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Neurons with dual axons in the substantia gelatinosa (SG) of the adult cat lumbosacral spinal cord[1]

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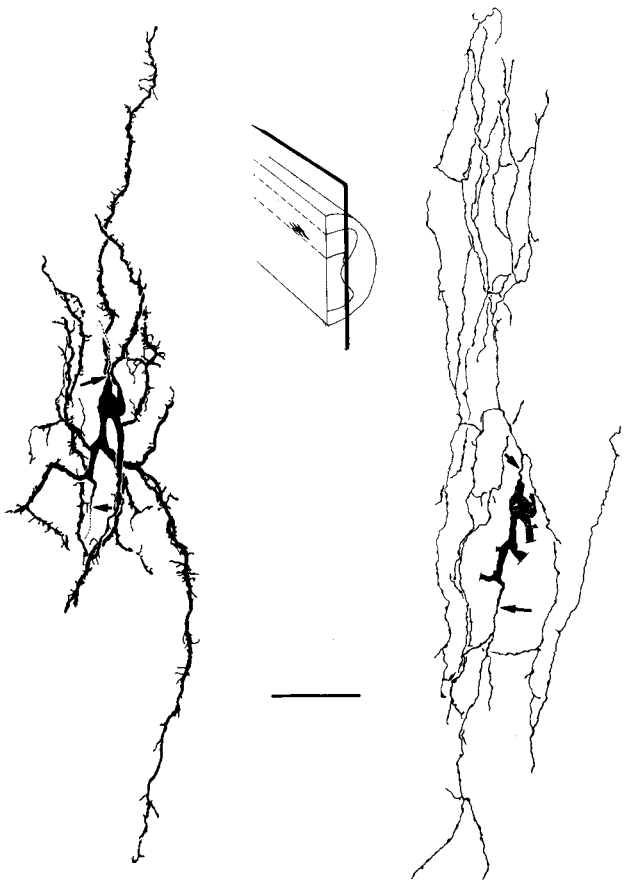
Summary. A small percentage of SG neurons possessing two separate and complete axons were observed in the lumbosacral spinal cord of the adult cat. Since they are found in small numbers and are structurally similar to single axon SG cells, dual axon cells may represent a developmental aberrancy rather than a functionally distinct cell type.

The concept that a neuron gives rise to one and only one axon is a maxime strongly established by Ramón y Cajal[3] and other classic anatomists. Modern anatomists, however, have periodically found exceptions to the 1-axon axiom. Several investigators have demonstrated interneurons with multiple somatic or dendritic appendages in the form of spines and beaded axon-like processes which have been shown ultrastructurally in several instances to be presynaptic processes (see Beal and Cooper[4] for references). The present report demonstrates a unique spinal neuron which generates 2 major, separate and complete axons. Although similar cells have been reported in the caudal trigeminal nucleus[5], this is the first report of dual axon cells in the SG of the spinal cord.

The present study is part of an extensive Golgi analysis of the morphological characteristics of neurons located in the SG, lamina II of Rexed[6], in the lumbosacral spinal cord of the adult cat. Spinal cord tissue was obtained from 15 sexually mature young adult cats weighing 1.5–2.0 kg. Each was processed according to a modified rapid Golgi procedure[7] and sectioned at 100–150 µm in the sagittal and transverse planes. Drawings of SG neurons were made with the aid of a light microscope with 100X objective and drawing tube attachment.

In the present analysis a small percentage of Ramón y Cajal's[8] 'central' cells were found to have 2 separate axons. The axons originate from the cell body or from dendrites at various distances from the cell body, either from the same pole or from opposite poles of the cell. The axons branch repeatedly and generate an extensive plexus in the vicinity of the cell body. The 2 axonal arbors display considerable overlap and have branching patterns similar to those produced by single axon cells. The axon collaterals are thin and characterized by numerous small 'boutons en passant' (fig.). Aside from an additional axon these cells have no distinctive structural features to separate them from other 'central' SG neurons. In fact, in the present study, single and dual axon cells have been found which are nearly identical with respect to cell size, shape, position, dendritic pattern and specializations.

The significance of neurons with dual axons is puzzling. One possibility is that each of the 2 axons has a different destination. In chick embryos of 10 days incubation, Ramón y Cajal[8] described SG neurons in the spinal cord with axons which left the gelatinous substance and entered the overlying white matter at 2 different and remote locations. These axons, however, did not originate from separate portions of the neuron, but rather were derived from a single stem fiber which branched near the cell body of origin into 2 distinct fibers with separate destinations. These cells were referred to as the 'cells of the combined axis-cylinder'. That such cells exist in the adult has been shown in several areas of the nervous system by Hayes and Rustioni[9] who reported dual projections of single neurons after double labeling of cells utilizing the retrograde transport of horseradish peroxidase (HRP) and (^{3}H) apo-HRP. Even though dual axon cells of the present study have collaterals in the vicinity of the cell body, the terminal ramifications of these fibers cannot be followed and may go on to separate and remote locations. One of the axons could conceivably project to brain stem centers, since some SG neurons of the spinal cord have been shown to project to higher levels[10,11]. The dual axon cells of the present study, then, could be a simple variation of the 'cells of the



Drawing of a single SG neuron exhibiting dual axons sectioned in the sagittal plane showing the full extent of its dendritic arbor (right) and plexus generated by the 2 axons (left). Arrows indicate origin of each axon. Bar: 50 µm.

combined axis cylinder'[8] with the only difference being an axon with a separate rather than a combined origin.

In support of this contention several examples of developing neurons with 2 separate axons (destined for different white fasciculi) were observed by Ramón y Cajal[8] in the spinal cords of very early (7–9-day) chick embryos. Cajal interpreted these as an early embryonic phase of the 'cells of the combined axis-cylinder'. Based on the observation that most SG neurons in the spinal cord of the adult possess only 1 axon, it would follow that the normal course of development of these cells involves the loss of 1 axon or the fusion of the 2 axons into a single fiber. In the case of persistent dual axons in the adult, the axons could have failed to fuse or the additional axon failed to degenerate, possibly due to the establishment of sufficient connections to warrant its retention. It seems possible then, that dual axon SG cells of the adult spinal cord may represent a developmental aberrancy rather than a functionally distinct class of neurons with special physiological properties. The rarity of the dual axon cell and its structural similarity to single axon 'central' cells would seem to support this.

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Spectral properties of methemoglobins prepared by the action of sodium nitrite and potassium ferricyanide[1]

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Summary. Methemoglobin was prepared by the addition of sodium nitrite or potassium ferricyanide to oxy or deoxyhemoglobin. The spectral properties of these methemoglobins were studied before and after extensive dialysis. It is shown that the methemoglobin formed by sodium nitrite has substantial spectral differences in visible and Soret band compared to that formed by potassium ferricyanide. These differences are proportional to the excess of sodium nitrite only. This suggests that both methemoglobins are similar compounds.

Hemoglobin is oxidized by oxygen (autoxidation) as well as by a great number of oxidants such as drugs or chemicals. The most common oxidizing agents used for preparation of methemoglobin either for research or for standards in clinical laboratory are sodium nitrite and potassium ferricyanide. Although certain spectral differences between these methemoglobins have been observed, the previous studies have been made on red cells or hemolysate[3–5]. This study was undertaken to elucidate the nature of these methemoglobins in pure form in the absence of organic phosphates.

Materials and methods. Pure hemoglobin was prepared by ion exchange chromatography[6]. Hemoglobin was stripped of all organic phosphates by the method already described[7]. Spectra were recorded on a double beam Beckman spectrophotometer model 35. The oxidation was carried out at 37 °C in a 1-cm light path quartz cuvette. Hemoglobin solutions were diluted with bis-tris buffer pH.6 to a concentration of 0.2 mM. Various amounts of sodium nitrite or potassium ferricyanide were added. Adequate time was allowed to elapse until oxidation reaction was completed and the spectra were recorded. The oxidation reaction was also carried out under anaerobic condition. This was performed by placing 2 ml of hemoglobin solution in a tonometer. After complete deoxygenation by vacuum, the hemoglobin was oxidized by the injection of various