THE JOURNAL OF COMPARATIVE NEUROLOGY 371:362–375 (1996)

Reactive Astrocyte Formation In Vivo Is Regulated by Noradrenergic Axons

RONALD GRIFFITH AND JEROME SUTIN
Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322

ABSTRACT

Beta adrenergic receptor antagonists greatly reduce reactive astrocyte formation induced by neuronal degeneration. To test the hypothesis that the density of noradrenergic innervation is a factor in the regulation of astrocytosis, we measured glial fibrillary acidic protein (GFAP) optical density after neuronal injury in central nervous system (CNS) regions with permanent noradrenergic sprouting or norepinephrine (NE) depletion. The injury model employs the injection of *Ricinus communis* lectin into a cranial or peripheral nerve to destroy CNS neurons without the blood-brain barrier disruption and lymphocyte infiltration associated with contusive or surgical lesions. We took advantage of the lack of an NE transporter in the terminals of certain classes of noradrenergic axons to produce noradrenergic sprouting in the trigeminal motor nucleus (MoV) with neonatal 6-hydroxydopamine (6-OHDA) treatment and to produce depletion of NE in the spinal cord dorsal horn with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4) administration. In each of these regions, GFAP optical density in the region of reactive astrocytes on the *Ricin* lectin-treated side was compared with the untreated contralateral (control) side in animals with NE hyperinnervation or NE depletion. GFAP density was increased about 55% in the injured NE-hyperinnervated MoV and was decreased about 35% in the injured NE-depleted dorsal horn. The degree of reactive astrocyte formation to injury is known to vary in different regions of the CNS, and our results suggest that differences in noradrenergic innervation may contribute to this variation. Along with earlier findings that β-adrenergic receptor blockade reduces reactive astrocyte formation, these data indicate that the noradrenergic innervation is a factor in the degree of astrocyte reactivity following injury.

Indexing terms: glia, microglia, brain injury, trigeminal, norepinephrine

Reactive astrocyte formation is a nearly universal reaction to injury in the adult mammalian central nervous system (CNS), but it varies in intensity in different regions. In addition to reactive astrocytes, the glial scar that develops progressively during the first week or two following traumatic injury also contains activated (amoeboid) microglia and newly formed vascular endothelium. Under some, but not all, conditions, axonal regeneration through a glial scar is limited, and the role of each cell type in regenerative growth is not well understood. We directed our attention to factors that influence the response of astrocytes to neuronal injury. The primary signals triggering the characteristic hypertrophy and increase of glial fibrillary acidic protein (GFAP) in reactive astrocytes have not been identified, but cytokines and trophic factors produced by microglia or invading monocytes and polymorphonuclear leukocytes can amplify the process (Perry and Gordon, 1988). In vitro studies identified a variety of neurotransmitter receptors and transporters (Shao and McCarthy, 1994) in astrocytes derived from the neonatal rat cortex or from C6 glioma cells. Astrocyte adrenergic receptors have been studied most extensively, but amino acid receptors are also well documented. The high affinity uptake of glutamate, aspartate, γ-aminobutyric acid (GABA), and taurine, and nonsaturable uptake of norepinephrine (NE), dopamine and 5-hydroxytryptamine, has also been characterized in primary astrocyte cultures (Hansson, 1989).

Beta adrenergic receptors are present in resting astrocytes and are up-regulated in reactive astrocytes isolated from the adult CNS (Salm and McCarthy, 1989; Shao and Sutin, 1992). Stone and Ariano (1989) were among the first to collate the evidence for activation of astrocytes by adrenergic neurons through paracrine transmission, emphasizing the role of astrocyte glycogenolysis in supporting local cellular energy demands during stress. Because reactive astrocyte formation, as reflected in GFAP labeling, is

Accepted April 9, 1996.

Address reprint requests to Dr. Jerome Sutin, Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

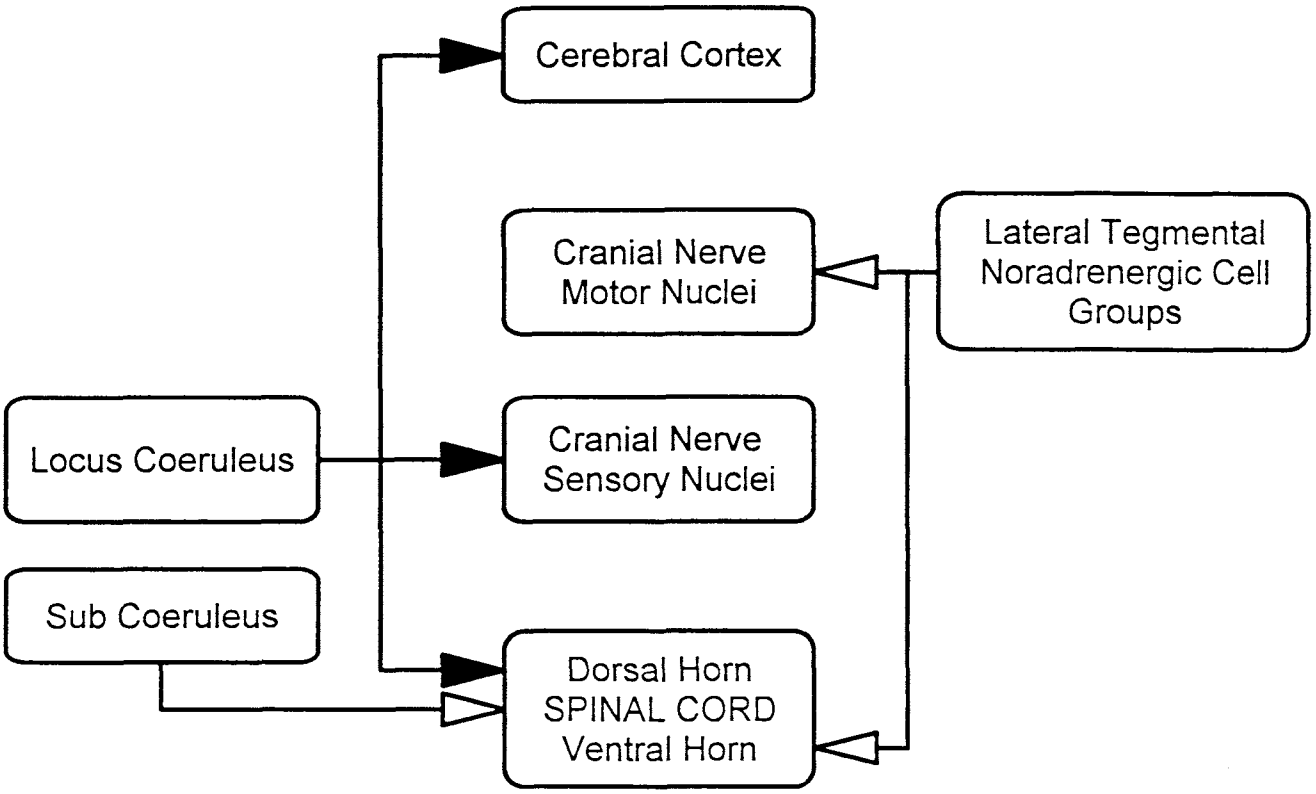

Fig. 1. A schematic of the pattern of noradrenergic innervation in the rodent central nervous system. The left and right columns show nuclei of origin of the major noradrenergic innervation for the target regions shown in the central column. Terminals with a norepinephrine (NE) transporter that makes them susceptible to destruction by N-(2-chloroethyl)-N-ethyl-bromobenzylamine hydrochloride (DSP-4) or 6-hydroxydopamine (6-OHDA) are indicated by the solid arrowheads. Terminals that are not destroyed by these agents are indicated by the open arrowheads. The spinal cord dorsal horn is depleted of NE after DSP-4 or neonatal 6-OHDA treatment, whereas the ventral horn is relatively unaffected. The trigeminal motor nucleus (MoV) shows marked NE sprouting (hyperinnervation) after neonatal 6-OHDA and is unaffected by DSP-4 treatment.

greatly reduced in vivo by treatment with β-adrenergic (Sutin and Griffith, 1993) or glutamate N-methyl-d-aspartate (NMDA; Garrison et al., 1994) receptor antagonists, we examined the relation between NE innervation density and injury-induced reactive astrocytes in the adult rodent CNS.

The pattern of NE innervation in the rodent CNS shows regional variation (Grzanna and Fritschy, 1991). Locus coeruleus neurons (A-6 group) supply the cerebral cortex, hypothalamus, cerebellum, sensory cranial nerve nuclei, and anterior horn of the spinal cord gray matter (Fig. 1). Most of the varicosities of these NE axons are destroyed by neonatal 6-hydroxydopamine (6-OHDA) treatment. NE fibers from cell bodies in the subcoeruleus nucleus that innervate the hypothalamus and spinal cord are not affected by 6-OHDA (McBride et al., 1985). The terminal axons of NE perikarya in the lateral pontine tegmentum (A-5 and A-7 groups) that innervate cranial nerve motor nuclei and the ventral horn of the spinal gray matter are not destroyed by the toxic catecholamine analogs 6-OHDA or N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4; Lyons et al., 1989; Fritschy and Grzanna, 1989), presumably because they do not possess a NE transporter. The NE innervation of the rat spinal cord described above shows some genetic variation. The scheme shown is Figure 1 is based on studies in the Sprague-Dawley substrain from Harlan, Inc. The Sprage-Dawley substrain from Sasco, Inc., differs, with a large NE projection of lateral tegmental (A-5 and A-7 groups) to the dorsal horn and a larger locus coeruleus projection to the ventral horn (Clark et al., 1991).

Neonatal 6-OHDA treatment results in permanent and extensive sprouting of NE axons in the trigeminal motor nucleus (MoV) and cerebellar cortex. MoV has the highest content of NE in the brain (Levitt and Moore, 1980), with levels about tenfold greater than the cerebral cortex or spinal cord. Compared with normal animals, the NE content of MoV is doubled after 6-OHDA treatment (Sutin and Minneman, 1985). Several other features make the MoV a favorable site to study neuronal injury-induced gliosis in addition to the ability to increase experimentally the NE innervation twofold or more. MoV consists of glia, vascular endothelium, and approximately 2,500 motor neurons innervating the jaw closing and opening muscles. Few interneurons are within MoV, most being found in the adjacent supratrigeminal nucleus and reticular formation. The primary afferent axons, which release aspartate or glutamate (Chandler, 1985), arise from perikarya in the mesencephalic trigeminal nucleus, with peripheral branches supplying muscle spindles in the jaw closing muscles.

In contusion or lesion models of CNS injury, infiltrating lymphocytes and monocytes with β-adrenergic receptor-mediated cytotoxic actions (Takamoto et al., 1991) complicate the analysis of adrenergic effects on reactive astrocyto-

sis. We chose an injury model in which the blood-brain barrier was not disrupted. Injection of the retrogradely transported lectin *Ricinus communis* into the masseter or sciatic nerve leads to neuron degeneration and glial scar formation in MoV and spinal cord, respectively.

MATERIALS AND METHODS

Surgical procedures

Adult Sprague-Dawley rats bred from Harlan, Inc., stock were used in all studies. All surgical procedures were carried out under sterile conditions using 70 mg ketamine and 6 mg xylazine per kilogram body weight. For studies of the MoV, the right masseter nerve was exposed in the infratemporal fossa by removal of the zygomatic arch. Care was taken to avoid injury to the orbitolachrymal duct. The nerve was ligated, injected with 1 μl volume of a solution containing 1.5 μg of *Ricin* lectin, and cut. After thorough flushing of the injection site with sterile saline, the incision was closed in layers with silk sutures. Three animals received intraperitoneal injection of 100 mg/kg cytosine β-D-arabinofuranoside hydrochloride (AraC) each morning and evening from the day of surgery until they were killed.

For studies of the lumbar spinal cord, the right sciatic nerve was exposed in the upper thigh and was injected in the manner described for the masseter nerve. The animals were observed periodically during recovery from anesthesia. Food and water intakes were monitored during the first 3 postsurgical days. After 7 days, the animals were deeply anesthetized with sodium pentobarbital, and those used for GFAP immunocytochemical examination were perfused through the heart with 150 ml of 0.85% saline followed by 500 ml 4% paraformaldehyde in cold 0.1 M phosphate buffer, pH 7.4. Animals used for dopamine-β-hydroxylase (DBH) immunocytochemistry were perfused with 150 ml of 0.85% saline followed by 500 ml of 2% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The brains and spinal cords were removed, and the pons and lumbar enlargement were excised and transferred to fresh fixative. The tissue was postfixed for 3 hours at 4°C, transferred to 0.1 M phosphate buffer, pH 7.4, and stored at 4°C until immunocytochemical processing.

To produce noradrenergic sprouting in the MoV, newborn rat pups were given subcutaneous injections on postnatal days 1 and 2 two of 6-OHDA (100 mg/kg body weight) dissolved in saline containing 100 mg/ml ascorbic acid. These pups were returned to the mother until weaning and were maintained in the animal quarters until used as adults (four females and six males ranging in age from 2 to 15 months, with a mean age of 9.15 months). Untreated rats (two females and four males ranging in age from 4 to 11 months, with a mean age of 5.17 months) served as controls. For the experiments in which a reduction of spinal cord NE was required, six rats (two females and four males ranging in age from 3 to 4 months, with a mean age of 3.7 months) were each given a single intraperitoneal injection of 50 mg/kg of DSP-4 24 hours (n = 2) or 48 hours (n = 4) before surgery for the *Ricin* lectin injection. Eight untreated rats (one female and seven males ranging in age from 4 to 8 months with, a mean age of 5 months) were used as controls and received only the *Ricin* lectin injection. The animals in each experimental group were maintained under the same environmentally controlled conditions and were handled in the same manner.

Immunocytochemistry

Forty micrometer Vibratome sections were prepared from the pons and lumbar spinal cord segments L3 and L4. Representative sections were stained with cresyl violet to confirm motor neuron degeneration. The other sections were processed for immunocytochemistry. Free-floating sections were incubated with monoclonal antibodies for GFAP (Sigma at 1:1,000 or Serotech at 1:800) overnight at 4°C. For DBH immunocytochemistry, sections were incubated for 1.5 hours at 37°C with a monoclonal antibody for DBH (Chemicon, at 1:750). Both antibodies were diluted in 0.01 M phosphate-buffered saline (PBS) containing 0.4% Triton X-100 and 1% normal horse serum. This was followed by incubation for 45 minutes at room temperature with a horse anti-mouse immunoglobin (IgG; H and L chain) biotinylated secondary antibody, which was preadsorbed against normal rat serum (1:250; Vector Laboratories). Sections were then incubated for 45 minutes at room temperature with avidin-horseradish peroxidase (HRP) complex (Vectastain Elite, Standard, Vector Laboratories). The chromogenic substrate was 0.05% 3'3'-diaminobenzidine added to 0.005% hydrogen peroxide in 0.05 M Tris buffer, pH 7.0. The reaction was stopped after 1.5 minutes by dilution with 0.01 M PBS. The sections were mounted on gelatin-coated slides, air dried overnight, and coverslipped with DPX mountant (BDH Laboratory Supplies). Several sections taken from each animal were processed in the same manner, but without exposure to the primary antibody, and exhibited no cellular labeling.

Data analysis

GFAP optical density (O.D.) was measured with the aid of a BioQuant IV (R & M Biometrics) image analyzer employing background linearity correction for the light source and optics. The pontine MoV and the spinal cord ventral horn contralateral to the side of *Ricin* lectin injection served as the control in each tissue section measured. The average pixel O.D. transform in a uniform measurement window on the ipsilateral, *Ricin* lectin-treated side is expressed as the percent change from the control side. [(control − experimental)/control] × 100. Distribution error due to the nonlinear relationship between transmittance and absorbance (Piller, 1977) is a potential problem when comparing measurements from areas of greatly differing density. Because GFAP-labeled normal astrocytes are surrounded by more unlabeled tissue than the hypertrophied reactive astrocytes, O.D. measurements are prone to distributional error. To minimize this type of error, transmittance was transformed to O.D. pixel by pixel before calculating the average O.D., and a uniform measurement field and interval between *Ricin* injection and death was used for all animals.

Fig. 2. **A:** Cresyl violet-stained section from the pons of an adult animal treated with 6-OHDA on postnatal day (P)1 and P2 to produce a permanent noradrenergic sprouting in the MoV. The right MoV on the control side is indicated by a single arrow. The left MoV (paired arrows) shows motor neuron degeneration and an increase in glial nuclei resulting from the injection of *Ricin communis* (*Ricin* lectin) into the masseter nerve 1 week earlier. **B:** Higher magnification of control MoV showing normal motor neurons and glial nuclei. **C:** Higher magnification of the MoV on the *Ricin* lectin-treated side showing the absence of motor neurons and an increase in glial cell nuclei. The asterisk in A indicates the cut marking the control side. Scale bar = 1 mm in A, 50 μm in B.

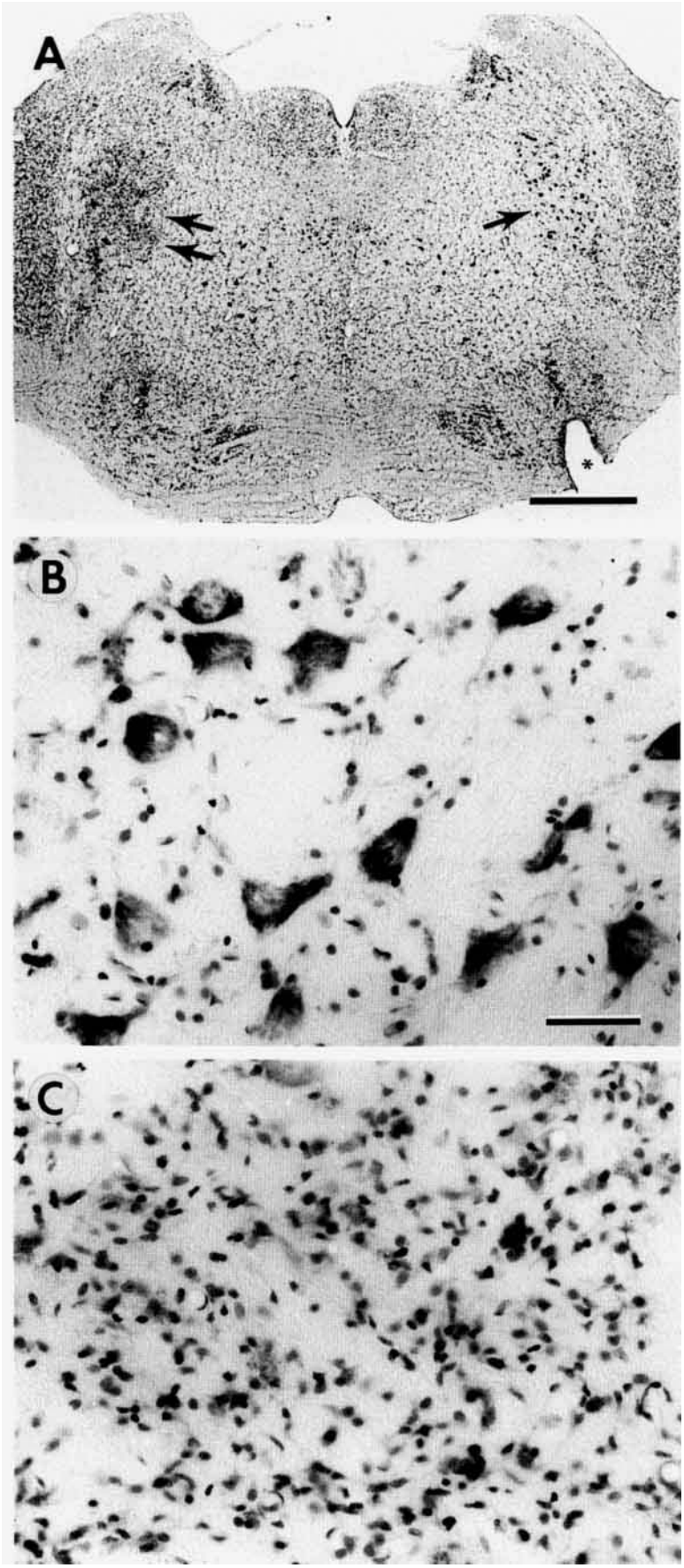

Figure 2

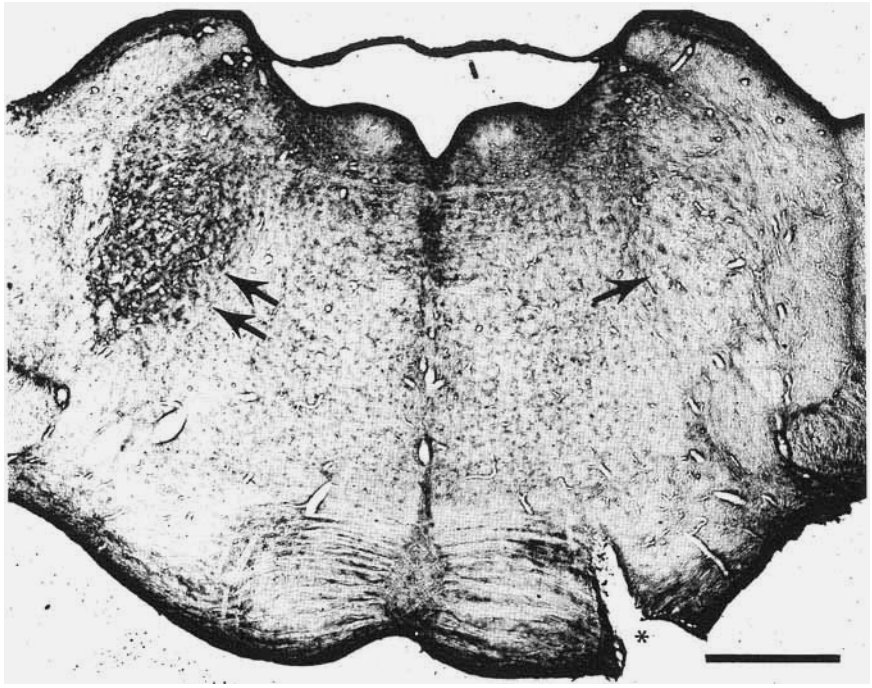

Fig. 3. Low-power view of a glial fibrillary acidic protein (GFAP)-immunolabeled section through the pons of a neonatal 6-OHDA-treated animal showing the normal MoV (single arrow) and reactive astrocytes in the contralateral, *Ricin* lectin-treated side, MoV (paired arrows). The asterisk indicates the cut marking the control side. Scale bar = 1 mm.

Statistical evaluations of the measurements were carried out by using a two-tailed Student's t test and analysis of variance (ANOVA).

RESULTS

Ricin lectin injected into the masseter nerve causes motor neurons in the masseter subnucleus of MoV to degenerate (Sasamoto, 1979; Fig. 2). The masseter subnucleus occupies the central part of MoV, but the glial reaction is distributed throughout a larger area of the nucleus (Shao and Sutin, 1991; Fig. 3), probably because dendritic processes of the degenerating neurons extend outside the subnucleus. Only a few GFAP-labeled astrocytes are present in the normal MoV (Fig. 4A), but, 7 days after neuronal injury, a dense network of reactive astrocytes is present everywhere except the region occupied by myelinated axons of the motor root (Fig. 4B). Activated microglia are also present (Fig. 5) in the nucleus after injury, but no infiltration of leukocytes or monocytes is seen in sections stained with cresyl violet. O.D. measurements were made in the MoV to quantitate changes in GFAP-labeled, reactive astrocytes. GFAP has been reported to increase in aged (24 months old) Long-Evans rats (O'Callaghan and Miller, 1991). To control for age and for any variations in immunohistochemical labeling in the Sprague-Dawley rats used, the MoV contralateral to the side injected with the cytotoxin served as an internal control in each section. GFAP density on the side with reactive astrocytes is expressed as the percent increase from the control side. Reactive astrocyte formation in the *Ricin* lectin-treated MoV of animals with normal NE innervation results in an average increase in GFAP density of 18% (Figs. 4, 7). Comparisons of the distributions of GFAP O.D. measurements from the control side MoV in normal and NE-hyperinnervated animals show no significant difference in a two-sample, two-tailed t test analysis (null hypothesis difference = 0; alpha = 0.05; $P = 0.14$).

Examples of the increase in DBH-labeled varicosities in the MoV of normal and neonatal 6-OHDA-treated animals are shown in Figure 8. The uninjured, NE-hyperinnervated MoV in 6-OHDA-treated animals has the same GFAP density as normally innervated animals. Following *Ricin* lectin treatment, GFAP density increases 55% ($P < 0.001$) over the normal NE-innervated nucleus response to *Ricin* lectin (Figs 6, 7). To decide whether the GFAP increase is due to astrocyte hypertrophy rather than proliferation, the mitosis inhibitor AraC was administered during the 7-day survival period. AraC treatment had no statistically significant effect on the increase in GFAP density (Fig. 7) in the hyperinnervated MoV. The increase in OX-42-labeled microglia in the injured MoV (Fig. 5B) probably accounts for most of the increase in glial nuclei seen in cresyl violet-stained sections (Fig. 2C).

The enhanced astrocyte reactivity in the NE-hyperinnervated region suggests a modulatory role for NE. To further test this possibility, we examined reactive astrocyte formation in a region partially depleted of NE. The methods available to produce chronic noradrenergic dennervation in the CNS depend on the uptake into varicosities of neurotoxic catecholamine analogs that cause axonal degeneration. 6-OHDA and DSP-4, the two drugs most commonly used for this purpose, both destroy only terminals originating from the A-6 (locus coeruleus) group of adrenergic

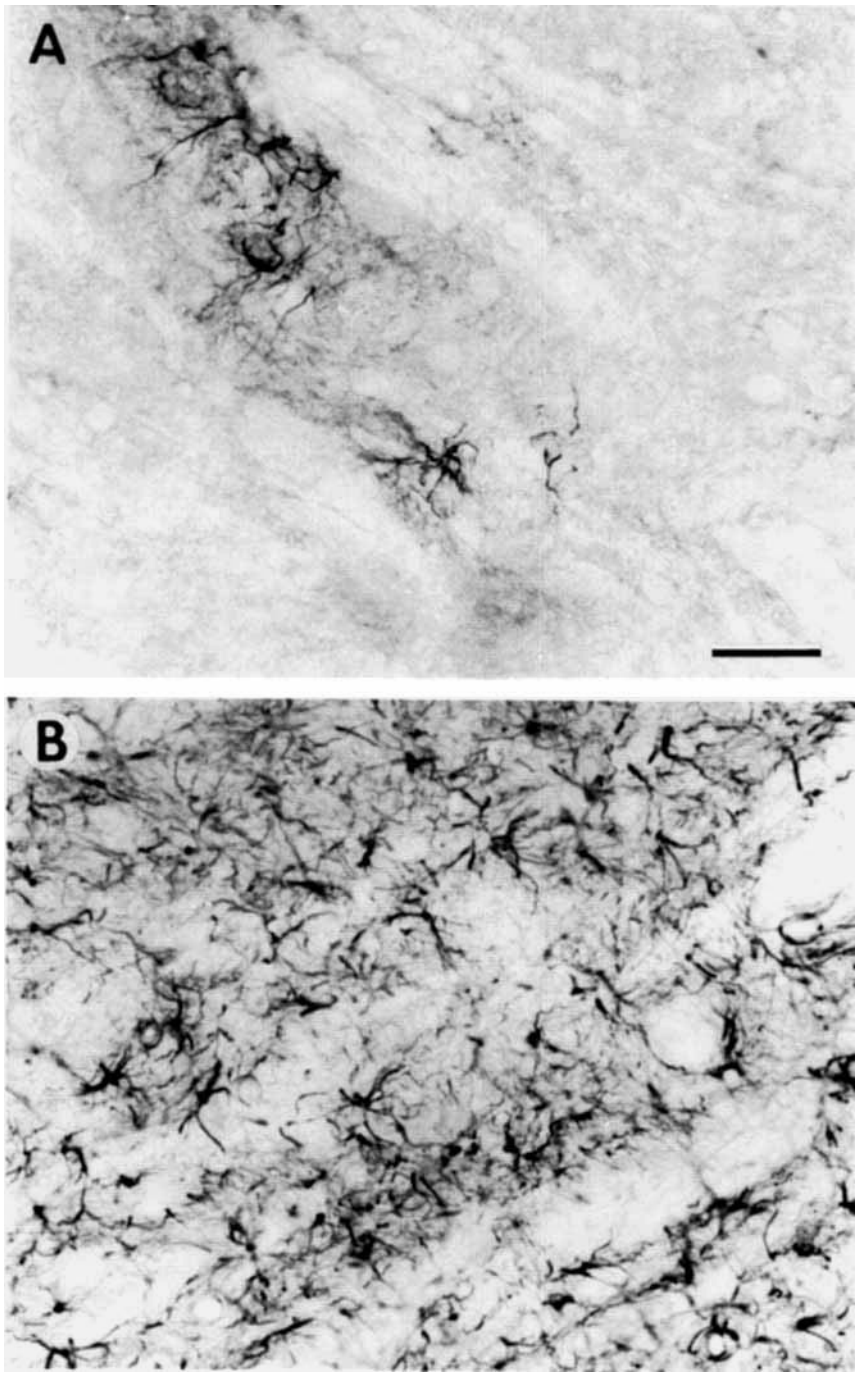

Fig. 4. GFAP immunolabeling of the control (**A**) and injured (**B**) MoV with normal NE innervation. The control nucleus has few GFAP-positive resting astrocytes, whereas the contralateral MoV, in which motor neurons were destroyed by masseter nerve injection of *Ricin* lectin, shows labeling of hypertrophied reactive astrocytes. The diagonal areas in MoV with fewer labeled processes contain the myelinated fibers of the trigeminal nerve motor root. Scale bar = 50 μm.

neurons. Only DSP-4 passes the blood-brain barrier and is effective when administered to adult animals. Because DSP-4 reduces DBH immunolabeling in the spinal cord dorsal horn within 24 hours (Fig. 9), we gave the drug 24 or 48 hours before the sciatic nerve injection of *Ricin* lectin and measured changes in reactive astrocyte formation. In the DSP-4-treated animals, the dorsolateral fasciculus and laminae 1 and 2 of the dorsal horn contralateral to the *Ricin* lectin-treated side show some astrocyte hypertrophy that is probably triggered by the degenerating NE axons (Fig. 10). Because it cannot be assumed, a priori, that the *Ricin* lectin- and DSP-4-induced astrocyte reactions were additive, the contralateral dorsal horn was not used for control measurements. Instead, GFAP O.D. was measured in both

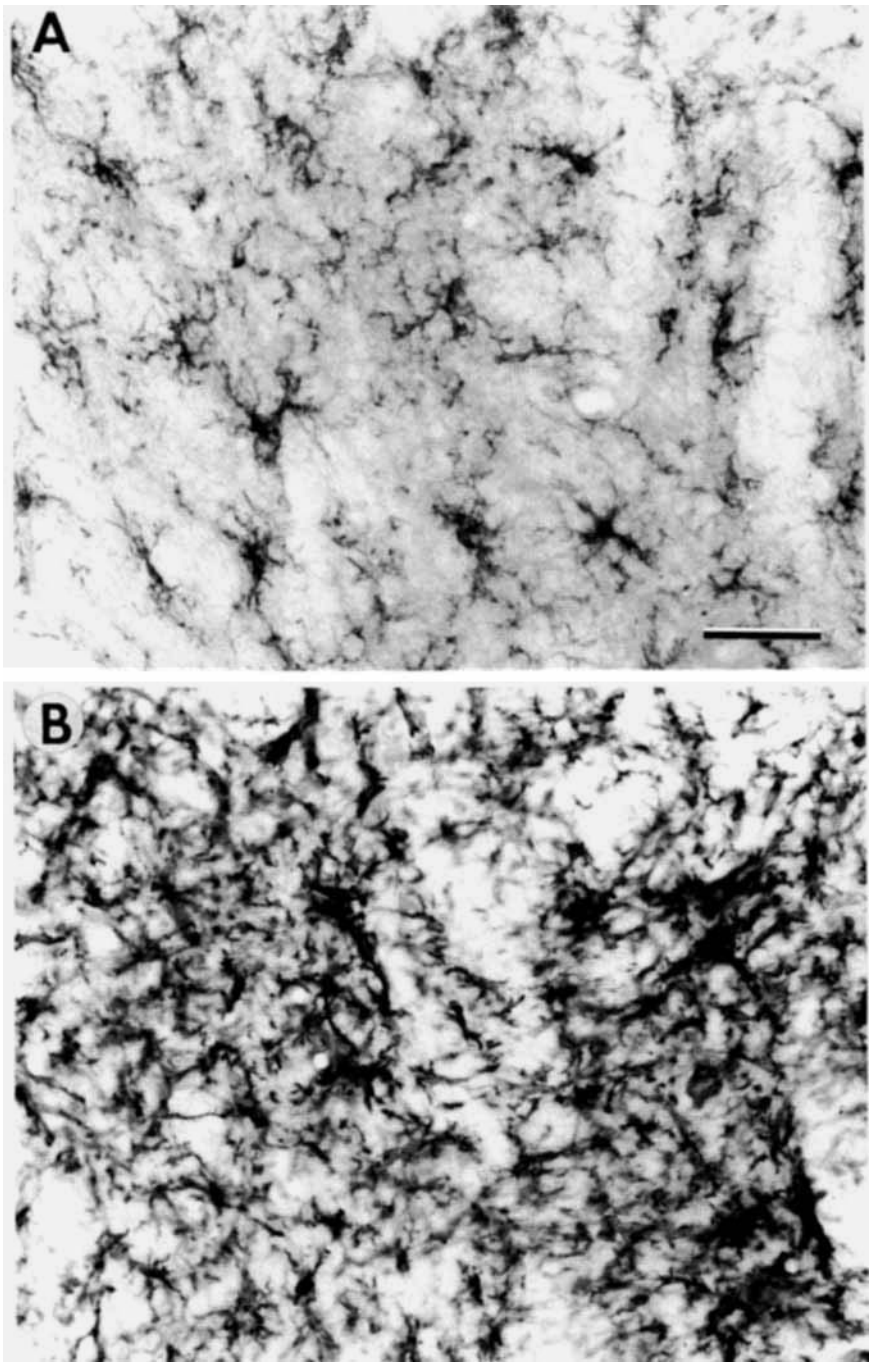

Fig. 5. OX-42 immunolabeling of the normal control side (**A**) and the *Ricin* lectin-treated injured side (**B**) of the MoV in an NE-hyperinnervated animal illustrating the increase in activated microglia. Scale bar = 50 μm.

the ipsilateral and contralateral dorsal horns (for examples, see Figs. 10, 11) and was compared with the GFAP density in a control area in the ventral white matter, a region where the NE axons are not affected by DSP-4 (Fig. 12). This method reveals the astrocyte reaction due to NE axon degeneration in the contralateral dorsal horn and subtracts it from that in the ipsilateral (*Ricin*) side. The mean increase in dorsal horn GFAP due to *Ricin* treatment in animals with depleted NE innervation is only 35% ($P < 0.01$) of that measured in animals with normal NE innervation. In the ventral horn, where NE axons are less affected by DSP-4 (Fig. 11), no change in reactive astrocyte formation is seen. A Scheffé confidence limits analysis of the data (Table 1) confirms the group differences seen with the t tests. These data from the NE-depleted spinal cord dorsal horn, the normally innervated ventral horn, and the NE

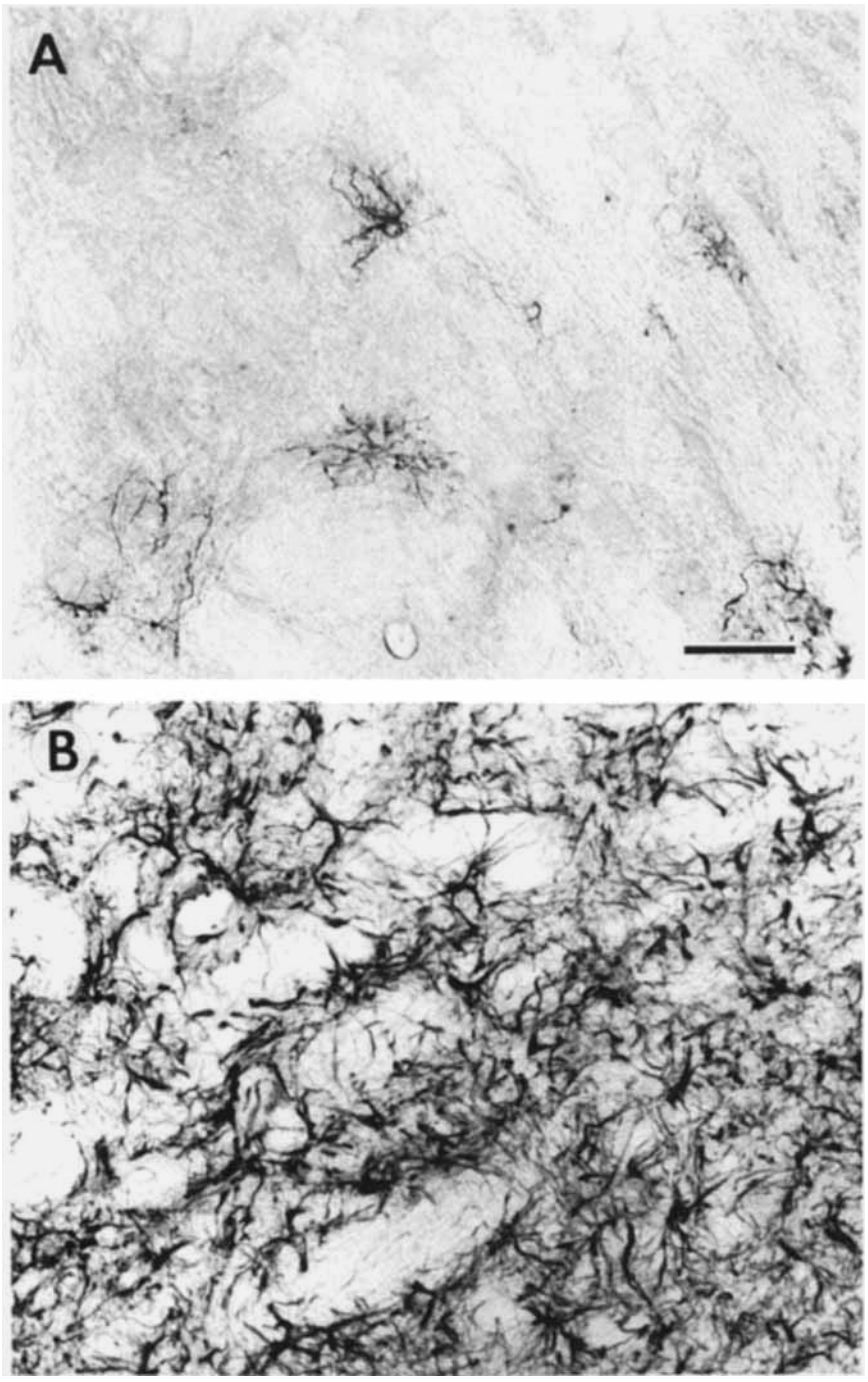

Fig. 6. GFAP immunolabeling of the control (**A**) and injured (**B**) NE-hyperinnervated MoV. Astrocyte density in the control nucleus is comparable to that in a normally innervated MoV, whereas, on the *Ricin* lectin-treated side, GFAP in reactive astrocytes is greater than that in the injured MoV of animals with a normal NE innervation. Scale bar = 50 μm.

hyperinnervated MoV support the hypothesis that adrenergic innervation modulates the response of astrocytes to neuronal injury.

DISCUSSION

Despite many advantages, in vitro models are not suitable for testing the hypothesis that NE modulates reactive gliosis. Adrenergic receptor agonists, which increase cAMP, cause stellation and GFAP synthesis in primary astrocyte cultures, giving them the morphological, but not all of the immunochemical, features of reactive astrocytes in vivo (Wandosell et al., 1993). Both cAMP-dependent kinase and protein kinase C can influence GFAP mRNA levels (Shafit-Zagardo et al., 1988), suggesting a possible mechanism for the effect of NE and glutamate on intermediate filament

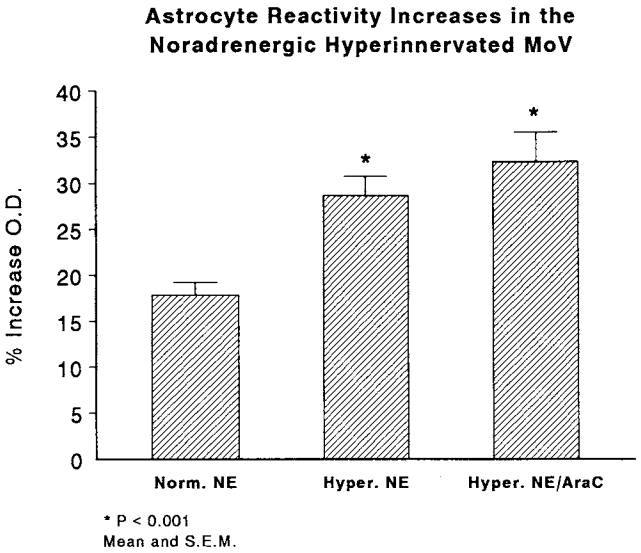

Fig. 7. Motor neuron degeneration-induced GFAP optical density changes in the normal and NE-hyperinnervated MoV. Density in the *Ricin* lectin-treated MoV is plotted as the percent increase over the contralateral control MoV in the same section. To test for the contribution of glial proliferation (in contrast to hypertrophy) to the GFAP density change, the mitosis inhibitor arabinofuranoside hydrochloride (AraC) was given. The *P* values (asterisks) are for the hyperinnervated NE group and the hyperinnervated plus AraC group compared with the normally innervated group. The hyper-NE and hyper-NE/AraC groups are not different from each other ($P > 0.05$). For the normal group, n = 6 animals; for the 6-OHDA group, n = 7; for the 6-OHDA/AraC group, n = 3.

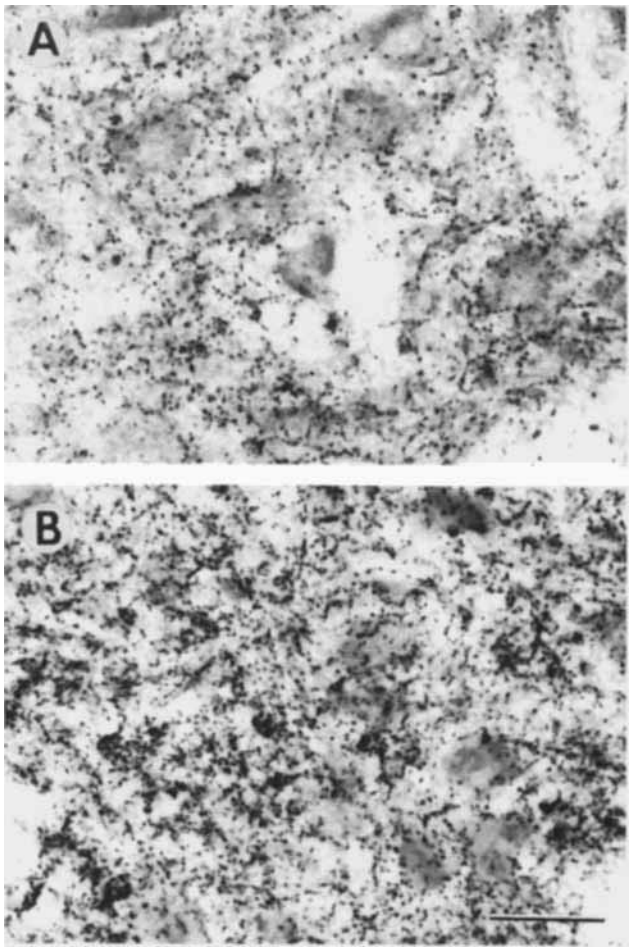

Fig. 8. Dopamine-β-hydroxylase (DBH) immunolabeling in the adult MoV in an animal with a normal NE innervation (**A**) and in a neonatal 6-OHDA-treated animal with an NE-hyperinnervated MoV (**B**). The increase in labeled varicosities in the 6-OHDA-treated animals is associated with a twofold increase in NE, which was measured by using high-performance liquid chromatography (HPLC). Scale bar = 50 μm.

synthesis in astrocytes. GFAP synthesis is increased several fold in primary astrocyte cultures grown in a defined medium containing hydrocortisone, putrescine, prostaglandin F-2α, and pituitary fibroblast growth factor (Morrison et al., 1985). The animals in each experimental series in this study were housed and handled in the same manner, and there should have been no environmental stress factors producing different adrenal steroid levels within or between groups.

Microglia express several antigens that are also found in monocytes/macrophages (Perry and Gordon, 1988), but not the nonspecific esterase (Hayes et al. 1988). Inflammation in the CNS is generally considered to require activated T-cells in addition to activated microglia, and a small number of these cells appear to be able to cross an intact blood-brain barrier (Hickey, 1991). We did not observe perivascular cuffing or dispersed lymphocytes or monocytes in the region of *Ricin* lectin-induced motor neuron degeneration. The microglial reaction that accompanies injury to the adult CNS may have a role in initiating or supporting astrocyte reactivity through the release of cytokines. Activated (amoeboid) microglia release IL-1 (Giulian et al., 1986). Human IL-1β administered to rats produces an increase in the accumulation of the monoamine oxidase-catalyzed NE-degradation product 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) in several brainstem and spinal cord regions in rats (Kabiersch et al. 1988). The MHPG/NE ratio increase in all regions examined indicates a generalized action of IL-1 in stimulating NE release. Microperfusion studies of the hypothalamic paraventricular nucleus also show an IL-1β-mediated NE release (Mohan-Kumar and Quadri, 1993). An essential role for amoeboid microglia in astrocyte hypertrophy and GFAP synthesis has not been demonstrated, but the action of IL-1 on NE release suggests the possibility of both β-adrenergic and IL-1 receptor-mediated effects in astrocytes.

If NE interacts with other signals to initiate astrocyte hypertropy, then changes in GFAP density should vary with CNS regional differences in adrenergic axon innervation. To test this relationship in the brain and spinal cord, GFAP immunolabeling must reflect the tissue concentration of the intermediate filament protein. Although this is generally the case, some exceptions exist (Goldmuntz et al., 1986; Le Prince et al., 1991; O'Callaghan, 1993). Short-term (48-hour) exposure of primary astrocytes to cAMP derivatives results in increased GFAP immunolabeling, but not in increased GFAP protein or mRNA. After exposure to the nucleotide for 7 days or more, increased antibody labeling is associated with increased protein and message (Le Prince et al., 1990). The lack of correlation between GFAP immunolabeling and protein level after short-term stimulation is thought to reflect posttranslational changes,

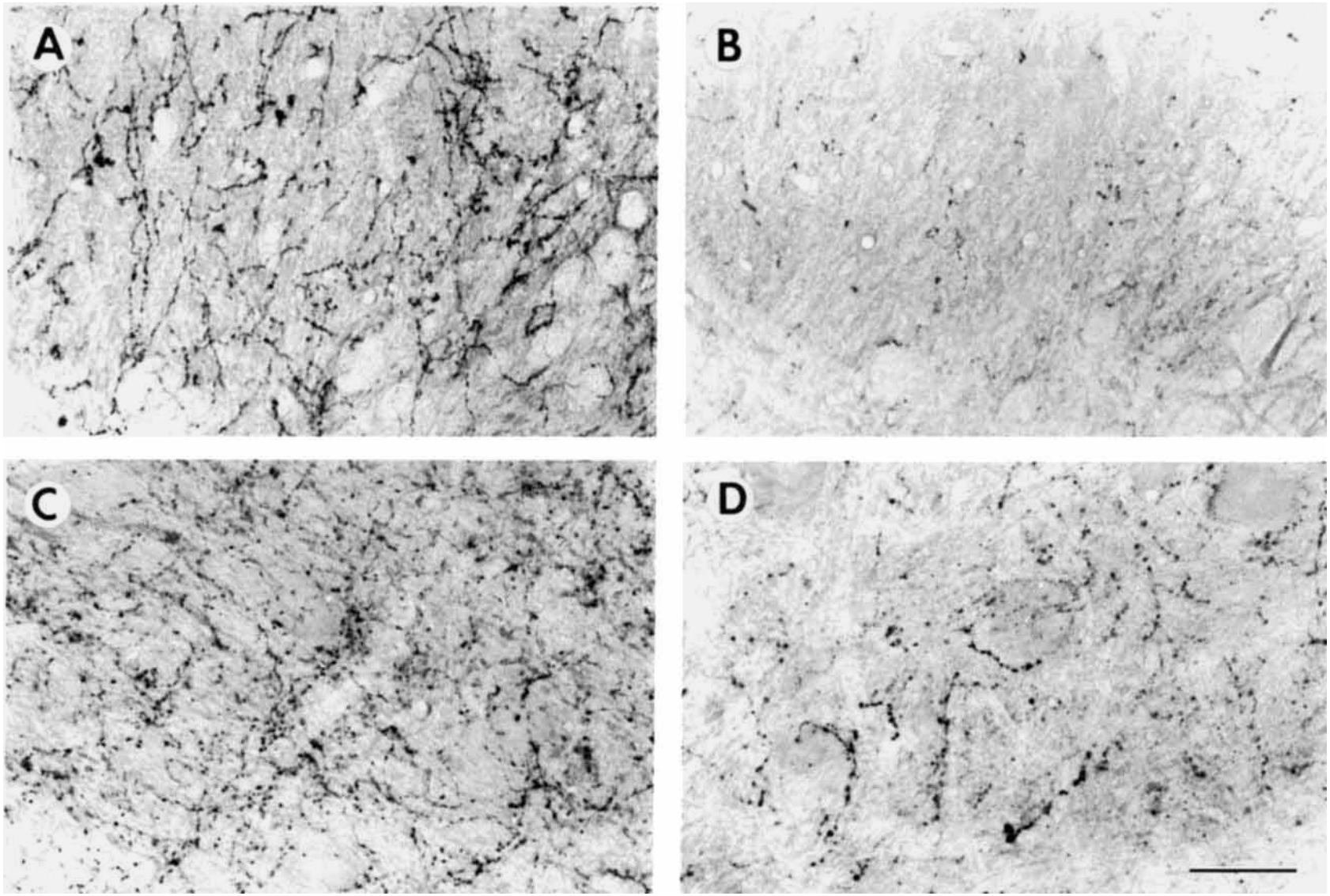

Fig. 9. DBH immunolabeling in the lumbar spinal cord dorsal (**A,B**) and ventral (**C,D**) horns of normal rats (A,C) and in rats treated with DSP-4 24 hours before euthanasia (B,D). DBH-positive fibers are largely eliminated in the dorsal horn and are reduced in the ventral horn of DSP-4-treated rats. Scale bar = 50 μm.

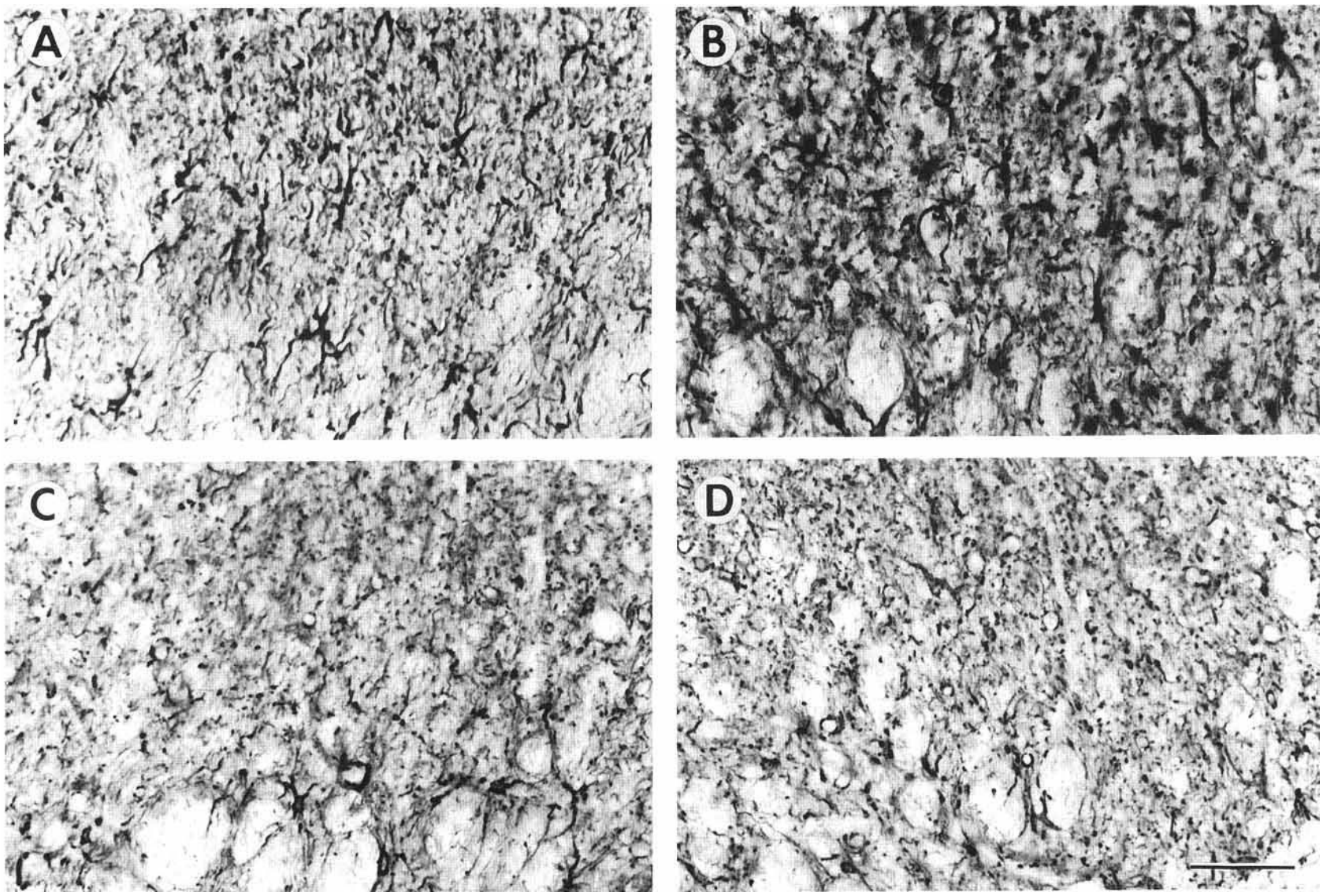

Fig. 10. GFAP immunolabeling in the dorsal horn of animals receiving a unilateral *Ricin* lectin injection in the sciatic nerve 7 days before euthanasia. **A,B:** Intact (control) side (A) and injured dorsal horns (B) of a normal (non-DSP-4-treated) rat. **C,D:** Intact (C) and injured (D) dorsal horns of a DSP-4-treated rat. The increased GFAP labeling usually seen in the injured dorsal horn of normal rats is attenuated in injured dorsal horn of NE-depleted, DSP-4-treated rats. Scale bar = 50 μm.

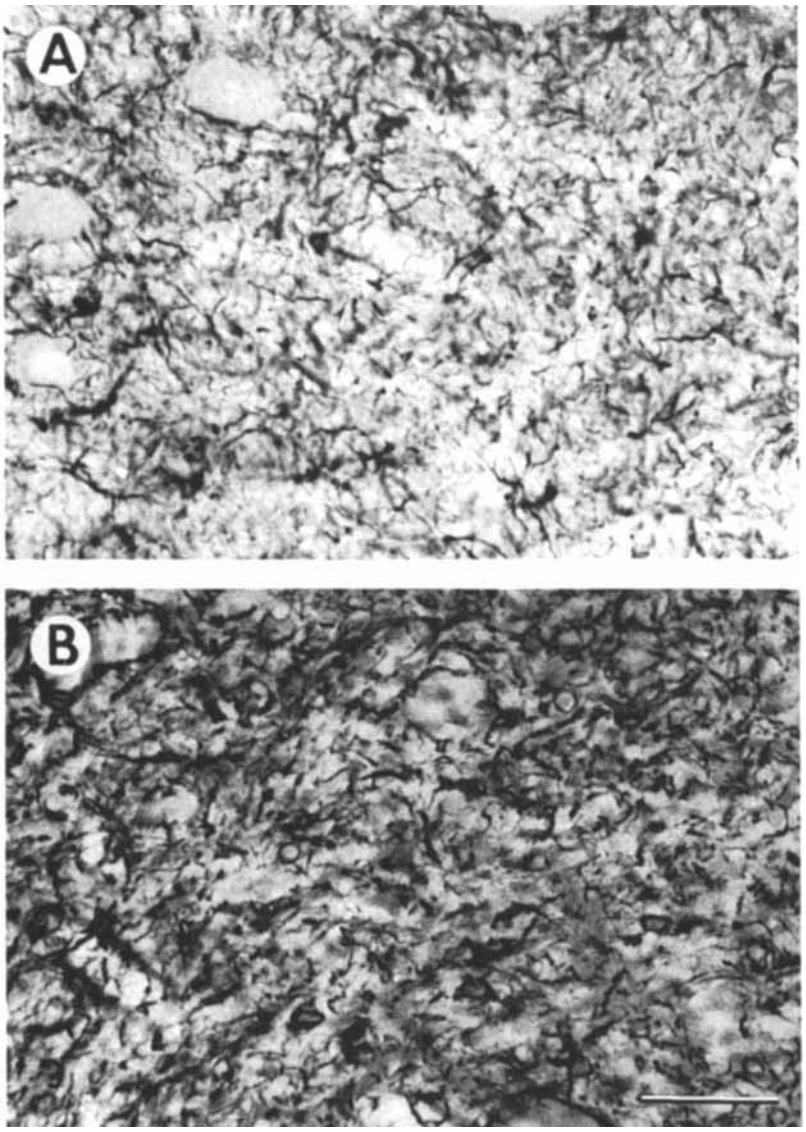

Fig. 11. Increase in GFAP immunolabeling in the lumbar spinal cord ventral horn of an animal treated with DSP-4. The DSP-4 was administered 24 hours before a unilateral injection of *Ricin* lectin into the sciatic nerve on the control side (**A**) and on the side receiving the *Ricin* lectin injection after 7 days' survival (**B**). Scale bar = 50 μm.

such as GFAP phosphorylation (Browning and Ruina, 1984), whereas the longer term positive correlation of GFAP immunolabeling, increased protein, and increased message is probably due to posttranscriptional regulation.

Regional differences in astrocyte GFAP expression and reactivity have been recognized (Hajos and Kalman, 1989; Malhotra et al., 1990). For example, stab wounds in the cerebral cortex produce a less extensive astrocyte reaction than similar wounds in subcortical structures (Mathewson and Berry, 1985). In the spinal cord, neuronal degeneration produces more severe reactions in the gray matter than in the white matter. A prominent response to axotomy- or toxin-induced neuropathy is the retraction of a substantial number of synapses from the soma (Sterman and Sposito, 1984). This synaptic disjunction is accompanied by the extension of astrocyte processes to surround the separated terminal and to occupy the exposed membrane of neuronal somata. The close association between retraction of presynaptic terminals and astrocyte reactivity may partially account for the more intense GFAP response in gray matter.

In animals with NE dennervation, the normal *Ricin* lectin-induced increase in dorsal horn GFAP is only about one-third of that found in animals with normal NE innervation. In the ventral horn, where NE axons are less affected by DSP-4, the astrocyte reaction did not differ from that in animals that did not receive DSP-4. These data demonstrate enhanced astrocyte reactivity in a region of increased NE innervation and attenuated reactivity in a region of reduced NE innervation.

The only previously known functional consequences of chronic NE hyperinnervation in the CNS are the facilitation of muscle afferent-evoked postsynaptic potentials in

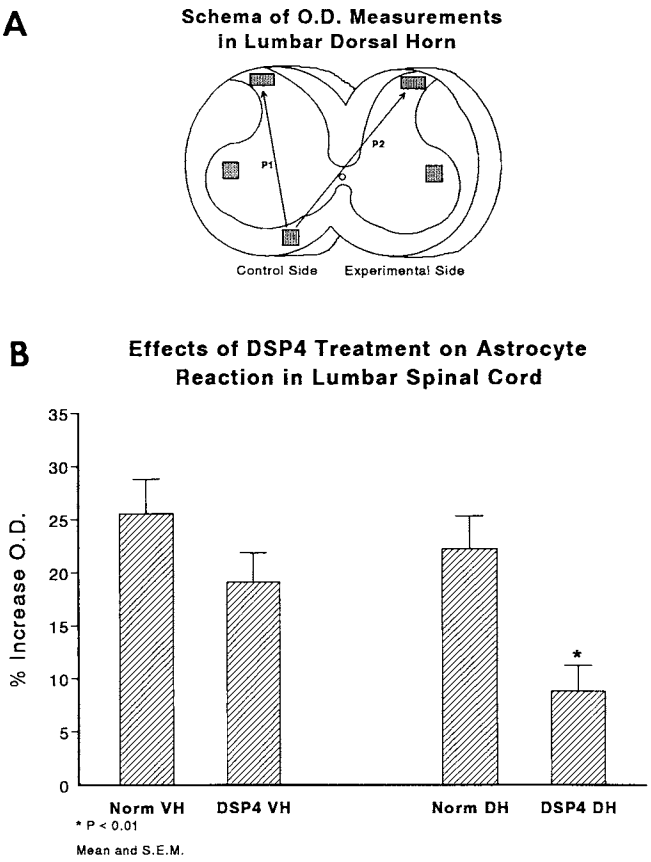

Fig. 12. **A:** Measurement scheme to determine the increase in GFAP in lumbar spinal cord dorsal horn due to primary afferent axon degeneration in animals treated with DSP-4 to deplete NE. The ventral white matter was used as the reference region for measurement of ipsilateral and contralateral dorsal horn optical density (see text). **B:** The effect of DSP-4 treatment on the increase in GFAP density in the ventral horn (VH) and dorsal horn (DH) on the *Ricin* lectin-treated side compared with the contralateral control side. In contrast to the dorsal horn, ventral horn NE fibers lack the NE transporter and are not destroyed by DSP-4. For the DSP-4 group, n = 6; for the control group, n = 8.

TABLE 1. A Statistical Analysis of the Change in GFAP O.D. in MoV and Lumbar Spinal Cord Using an Analysis of Variance[1]

Source	SS[2]	d.f.[3]	MS[4]	F-ratio	P
Analysis of Variance: % Change in GFAP O.D. in MoV					
Main Effects	1797.5	2	898.75	13.05	<0.001
Treatment	1797.5	2	898.75	13.05	<0.001
Multifactor Analysis of Variance: % Change in GFAP O.D. in Spinal Cord					
Main Effects	1941.19	2	970.59	7.58	0.0013
Location	533.64	1	533.64	4.169	0.0463
Treatment	1407.55	1	1407.55	10.99	0.0017
Location × Treatment	151.11	1	151.11	1.18	0.2823

[1]The variable Treatment includes untreated, 6-OHDA, and 6-OHDA + Arac treatments in the MoV analysis. In the spinal cord analysis, Location refers to the area of from which the measurement was taken (dorsal horn or ventral horn) and Treatment refers to no drug treatment or DSP4 treatment. Differences between treatment groups were identified with Sheffe's multiple comparisons.
[2]SS = sum of squares.
[3]d.f. = degrees of freedom.
[4]MS = mean squares.

trigeminal motor neurons and increased masseter muscle contraction (Vornov and Sutin, 1986; Stafford and Jacobs, 1990). Chronic CNS NE and dopamine depletion after 6-OHDA leads to increased sensitivity to intraventricular administration of NE and alterations in operant conditioned behavior, but, for the most part, animals compensate for the loss of transmitter (Breese and Cooper, 1977).

The biological role of reactive astrocytes is poorly understood. Although GFAP is a convenient marker for normal and reactive astrocytes, it is not always related to states of astrocyte morphology or function. Transgenic mice unable to produce GFAP still show reactive gliosis in response to intracerebral injections of Scrapie prions (Gomi et al., 1995) or stab wounds (Pekny et al., 1995). Under some circumstances, the glial scar inhibits regenerative axon growth (Stensaas et al., 1987) or neurite extension in vitro (McKeon et al., 1995), but, in the presence of certain trophic factors, astrocytes may support neurite extension (Kawaja and Gage, 1991). Judgments about the potential value of reducing astrocyte scar formation after injury must await clarification of the way reactive astrocytes effect axonal regeneration in vivo.

LITERATURE CITED

Breese, G.R., and R. Cooper (1977) Chemical lesioning: Catecholamine pathways. In R.D. Myers (ed): Methods in Psychobiology. Vol. 3. New York, Academic Press, Inc. pp. 28–40.

Browning, E.T., and M. Ruina (1984) Glial fibrillary acidic protein: Norepinephrine stimulated phosphorylation in intact C-6 glioma cells. J. Neurochem. *42:*718–726.

Chandler, S.H. (1985) Evidence for excitatory amino acid transmission between mesencephalic nucleus of V afferents and jaw-closer motorneurons in the guinea pig. Brain Res. *477:*252–264.

Clark, F.M., D.C. Yeomans, and H.K. Proudfit (1991) The noradrenergic innervation of the spinal cord: Differences between two substrains of Sprague-Dawley rats determined using retrograde tracers combined with immunocytochemistry. Neurosci. Lett. *125:*155–158.

Fritschy, J.-M., and R. Grzanna (1989) Immunohistochemical analysis of the neurotoxic effects of DSP-4 identifies two populations of noradrenergic axon terminals. Neuroscience *30:*181–197.

Garrison, C.J., P.M. Dougherty, and S.M. Carlton (1994) GFAP expression in lumbar spinal cord of naive and neuropathic rats treated with MK-801. Exp. Neurol. *129:*237–243.

Giulian, D., T.J. Baker, L.C. Shih, and L.B. Lachman (1986) Interleukin-1 of the central nervous system is produced by amoeboid microglia. J. Exp. Med. *164:*594–601.

Goldmuntz, E.A., C.F. Brosnan, F.-C. Chiu, and W.T. Norton (1986) Astrocytic reactivity and intermediate filament metabolism in experimental autoimmune encephalitis: The effect of suppression with prazosin. Brain Res. *387:*16–26.

Gomi, H., T. Yokoyama, K. Fujimoto, T. Ikeda, A. Katoh, T. Ito, and S. Itohara (1995) Mice devoid of the glial fibrillary acidic protein develop normally and are susceptible to Scrapie prions. Neuron *14:*29–41.

Grzanna, R., and J.M. Fritschy (1991) Efferent projections of different subpopulations of central noradrenaline neurons. Prog. Brain Res. *88:*89–101.

Hajos, F., and M. Kalman (1989) Distribution of glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes in the rat brain II. Mesencephalon, rhombencephalon and spinal cord. Exp. Brain Res. *78:*164–173.

Hansson, E. (1989) Co-existence between receptors, carriers, and second messengers on astrocytes grown in primary cultures. Neurochem. Res. *14:*811–819.

Hayes, G.M., M.N. Woodroofe, and M.L. Cuzner (1988) Characterization of microglia isolated from the adult human and rat brain. J. Neurol. Immunol. *19:*177–189.

Hickey, W.F. (1991) Migration of hematogenous cells through the blood-brain barrier and the initiation of CNS inflammation. Brain Pathol. *1:*97–106.

Kabiersch, A., A. del Rey, C.G. Honegger, and H.O. Besedovsky (1988) Interleukin-1 induces changes in norepinephrine metabolism in the rat brain. Brain Behav. Immunol. *2:*267–274.

Kawaja, M.D., and F.H. Gage (1991) Reactive astrocytes are substrates for the growth of adult CNS axons in the presence of elevated levels of nerve growth factor. Neuron *7:*1019–1030.

Le Prince, G., M.C. Copin, H. Hardin, M.F. Belin, J.P. Bouilloux, and M. Tardy (1990) Neuron-glia interactions: Effects of serotonin on the astroglial expression of GFAP and of its encoding message. Dev. Brain Res. *51:*295–298.

Le Prince, G., C. Fages, B. Rolland, J. Nunez, and M. Tardy (1991) DBcAMP effect on the expression of GFAP and of its encoding mRNA in astroglial primary cultures. Glia *4:*322–326.

Levitt, P., and R.Y. Moore (1980) Organization of brainstem noradrenaline hyperinnervation following neonatal 6-OHDA treatment in rat. Anat. Embryol. *158:*133–150.

Lyons, W.E., J.M. Fritschy, and R. Grzanna (1989) The noradrenergic neurotoxin DSP-4 eliminates the coeruleospinal projection but spares projections of the A5 and A7 groups to the ventral horn of the rat spinal cord. J. Neurosci. *9:*1481–1489.

Malhotra, S.K., T.K. Shnitka, and J. Elbrink (1990) Reactive astrocytes—A review. Cytobios *61:*133–160.

Mathewson, A.J., and M. Berry (1985) Observations on the astrocyte response to a cerebral stab wound in adult rats. Brain Res. *327:*61–69.

McBride, R.L., R.V. Ozment, and J. Sutin (1985) Neonatal 6-hydroxydopamine destroys spinal cord noradrenergic axons from the locus coeruleus, but not those from lateral tegmental cell groups. J. Comp. Neurol. *235:*375–383.

McKeon, R.J., A. Höke, and J. Silver (1995) Injury-induced proteoglycans inhibit the potential for laminin-mediated axon growth on astrocytes scars. Exp. Neurol. *136:*32–43.

MohanKumar, P.S., and J. Silver (1995) Systemic administration of interleukin-1 stimulates norepinephrine release in the paraventricular nucleus. Life Sci. *52:*1961–1967.

Morrison, R.S., J. De Vellis, Y.L. Lee, R.A. Bradshaw, and L.F. Eng (1985) Hormones and growth factors induce the synthesis of glial fibrillary acidic protein in rat brain astrocytes. J. Neurosci. Res. *14:*167–176.

O'Callaghan, J.P. (1993) Quantitative features of reactive gliosis following toxicant-induced damage of the CNS. Ann. NY Acad. Sci. *679:*195–210.

O'Callaghan, J.P., and D.B. Miller (1991) The concentration of glial fibrillary acidic protein increases with age in the mouse and rat brain. Neurobiol. Aging *12:*171–174.

Pekny, M., P. Leveen, M. Penka, C. Eliasson, C.-H. Berthold, B. Westermark, and C. Betsholtz (1995) Mice lacking glial fibrillary acidic protein display astrocytes devoid of intermediate filaments but develop and reproduce normally. EMBO J. *14:*1590–1598.

Perry, V.H., and S. Gordon (1988) Macrophages and microglia in the nervous system. Trends Neurosci. *11:*273–277.

Piller, H. (1977) Microscope Photometry. Berlin: Springer-Verlag.

Salm, A.K., and K. McCarthy (1989) Expression of beta-adrenergic receptors by astrocytes isolated from adult rat cortex. Glia *2:*346–352.

Sasamoto, K. (1979) Motor nuclear representation of masticatory muscles in the rat. Jpn. J. Physiol. *29:*739–747.

Schousboe, A., O.M. Larsson, and P. Krogsgaard-Larsen (1988) Uptake and release processes for neurotransmitter amino acids in astrocytes. In: The Biochemical Pathology of Astrocytes. New York: Alan R. Liss, Inc. pp. 381–394.

Shafit-Zagardo, B., A. Kume-Iwake, and J.E. Goldman (1988) Astrocytes regulate GFAP mRNA levels by cyclic AMP and protein kinase C-dependent mechanisms. Glia *1:*346–354.

Shao, Y.P., and K.D. McCarthy (1994) Plasticity of astrocytes. Glia *11:*147–155.

Shao, Y., and J. Sutin (1991) Noradrenergic facilitation of motor neurons: Localization of adrenergic receptors in neurons and non-neuronal cells in the trigeminal motor nucleus. Exp. Neurol. *114:*216–227.

Shao, Y., and J. Sutin (1992) Expression of adrenergic receptors in individual astrocytes and motor neurons isolated from the adult rat brain. Glia *6:*108–117.

Snow, D.M., V. Lemmon, D.A. Carrino, A.I. Caplan, and J. Silver (1990) Sulphated proteoglycans in astroglial barriers inhibit neurite outgrowth in vitro. Exp. Neurol. *109:*111–130.

Stafford, I.L., and B.L. Jacobs (1990) Noradrenergic modulation of the masseteric reflex in behaving cats. II. Physiological studies. J. Neurosci. *10:*99–107.

Stensaas, L.J., L.M. Partlow, P.R. Burgess, and K.W. Horch (1987) Inhibition of regeneration: The ultrastructure of reactive astrocytes and abortive axon terminals in the transition zone of the dorsal root. Prog. Brain Res. *71:*457–468.

Sterman, A.B., and N. Sposito (1984) Motorneuron axosomatic synapses are altered in axonopathy. J. Neuropathol. Exp. Neurol. *43:*201–209.

Stone, A., and M.A. Ariano (1989) Are glial cells targets of the central noradrenergic system? A review of the evidence. Brain Res. Rev. *14:*297–309.

Sutin, J., and R. Griffith (1993) β-Adrenergic receptor blockade suppresses glial scar formation. Exp. Neurol. *120:*214–222.

Sutin, J., and K.P. Minneman (1985) α_1- and β-Adrenergic receptors are co-regulated during both adrenergic dennervation and hyperinnervation. Neuroscience *14:*973–980.

Takamoto, T., Y. Hori, Y. Koga, and H. Toshima (1991) Norepinephrine inhibits human natural killer cell activity in vitro. Int. J. Neurosci. *58:*127–131.

Vornov, J.J., and J. Sutin (1986) Noradrenergic hyperinnervation of the motor trigeminal nucleus: Alterations in membrane properties and responses to synaptic input. J. Neurosci. *6:*30–37.

Wandosell, F., P. Bovolenta, and M. Nieto-Sampedro (1993) Differences between reactive astrocytes and cultured astrocytes treated with dibutyryl-cyclic AMP. J. Neuropathol. Exp. Neurol. *52:*205–215.