Cell Tissue Res (1987) 250:79–86

Cell and Tissue Research

Neurofilament-like and glial fibrillary acidic protein-like immunoreactivities in rat and guinea-pig sympathetic ganglia in situ and after perturbation

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Summary. The presence of neurofilament (NF)-like and glial fibrillary acidic protein (GFAP)-like immunoreactivities was studied in sympathetic ganglia of adult rats and guinea pigs during normal conditions and after perturbation. In the superior cervical ganglion (SCG) of normal rats, many ganglion cells and nerve fibers show NF immunoreactivity. Some of these nerve fibers disappear after preganglionic decentralization of SCG; this indicates the presence of a mixture of pre- and postganglionic NF-positive nerves in the ganglion. Cuts in both pre- and postganglionic nerves result in a marked increase in GFAP immunoreactivity in SCG, whereas NF immunoreactivity increases in nerve cell bodies after preganglionic cuts. Only a few ganglion cells show NF immunoreactivity in the normal SCG of guinea pig. All intraganglionic NF-positive nerves are of preganglionic origin; decentralization abolishes NF immunoreactivity in these nerve fibers. The inferior mesenteric ganglion, the hypogastric nerves and colonic nerves in guinea pigs contain large numbers of strongly NF-immunoreactive nerve fibers.

When the SCG of adult rat is grafted to the anterior eye chamber of adult rat recipients, both ganglionic cell bodies and nerve fibers, forming on the host iris from the grafted ganglion, are NF-positive. As only the perikarya of these neurons normally exhibit NF immunoreactivity, and the terminal iris arborizations are NF-negative, it appears that the grafting procedure causes NF immunoreactivity to become more widespread in growing SCG neurons.

Key words: Neurofilaments – Glial fibrillary acidic protein – Sympathetic ganglia – Intraocular transplantation – Rat – Guinea pig

Intermediate filaments have been observed in most cell types. Although structurally similar, they are made up of chemically and immunologically distinct proteins in cells of different embryological origin (Lazarides 1980). Neurofilament (NF) protein, which forms the neuronal intermediate filaments, has been shown by Hoffman and Lasek (1975) and Schlaepfer (1977) to consist of a triplet of polypeptides with molecular weights of approximately 70, 150 and 200 K daltons (see also Lazarides 1980). The development of an antiserum to NF by Dahl and Bignami (1977) has made it possible to study the appearance and distribution of NF in both the central and peripheral nervous system by means of immunohistochemical techniques. Most polyclonal antisera seem to react with all three polypeptides in the NF triplet (Dahl 1980, 1981; Dahl et al. 1981, 1982). The NF protein is chemically and immunologically distinct from the 50 K glial fibrillary acidic protein (GFAP).

In the central and peripheral nervous system, myelinated nerve fibers and certain nerve cell bodies have been shown to exhibit strong immunoreactivity to NF (Dahl and Bignami 1977; Schlaepfer and Lynch 1977; Dahl et al. 1981; Shaw et al. 1981; Björklund et al. 1984b; Seiger et al. 1984). In the peripheral nervous system, most of the NF-immunoreactive nerve fibers have been claimed to be of a sensory nature (Dalsgaard et al. 1984; Seiger et al. 1984), but NF-positive perikarya and nerve fibers have been demonstrated in the ciliary ganglion and the superior cervical ganglion of the rat (Seiger et al. 1984). However, in a study of the innervation of the iris, it has been shown that the dense network of NF-positive fibers is exclusively of sensory origin and that the autonomic terminal network is NF-negative (Seiger et al. 1984). Furthermore, by grafting adult trigeminal ganglia to the anterior eye chamber of adult recipients, it has been demonstrated that NF-positive nerve fibers can innervate the host iris even in the presence of the intrinsic NF-positive nerve plexus (Björklund et al. 1985b).

In astrocytes from the CNS, GFAP is the major component of the intermediate filament system (Bignami et al. 1980; Eng and DeArmond 1981). Recently, several papers reporting the presence of GFAP-like immunoreactivity in peripheral glial cells, such as cells in the rat enteric nervous system (Jessen and Mirsky 1980; Björklund et al. 1984b), some Schwann cells in rat sciatic nerve (Yen and Fields 1981; Dahl et al. 1982), and cells in the iris of several species (Björklund et al. 1984a) have been published.

In the present study, the appearance and origin of NF-like immunoreactivity was studied in the superior cervical ganglion (SCG) of rat and guinea pig. Pre- and postganglionic denervations and intraocular transplantation were used to perturb the system. In the guinea pig, the inferior mesenteric ganglion and related nerves were also analyzed. Furthermore, GFAP-like immunoreactivity was studied in intact and denervated SCG of both rat and guinea pig.

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Materials and methods

Adult albino rats (Sprague-Dawley) and albino guinea pigs of both sexes were used in all experiments. In one set of experiments, the SCG was subjected to a preganglionic or postganglionic denervation, i.e. the cervical sympathetic trunk was cut approximately 10 mm caudal to the ganglion, or the postganglionic nerves leaving the ganglion were cut as close to the ganglion as possible. The animals were allowed to survive for 7–11 days before sacrifice.

Intraocular grafting was performed as described previously (Olson et al. 1983). Superior cervical ganglia of rats were divided into two parts before grafting. Four weeks later the recipients were killed and the host iris with attached ganglion was grafted to the anterior eye chamber of adult recipients for four days in order to cause degeneration of intrinsic NF-positive iris nerves (cf. Seiger et al. 1984). Animals were sacrificed by cervical dislocation or exsanguination under deep ether anesthesia.

The SCG was removed from both species; the inferior mesenteric ganglion and the hypogastric and colonic nerves were also dissected from the guinea pigs. All specimens were sectioned on a cryostat. The sections (14 μm thick) were picked up on gelatin-coated slides. The iris grafts were stretch-prepared as whole mounts on gelatin-coated slides (Falck 1962; Malmfors 1965) after most of the ganglion graft had been removed. All slides were fixed in acetone for 3 min at room temperature and further processed for indirect immunohistochemistry according to Coons (1958). Briefly, the sections were incubated in a humid chamber at 4° C overnight with NF antibodies raised in rabbits against a 50000 MW degradation product of chicken NF protein (Dahl and Bignami 1977). Antiserum to GFAP was prepared in rabbits against degraded antigen from phosphate buffer extracts of autolyzed human spinal cord (Dahl and Bignami 1976). The NF antiserum was applied at a 1:1000 dilution, and the GFAP antiserum at 1:100 dilution in phosphate-buffered saline (PBS). The next day, the slides were rinsed in PBS, incubated with rhodamine-conjugated swine anti-rabbit antibodies (dilution 1:50–1:100 (Dakopatts, Denmark) at room temperature in darkness, rinsed in PBS and mounted in 90% glycerin in PBS. Control sections were incubated with preimmune serum. The sections were examined in a Zeiss fluorescence microscope equipped with an oil dark-field condenser and appropriate filter. For photography, Tri-X film (Kodak, Eastman, NY) was used.

Results

NF-like immunoreactivity in the SCG

In the SCG of unoperated rats and guinea pigs, a large number of NF-like immunoreactive nerve fibers was observed (Fig. 2A). In the rat, most neuronal perikarya were also NF-positive (Fig. 1A). Fluorescence was confined to the cytoplasm and was strongest in the peripheral part of the cell body. Between the ganglion cells, fluorescent fibers could be found running singly or in nerve bundles of variable diameter. In contrast to the SCG of the rat, the SCG of the guinea pig contained very weakly fluorescent ganglion cells. Therefore, the NF-positive nerve fibers were more easily visualized than in the rat (Fig. 2A). The fibers were present throughout the ganglion, although the density was variable in different parts of the ganglion. Nerve bundles containing varying numbers of fluorescent fibers were frequently observed. Some fibers in the SCG of both the rat and guinea pig closely surrounded the principal ganglion cells.

Transection of the cervical sympathetic trunk resulted in an almost complete disappearance of NF-positive fibers in the guinea pig SCG (Fig. 2B). Interestingly, a few strongly immunoreactive perikarya could be observed in such ganglia (Figs. 2B and 3A). In contrast to the guinea pig, preganglionic denervation of the SCG in the rat led to only a moderate decrease in the number of NF-positive fibers in the ganglion (Fig. 1B). However, as in the guinea pig, an increase in the fluorescence intensity was found in the cell bodies of the SCG following preganglionic denervation. This reaction was more uniform in the rat and seemed to affect all neurons (Fig. 1B). The numbers of NF-positive fibers observed in the SCG after transection of the postganglionic nerve bundles (Figs. 1C and 2C) did not decrease in either species. In the cervical sympathetic trunk, NF-positive fibers were present, running mostly as single fibers (Fig. 3B).

GFAP-like immunoreactivity in the SCG

GFAP-like immunoreactivity was observed throughout the intact SCG of the rat. The fluorescence intensity was relatively low and the immunoreactive structures were most clearly seen surrounding negative cell bodies (Fig. 1D). GFAP-positive fibers could also be found in the guinea pig, although the fluorescence intensity was very low. Following transection of either the pre- or postganglionic nerve trunk, a prominent increase in density and fluorescence intensity of GFAP-positive structures occurred thoughout the ganglion (Fig. 1E and F).

NF-like immunoreactivity in the inferior mesenteric ganglion

The inferior mesenteric ganglion (IMG) of the guinea pig displayed a very dense pattern of NF-positive nerve fibers. The NF-immunoreactive fibers occurred as single fibers, as a nerve network surrounding the principal ganglion cells or as nerve bundles containing varying numbers of nerve fibers (Fig. 4A). In general, the single nerve fibers and those forming the network appeared, in the IMG, to be more slender and more homogeneous in size and fluorescence intensity than those in the SCG. The nerve bundles ran in groups between the ganglion cells. The nerve cells were usually weakly fluorescent, but sometimes cells with strongly immunofluorescent cytoplasm were seen.

Sections through the hypogastric nerves revealed a large number of intensely fluorescent fibers (Fig. 4B). These fibers occurred in bundles with a varying number in each bundle. Small aggregates of sympathetic nerve cells containing fluorescent material in the cytoplasm were sometimes observed along the nerve bundles (Fig. 4B).

Many colonic nerve bundles of fluorescent fibers were seen in sections of the inferior mesenteric artery with surrounding tissue (Fig. 4C).

Intraocular transplantation of SCG

The grafts did not change in size in oculo. All ganglia contained large, strongly fluorescent perikarya. NF-positive cells could also be seen on the host iris after the major part of the ganglion graft had been removed (Fig. 5 inset).

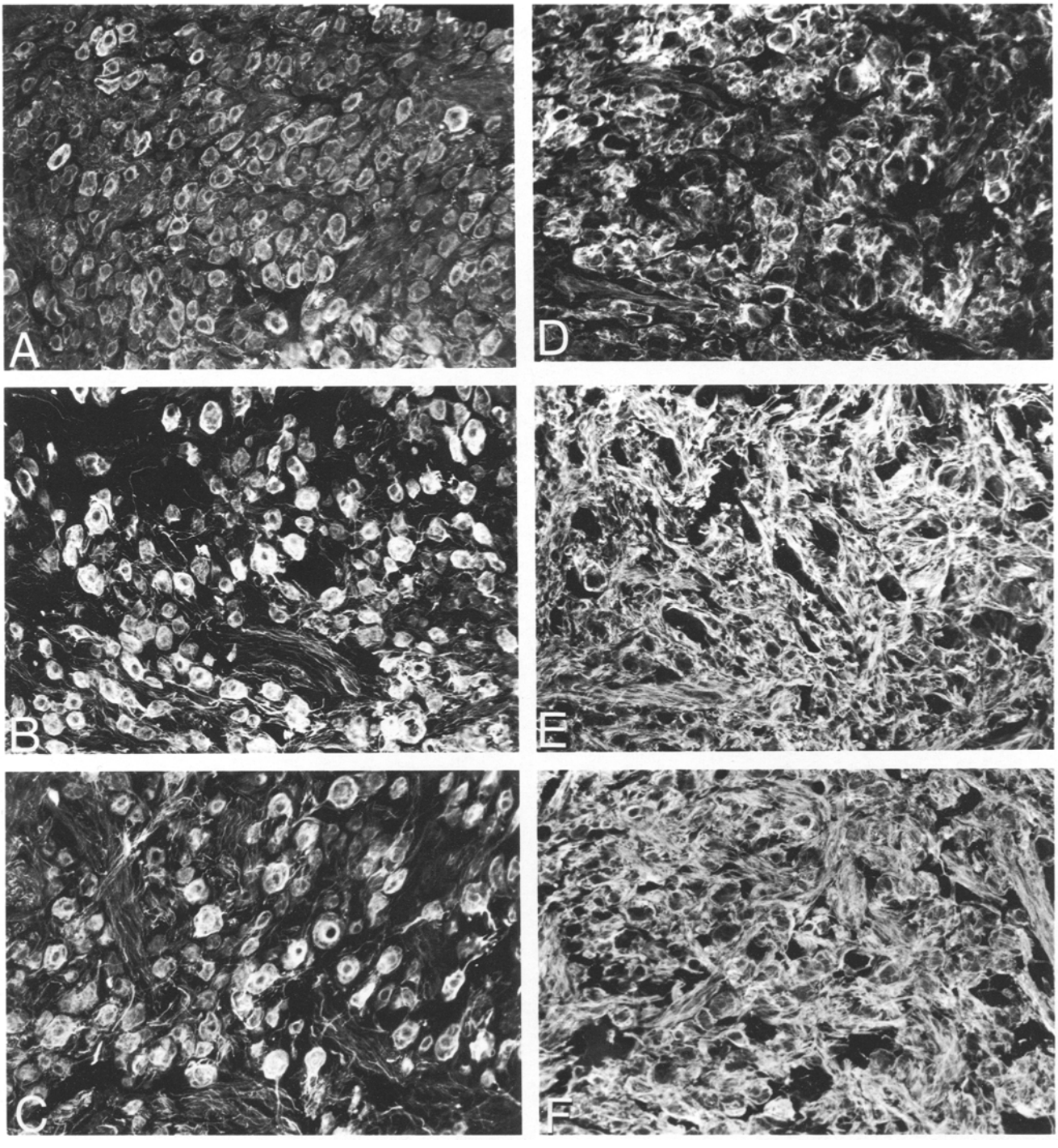

Fig. 1. NF- (**A–C**) like and GFAP- (**D–F**) like immunoreactivities in the intact (**A**, **D**), preganglionically (**B**, **E**) and postganglionically (**C**, **F**) denervated SCG of rat. In (A) weakly fluorescent NF-positive fibers are intermingled with NF-like immunoreactive perikarya. Following preganglionic denervation (**A**) a decrease in fluorescent nerve fibers is observed whereas no change in the number of positive fibers is seen after postganglionic denervation (**C**). In both **B** and **C**, the fluorescence intensity is increased in the neuronal cell bodies. Note a similar increase in amount and intensity of GFAP-like immunoreactivity when the intact SCG (**D**) is compared with the preganglionically (**E**) and postganglionically denervated SCG. Fluorescence micrographs, ×135

On stretch-prepared irides, which had been grafted together with the SCG graft 4 days earlier, the normal NF-immunoreactive nerve plexuses had almost completely disappeared. An extensive outgrowth of NF-positive fibers could be seen emanating from the ganglion graft (Fig. 5). The fibers ran mainly individually, although fiber bundles of variable diameter were occasionally observed. Outgrowing fibers could be seen at long distances from the ganglion graft. Fluorescent fibers were also seen entering both the sphincter and the ciliary body.

Discussion

In the present paper we have shown that almost all NF-positive nerve fibers in the SCG from normal guinea pigs are preganglionic, because transection of the cervical sym-

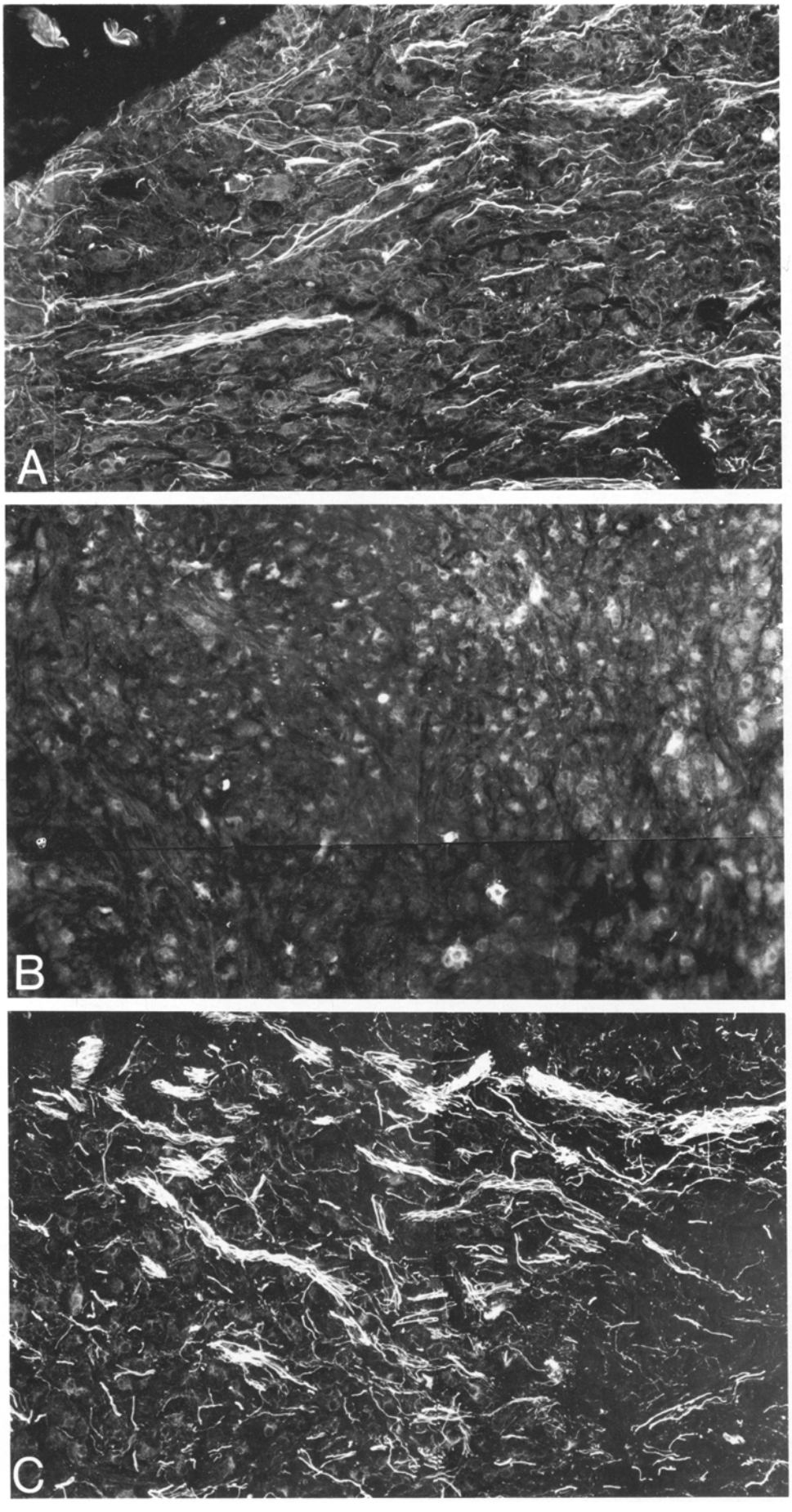

Fig. 2. NF-like immunoreactivity in the intact (**A**), preganglionically (**B**) and postganglionically (**C**) denervated SCG in guinea pig. A high density of fluorescent fibers is observed in **A** and **C**, whereas no NF-positive nerve fibers are present following preganglionic denervation. Fluorescence micrographs, × 131

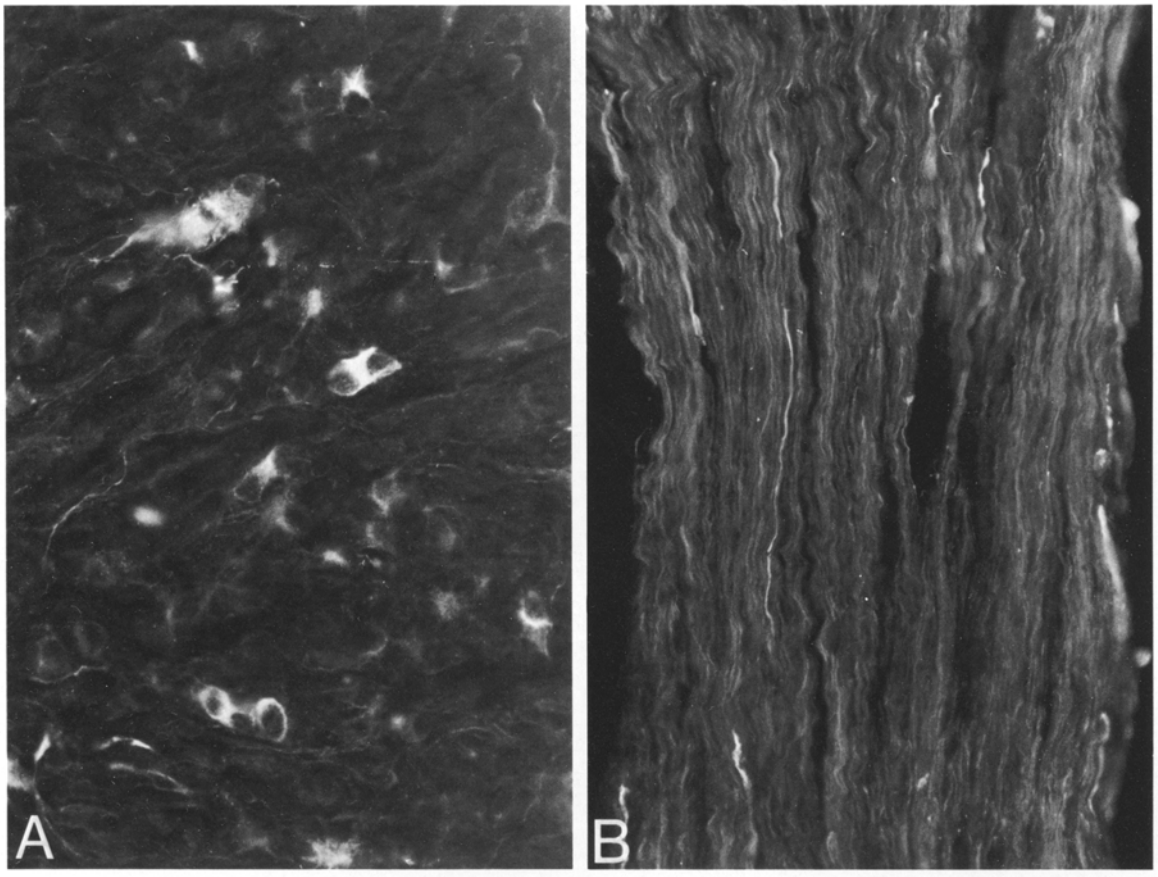

Fig. 3. **A** NF-positive neuronal perikarya in SCG from a guinea pig following preganglionic denervation. **B** Positive fibers in the cervical sympathetic trunk. Fluorescence micrographs, × 330

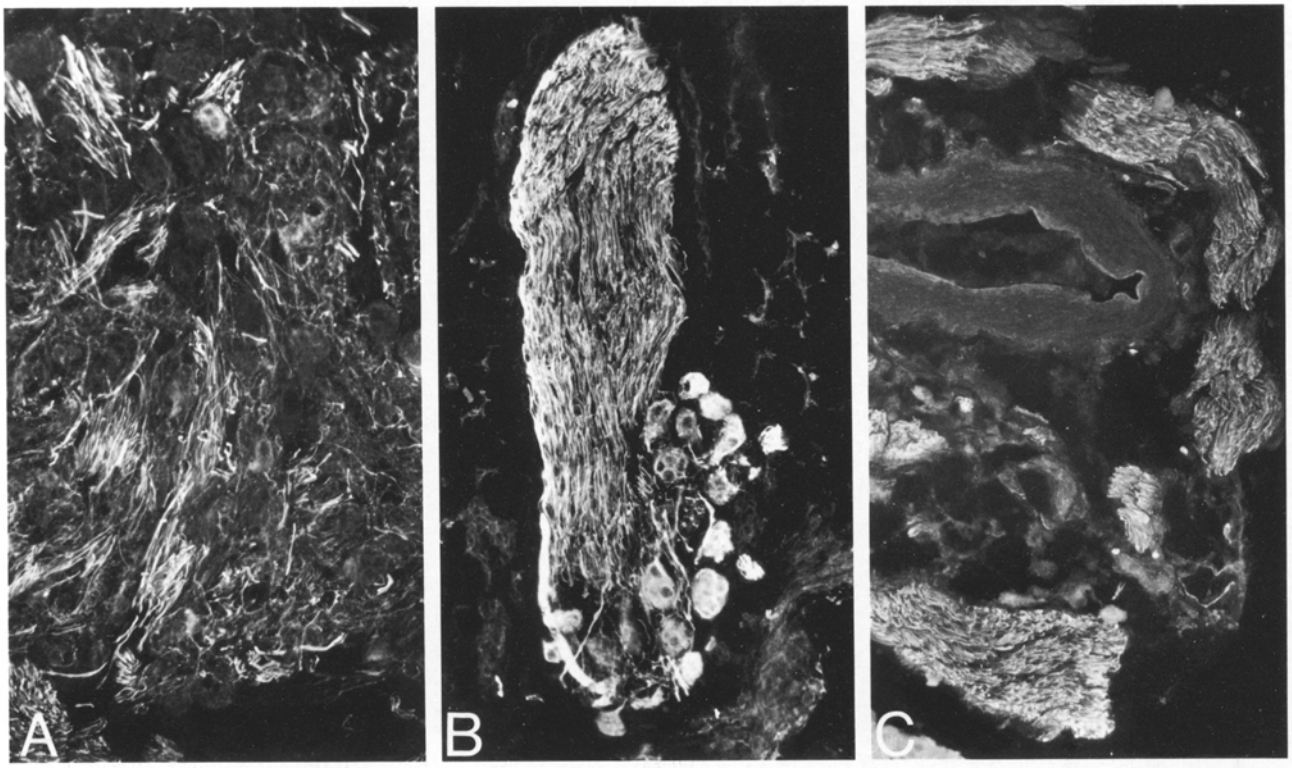

Fig. 4. NF-like immunohistochemistry. **A** A dense system of NF-positive fibers is seen in the inferior mesenteric ganglion of the guinea pig. **B** Large number of positive fibers in the hypogastric nerve surrounded by fluorescent perikarya. **C** Note large numbers of NF-like immunoreactive colonic nerve fibers surrounding the inferior mesenteric artery. Fluorescence micrographs, × 135

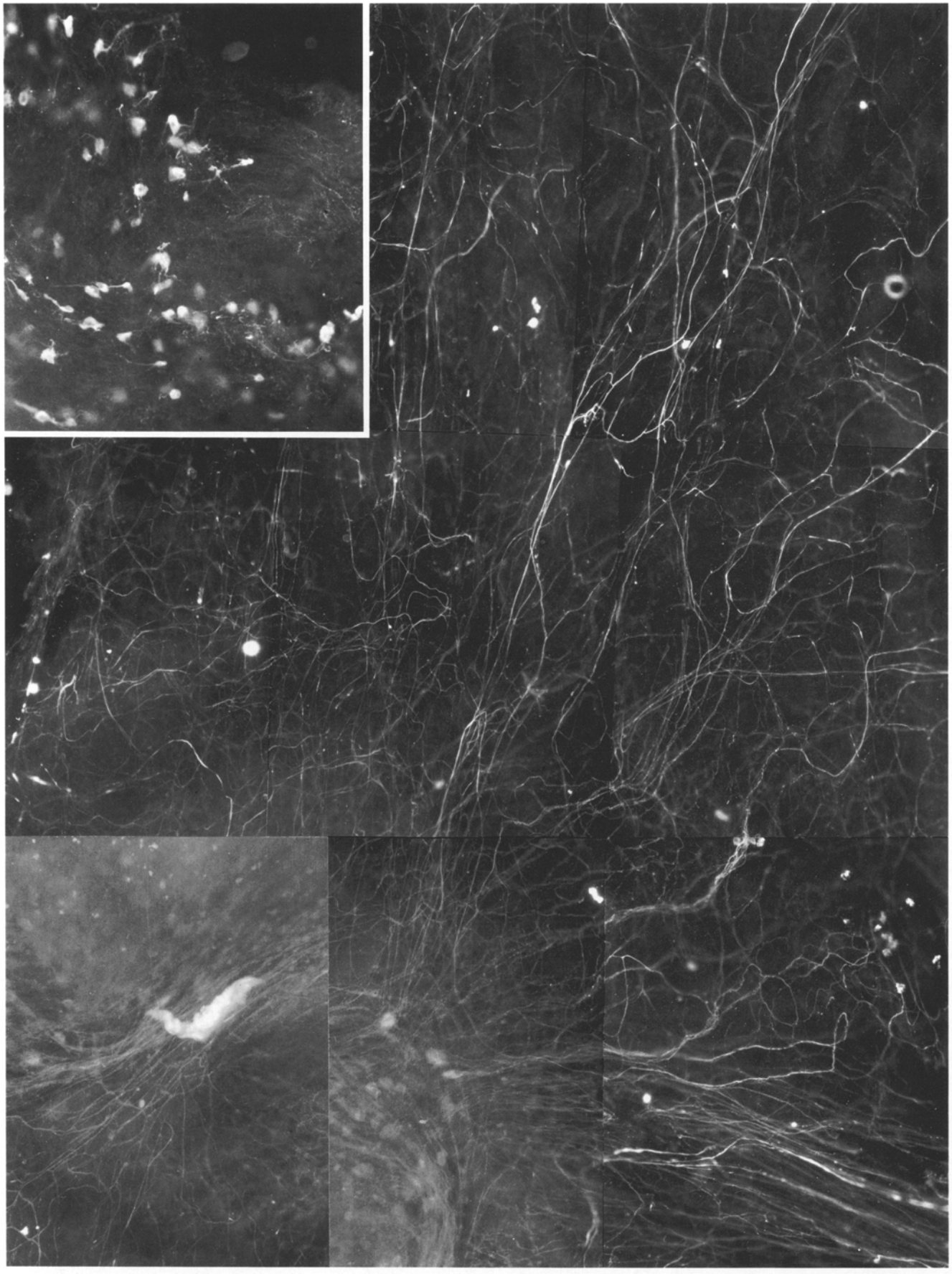

Fig. 5. Outgrowth of NF-positive fibers onto the host iris from a 4-week-old SCG graft (lower left corner). The iris with the attached ganglion graft was grafted to the anterior eye chamber of a normal rat 4 days earlier to cause degeneration of intrinsic NF-positive iris fibers. The *inset* shows NF-positive neuronal perikarya on the host iris after the SCG graft had been removed. Fluorescence micrographs, × 131

pathetic trunk leads to an almost complete disappearance of such fibers from the ganglion. However, in the SCG of the rat, NF-positive fibers seem to be both pre- and postganglionic because a preganglionic nerve cut causes only a moderate decrease in the number of positive nerve fibers. The situation in the inferior mesenteric ganglion in the guinea pig is probably more complicated, because it is known that this ganglion receives inputs from the spinal cord, dorsal root ganglia, other prevertebral ganglia and ganglia in the gut wall (Dalsgaard and Elfvin 1982). It seems reasonable to believe that all peripheral nerve fibers (sensory and autonomic) contain some NF protein, but that the concentration within individual nerve fibers is variable. It has been shown that in irides from normal rats, only sensory fibers are NF-positive (Seiger et al. 1984). Similarly, in the skin from the human finger, the NF immunoreactivity seems to be limited to sensory fibers (Dalsgaard et al. 1984; Björklund et al. 1986). It is thus interesting to note that various types of perturbations such as denervation, and transplantation to the anterior eye chamber, can increase the content of NF proteins within nerve cell bodies and fibers, respectively, that were previously almost undetectable using NF immunohistochemistry. In the present paper, we have demonstrated that SCG grafts can innervate the host iris with NF-immunoreactive fibers. This indicates a changed neuronal phenotype. It appears that a change in the environment of the neuronal cell bodies is necessary for the induction of increased neurofilament immunoreactivity, because complete sensory denervation of the iris in situ does not lead to increased NF immunoreactivity in the autonomic fibers in the iris (Seiger et al. 1984).

The presence of GFAP immunoreactivity in the peripheral nervous system has now been widely accepted. It is, however, believed that the central and peripheral GFAPs are not identical, although their molecular weights are comparable (Jessen et al. 1984). In the brain and spinal cord, it is well known that an increase in GFAP-like immunoreactivity occurs as a response to various kinds of trauma (Bignami and Dahl 1973; Björklund et al. 1983; Strömberg et al. 1986). Similarly, the weakly fluorescent Müller glial cells in the neuro-retina of the rat become strongly immunoreactive following various perturbations (Bignami and Dahl 1979; Björklund et al. 1985a). Also, transections of the olfactory nerve result in a marked increase in GFAP-immunoreactivity (Barber and Dahl, unpublished). Interestingly, using a monoclonal antibody, these authors have been able to demonstrate that this increased fluorescence intensity is a result of an increase in concentration of the peripheral-type GFAP. In the present paper, we demonstrate that, in the peripheral nervous system, denervation of the appropriate organ leads to a gliotic reaction as determined by GFAP immunohistochemistry. It seems reasonable that this gliotic reaction is similar to the one observed in the olfactory nerve.

In conclusion, we have shown that both GFAP immunoreactivity and NF immunoreactivity in the peripheral nervous system can increase as a response to various kinds of perturbations. Whether these changes in amount of intermediate filament protein have any functional consequences or are only a nonspecific response to disturbance remains to be elucidated.

Acknowledgements. This work has been supported by the Swedish Medical Research Council grants 14X-06555, 25P-6326, 12X-5189, the "Expressen" Prenatal Research Foundation, Torsten and Ragnar Söderbergs Foundation, and the Karolinska Institutet Foundations. Dr. D. Dahl was supported by the Veterans Administration. We thank Lena Holmberg, Anna-Stina Höijer, Barbro Standwerth and Karin Lundströmer for technical assistance, and Ida Engqvist for typing.

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Accepted February 9, 1987