.11 m/s for the first and second components, respectively ($n = 8$). We found statistically significant differences between the values of WM versus TM and WM versus HM for both the first and second components. We repeated these comparisons with optic nerves obtained from 16 day old animals. Typical recordings from the wild type, homo- and heterozygous PLP-deficient juvenile mice are shown in Fig 1B. Juvenile nerves usually presented three components. In the juvenile WM the first component was conducted with an apparent average velocity of 1.51 ± 0.49 m/s, the second with 0.71 ± 0.28 m/s and the third with 0.53 ± 0.06 m/s ($n = 8$). Optic nerves from juvenile TM presented also three components with conduction velocities of 0.94 ± 0.13 m/s, 0.58 ± 0.06 m/s and 0.43 ± 0.05 m/s for the first, second and third components, respectively. The conduction velocities of the optic nerves from juvenile HM were: 1.42 ± 0.25 m/s for the first component, 0.76 ± 0.14 m/s for the second and 0.57 ± 0.05 m/s for the third component. We found statistically significant differences between the values of juvenile WM versus juvenile TM but not versus HM only for the first component.

The present data suggest that the degree of myelination and the packing density of the myelin sheath affects conduction velocity in optic nerve fibres. The three groups of adult animals differ in packing density of the myelin sheath with a compact myelin sheath in normal mice and a completely disrupted loosely packed myelin in TM. Due to X-linked cellular mosaicism in HM the myelin sheath in some fibres is normal, while in others it is loosely packed as suggested by electronmicrographs [3]. The juvenile animals were studied at a time when myelination is still incomplete. We adjusted the stimulus intensity in such a way that is was always suprathreshold for two components of the compound action potential in adult animals and for all three components in the young animals. A near maximal stimulation was avoided for two reasons: we wanted to avoid contamination of the record by the stimulus artifact and we wanted to restrict the stimulation site to an area close to the stimulation electrode. It is likely that differences with respect to the site of stimulation exist between the different animal groups which makes calculation of action potential velocities inaccurate.

We studied the compound action potentials at an intensity where different fibre groups were activated as in-

dicated by different peaks. For a homogeneously conducting fibre population, the extracellular action potential is expected to be triphasic with a positivity preceding a larger negativity and a subsequent positivity again. Because of the limited length of the optic nerve and the fact that fibres with different conduction velocities were activated, different components were overlapping. The accurate determination of the peak of the extracellular action potentials was therefore impossible and this of course affects the exact determination of action potential conduction velocity in the three different conditions. Moreover, the action potentials in the juvenile animals with incomplete myelination and the action potentials in adult animals with different degrees of loose packing of the myelin sheath were preceded by a large positive component. Such positivities point to areas in which the axon membrane is either incapable of active action potential generation [10] or to a change in electronic length constant of the fibres [12]. If such positivities were preceding the negativity of the slower conducting nerve fibre group, the negative peak of the first action potential would be abbreviated, indicating a faster conduction velocity. In conclusion, since the conduction velocity is defined as the speed of propagation in homogeneously conducting fibres, we determined in our experiments only an apparent conduction velocity. However, due to the limited length of the nerve and the fact that the optic nerve contains differently large axons with relatively similar conduction velocities, it was impossible to make more precise measurements, at least with the present recording techniques.

In spite of these shortcomings the measured apparent conduction velocities in normal mice compare well to those measured in other adult and juvenile central fibre tracts [4,5]. The apparent conduction velocities were quite dissimilar in all groups of investigated animals. This suggests that the packing density of the myelin has a similar effect on conduction velocity as incomplete myelination, as evidenced by the slower conduction velocities in nerves from young animals. When studying nerves from 28 day old mice, which display still incomplete myelination, the conduction velocities were in between those from the young and adult age groups (data not shown).

The different components may reflect the existence of axons with different diameters. This is likely because optic nerves of adult wild type mice show complete myelination [3], but with considerable variations in the thickness of the myelin sheath which is known to increase with enlargement of the axon diameters [6,7]. Thus, the different components measured may refer to differently sized axons.

In wild type mice the process of myelination begins at day 2, reaches a maximum in 16–18 day old mice, and is completed at the age of 4 weeks. Consequently the conduction velocity in juvenile animals was expected to be smaller, which is indeed the case both in wild type and in homozygous mutant mice. Under these conditions the differences between homozygous mutant and wild type mice should be smaller, which is in accordance with our observations. Thus, the present findings show that defects in packing density of the myelin sheath lead to a functional deficit in conduction velocity in central fibres.

The apparent conduction velocities in adult transgenic mice were well above those observed in juvenile animals, implying that decompacted myelin is partly able to support conduction of action potentials. Electron microscopy and immunofluorescence analysis by confocal microscopy of optic nerve and different brain regions of these mutant mice [2] suggest that the axoglial contact, probably mediated by the myelin-associated glycoprotein (MAG) [8], is not affected by the PLP-deficiency.

Thus, this axoglial contact (i.e., one round of insulation) may be sufficient to provide for near half-maximal conduction properties as found in the PLP-deficient mice. In contrast to unmyelinated natural PLP-mutants [11] showing a severe neurological phenotype, PLP-deficiency, decompacted myelin, and suboptimal nerve conductivity are compatible with life and reproduction, and no overt phenotype results.

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