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The Effects of Decompression and Exogenous NGF on Compressed Cerebral Cortex

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ABSTRACT

Using a rat epidural bead implantation model, we found that compression alone could reduce the overall and individual layer thicknesses of cerebral cortex with no apparent cell death. The dendritic lengths and spine densities of layer II/III and V pyramidal neurons started to decrease within 3 days of compression. Decompression for 14 days resulted in near complete to partial recovery of the cortical thickness and of the dendritic lengths of layer II/III and V pyramidal neurons, depending on the duration of the preceding compression. The recoverability was better following short (3-day) than long (1- or 3-month) periods of compression. The loss of dendritic spines nevertheless persisted. An intraventricular infusion of NGF was performed after decompressing the lesions following 3 days of cortical compression, and this increased the recovery of the spines but not the dendritic length of the cortical pyramidal neurons, nor did it alter the recovery of the cortical thickness. NGF also promoted the increase of the dendritic spines, but not the dendritic length of the cortical pyramidal neurons of normal animals. In short, the data show that a few days of compression alone can cause permanent cortical damage. Exogenous NGF, if applied topically, may restore the dendritic spine density of cortical neurons subjected to compression.

Key words: brain tumor; hematoma; nerve growth factor; plasticity; trauma

INTRODUCTION

TRAUMATIC BRAIN INJURY or diseases such as tumors can damage the cortex through several mechanisms, including physical compression, release of chemical factors, and reactive tissue interactions. In order to gain insight into some of the pathophysiology associated with traumatic brain in relation to any subsequent cerebral cortic plasticity, the effect of physical compression alone on cerebral cortex was first investigated by developing a model that involved implanting a plastic bead into the epidural space overlying the sensorimotor cortex of rats (Chen et al., 2003). The bead was made of inert material, and its epidural localization prevented it from interacting with the cortex directly, thus minimizing complications from chemical or direct tissue interaction factors. The size and thickness of the bead were carefully chosen so that it caused only structural modification of the underlying cortex with no reduction of capillary density, cell death, or glial reaction. Changes occurred within 3 days of bead

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implantation, including thinning and reduction of the dendritic length and spine density within layer II/III and V pyramidal neurons of the underlying cortex (Chen et al., 2003). These findings also suggested that bead implantation did not cause local tissue ischemia or neuronal death emphasizing that the cortex can alter its morphology in response to mechanical compression alone.

In clinical practice, it is generally agreed that the removal of any compression source from the cortex, often rectifies some of the presumed compression-induced clinical manifestations such as seizure, hemiparesis, visual field loss, aphasia, and other focal symptoms (Reifenberger et al., 1996). However, whether the removal of a compression force reverses the anatomical changes caused by compression remains unknown. Further, the relationship between the timing of the decompression and the capacity for recovery of the compressed cortex remains largely unknown.

In this study, we sought first to study the recovery capacity of the compressed cerebral cortex. To this end, we explored the cortical cytoarchitecture and somatic-dendritic morphology of layer II/III and V pyramidal neurons of the sensorimotor cortex following decompression from short- and long-term compression. Dendritic branching, dendritic length, and dendritic spine density were analyzed because other studies have shown that they are altered following compression (Chen et al., 2003). Animals were examined 14 days following decompression, since preliminary experiments showed that the effect of compression stabilized by that time. An intracellular dye injection technique was used to reveal the somatic-dendritic arbors of the studied neurons because this technique offered the capacity to reveal the complete and detailed morphology of selected cells in isolation, with no background staining (Wang et al., 1996, 2002).

In recent years, various neurotrophic factors, especially nerve growth factor (NGF), have been shown to influence reparative changes after CNS injury (Cuello, 1994; Kolb et al., 1996). NGF has been shown to reduce dendritic atrophy of cortical neurons in aged rats (Mervis et al., 1991) and to prevent dendritic atrophy and promote functional recovery following cortical infarct (Kolb et al., 1996). In the view it would be of interest to learn whether exogenous NGF improves the recovery capacity of compressed cerebral cortex following decompression. To cumulate this intracerebroventricular infusions of NGF were administered for 14 days after decompression from 3 days of cortical compression since alterations of cortical morphology were prominent at this time.

The present study provides insight into the plasticity of the cerebral cortex subjected to compression and decompression and, in addition, explores the possibility of using exogenous NGF to enhance cortical recovery capacity following decompression.

MATERIALS AND METHODS

Epidural Compression of Cerebral Cortex

Twenty-five young adult female Wistar rats (Charles River) aged 6–7 weeks (150–175 g) were studied. Animals were housed and cared for according to guidelines of the animal research committee of the National Science Council. All efforts were taken to minimize animal suffering during and following surgery. Twenty-one of these rats were subjected to epidural bead implantation. A flat roundish polyethylene bead, 5 mm in diameter and 2.8 mm in thickness, was ground down on one of its flat sides to a thickness of 1.5 mm and was used to compress the cortex (Chen et al., 2003). Briefly, rats were anesthetized with 7% chloral hydrate (4.5 mL/kg; Merck, Darmstadt, Germany) and mounted on a stereotaxic device. An elliptical hole with a short diameter slightly smaller and a long diameter slightly greater than the diameter of the flat surface of the bead was drilled on the skull over the right sensorimotor cortex. The plastic bead was implanted into the epidural space with the flat surface up. One end was inserted in a direction paralleling the surface of the skull and along the short axis of the hole. After complete insertion into the hole, it was carefully pulled back so that the end of the bead could be lodged underneath the bone on the other side of the hole. This arrangement allowed the bead to seal the hole on the skull, since the short diameter of the hole was slightly less than the diameter of the bead.

Decompression of Cerebral Cortex

Three days ($n = 5$), 1 month ($n = 5$), and 3 months ($n = 5$) following bead implantation, rats were reanesthetized and mounted on a stereotaxic device for removing the implanted beads by reversing the implanting procedure. Following bead removal, the hole on the skull was covered with a thin layer of bone wax. There was no bulging of dura. In exploratory experiments, we determined the time needed for the effect of decompression to stabilize by examining animals 7, 14, and 30 days following bead removal. Based on the evaluation of cortical thickness and cytoarchitecture, we found that there was no further recovery of animals surviving longer than 14 days, while the recovery had not peaked by 7 days post implantation. Therefore, animals were examined 14 days following bead removal.

At the end of the survival period, rats were processed for *in vitro* intracellular dye injection (Wang et al., 2002). Briefly, rats were deeply anesthetized with chloral hydrate and perfused transcardially with 50 mL of lukewarm saline, followed by a low-strength fixative containing 2% paraformaldehyde in 0.1 M phosphate buffer

(PB), pH 7.3, at room temperature for 30 min. Brains were removed immediately following perfusion and sectioned with a vibratome (Technical Products International, St. Louis, MO) into 300-μm-thick coronal slices. About half of the slices encompassing the compressed cortical area were used for intracellular dye injection to reveal the morphology of selected cells. The rest were postfixed immediately in 4% paraformaldehyde in 0.1 M PB for 3 additional days, cryoprotected (Wang et al., 1996), and resectioned into 20-μm sections for studying their cytoarchitecture as detailed below. The remaining 6 bead-implanted rats were reserved for the NGF infusion study described below.

Intraventricular NGF Administration

After unilateral bead implantation for 3 days, rats ($n = 6$) were reanesthetized and mounted on a stereotaxic device. Following the removal of the implanted beads, a stainless steel cannula was implanted into the left lateral ventricle at the following coordinates relative to the bregma: anterior/posterior, -0.8 mm; lateral 1.3 mm; and ventral 3.5 mm from the skull. The cannula was connected via coiled flexible polyethylene tubing to a pretested, subcutaneously placed osmotic minipump (Alzet 2ML2, Palo Alto, CA) filled with NGF (6 μg/day for 14 days; Serotec, Oxford, UK) in artificial cerebrospinal fluid (ACSF). Four additional normal rats were subjected to the same minipump implantation but infused with the vehicle ACSF alone ($n = 2$) or with NGF ($n = 2$) in the same configuration. Rats survived for 14 days and were processed accordingly.

Histological Procedures to Reveal the Cytoarchitecture of Cerebral Cortex

Selected sections were stained with Cresyl violet or antiserum to PGP9.5, a neuronal marker, for evaluating cortical cytoarchitecture. For PGP9.5 immunohistochemistry, sections were reacted with rabbit polyclonal antibodies to the neuronal protein PGP9.5 (1:1000; Chemicon, Temecula, CA) for 18 h at 4¯C. Biotinylated goat anti-rabbit (1:200) immunoglobulins were used as the secondary antibodies and incubated for 1 h at room temperature. After rinsing three times in phosphate-buffered saline (PBS), they were incubated in standard avidin-biotin HRP reagent (Vector, Burlingame, CA) for 1 h at room temperature. Sections were then reacted in 0.05% 3-3′-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO) in 0.05 M Tris buffer, containing 0.01% H_2O_2. Reacted sections were mounted on subbed slides, air-dried, and coverslipped in Permount (Fisher, Fair Lawn, NJ).

Intracellular Dye Injection and Immunoconversion of the Injected Dye

Lucifer yellow (LY, Sigma) was used as the intracellular dye to reveal the somatic-dendritic arbors of selected cortical neurons (Wang et al., 2002). Briefly, slices were first treated with a solution containing 10^{-7} M 4′,6-diamidino-2-phenyl-indole (DAPI; Sigma) in 0.1 M PB for 30 min to label cell nuclei. A slice was then placed in a dish on the stage of a fixed-stage Zeiss Axioscop equipped with epifluorescence and covered with a thin layer of 0.1 M PB. Intracellular micropipette filled with LY solution (4% in water) mounted on a three-axial hydraulic micromanipulator (Narishige, Tokyo, Japan) was used. The slice was illuminated with a fluorescent light source and the fluorescence filter was set to visualize LY as yellow and DAPI as blue (390-420, FT 425, LP 450). The selected cell was impaled and a constant negative current was used to inject the LY until all terminal dendrites of the impaled cell fluoresced brightly. Several cells, well spaced apart, could be injected in each slice. At the end, the slice was removed and postfixed in 4% paraformaldehyde in 0.1 M PB for 3 days. It was then rinsed thoroughly in 0.1 M PB, cryoprotected, and carefully sectioned into 60-μm-thick serial sections with a sliding microtome (Wang et al., 1996).

The injected intracellular dye LY was converted into nonfading reaction product following the method described previously (Wang et al., 2002). Briefly, sections were treated in 1% H_2O_2 in PB for 30–60 min to remove endogenous peroxidase activity and then rinsed in PBS, followed by 2% bovine serum albumin and 1% Triton X-100 in PBS treatment for 1 h. Sections were then treated in biotinylated rabbit anti-LY (1:200; Molecular Probes, Eugene, OR) in PBS for 18 h at 4ßC. Following subsequent PBS rinses, sections were incubated with standard avidin-biotin HRP reagent at room temperature for 3 h. They were then reacted with 0.05% DAB and 0.01% H_2O_2 in 0.05 M Tris buffer at room temperature. Reacted sections were mounted onto slides, dehydrated in alcohol, cleared in xylene, and coverslipped with Permount.

Data Analysis

To determine how decompression alone or simultaneous NGF treatment affected the morphology of cortical output neurons subjected to different durations of epidural compression, the somatic-dendritic trees of layer II/III and V pyramidal neurons was reconstructed through serial sections using a Camera lucida drawing tube and a $\times 40$ objective lens in two-dimensional plane (Wang et al., 2002). Dendrites were divided into basal and apical compartments (Tseng and Prince, 1993) and their lengths were determined from a two-dimensional reconstruction

using PC-based software (Freemax Image-Pro, Media Cybernetics, Silver Spring, MD).

The extent of the dendritic arbor was analyzed following the method of Sholl by counting dendritic crossings with concentric circles drawn at 50-μm step increments in radii starting from the center of the soma (Tseng and Prince, 1993). Dendritic spines on selected proximal and distal segments of the basal and apical dendrites of

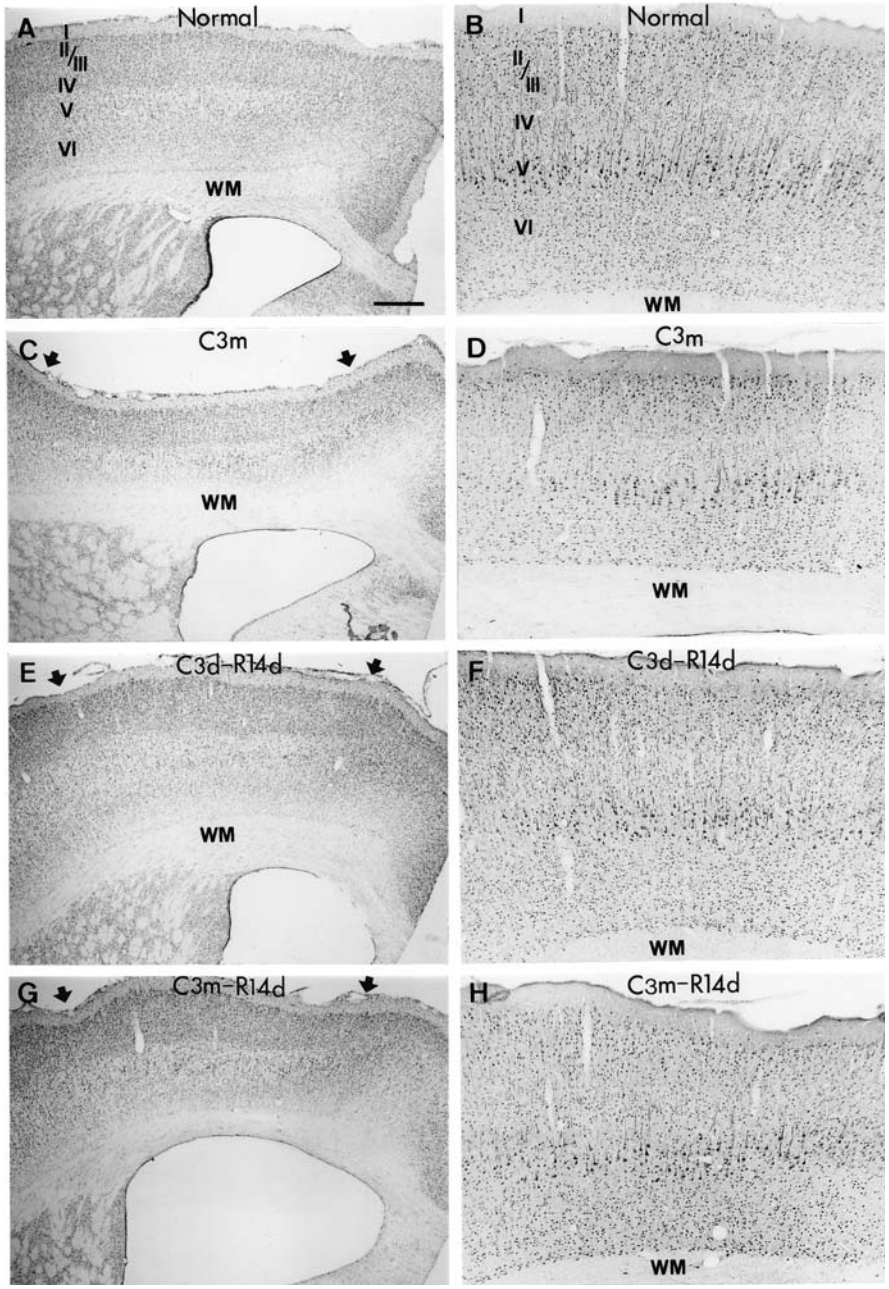

FIG. 1. Cytoarchitecture of the sensorimotor cortex of normal and experimental rats. Micrographs were taken from frontal sections stained with Cresyl violet (left column) and PGP 9.5 antiserum (right column), respectively. The latter reaction revealed only neurons and better illustrated the cortical layers. **A** and **B** were taken from the same normal animal, **C** and **D** from an animal 3 months following bead implantation (C3m), **E** and **F** from an animal surviving 14 days following the removal of a bead that had been implanted for 3 days (C3d-R14d), and **G** and **H** from an animal surviving 14 days following the removal of the bead that had compressed the cortex for 3 months (C3m-R14d). Roman numerals represent cortical layer. The compressed cortical area is located between the two arrows. WM: white matter. Bar = 0.5 mm (A,C,E,G), 250 μm (B,D,F,H).

the studied cells were reconstructed using a ×100 oil-immersion objective lens. For layer II/III pyramidal neurons, proximal and distal basal dendrites were the segment 25–75 and 100–150 μm from the soma, respectively. For the relatively large layer V pyramidal neurons, proximal and distal basal dendrites were defined as the segment 50–100 and 150–200 μm from where they originated from the soma, respectively. For both layer II/III and V pyramidal neurons, the proximal apical reconstructed dendrites were the first or second branch of the apical trunk, whereas the distal apical dendrites were the terminal dendrites of their apical tufts in layer I. No attempt was made to correct tissue shrinkage because the exact extent was not known. All tissue samples studied were subjected to similar shrinkage, therefore shrinkage was unlikely to affect group comparisons.

In order to determine the effect of decompression, data from bead-implanted animals that survived 3 days (n = 5), 1 month (n = 5), and 3 months (n = 2) and those of sham-operated animals (n = 4) were used for compartive purposes (Chen et al., 2003). Statistical significance between groups was determined using two-tailed Student's t-test.

RESULTS

The Effects of Decompression

The compressed cerebral cortex had a concave contour, yet, remained six-layered in structure (Fig. 1A–D). Its thickness was reduced by approximately 40% beginning at 3 days and persisted at this level up to 3 months

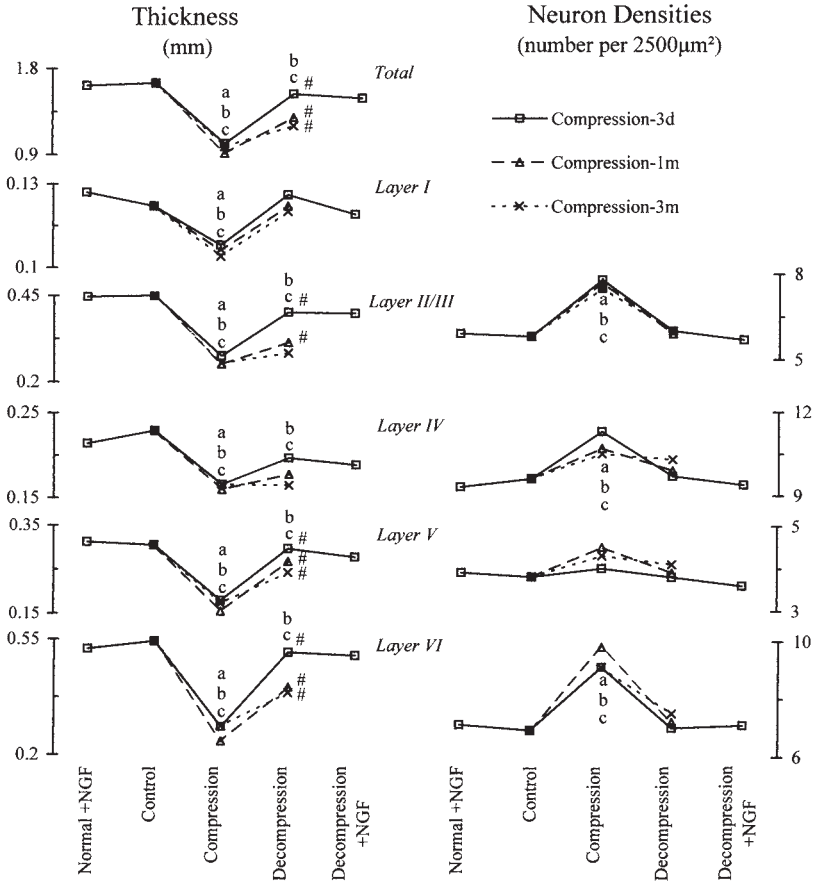

FIG. 2. The effect of compression, decompression, and exogenous NGF treatment on the thickness (left column) and neuron density (right column) of the rat sensorimotor cortex. Data plotted are means of the thicknesses of total and layers I–VI and the neuron densities of layer II/III–VI of the cortices of normal control, normal with NGF treatment, 3-day (3d), 1-month (1m), 3-month (3m) compression groups, decompression animals of the three compression groups, and 3-day compression followed by decompression with simultaneous NGF infusion. Number of areas analyzed was at least 30 in each group. For simplicity, the actual number of areas analyzed in each case is not shown. $^{a–c}p < 0.05$ between the 3d, 1m, and 3m groups and control, respectively; $^{\#}p < 0.05$ between the marked and its corresponding compression alone group (two-tailed Student's t tests).

following bead implantation (Fig. 2). The compressed cortical area regained its convex contour following bead removal; however, traces of the previously indented surface often remained visible at the edge of the compression (Figs. 1E,G; arrows). The compression-thinned cortex regained its thickness depending on the duration of the preceding compression: 3 days of compression allowed the cortex to return to near control levels (Figs. 1E,F and 2), while 1 or 3 months of compression limited the recovery of the cortex to approximately 70% to 80% of control (Figs. 1G,H and 2). The recovery capacity of each cortical layer was in general proportional to that of the overall cortex (Fig. 2). Increases in the neuronal densities of layers II/III, IV, and VI of the compressed cortices 3 days, 1 month, and 3 months postimplantation waned as the cortex regained its thickness following bead removal (Fig. 2). Neuronal densities of those compressed for 3 days returned close to control levels while those compressed for 3 months remained slightly higher than those of controls (Fig. 2). Alterations in the neuronal density of layer V followed the same trend however these changes were statistically insignificant, perhaps due to that layer's low neuronal density and large variability (Fig. 2).

The morphology of the somata of layer II/III (Fig. 3A–F) and V pyramidal neurons (Fig. 3A′–F′) of the affected cortex, that were not altered following 3 days and 1 month of bead implantation, remained unchanged following subsequent decompression (Fig. 4). However, their cell body area was reduced following decompression after 3 months of bead compression (Fig. 4). Decompression did not alter the numbers of the dendritic trunks of both pyramidal neurons (Fig. 4). Decompression rescued the reduction of the total dendritic lengths of layer II/III and V pyramidal neurons subjected to 3 days of compression to 94% and 87%, respectively, of their control values; however, the total dendritic length of layer V pyramidal neurons was still significantly lower than that of controls ($p < 0.05$; Fig. 4). Decompression failed to significantly restore the total dendritic lengths of both pyramidal neurons subjected to 1 or 3 months of bead compression. Neurons subjected to 1 month of compression did show some recovery of this dendritic length, yet this failed to reach statistical significance (Fig. 4). When dendrites were divided into apical and basal categories, the apical dendritic length of both neurons, that shortened prior to change is the basal dendrites' length following compression, showed a high degree of recovery following decompression after 3 days of compression. However, only the apical dendritic length of the layer II/III pyramidal neurons recovered to a level equivalent to that of the control neurons (Fig. 4).

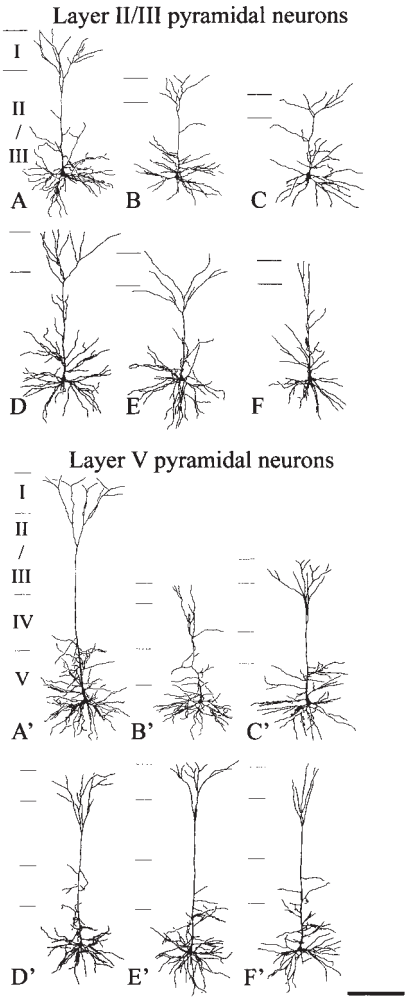

FIG. 3. Camera lucida drawings of layer II/III (**A–F**) and V pyramidal neurons (**A′–F′**) of the sensorimotor cortex of normal (**A** and **A′**) and experimental animals subjected to various treatments (**B–F** and **B′–F′**). **B** and **B′,** bead implantation for 3 days; **C** and **C′,** cortical compression for 1 month; **D** and **D′,** 3 days of compression followed by decompression with simultaneous NGF infusion for 14 days; **E** and **E′,** 3 days of compression followed by decompression for 14 days; **F** and **F′,** 1 month of compression followed by 14 days of decompression. Bars on the left of each drawing mark the demarcation between cortical layers as labeled. Bar = 200 μm (A–F), 250 μm (A′–F′).

Figure 5 plots the extent of the dendritic arbors of layer II/III (Fig. 5A) and V pyramidal neurons (Fig. 5B) of control, compressed, and compressed-decompressed cortices. For the sake of simplicity, since the curves of the 3-month compression with or without decompression were similar to those of the 1-month group, they were not plotted. Decompression failed to return the reduced

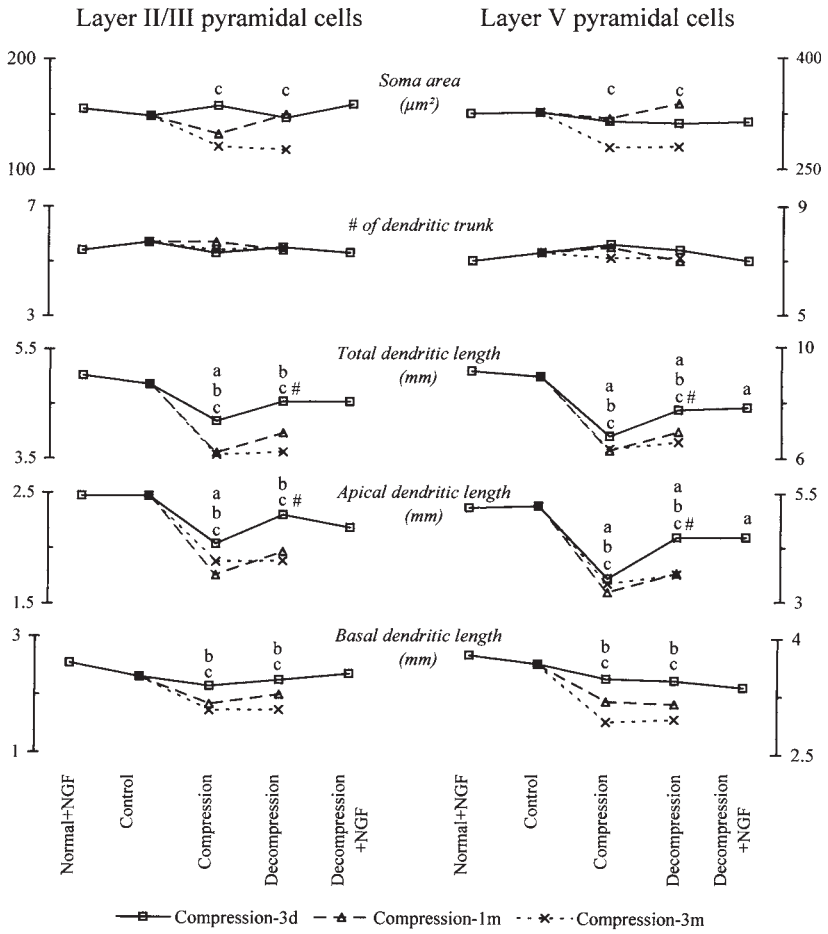

FIG. 4. The effect of compression, decompression, and NGF treatment on the somatic and dendritic arbors of layer II/III (left column) and V pyramidal neurons (right column) of the studied cortex. Mean somatic area, number of dendritic trunks, and total, apical, and basal dendritic lengths of each group are plotted from top to bottom as labeled. Data plotted include the 3-day (3d), 1-month (1m), and 3-month (3m) compression groups. Numbers of layer II/III and V pyramidal neurons reconstructed and analyzed were 27 and 26 for normal control, 28 and 21 for compression-3d, 25 and 21 for compression-3d + decompression, 26 and 24 for compression-1m, 21 and 20 for compression-1m + decompression, 18 and 15 for compression-3m, 14 and 13 for compression-3m + decompression, 20 and 16 for normal with NGF infusion, and 18 and 17 for compression-3d + decompression with simultaneous NGF infusion, respectively. Standard errors are excluded for simplicity. $^{a-c}p < 0.05$ between the marked 3d, 1m, and 3m groups and normal control neurons, respectively; $^{\#}p < 0.05$ between the marked and its corresponding compression alone group (two-tailed Student's *t* tests).

dendritic arbors of layer II/III pyramidal neurons to control levels, although those following 3 days of compression did show better recovery (Figs. 3 and 5A). However, decompression resulted in partial recovery of the dendritic profuseness of layer V pyramidal neurons, especially the apical dendritic tree, which was dramatically shortened following compression (Fig. 5B). The recovery in the vertical dimension of the apical dendritic arbors was especially remarkable in animals subjected to 3 days of compression since their longest apical dendrites had returned from no longer than 900 μm under compression to approximately 1100 μm following decompression (Figs. 3 and 5B). The densities of the dendritic spines on both proximal and distal basal and apical dendrites of layer II/III and V pyramidal neurons following decompression were not different from those subjected to bead implantation without decompression; both were significantly lower than those of controls, irrespective of the duration of the preceding compression (Figs. 6A,B and 7).

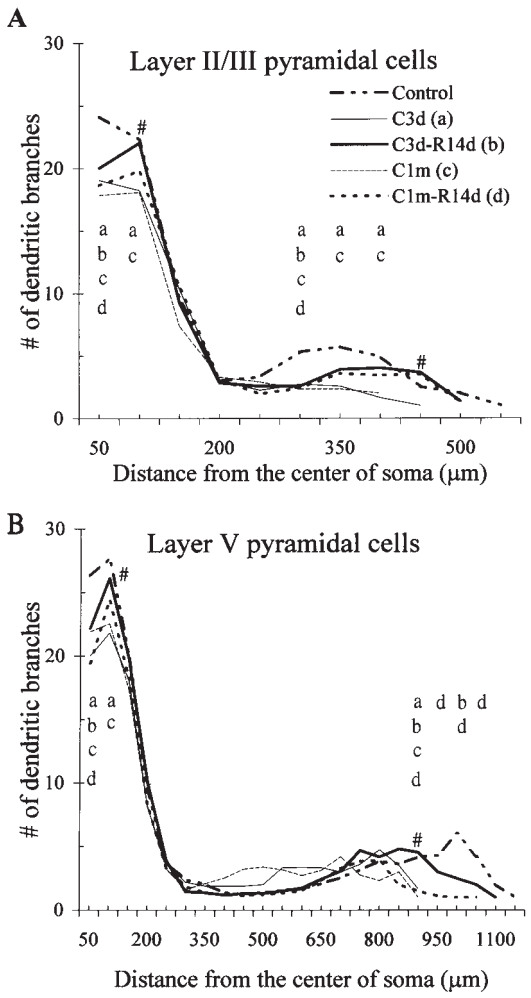

FIG. 5. The dendritic profuseness of layer II/III (**A**) and V (**B**) pyramidal neurons analyzed with the Sholl's method. Numbers of dendritic crossings on concentric circles drawn from the center of the soma at 50-μm increments in radii were plotted. Data from normal control (control), 3-day (C3d), and 1-month compression (C1m) groups, and decompression after 3 days (C3d-R14d) and 1 month (C1m-R14d) of compression were plotted. To enhance readability, data points are not marked and standard errors are not included. $^{a-d}p < 0.05$ between the labeled groups [(a), (b), (c), (d)] and normal control at each distance marked, respectively; $^{\#}p < 0.05$ between the 3d-compression and 3d-compression followed by decompression group at the location marked (two-tailed Student's *t* tests).

The Effects of Exogenous NGF on Compression-Induced Changes

Infusion of NGF into rats decompressed following 3 days of bead implantation failed to enhance the recovery of either the thickness or neuronal density of the compressed cortex (Figs. 2 and 6C). When the somatic-dendritic arbors of layer II/III and V pyramidal neurons were assessed (Fig. 3D,D′), exogenous NGF failed to change the somatic areas, numbers of dendritic trunks, or apical, basal, and total dendritic lengths of control animals (Fig. 4). Nor did it enhance the recovery of the dendritic length of layer II/III and V pyramidal neurons, that were shortened following 3 days of cortical compression (Fig. 4). Sholl's analysis also showed that NGF did not enhance the recovery of dendritic profuseness (not shown). Despite the lack of an enhancement in the recovery of dendritic lengths, exogenous NGF increased the spine densities to control levels on all segments of the dendrites of layer II/III and V pyramidal neurons examined (Figs. 6A,B); this was a significant improvement over the animals experiencing the same compression-decompression without NGF treatment (Fig. 7). Of note, however, NGF infusion also increased the dendritic spine densities in the control, non–bead-implanted animals (Figs. 6A,B and 7) as well as in the contralateral hemispheres of the compressed animals (not shown). The dendritic spine densities in these cases were significantly higher than those of controls (Fig. 7). This dendritic spine-enhancing effect of NGF on non–bead-implanted animals was specific; it failed to alter either the thicknesses or neuronal density of the cortices (Figs. 1, 6C) or the dendritic lengths of their layer II/III and V pyramidal neurons (Fig. 4). This enhancing effect was specific to NGF because these changes were not observed with vehicle injection (not shown).

DISCUSSION

The Effect of Decompression

In this study, we found that decompression could release cortex from most compression-induced structural changes depending on the duration of the preceding compression. The surface contour, overall thickness, and individual layer thickness and neuronal density of the affected cortex could recover to near control levels (93%) if the cortex was compressed for 3 days. Prolonged compression of 1 month or 3 months limited this recovery to 78% or 72%, respectively, of those of controls. The somatic size of layer II/III and V pyramidal neurons, that was not reduced until 3 months following compression, was unaffected by subsequent decompression. On the other hand, the compression-induced reduction of apical, basal, and total dendritic lengths and dendritic arbor profuseness could partially recover if the cortex was compressed for 3 days. In contrast compression for 1 month or 3 months aborted any dendritic-length recovery. Both

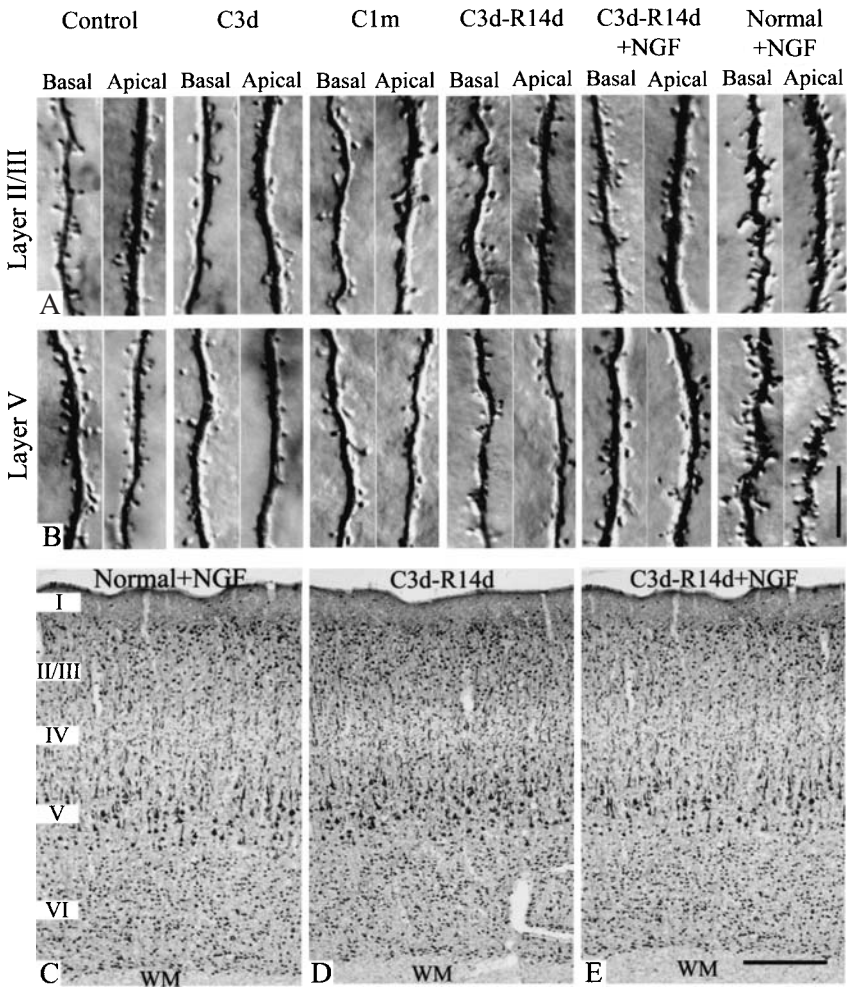

FIG. 6. Composite micrographs to show structural characteristics of sensorimotor cortices subjected to various compression, decompression, and/or NGF treatment. Top two rows (**A,B**) show the dendritic spines on represented dendritic segments of the layer II/III (row A) and V pyramidal cells (row B) of each treatment group indicated. Representative basal (left) and apical (right) dendritic segments of an intracellular dye-filled cell are illustrated in each group. In each set, the basal dendritic segment shown was ~100–150 μm away from the soma, while the apical dendritic segment was a terminal branch of the apical dendritic tuft of the same cell. Bottom row pictures (**C,D,E**), which were taken from frontal sections stained with PGP9.5 immunohistochemistry, show the cytoarchitecture of the cortex of the treatment group indicated. Roman numerals to the left indicate the cortical layers. C3d, compression 3 days; C1m, compression 1 month; C3d-R14d, compression 3 days followed by decompression for 14 days; C3d-R14d + NGF, C3d-R14d animals with simultaneous NGF infusion during decompression; Normal + NGF, normal animals infused with NGF for 14 days; WM, white matter. Bar = 10 μm (A,B), 300 μm (C–E).

layer II/III and V pyramidal cells failed to regain their dendritic spine densities even after only 3 days of compression, suggesting that clinical cases that involve cortical compression (such as epidural hematoma) could inflict enduring damage in cerebral output neurons in a matter of days.

It is known that dendritic spines are dynamic structures that can change form and density according to their environment (Horner, 1993); long-term potentiation and enriched environment increase dendritic spine densities (Globus et al., 1973), while aging (Feldman and Dowd, 1975) and pathological conditions such as dementia (Ferrer et al., 1990), temporal lobe epilepsy (Paul and Scheibel, 1986), and mental retardation states (Marin-Padilla, 1972) decrease them. The lack of recovery of dendritic spine density suggests that exposing the cortex to a few days of physical compression alone could lock the cortex into an altered functional status even after clinical manipulation removes

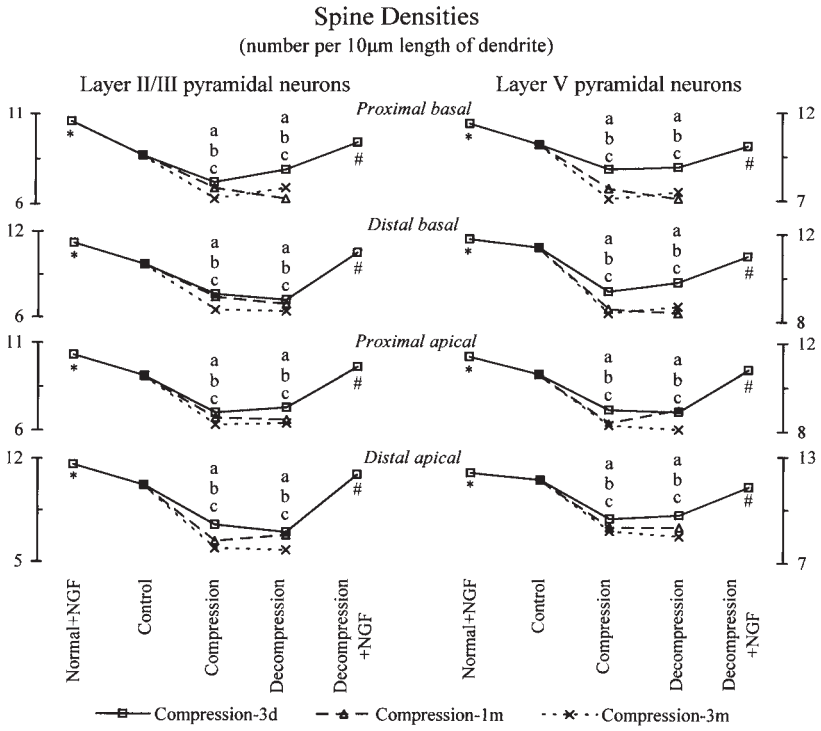

FIG. 7. The densities of dendritic spines on the proximal and distal apical and basal dendrites of layer II/III (left column) and V pyramidal neurons (right column) of the rat sensorimotor cortex. Data from animals subjected to 3 days (3d), 1 month (1m), and 3 months (3m) of compression, 3 days of compression followed by decompression, and 3 days of compression followed by decompression with simultaneous NGF infusion were plotted along with those of normal (control) with and without NGF treatments. Means of each group are plotted; standard errors are not included for the sake of readability. $^{a,b,c}p < 0.05$ between the labeled 3d, 1m, and 3m groups and normal; $^{\#}p < 0.05$ between the 3-day compression + decompression with and without simultaneous NGF infusion; $^{*}p < 0.05$ between control and normal with NGF infusion (two-tailed Student's *t* tests). The numbers of dendritic segments analyzed were different between groups, with an average of 41. The specific number of segments analyzed in each group is omitted for the sake of brevity.

the source of compression, such as surgical evacuation of epidural hematoma. In clinical practice, evacuation of epidural hematoma largely rectifies the associated symptoms. This suggests that partial loss, 15%–20%, of dendritic spines may be too subtle to be revealed behaviorally. Detailed tests may be required to reveal the impairments associated with the irreversible reduction of dendritic spines in cases involving cerebral compression. Since our model addresses only the pathophysiological aspect of physical compression on cerebral cortex, it in no way simulates clinical cases of epidural hematoma or meningioma, since these abnormalities involve much more than the fixed-sized physical compression that we imposed on the cerebral cortex in the present study.

The Effect of Exogenous NGF on Decompression

NGF infusion resulted in the recovery of the dendritic spine densities on layer II/III and V pyramidal neurons of cerebral cortex that had been compressed for 3 days. The rebound of dendritic spine densities on both layer II/III and V pyramidal neurons of the compressed cortex following NGF treatment suggests that these cells were capable of regrowing dendritic spines if appropriate factors and/or stimuli were available. These newly added dendritic spines are probably functional because the dendritic spines of pyramidal neurons were found to remain increased 4 months after 2 weeks of NGF treatment (Kolb et al., 1997). The specificity of the growth of dendritic spines may be achieved by confining stimulating factors and/or stimuli, such as the infusion of NGF, to the affected cortical area. NGF did not promote dendritic growth at the end of 14 days of continuous infusion; however, it promoted the dendritic branching of layer V, but not layer III, pyramidal neurons 4 months examined after 2 weeks of NGF infusion (Kolb et al., 1997).

In light of the finding that the regrowth of dendritic spines but not dendritic length was achieved following

14 days of exogenous NGF treatment, it is possible that the elaboration of dendritic spine and dendritic arborization are controlled by different mechanisms. It is known that NGF mediates its biological actions by activating TrkA receptors (Kaplan et al., 1991; Meakin and Shooter, 1992), which in the cerebral cortex are only expressed in forebrain cholinergic neurons (Bothwell, 1995). Although the mechanism of the action of NGF on non-cholinergic neurons is not known, NGF may stimulate terminal growth of basal forebrain cholinergic neurons (Heisenberg et al., 1994; Burgos et al., 1995), that, in turn, stimulate growth of dendritic spines on cortical pyramidal neurons. Another indirect action of NGF could be through enhanced synaptic activity, especially of cholinergic neurons, that could, in turn, stimulate spine density increases in pyramidal neurons.

This hypothesis seems plausible for several reasons. First, loss of basal forebrain cholinergic neurons, often associated with dementia or aging, is usually accompanied by loss of dendritic spines on cortical neurons (Feldman and Dowd, 1975; Ferrer et al., 1990). Second, activation of cholinergic pathways enhances the formation of long-term potentiation, which is often associated with an increase of dendritic spine density (Globus et al., 1973). Third, fibers in the cholinergic nerve bundle entering the cortex from the medial end of the white matter of each cerebral hemisphere become thicker following NGF treatment (Chen and Tseng, unpublished data). With this hypothesis, activation of cortical cholinergic pathways may benefit those subjected to cerebral compression or undergoing decompression surgeries by either preventing the loss or enhancing the recovery of dendritic spines on their cortical neurons.

In conclusion, using a rat epidural bead implantation model, we identified that the ability of the cortex to recover from compression-induced changes was dependent on the duration of the preceding compression. With the exception of a permanent loss of dendritic spines, near complete recovery of the compression-induced changes could be obtained only following short-term compression. NGF applied immediately following decompression restored dendritic spine densities without altering other parameters of the cortex that we had examined, at least in the short term. Thus, NGF may restore the dendritic spines of cortical neurons that were subjected to short-term compression. The specificity of this spine-generating effect may be achieved by topical NGF application or perhaps specific activation of the brain cholinergic pathways.

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