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Degeneration of anterior horn cell in neuronal type of Charcot-Marie-Tooth disease (hereditary motor and sensory neuropathy type II): a Golgi study

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Summary. A morphological study using the Golgi impregnation method was carried out on the anterior horn cells at cervical (C), thoracic (Th), and lumbar (L) levels of the spinal cord in a patient with neuronal type of Charcot-Maire-Tooth disease (hereditary motor and sensory neuropathy type II) and an age-matched control. The present study demonstrated an uneven cell body surface, loss of cells (particularly large cells), loss of dendrites, reduced dendritic extent and an irregular surface and shape of dendrites at the C and L levels. In contrast, hematoxylin and eosin and Klüver-Barrera staining showed only simple atrophy or no change. The Th level of the patient showed none of these changes. Our results suggest that the degeneration or loss of dendrites of anterior horn cells by the Golgi staining method, which is most severe at the L level, is closely related to clinical findings such as muscle atrophy and weakness in neuronal type of Charcot-Marie-Tooth disease.

Key words: Neuronal type of Charcot-Marie-Tooth disease – Hereditary motor and sensory neuropathy type II – Golgi method – Anterior horn cell – Dendrite

The peroneal form of inherited motor and sensory neuropathy was classified by Dyck and Lambert [6, 7] into two major types: a demyelinating hypertrophic form and a neuronal type of Charcot-Marie-Tooth disease (CMTD), which are designated hereditary motor and sensory neuropathy types I and II (HMSN types I and II), respectively. The most obvious differences between these two types are the motor nerve conduction velocities and the nerve biopsy findings. HMSN type II is usually characterized by normal or slightly reduced nerve conduction velocity, and axonal loss with little evidence of demyelination or hypertrophic changes in nerve bopsies [4–7, 9–11, 18]. Type II is apparently less frequent than type I [14].

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Because of the benign course of this disease, there have been relatively few postmortem studies [1–3, 12, 13, 17, 21], and no study of dendritic pathology in anterior horn cells of the spinal cord of CMTD. The present Golgi study describes specific pathological changes in their dendrites.

Case report

A 75-year-old man was admitted to our hospital in 1988. He complained of gait disturbance, which had progressed slowly over the last 20 years, having started at age of 55 years. There was no history of neuromuscular disease in his family. Examination revealed bilateral pes cavus deformities and weakness and atrophy of his distal lower extremity muscles. There was also some weakness of the intrinsic muscles of his hands. All modalities of sensation were slightly diminished below the elbows and knees. Ankle jerk was absent. The cranial nerves were normal. An electromyographic examination of upper and lower extremities showed high amplitude potentials of 4–9 mV and duration of 8–12 ms as well as a decreased interference pattern. Motor and sensory conduction velocities in the median and ulnar nerves were normal, while motor conduction velocity in the common peroneal nerve and sensory conduction velocity in the sural nerve could not be obtained.

Biopsy of the left sural nerve showed a decreased density of the myelinated fibers (3500/mm^2), particularly of the largest ones. Ultrastructural study showed numerous clusters of regenerating myelinated fibers. Onion bulb formations were not observed. The most peculiar feature was the presence of axonal lesions. Some axons were enlarged and were full of neurofilaments and a few mitochondria.

Materials and methods

The spinal cord was removed 10 h postmortem. As an age-matched control, we examined the spinal cord from a 73-year-old patient with chronic heart disease whose necropsy delay was also 10 h. The condition of each patient within the few days before death was similar.

The spinal cords were fixed in 10% formaldehyde. A paraffin section rostrally adjacent to the specimen blocks taken for the Golgi method at the cervical (C), thoracic (Th), and lumbar (L) levels in both cases was stained by hematoxylin-eosin (H&E) and Klüver-Barrera (K-B) staining methods.

Golgi staining

A modification of the rapid Golgi method was used on this material [8]. Specimen blocks, 5 mm thick, were taken from the ventral portion of the 10% formalin-fixed spinal cord including the anterior horn of the C7, Th6 and L1 levels, and the C7, Th6 and L2 levels, of the CMTD patient and control subject, respectively. After 3–4 h of washing in tap water, the blocks were immersed 0.1 M potassium dichromate for 1 week at 20 °C. They were then washed with distilled water for 5 min and placed 0.75% silver nitrate for 1 week at 20 °C. The blocks were then dehydrated in alcohol and embedded in 14% celloidin, and cut serially into 50 sections of 100-μm thickness. The sections were dehydrated in alcohol, cleared in toluene, and mounted.

The Golgi-stained neuron is usually cut into several sections by the microtome due to the remarkable spread of its dendrites. To follow the total dendritic course in successive Golgi-stained serial sections, we used a previously published tracing procedure [15, 16].

All Golgi-stained neurons were photocopied at 400-fold magnification by a Nikon Biophoto with FX-35A (Nippon Kogaku, Japan). The largest and the smallest diameters of the cross-sectional neuronal cell body were measured at each level using a Kontron image analyzer MOP10 (Kontrol, Eching/Munich, Germany). Based on cell size, the anterior horn cells counted were divided into three groups: large cells, for which the sum of the largest and the smallest diameters was more than 90 μm; a middle-sized cell, for which the sum of these two diameters was between 50 and 90 μm; and a small cells, for which the sum was below 50 μm.

Results

H&E- and K-B-stained sections showed a reduced number of anterior horn cells, with a preferential loss of large ones, at the C and L levels in the patient.

Morphological findings of Golgi staining

In the control the general cytoarchitectural features, including dendritic configurations and branching patterns, were normal at the all levels (Fig. 1A). Their somal shape was round to oval and occasionally polygonal. Three to five primary dendritic arbors originated from the cell body with one to three subsequent bifurcations (Figs. 2A, 3A, 4A). These dendritic branches radiated centrifugally in nearly all directions (Fig. 1A). The dendrites were uniform in width and devoid of any moniliform appearance, tapered gradually to a point at the distal end, and were as long as 0.5–2 mm (Figs. 2A, 3A, 4A).

In comparison to the control, there were striking abnormalities in the morphology of the neuron at the C and L levels in the patient: (1) irregularities of outline of the cell body were seen, possibly caused by remnants of dendrites (Fig. 1B); (2) there was a prominent decrease in the dendritic domain (Figs. 2B,4B); and (3) short, uneven projections were often seen stemming directly from the cell body but terminating bluntly a short distance from their origin, some of which had the appearance of nodular enlargement (Fig. 1B). In contrast to the C and L levels, the above-mentioned alterations were not observed at the Th level in the patient which showed normal findings (Fig. 3B).

There were no large neurons, and the density of middle-sized and small neurons was markedly reduced at the C and L levels in the patient (Figs. 2B, 4B). At the Th level there was no difference between the patient and the control in the number of each type of neuron.

Discussion

We consider the clinical and pathological features of our case to be most consistent with those of the neuronal type of CMTD (HMSN type II) [4–7, 9–11 18]. Sporadic cases with similar clinical and pathological findings are usually included in this group, since they probably have an autosomal dominant pattern of inheritance with poor expressivity in one of the parents [4, 9, 10].

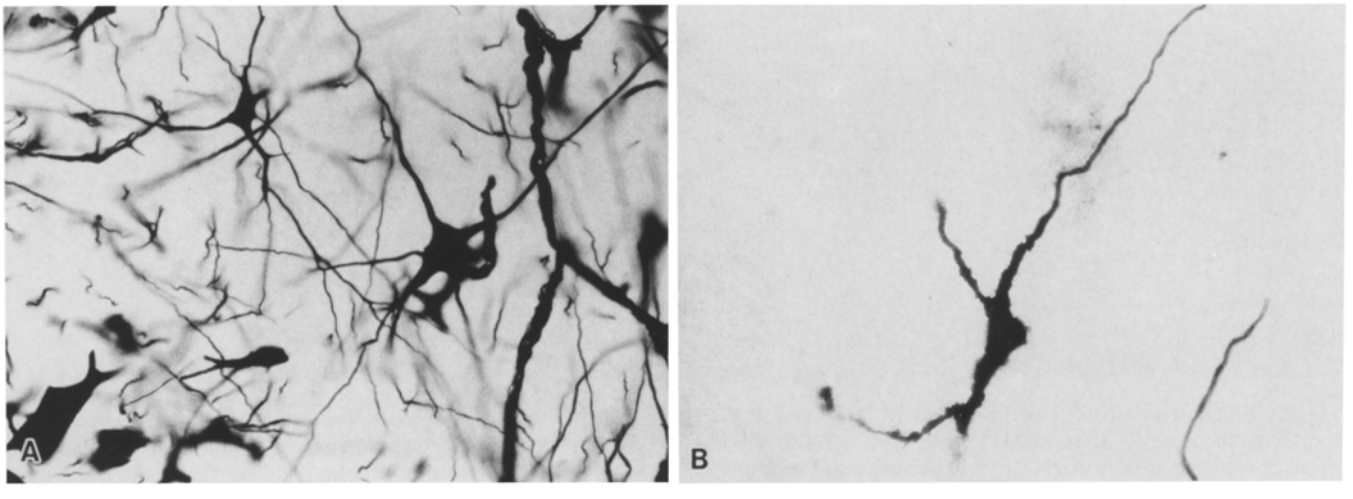


Fig. 1A,B. Lumbar (L) level of the spinal cord. **A** The L2 level of the control. Four to five primary dendrites radiating centrifugally in all directions from the cell body with subsequent bifurcations. **B** The L1 level of Charcot-Marie-Tooth disease (CMTD) patient. Two short and irregular dendrites stem from the cell body but terminate bluntly a short distance from their origin. Golgi stain, **A** × 98; **B** × 196

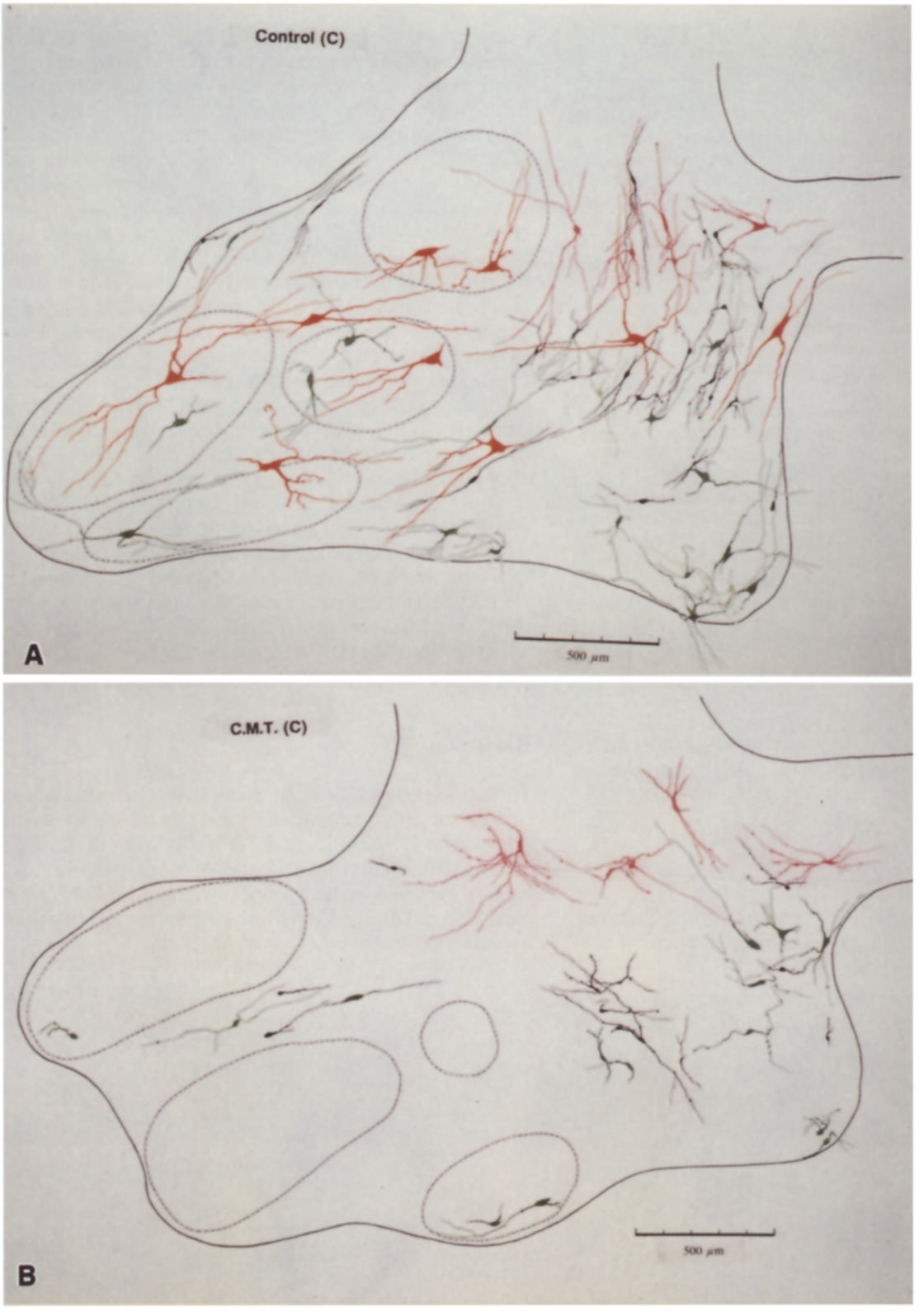


Fig. 2A,B. Drawing of anterior horn cells of the cervical (C) 7 level from the control (**A**) and CMTD patient (**B**). **A** Three to five primary dendritic arbors originate from the cell body with one to three subsequent bifurcations. The dendrites taper gradually to a point at the distal end, and are as long as 0.5–2 mm. **B** The loss of dendrites is dramatic with the reduced dendritic domain, and primary branchings are markedly diminished in number with virtually no secondary branching. *Red-, green-, black-,* and *purple-colored* cells correspond to large, middle and small anterior horn cells, and cells of the intermediary zone, respectively

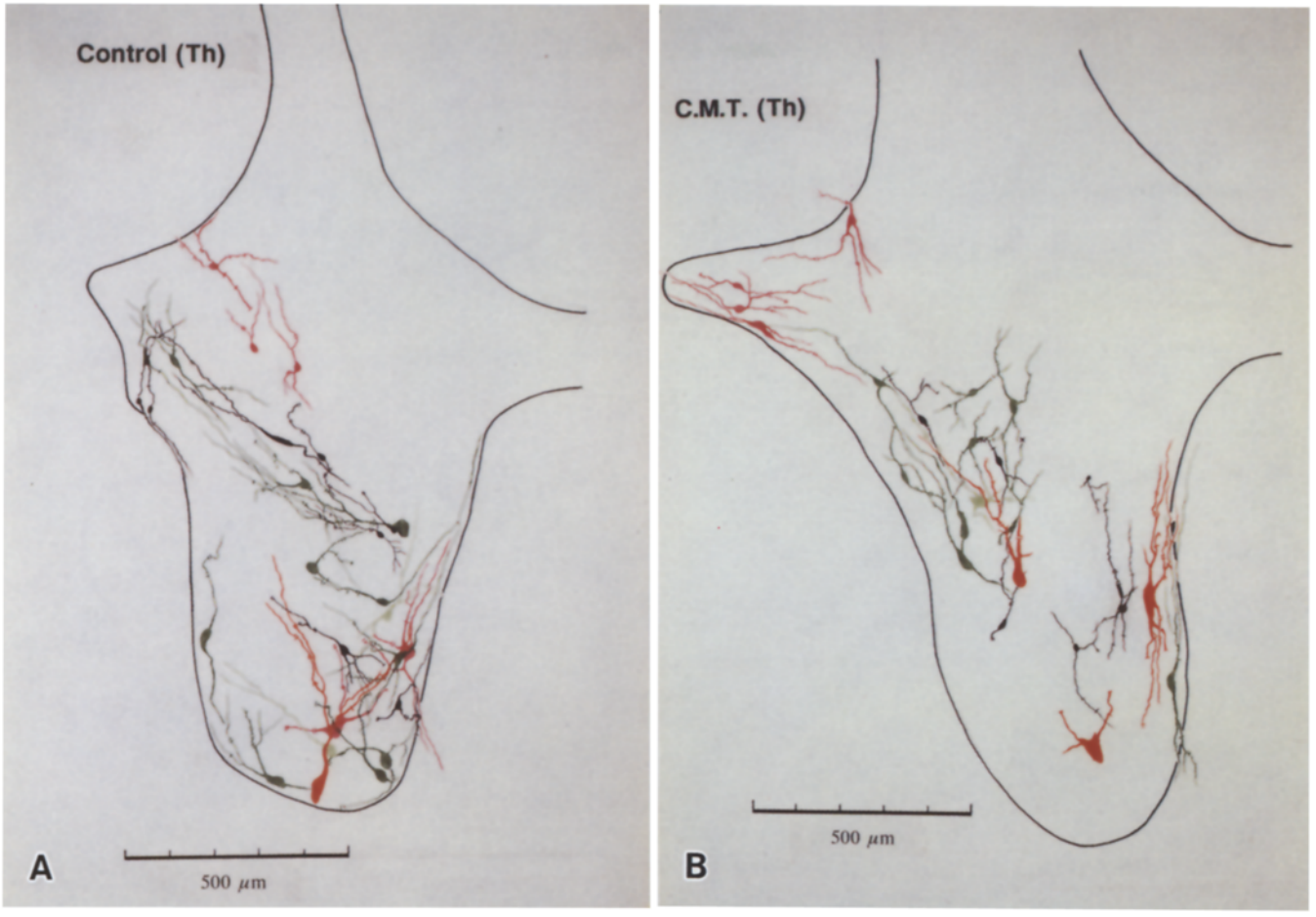


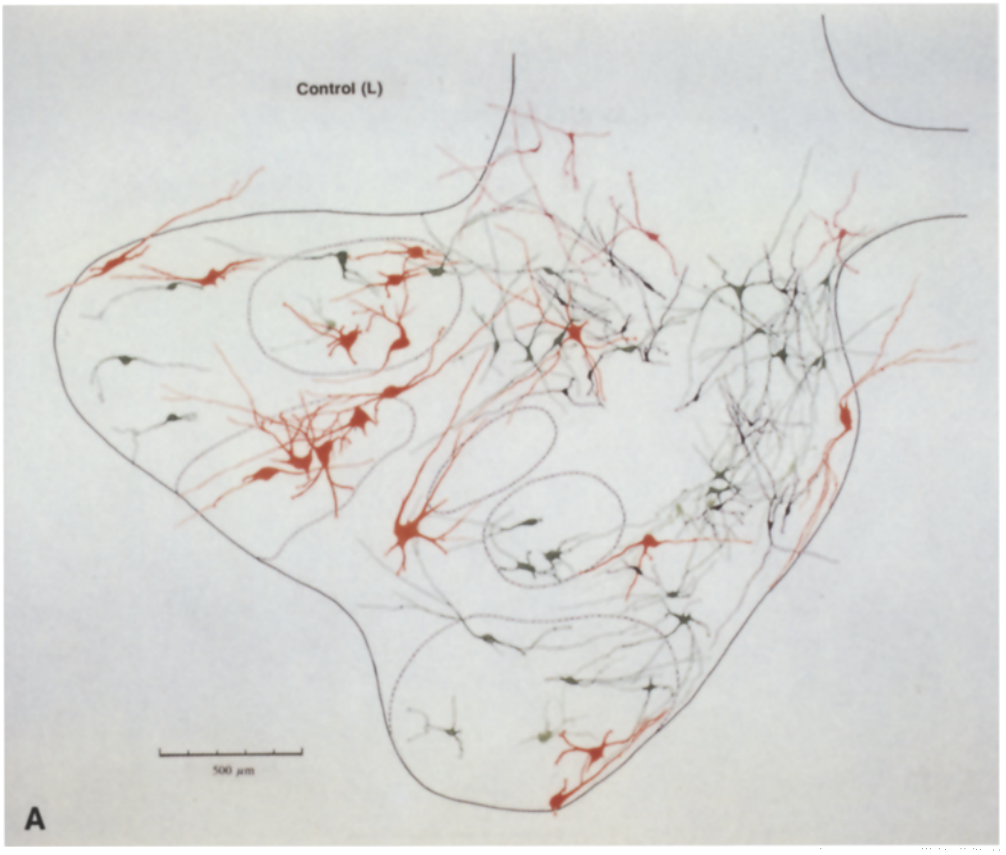
Fig. 3A,B. Drawing of anterior horn cells of the thoracic (Th) 6 level from the control (**A**) and CMTD patient (**B**). There are no morphological changes of the cell body and dendrites in either **A** or **B**. *Red-, green-, black-,* and *purple-colored* cells correspond to large, middle and small anterior horn cells, and cells of the intermediary zone, respectively

Only three postmortem studies of HMSN type II have been published [2, 3, 12]. Hughes and Brownell [12] described four autopsied cases. Constant findings in their study were demyelination in the posterior columns, loss of motor neurons of the anterior horns, loss of dorsal root ganglion cells and distally accentuated axonal loss in spinal roots and peripheral nerves. Dupuis et al. [3] found integrity of the posterior columns, of anterior and posterior spinal roots and of a thoracic dorsal root ganglion. In the anterior horns there was a loss of motor neurons. Postmortem study of one case by Berciano et al. [2] showed loss of anterior horn and dorsal root ganglion neurons in the lumbar and sacral levels and degeneration of the fasciculus gracilis. They suggested that these anatomical findings were consistent with the hypothesis that HMSN type II represents a primary neuronopathy affecting motor and sensory neurons.

The present study demonstrated an uneven surface of the neuronal cell body, loss of anterior horn cells (especially large cells), loss of dendrites, reduced extent of the dendritic domain, together with distortion, rough surface and irregular shape of dendrites at the C and L levels in the patient. However, the Th level of the patient showed none of these abnormalities.

This is the first report that a distinct pattern of abnormal dendritic and neuronal cell body pathology has been demonstrated in HMSN by the Golgi staining method. In our case, routine H&E and K-B staining which showed simple atrophy or almost normal appearance is contrasted by an absolute decrease in number of dendrites, with "stumping" of branches, and nodularities or other irregularities of dendrites shown using the Golgi method. These results indicate that cellular microscopical findings using techniques such as H&E staining cannot detect most of the cells that have lost dendrites, and that dendritic changes may exist in neurons considered not to have been involved.

It is noteworthy that an ongoing process, which may produce little obvious change in a nerve cell body as viewed with routine neuropathological methods, may have already crippled the neuron as a functioning element in a synaptic pool through impairment of the dendritic domain [20]. It is recognized that dendrites form a specialized postsynaptic apparatus either for excitatory or inhibitory synaptic input and that destruc-



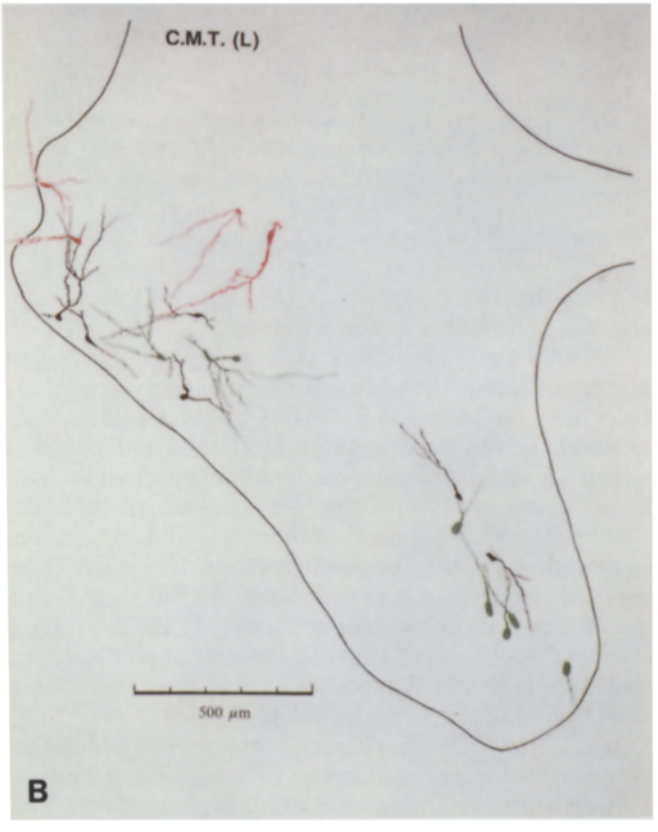


Fig. 4A,B. Drawing of anterior horn cells of the L2 level from the control (**A**) and the L1 level from CMTD patient (**B**). **A** Three to five primary dendritic arbors originate from the cell body with one to three subsequent bifurcations. The dendrites taper gradually to a point at the distal end, and are as long as 0.5–2 mm. **B** The loss of dendrites is dramatic with the reduced dendritic domain, and primary branchings are markedly diminished in number with virtually no secondary branching. *Red-*, *green-*, *black-*, and *purple-colored* cells correspond to large, middle and small anterior horn cells, and cells of the intermediary zone, respectively

tion of these might disrupt neuronal integration mechanisms [19]. The degree of clinical abnormalities, such as muscle weakness and atrophy, was closely correlated with the severity of deterioration in the corresponding anterior horn cells, which was most severe at the L level. Thus, the findings of our case suggest that degeneration and loss of dendrites may interrupt communication between the neurons, and that the decreased inputs to cells from other neuron systems through the loss of axo-dendritic synapses cause dysfunction of the cell.

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References

1. Alajouanine T, Castaigne P, Cambier J, Escourolle R (1967) Maladie de Charcot-Marie. Presse Med 75:2745–2750
2. Berciano J, Combarros O, Figols J, Calleje J, Cabello A, Silos I, Coria F (1986) Hereditary motor and sensory neuropathy type II. Clinicopathological study of a family. Brain 109:897–914
3. Dupuis M, Brucher JM, Gonsette R (1983) Étude anatomo-clinique d'une forme neuronal de la maladie de Charcot-Marie-Tooth. Rev Neurol (Paris) 139:643–649
4. Dyck PJ (1975) Inherited neuronal degeneration and atrophy affecting peripheral motor, sensory and autonomic neurons. In: Dyck PJ, Thomas PK, Lambert EH (eds) Peripheral neuropathy, 1st edn. Saunders, Philadelphia, pp 825–867
5. Dyck PJ (1984) Inherited neuronal degeneration and atrophy affecting peripheral motor, sensory, and autonomic neurons. In: Dyck PJ, Thomas PK, Lambert EH (eds) Peripheral neuropathy, 2nd edn. Saunders, Philadelphia, pp 1600–1625
6. Dyck PJ, Lambert EH (1968) Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. 1. Neurologic, genetic and electrophysiologic findings in hereditary polyneuropathies. Arch Neurol 18:603–618
7. Dyck PJ, Lambert EH (1968) Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. 2. Neurologic, genetic and electrophysiologic findings in various neuronal degenerations. Arch Neurol 18:619–625
8. Hara K, Kajita N (1984) A modification of Golgi's rapid method. Its application to the human brain. J Saitama Med Sch 11:57–65
9. Harding AE, Thomas PK (1980) The clinical features of hereditary motor and sensory neuropathy types I and II. Brain 103:259–280
10. Harding AE, Thomas PK (1980) Genetic aspects of hereditary motor and sensory neuropathy (types I and II). J Med Genet 17:329–336
11. Harding AE, Thomas PK (1980) Autosomal recessive forms of hereditary motor and sensory neuropathy. J Neurol Neurosurg Psychiatry 43:669–678
12. Hughes JT, Brownell B (1972) Pathology of peroneal muscular atrophy (Charcot-Marie-Tooth disease). J Neurol Neurosurg Psychiatry 35:648–657
13. Julien J, Vital C, Vallat JM, Coquet M, Blanc M (1974) Maladie de Charcot-Marie et diabète. Etude clinique, ultrastructurale et autopsique d'une observation. Nouv Press Med 3:139–142
14. Julien J, Vital C, Lagueny A, Ferrer X (1988) Hereditary motor and sensory neuropathy type II with axonal lesions. J Neurol 235:254–255
15. Mannen H (1966) Contribution to the quantitative study of the nervous tissue. A new method for measurement of the volume and surface area of neuron. J Comp Neurol 126:75–90
16. Mannen H (1975) Reconstruction of axonal trajectory of individual neurons in the spinal cord using Golgi-stained serial sections. J Comp Neurol 159:357–374
17. Mathews T, Moosy J (1972) Mixed glioma, multiple sclerosis, and Charcot-Marie-Tooth disease. Arch Neurol 27:263–268
18. Ouvrier RA, McLeod JG, Morgan GJ, Wise GA, Conchin TE (1981) Hereditary motor and sensory neuropathy of neuronal type with onset in early childhood. J Neurol Sci 51:181–197
19. Purpura DP (1974) Dendritic spine "dysgenesis" and mental retardation. Science 186:1126–1128
20. Scheibel ME, Davies TL, Lindsay RD, Scheibel AB (1974) Basilar dendrite bundles of giant pyramidal cells. Exp Neurol 42:307–319
21. Smith TW, Bhawan J, Keller RB, DeGirolami U (1980) Charcot-Marie-Tooth disease associated with hypertrophic neuropathy. A neuropathologic study of two cases. J Neuropathol Exp Neurol 39:420–440