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A Golgi Study of the Proximal Portion of the Human Purkinje Cell Axon

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Summary. The proximal portion of the Purkinje cell axon in normal cerebellum was investigated using the Golgi-Cox method. The axon emerging from the axon hillock tapered as it proceeded distally along the initial segment. The most distal portion of the initial segment was the narrowest (about 1 μm). Then the axon became thicker again in the probable myelinated portion. The length of the axon hillock plus the initial segment ranged from 21 μm to 52 μm, 35 ± 6 μm on average ± SD. The axon arose from any site of the soma and the primary dendrite of the Purkinje cell. Almost half of the axons emanated from a lateral surface of the soma. The dendritic arbores of the Purkinje cell with a torpedo were atrophic.

Key words: Purkinje cell axon – Initial segment – Torpedo – Somatic spine – Golgi method

Introduction

Recently, a Golgi impregnation method applied to pathologic materials yielded some interesting results (Scheibel and Scheibel 1975; Marin-Padilla 1976; Purpura and Suzuki 1976; Purpura et al. 1976; Scheibel and Tomiyasu 1978; Williams et al. 1978; Landis et al. 1981; Fujisawa and Nakamura 1982). The application of the Golgi method made it possible to examine almost the entire extent of a neuron including neural processes. In Golgi preparations, every neuron within a single set is considerably different from one another even in normal conditions. Therefore, knowledge of the extent of variation of individual neurons in non-pathologic conditions is essential to a correct recognition of pathologic neurons. Although the Golgi method has a long history of over 100 years, most of the studies on the normal morphology of neurons have been carried out using animal materials.

In the present study, we have concentrated our attention on the variation of human Purkinje cells in non-pathologic cerebellum. We report the morphology of the proximal portion of the human Purkinje cell axon.

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Materials and Methods

The specimens were obtained from three patients who had no abnormality of the cerebellum both neurologically and neuropathologically. Patient 1, an 86-year-old woman, died of sepsis. Only mild senile changes (neurofibrillary tangles and senile plaques) were present in the cerebral cortex. The cerebellum was unremarkable except for a small number of torpedoes in the granular layer. Patient 2, a 70-year-old woman, died of breast cancer. No metastasis of the cancer to the brain was seen. Patient 3, a 64-year-old man, died of esophageal carcinoma. The brain was unremarkable.

Several blocks of tissue were taken from the cerebellar hemisphere on the autopsy table for a Golgi-Cox study (Sholl 1953). The interval between death and tissue fixation was 8 h (patient 1), 39 h (patient 2) and 19 h (patient 3). Each block was immersed for 9–10 weeks in a solution containing 5% potassium dichromate (20 ml), 5% mercuric chloride (20 ml), 5% potassium chromate (8 ml), and distilled water (40 ml). Then the block was embedded in celloidine and cut serially into 100 μm thick sections. After immersion in 5% potassium sulfite solution, these sections were dehydrated and mounted. A total of 167 sections were used for light-microscopic observation.

Results

The Morphological Features of the Proximal Portion of the Purkinje Cell Axon

In most Purkinje cells, even in excellently impregnated cells, only the axon hillock and the initial segment

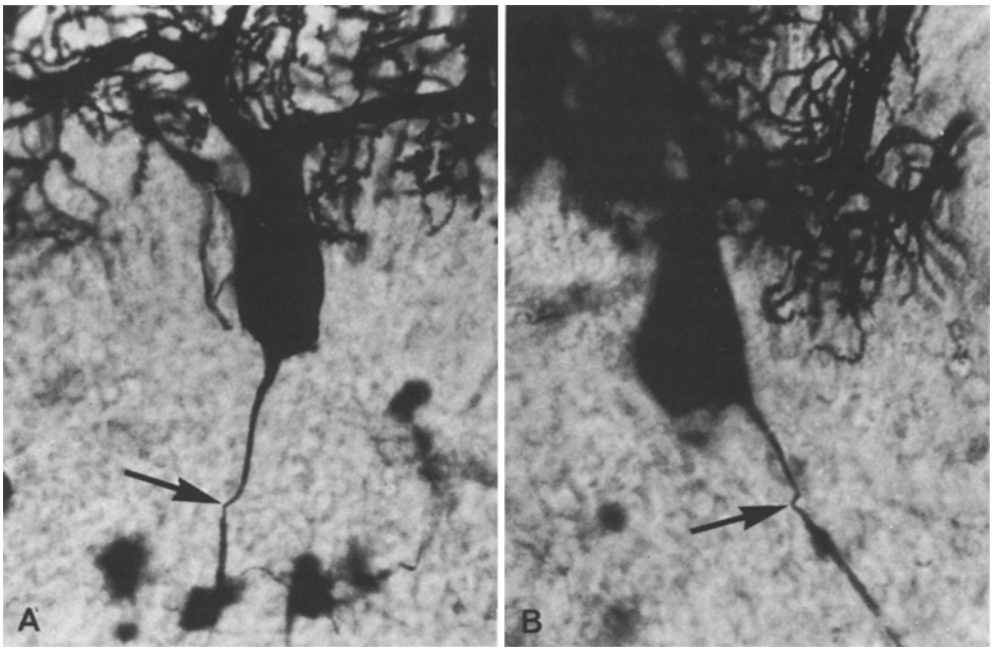

Fig. 1. A A Purkinje cell the axon of which arises from a basal pole of the soma just opposite to the primary dendrite (Type I). After emerging from the soma, the axon is tapering gradually as it proceeds distally along the initial segment. The most distal portion of the initial segment is narrowest, about 1 μm in diameter (*arrow*), and then the axon becomes thicker again; ×440. **B** A Purkinje cell the axon of which arises from a corner between a basal pole and a lateral surface of the soma (Type II). An *arrow* points to the most distal narrowest point of the initial segment; ×440

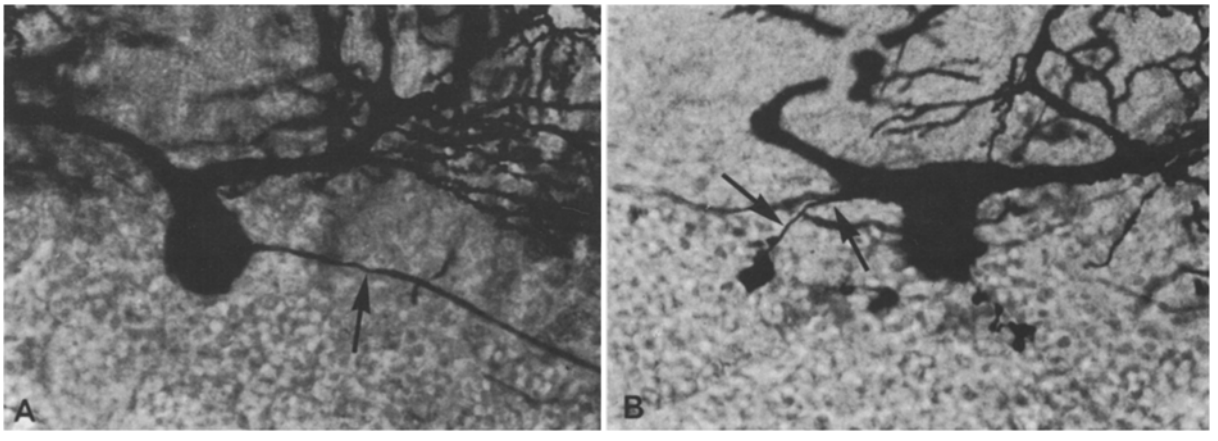

Fig. 2. A A Purkinje cell the axon of which arises from a lateral surface of the soma (Type III). An *arrow* indicates the most distal narrowest point of the initial segment; ×380. **B** A Purkinje cell the axon of which arises from one of the primary dendrites (Type IV). The tapering axon hillock and initial segment are seen (*arrows*); ×380

were impregnated, and the more distal portion of the axon was not visualized. By careful observation, however, axons which were sufficiently impregnated over 100 μm or longer from the soma were sometimes encountered. We evaluated 32 such axons for the purpose of clarifying the morphological features of the proximal portion of the Purkinje cell axon.

Every axon emerging from the axon hillock of a soma tended to taper as it proceeded distally along the initial segment. The most distal point of the initial segment of the axon was the narrowest, and then the axon expanded again probably at the beginning of the myelinated portion (Figs. 1, 2). The length of the axon hillock plus the initial segment was 35 ± 6 μm on average ± SD, ranging from 21 μm to 52 μm. The caliber of the most distal portion of the initial segment was approximately 1 μm, and that of the myelinated portion of the axon was about 2 μm.

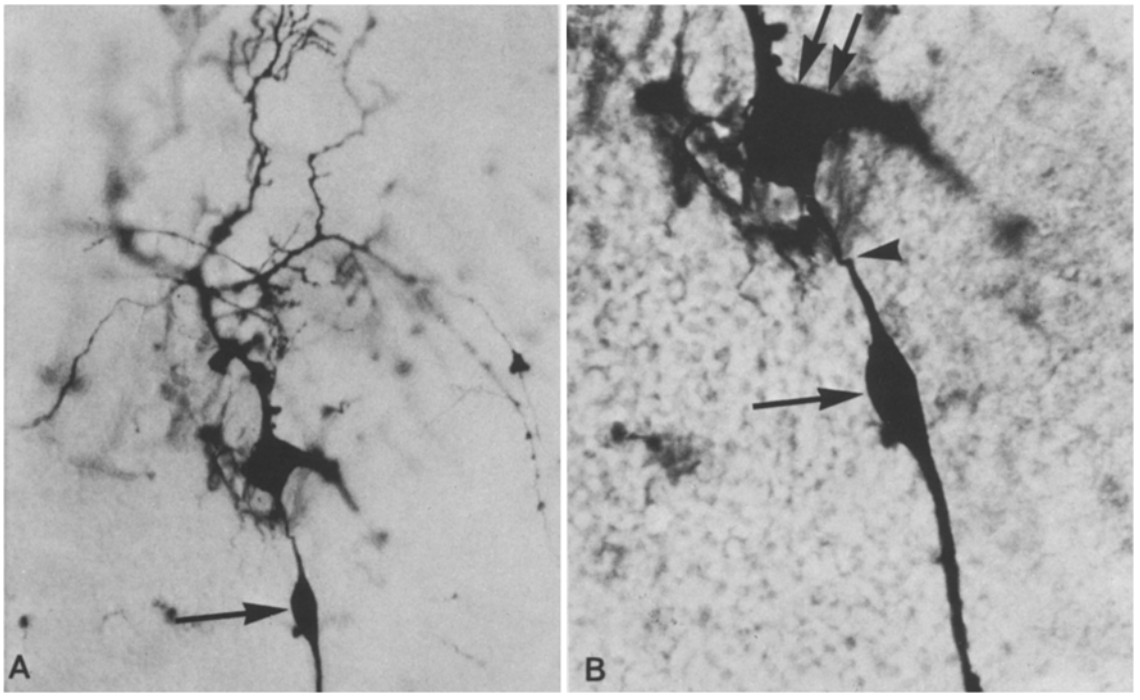

Fig. 3. A The dendritic arbores of the Purkinje cell the axon of which has a torpedo (*arrow*) are deformed and atrophic; ×220. **B** A higher power picture of the torpedo (*arrow*) and the soma (*two arrows*) in **A**. The soma is not atrophic. An *arrowhead* points to the narrowest portion of the initial segment of the axon; ×440

The Site of Axonal Outgrowth from the Purkinje Cell

A Purkinje cell axon emerged from any portion of the soma or, rarely, from a proximal portion of the primary dendrite. We classified the Purkinje cells into four types based on the site of axonal outgrowth. The Purkinje cell whose axon arose from the basal pole of the soma just opposite to the primary dendrite(s) was Type I (Fig. 1A). A cell with the axon emerging from a corner between the basal pole and a lateral surface of the soma was Type II (Fig. 1B). A cell with the axon arising from a lateral surface of the soma was Type III (Fig. 2A), and a cell with the axon from a proximal portion of the primary dendrite was Type IV (Fig. 2B). Of 200 Purkinje cells we examined, 39 cells (19.5%) belonged to Type I, 74 cells (37.0%) to Type II, 83 cells (41.5%) to Type III, and four cells (2.0%) to Type IV. In the depth of a folium of the cerebellar cortex, Type III cells were most frequently observed among other types. Type I cells were located mainly in the crest and slope of a folium. Type IV cells were recognized exclusively in the slope of a folium.

The Torpedo

A focal, fusiform expansion of the axon, or so-called "torpedo", was recognized in two Purkinje cells of patient 1 (86 years of age). The larger torpedo was 37 μm in length and 16 μm in the largest diameter (Fig. 3A, B), and the smaller one was 30 × 21 μm. The size of the somata of the Purkinje cells with the torpedoes was normal (33 × 25 μm and 35 × 26 μm each), but the dendritic system of such cells was atrophic and did not show a normal fan-like configuration (Fig. 3A).

A Purkinje Cell with Unusual Configuration

We encountered one Purkinje cell with unusual configuration in patient 2 (Fig. 4A). This Purkinje cell was studded with many spine-like structures all over the surface, including the soma, the primary and secondary dendrites and, of course, the distal dendrites (Fig. 4B). In addition, this cell protruded a thick rod-like process from a corner between a basal and a lateral surface of the soma. The process had small and large spine-like structures also and an axon emerged from its distal tip (Fig. 4C).

Discussion

The Shape of the Proximal Portion of the Purkinje Cell Axon

According to Palay and Chan-Palay (1974), using a rapid Golgi method, the most proximal portion of the initial segment of the rat Purkinje cell axon is about 1μm in diameter, and the axon becomes narrower

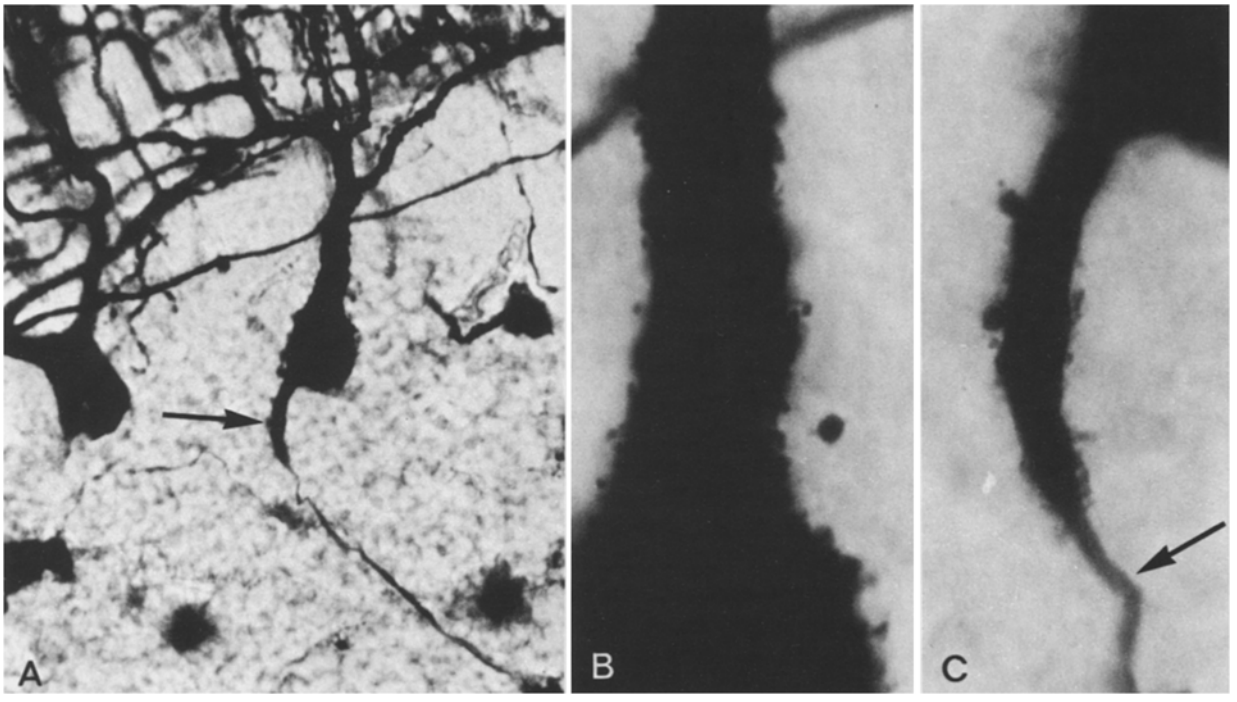

Fig. 4. A A Purkinje cell with an irregular cell surface and a rod-like protrusion (*arrow*) from a corner between a basal and a lateral surfaces of the soma. An axon arises from a distal tip of the protrusion; ×396. **B** A higher power picture of the primary dendrite and the *upper portion* of the soma in **A**. Many spine-like structures are present on the cell surface; ×1800. **C** A higher power picture of the protrusion in **A**. Small and large spine-like structures are seen on the surface. An *arrow* points to the axon; ×1800

gradually as it proceeds distally. At the most distal point of the initial segment, the axon attains its smallest diameter of about 0.5 μm and then it becomes thicker again in the myelinated portion. The length of the initial, unmyelinated part of the axon is about 40 or 50 μm. In the present study, the length of the axon hillock plus the initial segment was 35 ± 6 μm on average $\pm$ SD, and the narrowest diameter of the initial segment was almost constantly about 1 μm. As compared with those in the rat, the initial segment of the human Purkinje cell axon seems stouter and thicker although, in a strict sense, it is impossible to compare both values because of the difference in the methods used.

An axon of a large anterior horn cell in the spinal cord also has morphological features similar to that of the Purkinje cell (Nakano and Hirano 1983; Carpenter and Sutin 1983). At least concerning the Purkinje cells of the cerebellum and the large anterior horn cells of the spinal cord, these morphological features mentioned above are very important in differentiating between an axon and a long, thin dendrite directly arising from a soma.

The Site of Axonal Outgrowth from the Purkinje Cell

It has been generally believed that the axon of the Purkinje cell arises from a basal pole of the soma (Palay and Chan-Palay 1974). However, the present study demonstrated that such Purkinje cells were in the minority in the human, and that almost half of the human Purkinje cells grew out their axons from a lateral surface of the soma.

Most of the axons of the pyramidal neurons in the cerebral cortex are known to emanate from the basal surface of the soma. However, some pyramidal cell axons have been reported to arise from a lateral surface of the soma or from the apical or basal dendrite (Van der Loos 1965; Stensaas 1967; Globus and Scheibel 1967). About 10% of the axons of large neurons in the spinal anterior horn have been also reported to emerge from a dendrite (Nakano and Hirano 1983). In the light of these reports and the present results, the variability of the site of axonal outgrowth from a neuron seems a general phenomenon in the central nervous system.

The Torpedo

A torpedo of the Purkinje cell axon is seen in various disorders involving the cerebellum (Menkes et al. 1962; Hirano et al. 1973; Williams et al. 1978; Hirano 1981). It can also be observed in a normal aged cerebellum (Hirano et al. 1980), as demonstrated in this study. The soma of the Purkinje cell with a torpedo

has been described to undergo a simple atrophy (Blackwood 1977). In the present study, however, the Purkinje cells with a torpedo did not show any picture of simple atrophy of the soma but an atrophy of dendritic arbores.

The Purkinje Cell with Unusual Configuration

In the observation of thousands of Purkinje cells, another cell of this kind was not detected.

Somatic spines of the Purkinje cell are present in kinky hair disease (Menkes et al. 1962; Aguilar et al. 1966; Hirano et al. 1977; Williams et al. 1978) and in the immature cerebellum of the fetus (Zecevic and Rakic 1976). In these conditions, however, many Purkinje cells show similar morphology.

The rod-like protrusion with spine-like structures resembles a meganeurite of a cortical pyramidal neuron in several neuronal storage diseases (Purpura and Suzuki 1976; Purpura and Baker 1977; Walkley et al. 1981). However, the present patient did not show any evidence of neuronal storage diseases.

At the present time, we cannot offer any satisfactory explanation about the Purkinje cell with unusual configuration.

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