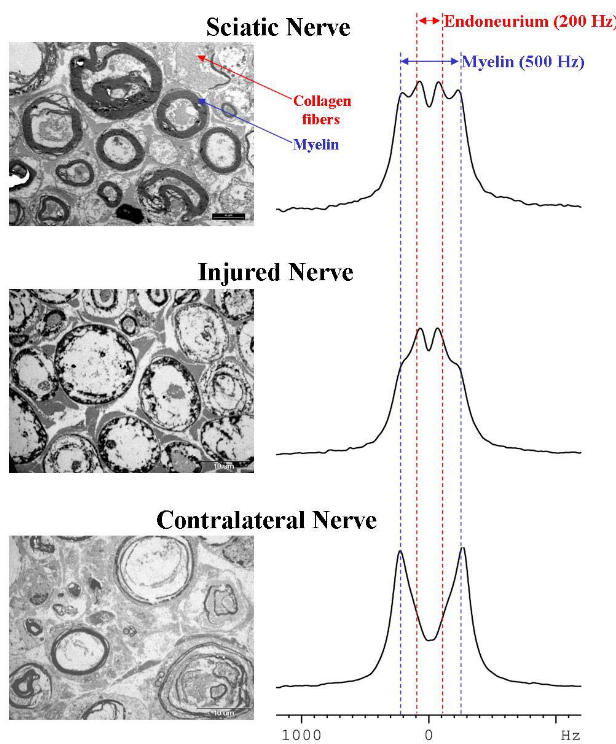
Histological Confirmation of the Bilateral Effect of Unilateral Nerve Injury Detected by ^{2}H NMR

T. Ben-David[1], G. Saar[1], H. Shinar[1], and G. Navon[1]
[1]School of Chemistry, Tel Aviv University, Tel Aviv, Israel

Introduction: ^{2}H double quantum filtered (DQF) spectra of nerves equilibrated in deuterated saline consist of quadrupolar split satellites representing the water in the different compartments of the nerve (1, 2). This technique gives enhanced resolution as a result of the elimination of the isotropic water signal. We have recently used this technique to monitor the Wallerian degeneration in rat sciatic nerve (3) and found that not only the spectrum of the injured nerve is affected but also that of the contralateral nerve. There are very few reports in the literature (4-6) indicating a bilateral effect of nerve injury and the causes of this effect are not understood. We present here, along with the NMR results, results obtained by histology, strongly corroborating our initial finding. Moreover, the combination of these two modalities enables us to explain the processes affecting the injured and contralateral nerves.

Materials and Methods: The right sciatic nerve of 6 months old Wistar rats was injured either at the proximal end or at the center of the nerve and both the injured and the contralateral nerves were excised 4 days after the procedure. The nerves were equilibrated in deuterated saline and were measured using ^{2}H in-phase DQF NMR pulse sequence: $90^0 - \tau - 90^0 - t_{DQ} - 90^0 - \tau - 90^0 - t_{ZQ} - 90^0 -$ Acq (4). τ is the creation time of the double quantum coherences, t_{DQ} is the DQ evolution time and t_{ZQ} is the evolution time of the zero quantum (ZQ) coherences

Results: Striking differences were observed between the ^{2}H DQF spectra of injured, control and contralateral rat sciatic nerves. At a creation time of τ=1ms two pairs of quadrupolar split satellites are observed in the control nerve with splittings of about 200 Hz and 500 Hz, which are tentatively assigned to water at the endoneurium and the myelin compartments. The ratio of the intensities of the 200 Hz satellites to the 500 Hz ones was 0.87±0.05 (n=10). Four days after the injury at the proximal end of the sciatic nerve, the intensity ratio for same two pairs of satellites was 1.38±0.30 (n=9). The reduction of the signal associated with the myelin agrees with the damage to the myelin seen in the TEM image (x3000) of the injured nerve. The uninjured contralateral nerve shows very different spectrum than that of the control. The intensity of the 200 Hz satellites is greatly reduced and the ratio between the split signals is 0.38±0.09 (n=7). The TEM image of the contralateral nerve supports this finding, as a much smaller amount of collagen fibers are observed in the endoneurium. Although the myelin layer in the TEM image of the contralateral nerve is different from that of the control, its amount appears to be similar. In another set of experiments, the sciatic nerve was cut in the center of the nerve rather than at the upper end. The resulted damage to the distal part of the injured nerve assessed from the ratio of the two satellites was more significant (1.62±0.22, n=5). No effect on the spectrum of to either the proximal part of the injured nerve (1.03±0.13, n=5) or the contralateral nerve (0.91±0.08, n=5) was observed.



Discussion: The assignment of the 500 Hz split signal to the water in the myelin layer is supported by the reduction in both the level of this signal in DQF spectrum and that of myelin tissue in the TEM, in the injured nerve relative to the control. Similarly, the 200 Hz split signal is a reliable representative of the endoneurium component, as seen in the injured nerve as well as in the contralateral nerve. This conclusion is also supported by the MRI experiments that were performed on bovine optic and porcine sciatic nerves. The level of the 200 Hz split signal is barely observed in the spectrum of the optic nerve compared to the sciatic spectrum. This result is in agreement with the smaller amount of endoneurium observed by ^{1}H MRI in the optic elative to the sciatic.

Conclusions: Our original finding of the surprising effect of the unilateral sciatic nerve injury on the contralateral nerve is now confirmed by the histological finding. The comparison of the NMR and the histology helps us in the assignment of the satellites with the quadrupolar splittings of 200 and 500 Hz to the water of the endoneurium and the myelin respectively.

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