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Electron microscopic study of sprouting dendrites in the ciliary ganglia of cat and monkey (*Macaca fascicularis*) following pre- and post-ganglionic axotomy

Y.L. Zhang, C.K. Tan and W.C. Wong

Department of Anatomy, Faculty of Medicine, National University of Singapore, Kent Ridge, Singapore

Summary. The present paper reports the ultrastructure of dendritic sprouting and formation of associated synapses in the ciliary ganglion of cat and monkey induced by pre- and post-ganglionic axotomy. In both series of experiments, sprouting dendrites were observed mostly at 3-5 days postoperatively; such profiles were identified by their dense packing of mitochondria and glycogen-like granules. In longitudinal section, such profiles appeared as expanded extensions from the normal-looking dendritic trunks. None were observed to arise directly from the neuronal soma. After preganglionic nerve section, the cross-sectional diameters of such profiles measured 2.2±1.0 μm (range: 0.9-6.2 μm) in cat and 2.4±0.7 μm (range: 0.9-5.5 μm) in monkey. After postganglionic nerve section, the cross-sectional diameters of such profiles measured 2.1±0.7 μm (range: 0.8-4.5 μm) in cat and 2.8±1.4 μm (range: 1.1-7.0 μm) in monkey. After preganglionic axotomy, in both cat and monkey, the axon terminals began to degenerate at 3 days postoperatively and disappeared by 5 days postoperatively. However, at later postoperative survival periods, the axon terminals reappeared and were observed to make synaptic contacts with the sprouting dendrites. Some of the sprouting dendrites were observed to degenerate, some as early as 3 days postoperatively; such profiles did not appear to have any synapse on them. After postganglionic axotomy, such sprouting dendritic profiles were also observed to make synaptic contacts with axon terminals; some were only closely associated with profiles filled with synaptic vesicles. The results thus suggest that through the formation of new synapses, sprouting of dendrites may have a role to play in neuronal survival after axotomy.

Key words: Ciliary ganglion, Dendritic sprouting, Axotomy, Cat, Monkey (*Macaca fascicularis*)

Offprint requests to: Dr. C.K. Tan, Department of Anatomy, Faculty of Medicine, National University of Singapore, Kent Ridge, Singapore 0511

Introduction

Neuronal plasticity is a common phenomenon in both the central and peripheral nervous systems of adult animals (Raisman, 1969; Courteney and Roper, 1976; Roper and Ko, 1978; Greenough et al., 1986; Walkley et al., 1988; Hamori, 1990; Goodman et al., 1991). Dendritic sprouting or growth induced by axotomy has been reported in peripheral ganglia (Ceccarelli et al., 1971; Matthews and Raisman, 1972; Johnson, 1983, 1988; Thanos, 1988; Hadley, 1990; Cho and So, 1992; Tabata and Fukuda, 1992) and central neurons of lamprey and cricket (Hall and Cohen, 1983a,b, 1988a,b; Roederer and Cohen, 1983a,b). However, little information is known about the ultrastructure of such sprouting dendrites induced by axotomy and, if any formation of synapses with them.

In addition to its cholinergic nature (Koelle, 1955; Koelle and Koelle, 1959; Huikuri, 1966; Robinson and Heine, 1974), the ciliary ganglion has been shown to contain neuropeptide Y-, tyrosine hydroxylase-, substance P-, calcitonin gene-related peptide- and vasoactive intestinal polypeptide-positive neurons or fibres (Leblanc et al., 1987; Stone et al., 1988; Grimes et al., 1990; Hardebo et al., 1992; Zhang et al., 1994a,b; Kirch et al., 1995; Tan et al., 1995). These studies have indicated that several substances are involved in the neuronal transmission and modulation. At light microscopic level, Johnson (1983, 1988) and Hadley (1990) have reported the changes of the dendritic geometry after both pre- and post-ganglionic nerve sections in the ciliary ganglion of the rabbit. The present study aims to study the ultrastructural features of sprouting dendrites and their synaptic connections with sprouting axon terminals in the ciliary ganglia of adult cat and monkey (*Macaca fascicularis*) following pre- and post-ganglionic nerve sections and extends our previous studies of the normal structure and synaptic organisation of the ciliary ganglion of cat and monkey (Zhang et al., 1993, 1994a,b).

Materials and methods

46 adult cats, weighing between 2.2-3.8 kg, and 17 adult monkeys (*Macaca fascicularis*), weighing between 1.6-2.8 kg of both sexes, were used in the present study, 20 cats and 8 monkeys were subjected to pre-ganglionic nerve section, 24 cats and 8 monkeys to postganglionic axotomy; 2 cats and 1 monkey were used as normal control. The animals were anaesthetized by intraperitoneal injection of sodium pentobarbitone (30 mg/kg body weight). The operative procedures were identical for both species of animal. All operations were consistently carried out on the right side under aseptic conditions. The ganglion of the left side was used as control. The temporalis muscle was excised and the coronoid process of the mandible and roof of the orbit were nibbled off with bone rongeur to expose the orbital contents. The short ciliary nerves were identified and cut near the sclera; the oculomotor nerve was cut near the ganglion. The animals were sacrificed 1, 3, 5, 7, 12, 14, 21, and 28 days after operation. For sacrifice, the animals were anaesthetized by intraperitoneal injection of sodium pentobarbitone (30 mg/kg body weight) before they were perfused intracardially by using the two-stage method of Peters (1970). Artificial respiration was maintained with an animal ventilator via a tracheostomy. The thorax was then opened and a mixture of 1000 units of heparin and 1 ml of 1% sodium nitrite solution were injected into the left ventricle of the heart. Five minutes later, the animals were perfused intracardially with 300-500 ml of Ringer's solution at room temperature (25 °C), followed by fixative. Initially, the animals were perfused intracardially with 1000 ml of dilute fixative consisting of 1% paraformaldehyde and 1.25% glutaraldehyde. This was followed by 1000 ml of concentrated fixative consisting of 4% paraformaldehyde and 5% glutaraldehyde. In both instances, the fixative was made up in 0.1M cacodylate buffer, pH 7.4.

At the end of the perfusion, the ciliary ganglia of both the operated and unoperated sides were removed and kept in concentrated fixative for 4 hours at 4 °C before they were rinsed in 0.1M cacodylate buffer (pH 7.4) containing 5% sucrose. They were then cut into small pieces and postfixed in 2% osmium tetroxide in 0.1M cacodylate buffer (pH 7.4) before being dehydrated in an ascending series of ethanol followed by embedding in Araldite. Ultrathin sections were cut on a Reichert Ultracut E ultramicrotome and double-stained with uranyl acetate and lead citrate. All sections were viewed under either a Philips 400T or JEOL 1200 EX electron microscope.

Results

Pre-ganglionic nerve section

In both cat and monkey, sprouting dendritic profiles were observed as early as 1 day after operation. These profiles were more frequently observed 3 days after operation; such profiles were identified by their dense packing of a large amount of mitochondria and glycogen-like granules (Figs. 1-8). In longitudinal or oblique sections, such profiles appeared as expanded extensions from the normal-looking dendritic trunks (Figs. 5, 6). None were observed to arise directly from the neuronal soma. The mean cross-sectional diameter of such profiles measured 2.2± 1.0 μm (range: 0.9-6.2 μm) in cat and 2.4± 0.7 μm (range: 0.9-5.5 μm) in monkey. Most axon terminals, if not all, degenerated 3 days after operation and disappeared by 5 days after operation. After 14 days, axon terminals reappeared and were observed to synaptically contact (Figs. 3-6) or to be closely associated (Fig. 7) with profiles of sprouting dendrites. Such profiles could still be observed 21 and 30 days after operation. Some of these profiles showed signs of degeneration as shown by the presence of dense bodies and dilated mitochondria (Fig. 8). However, no axon terminal was observed to synaptically contact with such degenerated profiles.

Post-ganglionic nerve section

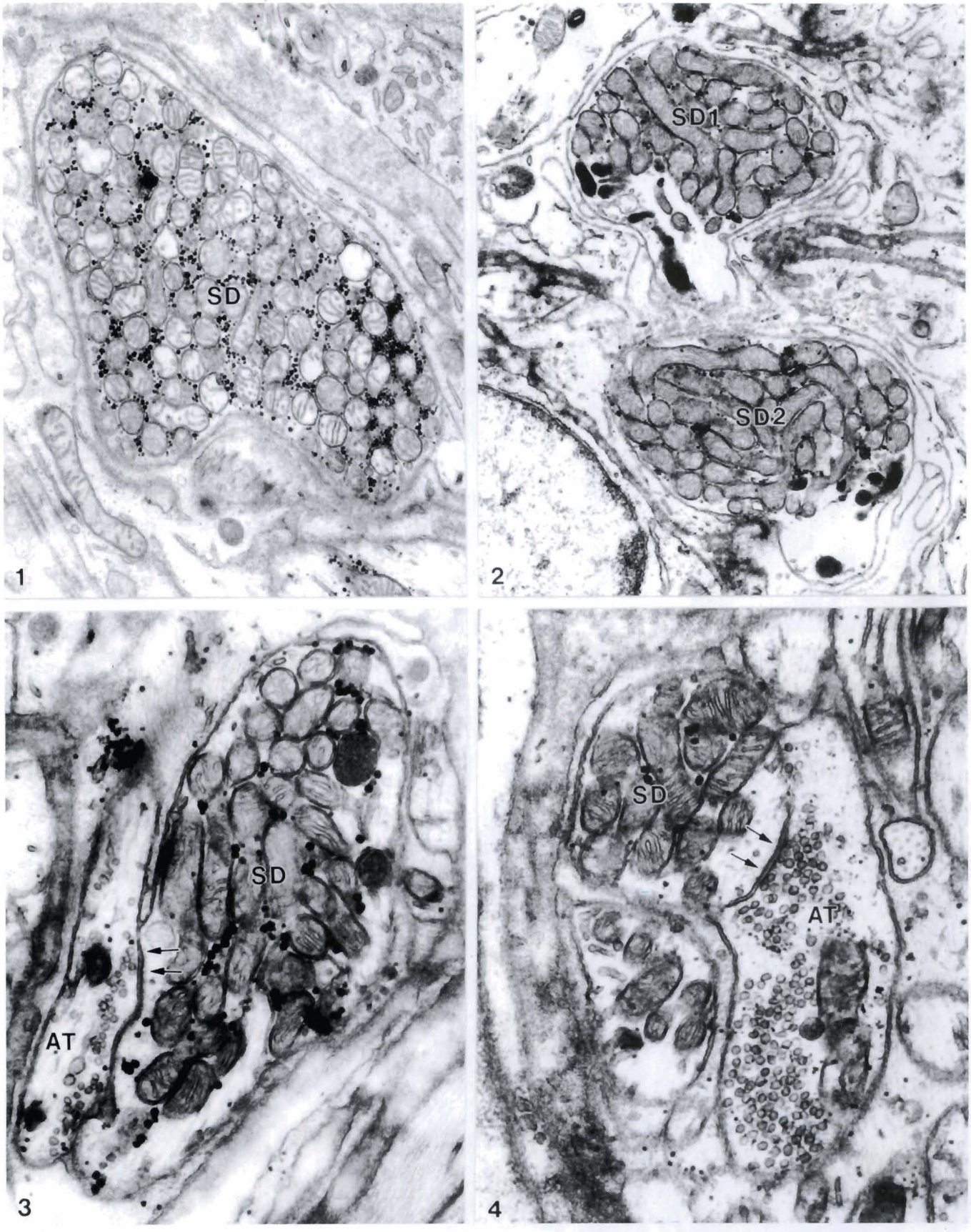
In both cat and monkey, similar profiles of sprouting dendrites packed with mitochondria and glycogen granules were also observed in the ciliary ganglion following postganglionic nerve section (Figs. 9-11). They were observed as early as 1 day after operation and more frequently 3 days after operation. Similar to those in the preganglionic axotomy, these dendritic profiles were also found to arise only from dendrites; none were observed to arise from neuronal soma. In some cases, such profiles were synaptically contacted by axon terminals (Fig. 9). In others, they were closely, but not synaptically, associated with axonal profiles containing synaptic vesicles (Fig. 10). The mean cross-sectional

Fig. 1. Electron micrograph showing a cross-section of a sprouting dendrite (SD) densely packed with mitochondria and glycogen-like granules in the ciliary ganglion of cat. 3 days after preganglionic axotomy. x 23,000

Fig. 2. Electron micrograph showing two sprouting dendrites (SD1, SD2) densely packed with mitochondria and glycogen-like granules in the ciliary ganglion of monkey. 5 days after preganglionic axotomy. x 23,000

Fig. 3. Electron micrograph showing a sprouting dendrite (SD) packed with mitochondria and glycogen-like granules receiving a synaptic contact (arrows) with an axon terminal (AT) in the ciliary ganglion of cat. 12 days after preganglionic axotomy. x 38,500

Fig. 4. Electron micrograph showing a sprouting dendrite (SD) packed with mitochondria and glycogen-like granules making synaptic contact (arrows) with an axon terminal (AT) in the ciliary ganglion of monkey. 14 days after preganglionic axotomy. x 38,500


SD
1
SD1
SD2
2
SD
AT
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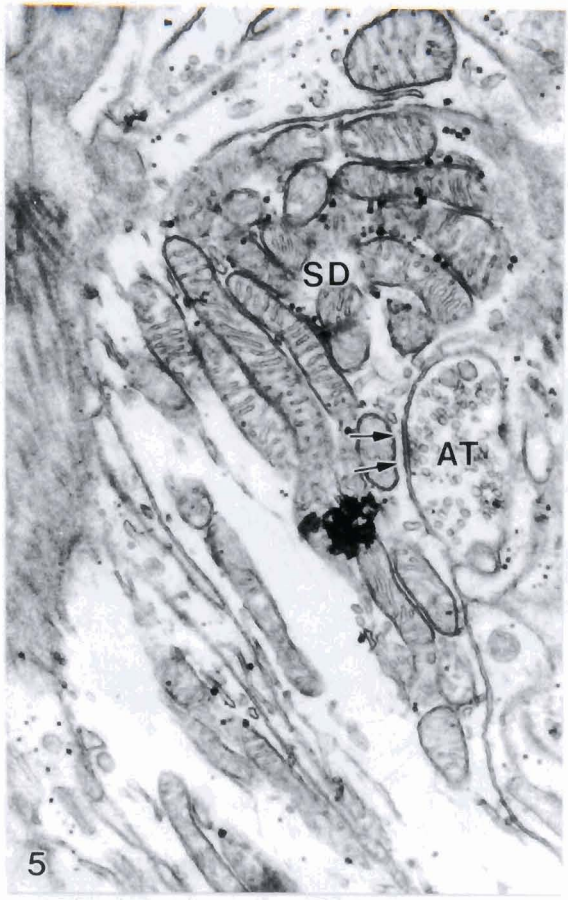
AT
4



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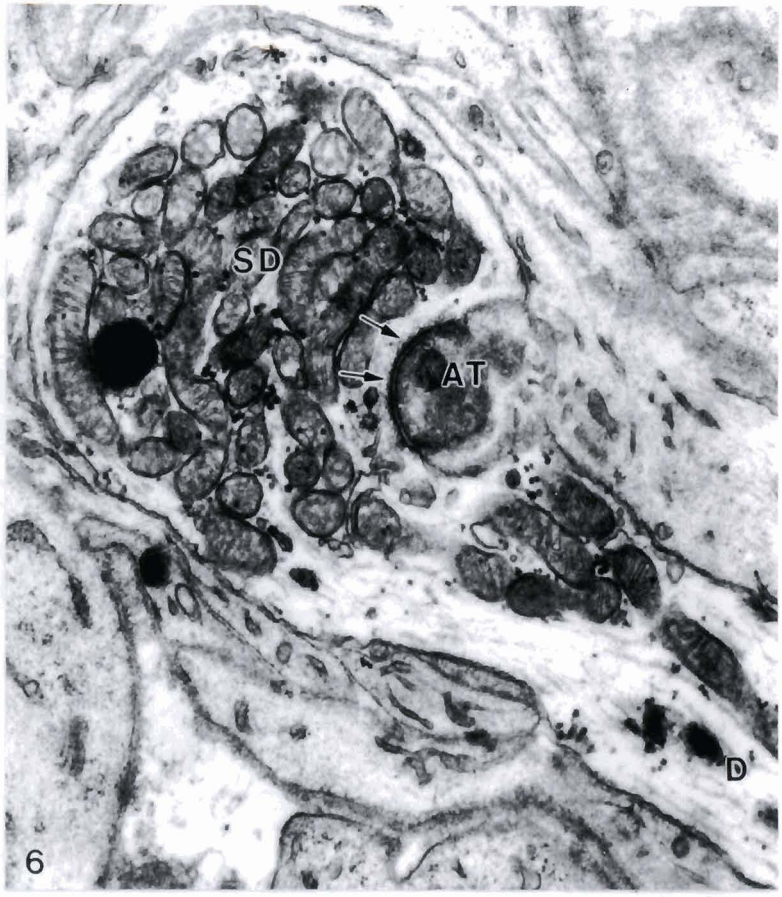

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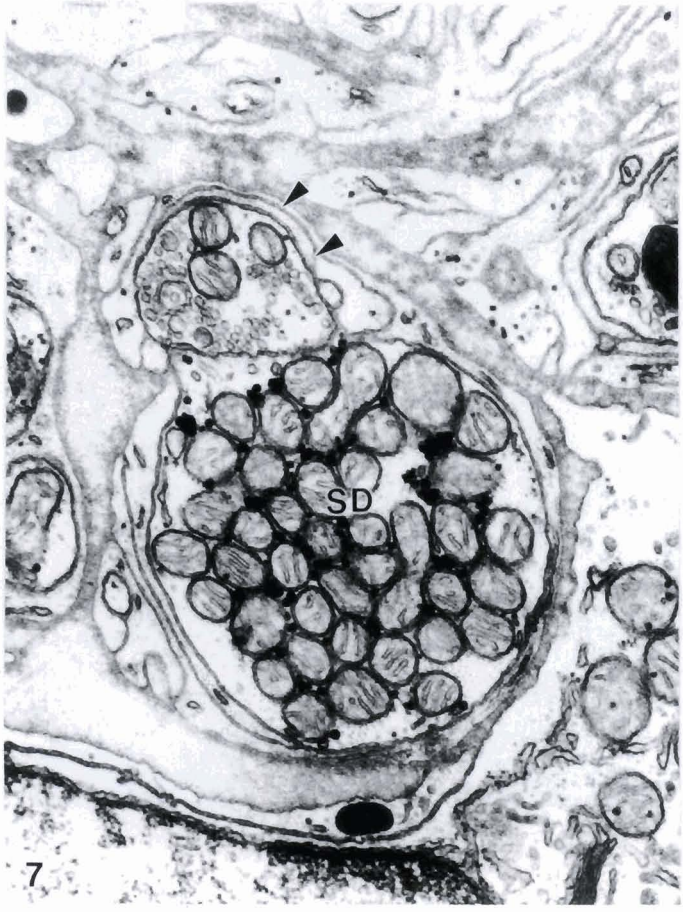

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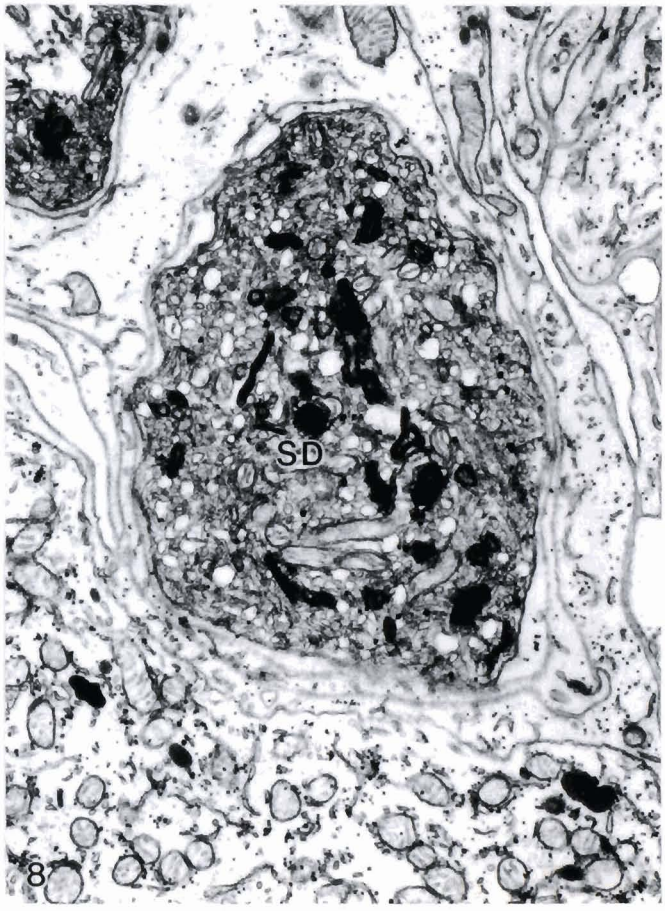

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Fig. 5. Electron micrograph showing a sprouting dendrite (SD) packed with mitochondria and glycogen-like granules making synaptic contact (arrows) with an axon terminal (AT). 14 days after preganglionic axotomy. Ciliary ganglion of cat. x 26,000

Fig. 6. Electron micrograph showing a sprouting dendrite (SD) packed with mitochondria and glycogen-like granules in the ciliary ganglion of monkey. The sprouting dendrite appears to be an expanded part of a normal-looking dendrite (D). It synapses (arrows) with an electron-dense axon terminal (AT). 14 days after preganglionic axotomy. x 30,000

Fig. 7. Electron micrograph showing a sprouting dendrite (SD) packed with mitochondria and glycogen-like granules closely associated with a profile containing synaptic vesicles (arrowheads). 12 days after preganglionic axotomy in the ciliary ganglion of cat. x 30,000

Fig. 8. Electron micrograph showing a sprouting dendrite (SD) packing with dilated mitochondria, dense bodies and a few glycogen-like granules in the ciliary ganglion of monkey. 21 days after preganglionic axotomy. x 17,500

diameter of these profiles measured 2.1±0.7 μm (range: 0.8-4.5 μm) in cat and 2.8±1.4 μm (range: 1.1-7.0 μm) in monkey. Dense bodies and dilated mitochondria were sometimes observed in these dendritic profiles (Fig. 11) on which no axon terminals were observed; such profiles were considered to be degenerating and were observed as early as 3 days postoperatively.

Control study

In the ciliary ganglia of normal animals and on the unoperated side of operated animals, such dendritic profiles packed with mitochondria and glycogen-granules were only rarely found in some animals. No axon terminals were observed to synapse with such profiles.

Discussion

The present study describes the ultrastructure of sprouting dendrites induced by axotomy. The profiles of sprouting dendrites were characterized by expanded profiles fully packed with mitochondria and glycogen-like granules. Such profiles were most frequently found at 3-5 days after both pre- and post-ganglionic axotomy. In longitudinal section, such profiles appeared as expanded extensions from the dendritic trunks. These structures were not observed in the ciliary ganglion of normal animals in our previous studies (Zhang et al., 1993, 1994a,b) and were only occasionally observed in some animals on both the unoperated sides and control animals. Such profiles were similar to the sprouting dendritic tips reported in the lateral vestibular nucleus of normal mature rats and cats (Sotelo, 1967; Mugnaini et al., 1967; Sotelo and Palay, 1968; Peters et al., 1991) and in the cerebellar central nuclei of the cat (Sotelo and Angaut, 1973). In the ciliary ganglion of the rabbit, Johnson (1983, 1988) and Hadley (1990) have reported the changes of the dendritic geometry after both pre- and post-ganglionic nerve sections in their light microscopic studies. Ultrastructurally, similar sprouting dendrites have also been reported in the superior cervical ganglion of cat after a vagus-sympathetic anastomosis (Ceccarelli et al., 1971) and in the stellate ganglion of normal pig-tailed monkey (Leong and Wong, 1988).

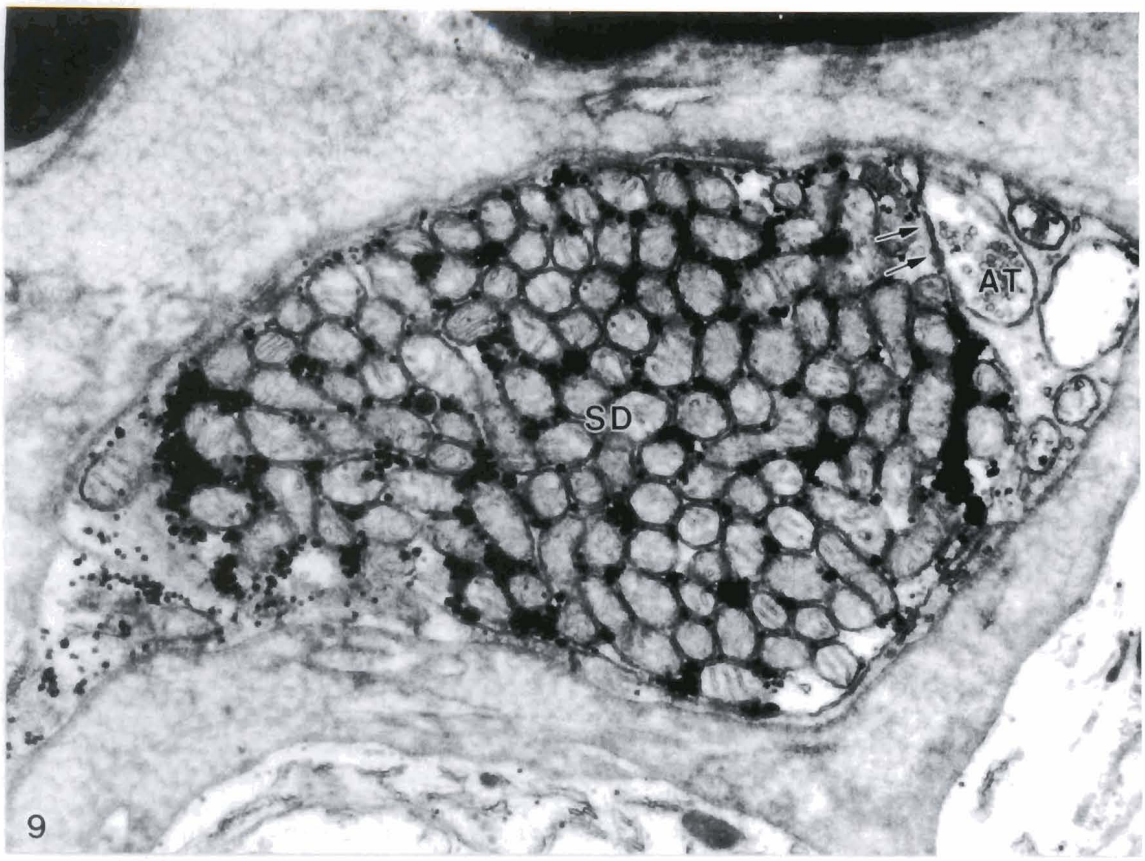
Johnson (1988) reported sprouting fine processes arising from both dendrites and cell bodies after preganglionic axotomy. In rat superior cervical ganglion, sprouting dendrites arising from cell bodies have also been reported (Matthews and Raisman, 1972). In contrast, in the present study, no sprouting dendrites were observed to arise directly from the neuronal soma. On the other hand, the dendritic length and complexity were reduced by 60-70% after postganglionic axotomy in the mouse superior cervical ganglion (Yawo, 1987). In Lamprey central neurons, axotomy could induce both dendritic sprouting and retraction (Hall and Cohen, 1983a,b, 1988a,b). In the present study, dense bodies and dilated profiles resembling degenerating mitochondria were observed in some of these profiles in animals with longer postoperative survival time, suggesting that some of these profiles might undergo degeneration.

The axon terminals have been frequently found to form synapses with such dendritic profiles. Such axon terminals were considered as newly formed. These were more evident in the denervated ciliary ganglion. Since almost all axon terminals were degenerated 3 days after operation and disappeared 5 days after operation, the reappearance of axon terminals suggests that they must be newly formed. The electrophysiological study of Johnson (1988) has also shown that there was a rapid formation of large numbers of functional synapses and intraganglionic connections after denervation. New formation of synapses has also been reported in frog

Fig. 9. Electron micrograph showing a large sprouting dendrite (SD) densely packed with mitochondria and glycogen-like granules making a synaptic contact with an axon terminal (AT) in the ciliary ganglion of cat. 5 days after postganglionic axotomy. x 30,000

Fig. 10. Electron micrograph showing a sprouting dendrite (SD1) packed with mitochondria and glycogen-like granules in the ciliary ganglion of monkey. 5 days after postganglionic axotomy. x 26,000

Fig. 11. Electron micrograph showing a sprouting dendrite (SD1) packed with mitochondria and glycogen-like granules lying adjacent to another sprouting dendrite (SD2) packed with mitochondria and dense bodies and a few glycogen-like granules in the ciliary ganglion of cat. 7 days after postganglionic axotomy. x 14,000



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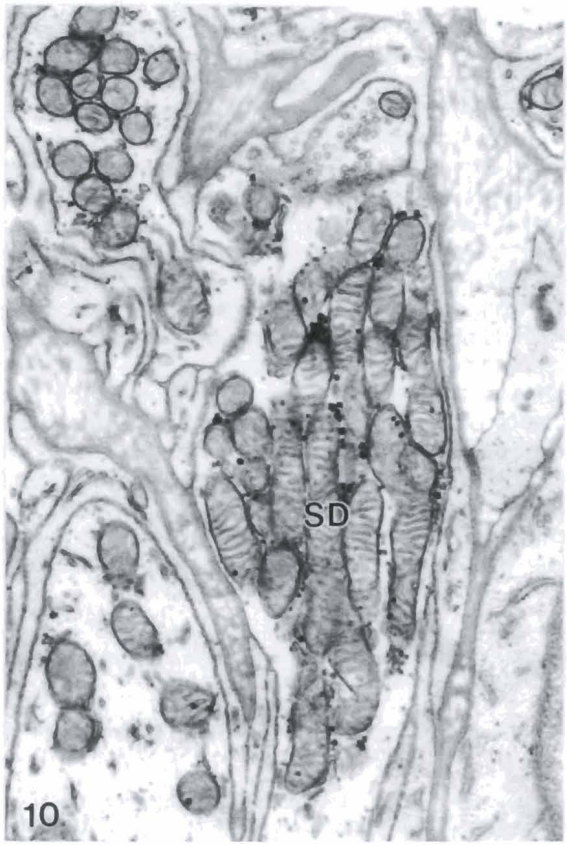

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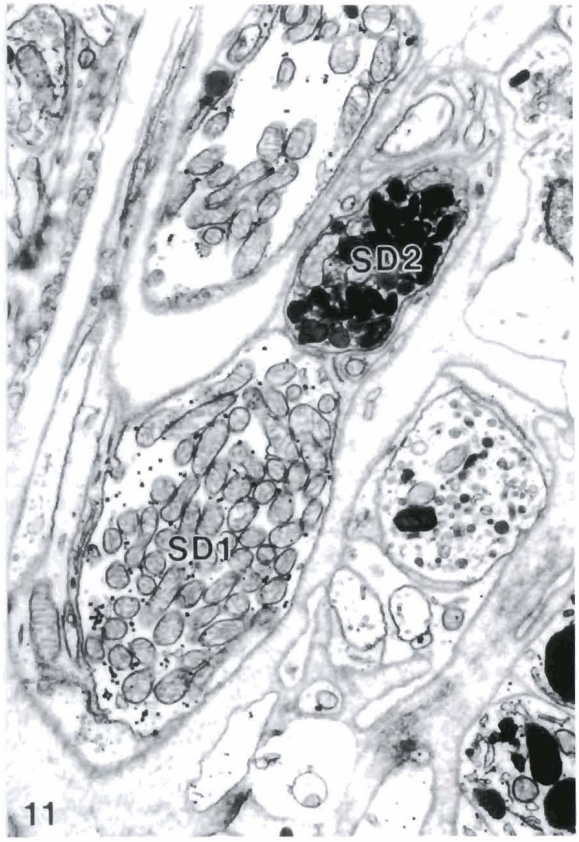

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cardiac ganglion after partial denervation (Courteney and Ropper, 1976; Roper and Ko, 1978). However, in the present study, dendritic profiles containing dense bodies and dilated mitochondria were never observed to be synaptically contacted by any axon terminals. It was thus assumed that those dendritic profiles which failed to receive synaptic contact would undergo degeneration. Hence the fate and survival of the sprouting dendrites would depend on whether there was synaptic reinnervation.

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