

Improvement of cognitive deficits and decreased cholinergic neuronal cell loss and apoptotic cell death following neurotrophin infusion after experimental traumatic brain injury

GRANT SINSON, M.D., BRIAN R. PERRI, B.A., JOHN Q. TROJANOWSKI, M.D., PH.D.,
EUGENE S. FLAMM, M.D., AND TRACY K. MCINTOSH, PH.D.

Division of Neurosurgery and Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

✓ This study explores the effects of infusion of nerve growth factor (NGF) on behavioral outcome and cell death in the septal region using the clinically relevant model of fluid-percussion brain injury in the rat. Animals were subjected to fluid-percussion brain injury and 24 hours later a miniosmotic pump was implanted to infuse NGF (12 animals) or vehicle (12 animals) directly into the region of maximum injury for 2 weeks. Four weeks postinjury the animals were tested for cognitive function using a Morris Water Maze paradigm. Neurological motor function was evaluated over a 4-week postinjury period. The rats receiving NGF infusions had significantly higher memory scores than vehicle-treated animals. Examination of the cholinergic neurons in the medial septal region using choline acetyltransferase immunohistochemistry demonstrated significant cell loss after injury. Infusion of NGF significantly attenuated loss of these cholinergic neurons.

A second group of animals was subjected to fluid-percussion brain injury alone (23 rats) or injury followed by NGF infusion (18 rats). These animals were killed between 24 hours and 2 weeks postinjury and the septal region was examined for the presence of apoptotic cells using the terminal deoxynucleotidyl transferase-mediated biotinylated-deoxyuridinetriphosphate nick-end labeling technique. Apoptotic cells were identified as early as 24 hours postinjury; their numbers peaked at 4 and 7 days, and then declined by 14 days. The NGF-treated animals had some apoptotic cells; however, even at 7 days there were significantly fewer of these cells. No significant motor differences were observed between the NGF- and vehicle-treated groups.

These data indicate that NGF administration beginning 24 hours after fluid-percussion brain injury has a beneficial effect on cognition and results in sparing of cholinergic septal neurons. These improvements persist after cessation of NGF administration. The beneficial effects of NGF may be related to its ability to attenuate traumatically induced apoptotic cell death.

KEY WORDS • apoptosis • fluid-percussion injury • nerve growth factor • cognitive dysfunction • traumatic brain injury • rat

AN estimated 420,750 people per year are discharged from hospitals in the United States after sustaining a head injury and approximately 20% of these people have residual neurological disabilities.²⁹ Even subtle alterations in cognitive function can significantly disable those who otherwise have made good recoveries.^{4,32,41} The lateral fluid-percussion model of brain injury has been extensively studied because of the pathological and behavioral similarities it shares with clinical head injury.^{16,20,40} Cognitive deficits of learning and memory can be demonstrated using the Morris Water Maze (MWM) and have been shown to persist for months after brain injury.^{20,45,59}

Administration of various growth (neurotrophic) factors has been shown to support neuronal cells in a variety of models of central nervous system (CNS) injury.^{28,36} Nerve growth factor (NGF) remains the most extensively studied neurotrophic factor, and treatment with NGF has been shown to attenuate cell death after ischemic, excitotoxic,

or hypoglycemic injury and after axonal transection.^{2,7,13,18,53,56} Although little is known concerning the response of endogenous trophic factors to CNS injury, Patterson, et al.,⁴⁴ have demonstrated the presence of NGF in the cerebrospinal fluid of brain-injured human patients.⁸ Previously we have shown that NGF infusion can significantly improve the cognitive deficits normally associated with fluid-percussion brain trauma.⁵⁸ However, no histopathological differences were noted that correlated with this improvement.

The most common type of programmed cell death is apoptosis.²⁷ Apoptotic cell death has been shown to occur after experimental brain injury,⁵⁰ and neurons can be induced to undergo apoptosis in vitro by depriving them of NGF and can then be rescued by returning NGF to the culture medium.^{6,47} Recently apoptotic cell death has also been described in numerous models of CNS disease including ischemia and excitotoxic injury.^{10,35,37,48} This study was performed to evaluate the presence of lasting cogni-

tive improvements induced by NGF infusion after fluid-percussion brain injury in rats and to identify the histological correlates of these behavioral changes.

Materials and Methods

Surgical Procedures

Twenty-four male Sprague–Dawley rats, each weighing between 350 and 400 g, were anesthetized with 60 mg/kg sodium pentobarbital administered intraperitoneally. The lateral (parasagittal) fluid-percussion model of brain injury was used.⁴⁰ Briefly, a 5-mm craniectomy was performed over the left parietal cortex midway between the lambda and bregma. A hollow Leur-loc fitting was cemented to the craniectomy site, and the injury was delivered after attaching the Leur-loc to the fluid-percussion device, which rapidly injects a bolus of saline into the epidural space. All animals received brain injury of moderate severity (2.1–2.3 atm).

Twenty-four hours after brain injury, animals were reanesthetized with 60 mg/kg sodium pentobarbital and a 1-mm burr hole was made 2 mm lateral to the craniectomy site (corresponding to the area of maximum cortical injury observed in this model).⁴⁰ The dura was exposed via the burr hole and then incised with a 26-gauge needle. A brain infusion cannula was placed stereotactically to a depth of 1.5 mm in the cortex at the site of maximum cortical injury, secured with dental cement, and the skin was sutured over the apparatus. Miniosmotic pumps with an infusion rate of 0.5 μ l/hour for 14 days were filled with NGF and vehicle or vehicle alone 4 to 6 hours prior to implantation. A brain infusion cannula was then attached to the pumps and the units were primed in sterile saline at 39°C, after which the pumps were implanted subcutaneously in the animals. The animals were separated into two groups: Group 1 (12 rats) received infusions containing NGF (artificial cerebrospinal fluid, 0.1 mg/ml rat serum albumin, 25 μ g/ml 7S NGF, and 0.05 mg/ml gentamicin); Group 2 (12 rats) received infusions of the same solution without NGF. Two weeks postinjury the brain cannula and pump were removed to confirm complete delivery of the infusate during that time period. Procedures were performed under sterile conditions in all groups and normothermia was maintained with the animals on warming pads. Brain temperature was not directly monitored because it has been shown previously that use of warming pads maintains normal brain temperature in this animal model of brain injury.⁴²

Evaluation of Motor and Cognitive Function

The evaluation of memory and motor function in rats undergoing a fluid-percussion brain injury has been described previously in detail.^{40,60,61} All animals were tested in the MWM 4 weeks postinjury. The maze is a circular tank 1 m in diameter that is filled with water maintained at 18°C. The water surface was made opaque with a covering of styrofoam pieces. During training of the animals, a submerged platform was present in the maze. Each animal underwent 20 training trials over a 2-day period during which it learned to locate the platform using external visual cues. Uninjured sham-treated animals (12 rats) were also trained using the same paradigm. The time needed to find the platform was recorded for each of 20 trials. At 48 hours after training, animals in all groups were assessed for their ability to remember the learned task of locating the platform in the MWM. For this evaluation the platform was removed from the maze and the animal's swimming pattern recorded with a computerized video system for 1 minute. The maze was separated into zones that were weighted according to their proximity to the platform's location during training. A memory score was generated by multiplying the weighted numbers by the time the animal spent in each zone and then adding these products.^{23,42,60,61}

An investigator blinded to treatment group evaluated the animals' neurological motor function at 1, 2, and 4 weeks postinjury.^{40,60} Animals were scored from 0 (severely impaired) to 4 (normal) for each of the following: 1) left and 2) right forelimb flexion during suspension by the tail; 3) left and 4) right hindlimb flexion when the forelimbs remain on a surface and the hindlimbs are lifted up and

back by the tail; 5) the ability to resist lateral pulsion to the left and 6) right; and 7) the ability to stand on an inclined plane in the left, 8) right, and 9) vertical positions. For the inclined plane (angle board) test, animals received a score of 4 by standing at a 45° angle; 3 at 42.5°; 2 at 40°; and 1 at 37.5°. A composite motor score (0–36) was generated by combining the scores for each of these tests.

Histological Evaluation

The animals were killed at 4 weeks postinjury (after behavioral testing) with an overdose of 200 mg/kg sodium pentobarbital administered intraperitoneally and perfused with intracardiac heparinized saline followed by 4% paraformaldehyde. The brains were removed, postfixed overnight in paraformaldehyde, and stored in phosphate buffer before being cut into 50- μ m sections on a vibratome.

Hippocampal CA3 damage was characterized in all animals at two selected brain regions (one rostral and one caudal hippocampal segment) using toluidine blue Nissl staining. A score of 0 to 3 was derived for each region, with a score of 3 representing a normal-appearing CA3 region; a score of 2 representing a distinguishable region of cell loss due to a thinning of the pyramidal cell layer; a score of 1 representing a region in which continuity of the CA3 pyramidal cell layer was maintained by only a single cell in at least one portion; and a score of 0 representing those CA3 regions in which cell loss was severe enough to disrupt the continuity of the pyramidal cell layer completely in at least one segment. The scores from the rostral and caudal sections were combined to provide a score of 0 to 6 for CA3 cell survival in each animal.

Sections were taken through the septal nuclei 350 μ m rostral to the anterior commissure decussation. Free-floating sections were incubated in choline acetyltransferase (ChAT) antibody (1:1000 dilution) at 4°C for 36 hours. Subsequently, these sections were exposed for 1 hour to goat anti-rabbit immunoglobulin G followed by rabbit peroxidase–antiperoxidase. Finally, sections were incubated in 3,3'-diaminobenzidine for 10 minutes. The border of the medial septal area was identified laterally and dorsally by the presence of ChAT-positive cells and ventrally by a line drawn through the center of the anterior commissure, after the method of Hagg, et al.¹⁸ Cholinergic cells were defined as those cells that stained with the ChAT antibody and had a diameter greater than 12 μ m.¹⁸ Measurements of the area of the septal nuclei were made using image analysis on the same sections used for ChAT immunohistochemistry. The borders for the septal nuclei were the corpus callosum dorsally, the ventricles laterally, and a line drawn through the center of the anterior commissure ventrally.

Apoptosis Studies

A second group of animals was subjected to fluid-percussion brain injury of moderate severity (2.1–2.3 atm). One set of these animals (23 injured and four sham-injured) was killed at 1, 2, 4, 7, or 14 days postinjury and prepared as noted above, with the exception that the brains were embedded in paraffin blocks. Three 6- μ m-thick sections were taken from the area between 300 and 350 μ m rostral to the anterior commissure decussation for each brain. These sections were then stained for apoptotic cells using the terminal deoxynucleotidyl transferase (TdT)-mediated biotinylated-deoxyuridinetriphosphate (dUTP) nick-end labeling (TUNEL) technique.^{15,50} Sections were heated for 15 minutes at 60°C, deparaffinized, and rehydrated. After digestion with 0.02% trypsin in phosphate-buffered saline followed by washing in phosphate-buffered saline, the sections were incubated in Buffer A (200 mM potassium cacodylate, 0.025 M Tris, and 0.25 mg/ml bovine serum albumin, pH 6.6). Sections were then incubated at 37°C for 60 minutes with the labeling solution: TdT (0.3 U/ μ l), biotinylated-16-dUTP (20 μ M), and 1.5 mM cobalt chloride in Buffer A. Reactions were halted by rinsing in Buffer B (300 mM sodium chloride and 30 mM sodium citrate, pH 7). Sections were washed with 0.01 M Tris at pH 7.4, blocked with 10% goat serum in 0.01 M Tris, incubated with streptavidin-conjugated alkaline phosphatase (1:40 dilution), and stained with Fast Red. Slides were then washed, counterstained, and mounted.

Because we have previously observed that the TUNEL technique

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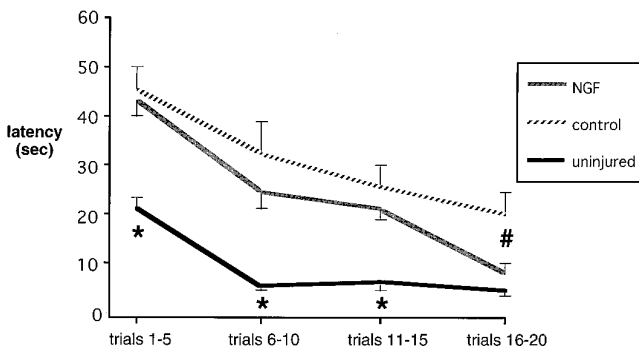


FIG. 1. Graph showing comparison of latencies to find the MWM platform during learning trials. Average latencies for each set of five trials are shown (*bars* represent standard error of the mean [SEM]). During the first three sets (Trials 1–15) all injured animals displayed latencies that were significantly longer than those seen in uninjured animals (* $p < 0.05$). Although the latencies of animals that had received NGF were better than those in injured control animals during these sets (45 ± 4.6 , 33 ± 5.2 , and 26 ± 3.5 versus 43 ± 3.4 , 26 ± 4.7 , and 21 ± 2.8 , respectively; mean \pm SEM), the differences were not significant. The last set of trials shows that the latencies for NGF-treated animals (8 ± 1.7) improved and were not significantly different from uninjured sham-treated animals (5 ± 0.6). The latencies for the NGF-treated animals (8 ± 1.7) were significantly better than for the injured control animals (20 ± 4.8) during this last set of trials ($\#p < 0.05$).

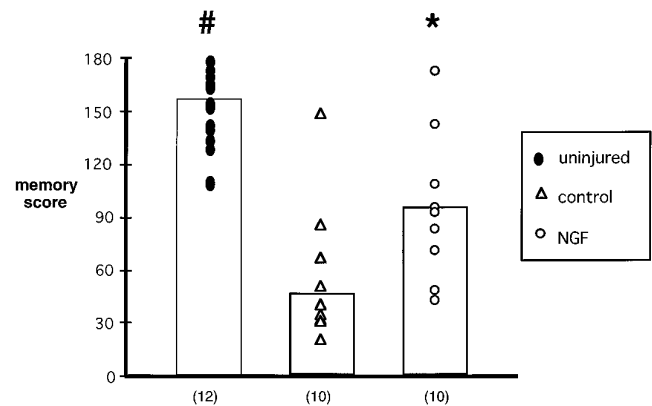


FIG. 2. Graph showing comparison of memory scores 48 hours after completion of the MWM training of vehicle-treated (median score 46), NGF-treated (median score 94), and uninjured animals (median score 154). The NGF-treated animals had significantly higher memory scores compared with vehicle-treated control animals (* $p < 0.05$). Uninjured animals had significantly higher memory scores compared with both groups of injured animals ($\#p < 0.05$). Numbers in parentheses represent the number of animals tested.

will stain cells undergoing both necrotic and apoptotic cell death, only those cells that were TUNEL positive and displayed apoptotic morphology were counted in the entire septal region (as defined above) on all three sections from each brain. Apoptotic morphology was characterized by small round cells with round, condensed, and often fragmented nuclei.⁵⁰

Based on the results of these studies, a third group of animals (18 rats) was subjected to fluid-percussion brain injury of moderate severity (2.1–2.3 atm), followed by the placement of an osmotic pump to infuse NGF beginning 24 hours postinjury (as above). The animals were then killed at 2, 7, or 14 days postinjury and the brains were processed, sectioned, TUNEL stained, and evaluated for morphological evidence of apoptotic cell death.

Statistical Analysis

Ordinal measurements from neurobehavioral motor function tests, memory tests, and hippocampal CA3 cell loss were evaluated using Kruskal–Wallis analysis of variance followed by individual Mann–Whitney U-tests. Mean values, including latencies to find the platform during learning in the MWM, ChAT-positive cells, septal area values, and apoptotic cells were all compared using analysis of variance followed by post-hoc Student's t-tests. A probability value of less than 0.05 was considered statistically significant.

The procedures used throughout this study were approved by the institutional animal care and use committee of the University of Pennsylvania and were performed in accordance with standards published in the *Guide for the Care and Use of Laboratory Animals*, U.S. Department of Health and Human Services, Publication 85–23, 1985.

Sources of Supplies and Equipment

The brain infusion cannulas and miniosmotic pumps (model 2002) were purchased from Alza Corp., Palo Alto, CA. The ChAT antibody was acquired from Chemicon International, Houston, TX, and the 3,3'-diaminobenzidine and Fast Red stain were from Sigma Chemical Co., St. Louis, MO. The TdT and dUTP were obtained from Boehringer Mannheim, Mannheim, Germany. The

streptavidin-conjugated alkaline phosphatase was purchased from BioGenex Corp., Framingham, MA.

Results

The learning latencies and memory scores are illustrated in Figs. 1 and 2. Two animals from each group were killed before completion of the study because of a failure to thrive and are not represented in the cognitive or histological data. All injured animals learned significantly more slowly than the uninjured control animals during the first three sets of five trials ($p < 0.05$). However, by the fourth set of five trials the latencies in the injured, NGF-treated animals had improved and were significantly better than those in the injured vehicle-treated animals ($p < 0.05$) and not significantly different from those in the uninjured animals. The NGF-treated animals also performed significantly better than vehicle-treated controls during memory testing ($p < 0.05$; Fig. 2). The results of motor testing are shown in Fig. 3. There was a gradual improvement in neurobehavioral motor scores over the course of the experiment but no significant motor differences were observed between the NGF- and vehicle-treated injured groups.

Figure 4 shows the results of calculations for septal area nuclei and cholinergic cell counts. A significant loss of septal area nuclei and cholinergic cells was identified in all injured animals compared with sham-treated (uninjured) animals ($p < 0.05$). The NGF-treated animals displayed less cholinergic cell loss than vehicle-treated animals (Fig. 5). The difference was statistically significant only when comparing cell loss on the side contralateral to the injury site ($p < 0.05$). Histological evaluation of the cortical injury cavity and hippocampal CA3 cell loss revealed no differences between the vehicle- and NGF-treated animals (data not shown). All injury cavities communicated with the ventricular system.

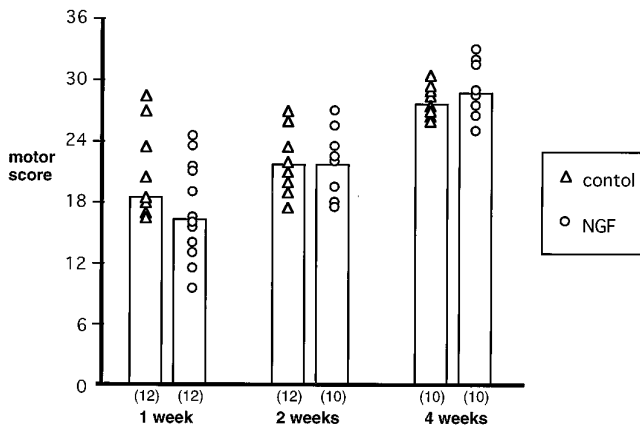


FIG. 3. Graph showing neurological motor scoring in injured vehicle-treated controls (medians of 18.5, 22.8, and 28.0) and injured NGF-treated animals (medians of 16.3, 22.5, and 28.8) at 1, 2, and 4 weeks postinjury. There is a gradual improvement in motor scores over time in both groups and no significant differences between these groups. Numbers in parentheses represent the numbers of animals tested.

Evaluation of sections stained using the TUNEL technique demonstrated that cells in the septal region showed morphological evidence of apoptotic cell death at the earliest time point tested (24 hours postinjury; Fig. 6). This cell death was at maximum levels at the 4- and 7-day postinjury time points and had decreased by 14 days postinjury. Cells undergoing apoptosis were not confined to a specific location or septal nucleus but rather were distributed bilaterally throughout the septal region (data not shown). Although treatment with NGF resulted in slightly fewer apoptotic cells at the 2- and 14-day time points ($p =$

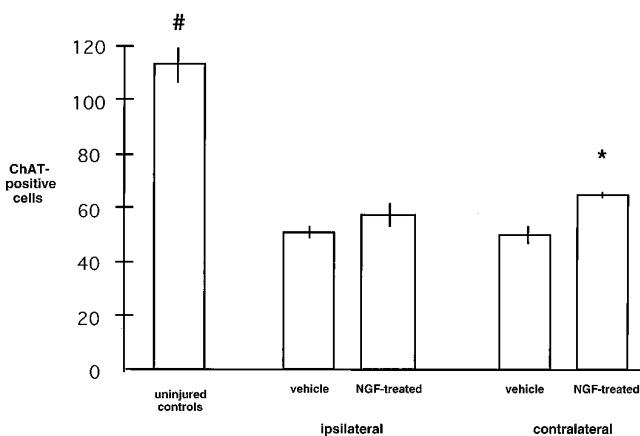


FIG. 4. Graph showing ChAT-positive cells in the medial septal nucleus in uninjured, injured vehicle-treated, and injured NGF-treated animals. Uninjured animals exhibited significantly higher numbers of ChAT-positive cells (114 ± 5.7 , mean \pm SEM) compared to both injured groups ($\#p < 0.05$). Ipsilateral to the side of injury the NGF-treated animals displayed more cells (58 ± 5.1) than did vehicle-treated animals (51 ± 2.7), but this was not a significant difference. Contralateral to the side of injury the NGF-treated animals exhibited significantly more ChAT-positive cells (65 ± 2.1) than did vehicle-treated animals (50 ± 3.3 ; $*p < 0.05$).

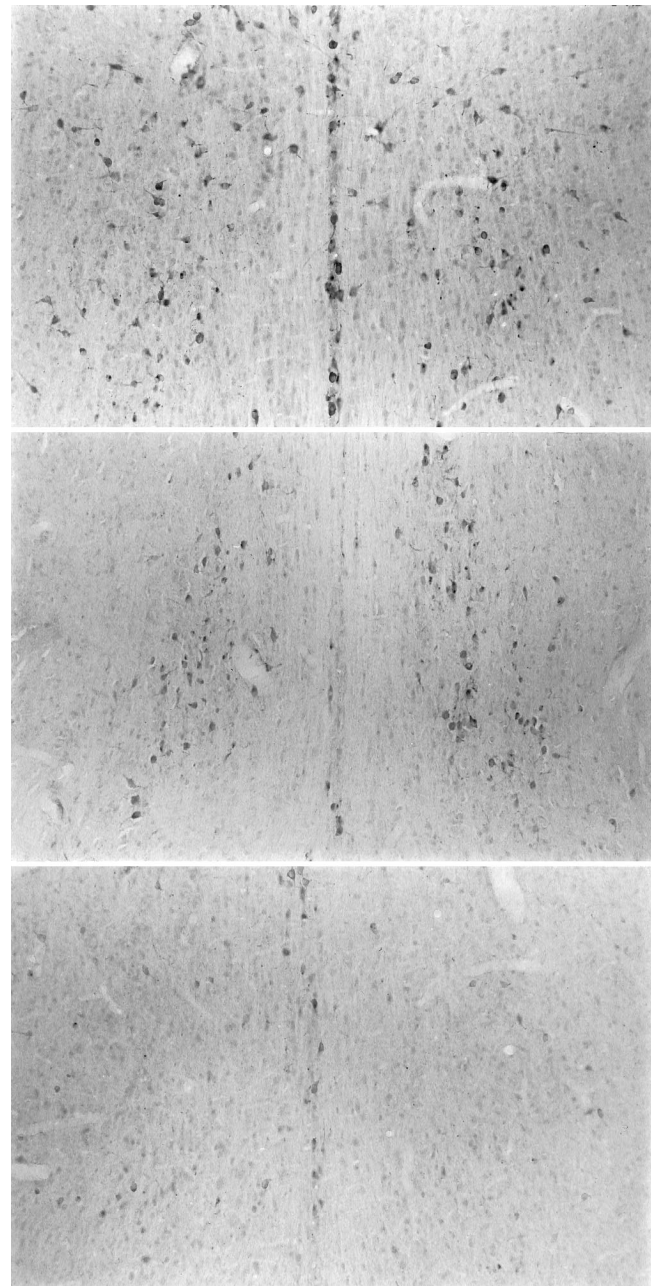


FIG. 5. Photomicrographs showing coronal 50- μ m sections of rat brain cut through the medial septal nucleus. The center of each photomicrograph is midline and the left side is ipsilateral to the injury. *Upper*: Photomicrograph of brain section from an uninjured animal with diffuse robust ChAT-staining neurons. *Center*: Photomicrograph of brain section from an injured NGF-treated animal showing a significant number of ChAT-staining neurons, although there are fewer than in the uninjured animal. *Lower*: Photomicrograph of brain section from an injured vehicle-treated animal demonstrating far fewer ChAT-staining neurons. ChAT antibody, original magnification $\times 100$.

not significant; Fig. 6), a significant diminution of cells undergoing apoptosis was observed in injured NGF-treated animals at 7 days postinjury when compared to injured vehicle-treated animals ($p < 0.05$).

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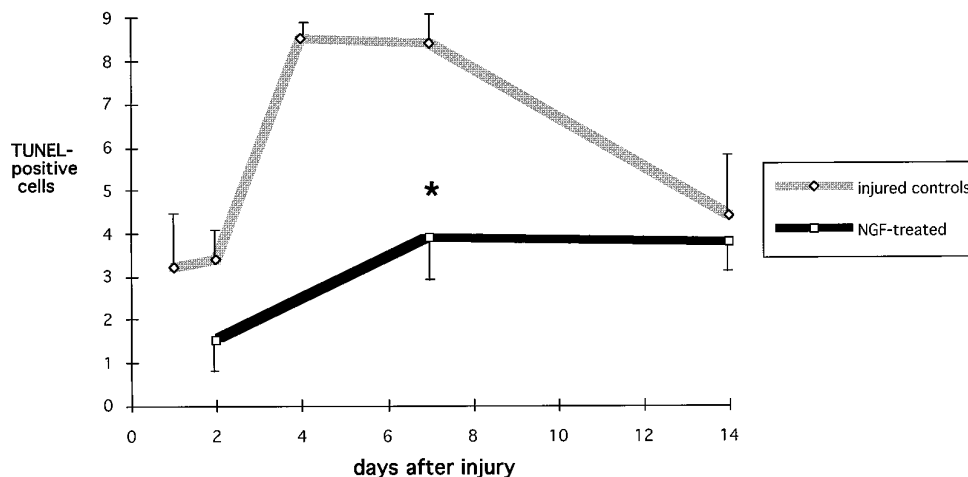


FIG. 6. Graph showing apoptotic (TUNEL-positive) cells in the septal region after fluid-percussion brain injury. Apoptotic cells were identified as early as 24 hours postinjury (3.2 ± 1.3 per brain [mean \pm SEM]). The number of cells increased significantly at 4 and 7 days postinjury (8.5 ± 0.3 and 8.4 ± 0.9), and declined by 14 days (4.4 ± 1.4). Although some apoptotic cells were seen in NGF-treated animals, even at 7 days there were significantly fewer cells (3.9 ± 0.9 ; * $p < 0.05$). Sham animals had 2.3 ± 0.7 apoptotic cells. The number of apoptotic cells in NGF-treated animals was never significantly different from that in the sham-treated animals.

Discussion

These studies demonstrate that NGF infusion initiated 24 hours after fluid-percussion brain injury in rodents can attenuate learning deficits. These improvements persist for a prolonged period after NGF infusion has been discontinued. Also, sparing of cholinergic neurons and attenuation of apoptotic cell death in the septal region appear to correlate with these NGF-induced cognitive changes.

Cognitive Outcomes in Experimental Brain Injury

Previous studies have characterized the cognitive deficits that result from fluid-percussion brain injury in the rat.^{20,23,46,59,61} The earliest studies using the MWM as a measure of cognitive function after fluid-percussion brain injury focused on the measurement of retrograde amnesia.⁶¹ This paradigm has proved useful in the rapid evaluation of novel treatments for brain injury because animals can be tested in the acute posttraumatic period.^{58,60} In an attempt to model posttraumatic learning deficits accurately and to evaluate the long-term cognitive outcomes associated with experimental brain injury, other investigators have used MWM learning paradigms similar to the one used in the current study.^{20,21,34,45,46} In these studies, learning deficits in rats subjected to fluid-percussion brain injury have been demonstrated months after injury.^{20,46} In the present study NGF administration significantly improved the ability of animals to learn a new task after traumatic brain injury (TBI).

Loss of ipsilateral cortical and hippocampal CA3 pyramidal neurons is the most striking histological change in rodents undergoing lateral fluid-percussion brain injury.^{3,23} The identification of bilateral hippocampal cell loss in the region of the dentate hilus also suggests a potential mechanism underlying posttraumatic memory dysfunction in this model.⁵⁹ How bilateral hippocampal damage is produced in this model remains an important

mechanistic question. After the observation of the loss of cholinergic cells in the medial septal region after fluid-percussion brain injury in the rat, Leonard, et al.,³¹ and Schmidt and Grady⁵² have proposed that these cognitive deficits may be, in part, the result of damage to the septo-hippocampal pathway. Because other models of CNS injury have shown that axotomized cholinergic neurons in the medial septal region can be salvaged by administration of NGF,¹⁹ our previous studies demonstrating improved cognition after fluid-percussion brain injury in rats receiving NGF infusions suggested that improvements in cognition were the result of NGF effects on cholinergic medial septal neurons.^{57,58} In our model NGF may reach sites remote from the infusion/injury site via the ventricular system.

To our knowledge, the present study is the first to correlate neuronal loss in the septal nucleus after fluid-percussion brain injury with posttraumatic cognitive dysfunction. We have also demonstrated the ability of NGF to attenuate this neuronal loss significantly and improve cognitive outcome. These results occurred 2 weeks after the termination of NGF infusions, suggesting that treatment with NGF may induce permanent changes. The results of our detailed histological analysis further indicate that the mechanism for this improvement may be an NGF-induced rescue of septal cells that would otherwise undergo apoptotic cell death. In addition, NGF can induce significant sprouting in cholinergic neurons.^{14,68} Remodeling of hippocampal afferents and reduction of septal cell loss may contribute to the observed functional improvements.

Cognitive Outcomes in Human Brain Injury

An increased understanding of the biomechanics of human head injury has provided valuable information about the relationship between diffuse axonal injury and poor cognitive outcomes.^{1,17,64} However, the paucity of histopathological specimens from human patients who have

well-defined posttraumatic cognitive deficits has made the correlation of neuronal damage and specific types of cognitive dysfunction difficult. Whereas bilateral hippocampal injuries have been shown to result in significant memory disturbances in humans,^{54,69} there is evidence that septohippocampal cholinergic pathways may be as important. In a series of classic monographs on the pathology of human head injury, Strich⁶³⁻⁶⁵ noted that the presence of axonal degeneration in the fornices is a common sequela of TBI. In a review of a group of head-injured patients who developed posttraumatic dementia, Hillbom and Jarho²⁴ observed that lesions causing memory disturbances are always bilateral and damage the limbic system and its connections with the frontal lobes. Damage to the fornices from nontraumatic mechanisms (surgical disruption or tumor infiltration) also results in memory disturbances in humans.⁹ This further implicates the septohippocampal pathway as a potential area in human head injury which, when damaged, may result in cognitive deficits.

More recently, associations between Alzheimer's disease and head trauma have been identified, including the suggestion that patients with the apolipoprotein-e4 allele have a much greater risk of Alzheimer's disease if they also have a history of traumatic head injury.^{38,39,51} The loss of cholinergic neurons in Alzheimer's disease is well established^{5,12} and has even resulted in a clinical trial of NGF administration as a treatment for Alzheimer's disease.^{43,55} Initial reports do not appear to show dramatic improvement in the first patient described from this series.⁵⁵ The similarities between the pattern of neuronal loss in Alzheimer's disease and that seen in experimental brain injury also support the hypothesis that the loss of cholinergic septal neurons contributes to the memory dysfunction observed in both of these disease processes. However, whereas Alzheimer's disease is a chronic, progressive ailment, there is no evidence to suggest that the neuronal injury that results in posttraumatic cognitive dysfunction continues over a similar time course of months and years. Therefore, the possibility that a therapeutic regimen of NGF treatment after TBI could lead to more lasting improvements should be studied further.

Septal Cell Death in TBI

Programmed cell death is a transcription-dependent event that occurs normally during neural development.^{25,33} Apoptosis is the most common form of programmed cell death.²⁷ Apoptosis occurs in various pathological states including: ischemia,^{10,35,37} retinitis pigmentosa,⁴⁹ Huntington's disease,⁴⁸ and fluid-percussion brain injury.⁵⁰ Apoptotic cell death has recently been described as occurring during cycles of recurrence and remission in human lymphoma.²⁶ Withdrawal of NGF from neuronal cultures is a useful in vitro model of apoptosis that has demonstrated that these neurons can be rescued by the reapplication of NGF to the culture medium.^{6,47} The present study is the first to demonstrate that traumatically induced apoptotic cell death in the septal nuclei occurs in vivo and can be attenuated with NGF treatment.

The improvements in cognition and ChAT staining seen after fluid-percussion brain injury and NGF infusion parallel the effects of NGF in models of fimbrial transec-

tion.^{22,30,66,67} In these models it has been shown that the apparent loss of cholinergic septal neurons can be reversed by treatment with NGF, even when initiation of treatment was delayed.^{11,18,62} This indicates that neurons in this model may not die but simply atrophy and stop staining for ChAT. In contrast, the loss of ChAT-positive cells observed in the fluid-percussion model of brain injury appears to be the result of neuronal death that would not respond to treatment months or years postinjury.

Conclusions

These data support the hypothesis that damage to the septohippocampal pathway results in cognitive deficits in the clinically relevant model of fluid-percussion brain injury in the rat. Infusion of NGF beginning 24 hours postinjury results in significant improvements in posttraumatic learning deficits, and these benefits persist after cessation of the NGF treatment. This model of NGF infusion into animals undergoing TBI suggests that neurotrophin therapy may be a useful option for the prevention of clinical posttraumatic cognitive deficits. Further evaluation of this hypothesis is warranted.

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Address reprint requests to: Tracy K. McIntosh, Ph.D., Division of Neurosurgery, University of Pennsylvania School of Medicine, 3400 Spruce Street, Philadelphia, Pennsylvania 19104–4283.