European Review for Medical and Pharmacological Sciences 2024; 28: 4302-4312

Intranasal human-recombinant nerve growth factor administration improves cognitive functions in a child with severe traumatic brain injury

L. CAPOSSELA[1], B. GRAGLIA[1], S. FERRETTI[1], L. DI SARNO[1], A. GATTO[2], M.L. CALCAGNI[3], D. DI GIUDA[3], F. COCCIOLILLO[4], D.M. ROMEO[5], L. MANNI[6], M. SOLIGO[6], S. STACCIOLI[7], E. NAPOLI[8], A. CHIARETTI[7]

[1]Institute of Pediatrics, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica Sacro Cuore, Rome, Italy
[2]Institute of Pediatrics, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy
[3]UOC di Medicina Nucleare, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy
[4]UOC di Medicina Nucleare, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy
[5]Unità di Neurologia Pediatrica, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy
[6]Istituto di Farmacologia Traslazionale, Consiglio Nazionale delle Ricerche (CNR), Rome, Italy
[7]Dipartimento di Neuroriabilitazione Intensiva, Ospedale Pediatrico "Bambino Gesù", Rome, Italy
[8]Department of Neurosciences, Unit of Neurorehabilitation, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

ABSTRACT. – BACKGROUND: Behavioral and neuropsychological functions are frequent long-term sequelae of severe traumatic brain injury (TBI). Neuropeptides, such as nerve growth factor (NGF), can enhance neurogenesis and improve cognitive functions after TBI, playing a pivotal role in neuroplasticity. A limited number of studies documented the safety and efficacy of intranasal NGF administration in children with severe TBI.

CASE REPORT: A fourteen-year-old boy with a diffuse axonal injury secondary to severe TBI was treated with human-recombinant NGF administration. This patient underwent treatment with intranasal hr-NGF administration at a total dose of 50 gamma/kg, three times a day for seven consecutive days. The treatment schedule was performed for 4 cycles, at one month distance each. NGF administration improved radiologic functional assessment evaluated with positron emission tomography scan (PET) and single photon emission computed tomography (SPECT), with an important improvement in clinical conditions. Significant improvements were also observed, mainly in cognitive processes, memory, the planning of a communication strategy, execution skills, attention, and verbal expression. No side effects were reported.

CONCLUSIONS: Additional studies are required to gain a deeper insight into this neurotrophin's neuroprotective function, but our findings reveal a potential efficacy of intranasal hr-NGF administration in enhancing cognitive and clinical outcomes among children with diffuse axonal injury after severe TBI.

Key Words:
Nerve growth factor, Brain injury, Children, Diffuse axonal injury, Cognitive functions.

Introduction

The primary cause of disability and mortality among pediatric patients is traumatic brain injury (TBI)[1]. Some groups face a higher risk of experiencing long-term neurological issues following such injuries, including children aged 0 to 4 years and adolescents/young adults (15-24 years)[2]. The mechanical injury leads to disruptions in ion balance, excitotoxicity, and cell depolarization, along with damage to endothelial tissue and the release of proinflammatory chemokines and cytokines. Additionally, there is mitochondrial damage, resulting in calcium buildup, energy depletion, and the release of oxygen species. These processes lead to demyelination, axonal degeneration, and protein aggregation. These cerebral reactions contrib-

Corresponding Author: Antonio Chiaretti, MD; e-mail: antonio.chiaretti@policlinicogemelli.it

ute to significant brain inflammation and reduced cerebral blood flow, resulting in hypoglycemia, hypoxia, and blood-brain barrier dysfunction[3]. Furthermore, secondary brain injury, caused by reperfusion, oxidative stress, edema, and ischemia, often results in neuronal loss and progressive impairment of motor and cognitive functions[3,4].

Behavioral and neuropsychological impairments are common long-term consequences of severe TBI in children and young adults[5]. Currently, there is limited evidence regarding effective therapies for neuronal restoration following TBI[6]. However, some studies suggest that neurotrophic factors may promote neurogenesis and improve cognitive functions post-TBI, playing a crucial role in neuroplasticity[7,8]. Specifically, nerve growth factor (NGF) has been shown to reduce neuroinflammation, protein aggregation, and mitochondrial dysfunction while enhancing angiogenesis and protecting oligodendrocytes[9]. Its neuroprotective effects have been demonstrated in both animal models and human studies, with promising results from intranasal NGF delivery in children with severe neurological sequelae following conditions such as meningitis, chronic vegetative state after cardiac arrest, and unresponsive wakefulness syndrome post-TBI[10-13]. Intranasal administration offers a safe and non-invasive delivery method, ensuring effective NGF concentration in specific brain areas[14]. Based on these encouraging findings, we present a case study of a child with neurological sequelae after severe TBI who underwent intranasal administration of human-recombinant NGF (hr-NGF).

Case Presentation

A fourteen-year-old boy was admitted to the emergency room and then to the Pediatric Intensive Care Unit for severe TBI secondary to a car accident. He immediately underwent tracheal intubation and sedation. A total body computed tomography (CT) scan showed a pneumoencephalus in the frontal left side with multiple hemorrhagic petechiae in frontal and parietal areas with loss differentiation in grey and white matter. Brain MRI confirmed cortico-subcortical hemorrhagic foci also involving the corpus callosum, corona radiata, mesencephalon, pons, and right basal ganglia, confirming a diffuse axonal injury (DAI). An intracranial pressure monitoring was carried out through sensor positioning. Additionally, computed tomography scans revealed maxillofacial trauma, including fractures of the left zygomatic-orbital complex and mandible. These injuries were treated with emergency surgery, with no alteration in ocular alignment. Post-traumatic sinus venous thrombosis was developed and treated with Enoxaparin.

Somatosensory evoked potentials (SSEP) performed in acute phase showed bilateral absence of N20 responses from cortical median nerve. Auditory evoked potentials (AEPs) that assess specific areas of the brainstem, basal ganglia, and auditory cortices revealed a normal signal for right side auditory stimulus and abnormal signal in III-IV peaks for left side auditory stimulus. Visual evoked potential showed increased bilateral stimulus latency. EEG recording showed a general slowing of electrical activity in both hemispheres, with good reactivity to stimulations.

Fifteen days after TBI, the child was admitted to the Neurorehabilitation Unit to receive neurorehabilitation treatment and medical care. The duration of inpatient rehabilitation was 2 months. The neurological examination at the admission revealed an alert, mild alteration of consciousness (Glasgow coma scale 11), good motor strength (better on the right than on the left side), and chaotic motility but adequate response to painful stimuli. Spontaneous eye opening, symmetric pupil size with partial reactivity, and divergent strabismus in the right eye due to superior rectus muscle hypofunction were reported. Verbal communication was absent, revtaealing an aphasic disorder with moments of agitation.

The child progressively improved his state of consciousness in a few days after the treatment. His functions progressively stabilized, with normal blood pressure, heart rate, and respiratory function recovery. Bowel and bladder functions were restored. During his stay in an inpatient rehabilitation setting, the therapeutic program included physical therapy, speech therapy for feeding and for language, occupational therapy, and serial orthoptics evaluations. Given the improved oral motor skills noted by the speech therapist, the nasogastric tube was removed, maintaining caution regarding solid foods. One month after the admission he can control the trunk and stays sit alone. Functional measures through gross motor function measure (GMFM) performed one week following the admission and at discharge revealed respectively 11% and 98% scores, while self-care assessment through functional independence measure (WeeFim) revealed 14.28% and 95.23% scores confirming a good self-care level achieved.

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Fifteen days after admission, a speech and language therapy assessment was carried out, which showed reduced verbal initiative, incorrect answers to some questions, and attention problems. In inpatient rehabilitation, a progressive recovery of aphasic disorder and orientation was observed. At discharge, he claimed oriented and correct responses to questions. To guarantee a natural rehabilitation, participation also in his usual context, as a hospital school, was provided.

EEG recording at discharge showed good background activity in both hemispheres without epileptic abnormalities. The somatosensorial evoked potential was finally normal. Orthoptics evaluation disclosed a complete recovery of binocular vision in some gaze positions (up and right), and transient diplopia persisted.

A CT scan at the end of admission showed a small flap of subdural hematoma, which completely disappeared at the MRI exam. This is mainly related to mesencephalic involvement, the absence of N20 response at SSEP in the acute phase, persistence of inhibition, slow execution abilities, and other neuropsychological functions. He was selected for NGF treatment, and a cycle of outpatient rehabilitation was prescribed.

Intranasal Human-Recombinant NGF (hr-NGF) Administration

Four months post-TBI, due to the enduring cognitive dysfunction, the possibility of initiating therapy involving the intranasal administration of human recombinant NGF was contemplated. Parents were asked to sign written informed consent before starting the therapy. The study was approved by the Ethics Committee (approval No. 5169/20, ID 2989) of the Fondazione Policlinico Universitario Agostino Gemelli – IRCCS, Rome, Italy. This study is also a part of a registered clinical trial (EudraCT number 2019-002282-35). The child underwent intranasal administration of hr-NGF (Oxervate by Dompè Farmaceutici, Milan, Italy) in four cycles of seven days each.

Intranasal clinical grade hr-NGF aqueous solution was administered at a total dose of 50 gamma/kg. This amount is considered sufficient to reach and stimulate NGF receptors, mainly Tyrosine receptor kinase A (TrkA), in most cerebral cholinergic and serotoninergic areas of the brain, as previously reported in the literature[15,16].

The product used was Oxervate, consisting of cenegermin-bkbj (hr-NGF) ophthalmic solution 0.002% (20 µg/mL) (Dompè Farmaceutici SpA, Milano, Italy). The vial of Oxervate was stored at 4°C until its usage. The total dose of hr-NGF (50 µg/kg) was divided into four cycles of treatment. In each cycle, ¼ of the total dose was distributed in 21 doses, and each dose was divided into 2 nostrils and administered 3 times a day for 7 consecutive days. The cycles of administration started on days 1, 31, 61, and 91 of treatment. In this way, the dose and final volume of each administration were calculated according to the patient's weight. MAD Nasal™ (Intranasal Mucosal Atomization Device from Teleflex – MAD100, Version Model Number IPN048826) was used to deliver hr-NGF without any process of dilution or lyophilization via a fine spray (30 microns) to facilitate its absorption by olfactory and trigeminal nerve fibers.

Prior to administering NGF, the nostrils were rinsed with 1 mL of saline solution and then gently suctioned to prevent any potential interference with drug absorption.

Results

Neurological Assessment

A comprehensive and detailed examination of cognitive, neuropsychological, and academic aspects was performed by the patient 2-4 months post-TBI after the inpatient rehabilitation. The same neuropsychological assessment was administered to the patient one month after the four cycles of intranasal NGF, approximately 6 months following the initial evaluation.

The Wechsler Intelligence Scale for Children was used to assess cognitive functioning. The Wisconsin card sorting test was utilized to measure set-shifting, cognitive flexibility, and perseverative responses. Executive attention and control were examined using the continuous performance test-III. The NEPSY-II evaluated auditory attention, vigilance, the ability to shift and maintain response sets, inhibition of automatic responses, visuospatial memory, verbal and learning memory, and narrative memory. Reading, writing, and orthographic competence were assessed with the MT-2 test, while math skills were evaluated using the advanced MT-2 test.

Table I and Table II show results obtained by the patient in the specific domains before NGF treatment compared to the scores obtained after NGF treatment[17-21]. Additionally, post-NGF treatment evaluation included an assessment of adaptive functioning using the VINELAND-II interview and an evaluation of the ability to integrate motor and visual skills through the administration of the

Table I. Specific domain's results at baseline and follow-up.

Task	Skills investigated	Domains	Scores pre NGF treatment	Scores post NGF treatment
Wechsler intelligence scale for children[17]	Cognitive functioning	Full scale intelligence quotient (FSIQ) (M=100±15)	81	**95**
		Verbal comprehension index (VCI) (M=100±15)	84	84
		Perceptual reasoning index (PRI) M=100±15)	108	106
		Working memory index (WMI) M=100±15)	88	**103**
		Processing speed index (PSI) M=100±15)	56	**76**
Wisconsin card sorting test[18]	Set-shifting, cognitive flexibility and perseverative response	Percent errors (T score; M=50)	58	unavailable
		Percent perseverative response (T score; M=50)	39	
		Percent perseverative errors (T score; M=50)	47	
		Percent non-perseverative errors (T score; M=50)	67	
Continuous performance test-III[19]	Executive attention and control	Detectability (T score; M=50±10)	35	34
		Omission (T score; M=50±10)	43	46
		Commission (T score; M=50)	40	32
		Perseveration (T score; M=50±10)	45	45
		HRT (T score; M=50±10)	61	**74**
		HRT SD (T score; M=50±10)	48	49
		Variability (T score; M=50±10)	42	44
		HRT block change (T score; M=50±10)	51	40
		HRT ISI change (T score; M=50±10)	55	58

NGF: nerve growth factor.

Developmental Test of Visual Motor Integration. Table III presents the obtained results[22,23].

Positron Emission Tomography Scan results (Details Regarding the Protocol Employed in the Supplementary Materials)

The first Positron Emission Tomography/Computed Tomography (PET/CT) study was performed four months after the head injury and 2 days before the beginning of intranasal NGF administration. The second PET/CT assessment was repeated one month after the end of the treatment. At visual analysis, the first PET/CT study showed a moderate reduction in uptake of the metabolic tracer in correspondence with the mesencephalic structures. It was associated with an asymmetrical uptake of the radiopharmaceutical in correspondence with the right thalami, which was less than the left. There was a slight reduction in metabolic tracing in the left mesial temporal cortex and a further slight reduction in radiopharmaceutical uptake in the bilateral cerebellum. In conclusion, it showed a mild reduction of 18F-FDG

Table II. Specific domain results at baseline and follow-up.

NEPSY-II[20]	Auditory attention, vigilance and ability to shift and maintain set of response	Auditory attention (percentile rank)	6-10°	26-50°
		Response set (percentile rank)	11-25°	**51-75°**
	Inhibition of automatic response	Denomination (combined scaled score)	6	10
		Inhibition (combined scaled score; M=10±3)	2	9
		Shifting (combined scaled score; M=10±3)	5	9
	Visuo-spatial memory	Memory for design (scaled score; M=10±3)	6	9
		Memory for designs delayed (scaled score; M=10±3)	1	**10**
	Verbal and learning memory (short and long term)	Memory for list (scaled score; M=10±3)	3	3
	Narrative Memory	Memory for list delayed (scaled score; M=10±3)	1	1
(pre-NFG) MT-2 test (post-NFG) advanced MT-2[21]	Reading[§]	Comprehension (percentile rank)	<5°	<5°
		Speed (percentile rank)	<5°	**5-10°**
		Decoding accuracy (percentile rank)	10°	**50°**
(pre-NFG) learning test for writing and orthography (BVSCO-2); (post-NFG) MT-2 test[21]	Writing and of orthographic competence[§]	Orthographic competence (percentile rank)	<5°	**50°**
(pre-NFG) learning test for calculation in developmental age (BDE-2) (post-NFG) advanced MT-2 test[21]	Math skills[§]	General number knowledge (quotient; M=100±15)	<49	unavailable
		Calculation (quotient; M=100±15)	<49	unavailable
		Number Sense (quotient; M=100±15)	<49	unavailable
		Mental calculation test (percentile)	unavailable	40°
		Mental calculation time (percentile)	unavailable	30°
		Arithmetical facts (percentile)	unavailable	10°

[§]Academic abilities were evaluated from pre- and post-NGF treatment with different tools since the boy, after hospitalization, began a new school cycle (from middle school to high school). NGF: nerve growth factor.

Table III. Vineland-II interview and visual-motor integration test.

Tasks	Domains	Scores
Vineland-II interview[22]	Communication (deviation quotient; M=100±15)	84
	Daily living skills (deviation quotient; M=100±15)	97
	Socialization (deviation quotient; M=100±15)	98
	Adaptive behavior composite (deviation quotient; M=100±15)	91
VMI test (visual motor integration)[23]	Visual task (standard score; M=100±15)	105
	Motor task (standard score; M=100±15)	85
	Visual motor integration (standard score; M=100±15)	96

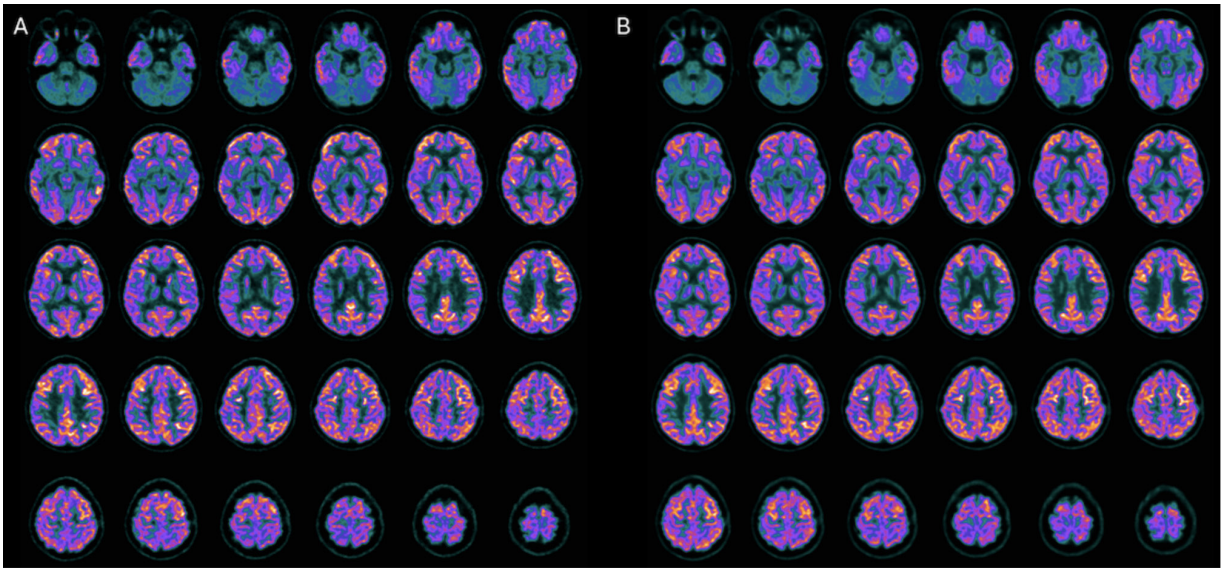

Figure 1. 18F-FDG PET brain axial slices performed before (**A**) and after (**B**) NGF treatment. **A**, Mild reduction of 18F-FDG uptake in bilateral parietal cortex, subcortical regions, and a slightly more severe reduction in cerebellum. **B**, After the NGF administration, an increase of the radiotracer uptake in the bilateral parietal cortex (right: +7%; left: +6%), right and left caudate nucleus (right: +3%; left: +5%), and cerebellum (+6%) was observed.

uptake in the bilateral parietal cortex and subcortical regions and a slightly more severe reduction in the cerebellum (Figure 1A).

After one month from the last cycle with intranasal NGF administration, PET-TC showed an increase in radiotracer uptake in the bilateral parietal cortex (right: +7%; left: +6%), as well as in the right and left caudate nucleus (right: +3%; left: +5%), and the cerebellum (+6%) (Figure 1B).

Single photon emission computed tomography results (Supplementary Materials)

The timing of execution of SPET/CT assessments was the same as the PET/CT studies. Fig-

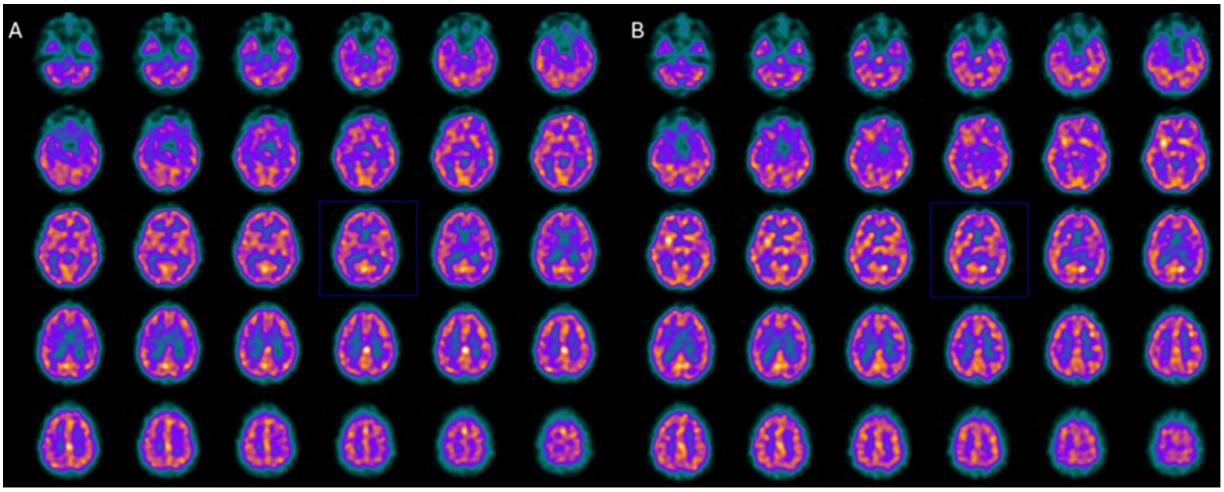

Figure 2. Perfusion SPECT images before (**A**) and after (**B**) hr-NGF administration. **A**, ^{99m}Tc-HMPAO SPECT images (transaxial slices) before hr-NGF treatment showed a mild reduction in radiotracer uptake (hypoperfusion) in the right and left caudate nucleus, right putamen, right and left thalamus. **B**, After hr-NGF treatment, a slight increase in ^{99m}Tc-HMPAO uptake was detected in the right caudate nucleus (+8%), left caudate nucleus (+7%), right putamen (+10%), right and left thalamus (+8%, respectively).

ure 2 shows the evolution of cerebral perfusion before and after the intranasal NGF administration. The baseline SPECT/CT scan (Figure 2A) revealed a mild reduction in radiotracer uptake (hypoperfusion) in the right and left caudate nucleus, right putamen, and right and left thalamus.

In the second SPECT/CT scan after one month of the last cycle of NGF treatment (Figure 2B), it was evaluated a slight increase in ^{99m}Tc-HM-PAO uptake in the right caudate nucleus (+8%), left caudate nucleus (+7%), right putamen (+10%), right and left thalamus (+8%, respectively).

EEG Evaluation

EEG examinations performed before intranasal NGF administration showed a normal electro-physiological rhythm. This exam was repeated after one month of the last cycle of NGF treatment, confirming a normal rhythm.

Discussion

In this study, we report the effects of treatment with intranasal hr-NGF in a fourteen-year-old boy with a diffuse axonal injury caused by a severe TBI. Diffuse axonal injury (DAI) has emerged as a widespread and significant pathological feature of traumatic brain injury. Axons in the white matter are particularly prone to harm due to the mechanical forces applied to the brain during TBI. As a result, DAI has been observed across all degrees of TBI severity and might potentially function as a primary pathological foundation for mild TBI (concussion). From a pathological perspective, DAI covers a variety of irregularities, beginning with the initial mechanical disruption of the axonal cytoskeleton, followed by transportation, swelling, and proteolysis disruptions, ultimately leading to secondary physiological changes. Depending on the seriousness and scope of the injury, these alterations can immediately present as loss of consciousness or confusion and persist as coma and/or cognitive dysfunction[24].

The continued occurrence of negative consequences years after the incident, despite improvements in cardio-pulmonary resuscitation techniques, led the American Heart Association to identify brain injury as a crucial focus for both clinical and experimental investigation. Presently, there are no effective treatments or medications accessible to improve neurological outcomes in these patients[25].

Despite receiving innovative and current critical, neuro-intensive, and rehabilitative care, our patients exhibited only minimal neurological improvement. Therefore, we decided to begin an experimental therapy using intranasal hr-NGF. The extensive involvement of NGF's neuroprotective mechanisms in both the central and peripheral nervous systems has been considerably documented, particularly concerning neuronal damage resulting from severe TBI[16].

In the past, delivering neurotrophic factors to the brain has been a significant challenge because of the blood-brain and blood-cerebrospinal fluid barriers, which impede the entry of drugs into the central nervous system (CNS). Accumulated knowledge suggests the presence of a direct route from the nasal cavity to the brain, allowing for drug delivery into the CNS. While the precise mechanisms are not fully comprehended, emerging evidence indicates that these pathways include olfactory nerve endings, trigeminal nerve fibers, as well as vascular and lymphatic routes, facilitating the active transport of NGF into the CNS[26]. In experimental animal models, the intranasal administration of NGF has shown various effects following severe TBI. These include reducing amyloid β42 (Aβ42) deposits and brain edema, suppressing the transcription and expression of pro-inflammatory cytokines, decreasing mitochondria-mediated apoptosis, and lowering TBI-induced elevation of interleukin 1β (IL-1β). As a consequence, this significantly increases neurological outcomes in injured brains[27].

This novel therapeutic intervention resulted in significant enhancements in functional outcomes, as indicated by PET/CT and SPECT/CT results, accompanied by a simultaneous improvement of the patient's clinical and neurological conditions. In fact, before this treatment, the boy showed slow execution abilities and cognitive, memory, and attention problems. By analyzing the clinical and neurological scores emerging from pre- and post-NGF treatment cognitive assessments, it is possible to highlight a significant improvement in the aspects of processing speed and working memory capacity. Going into detail about neuropsychological skills, we can see more accurate scales in the test that evaluate executive attention and control, even if in a more conservative response style. It is also possible to emphasize better performances in tests of auditory attention, the ability to inhibit automatic responses, and visuospatial memory. Regarding academic skills, better decoding, reading, and spelling skills were observed. From the

interview on adaptive skills, we noted skills in line with age expectations both in terms of daily living and socialization.

This result correlates well with the increased radiotracer uptake in glucose metabolism in the bilateral parietal cortex, right and left caudate nucleus, and cerebellum, and with the mild increased vascularization of the mesial temporal cortex documented by PET and SPECT, respectively, after the NGF treatment. In this context, it is important to emphasize that the mesial temporal lobe (MTL) is widely recognized as essential for memory function. Prior studies[28,29], which leveraged a thorough anatomical delineation of MTL structures and a meticulous examination of memory-related phenomena, have endeavored to delineate the distinct role of MTL structures in brain plasticity and the regulation of human behavior. The distinctiveness of MTL contributions to long-term memory has been demonstrated by the preservation of short-term memory and non-mnemonic perceptual, motor, and cognitive abilities[30]. Current anatomical investigations reveal that the cerebellum's output extends to various nonmotor regions in the prefrontal and posterior parietal cortex, in addition to cortical motor areas. The breadth of tasks associated with cerebellar activation is noteworthy, encompassing assessments of attention, executive control, language, working memory, learning, pain processing, emotion regulation, and addiction-related behaviors[31,32]. PET and SPECT investigations have documented both changes at the level of the two aforementioned structures. It is now recognized that severe TBI triggers a cascade of regenerative processes that may persist over several weeks[33]. The improvement of children's neurological conditions may be partly attributed to the intrinsic physiological recovery following TBI. Various adaptive mechanisms likely initiate shortly after the injury, resulting in neuroplastic changes that aid in the restoration of certain neurological functions. Post-TBI recovery seems to depend significantly on oligodendrogenesis, the generation of new oligodendrocytes[34]. Oligodendrocytes fulfill various roles in the healthy brain, with their primary function being the production of myelin around axons, thereby supporting their essential role in post-TBI neuroplasticity and circuit function[35]. The interplay between axons and oligodendrocytes governs the velocity of signal propagation in a manner contingent on activity, underscoring the role of oligodendrocytes in augmenting adaptability. Thus, it is plausible that our patient's neurological amelioration arises partly from this innate recuperative mechanism, although gauging its magnitude would pose a challenge[33]. Our patient's significant neuroradiological and clinical changes over a relatively brief period are likely attributed more to NGF treatment than to spontaneous recuperation. Additional investigations conducted by our group have examined the impact of intranasal hr-NGF on individuals enduring extensive brain damage following severe brain injury. Primarily, these studies have highlighted improvements in motor functions, characterized by a notable decrease in muscle hypertonicity and spasticity, illustrating the crucial role of NGF in striatal cholinergic dysfunction[11,12].

In this patient, we want to underline the important results obtained in the field of higher cognitive functions. Indeed, after NGF treatment, significant improvements were observed mainly in cognitive processes, memory, in the planning of a communication strategy, execution skills, attention, and verbal expression. Finally, we would like to underline that on a qualitative level both the child and the parents report a substantial general improvement in performance and reactivity in everyday life.

Conclusions

The administration of intranasal NGF shows promise as a safe and effective intervention for treating children with neurological sequelae resulting from severe TBI. Our results are promising and expected to pave the way for future clinical studies aimed at evaluating the potential effectiveness of intranasal hr-NGF administration in improving cognitive and clinical outcomes in children with diffuse axonal injury. This may serve as an initial phase in the development of a larger-scale clinical program aimed at assessing the potential efficacy of intranasal NGF administration in enhancing neurological outcomes and clinical functions among children with severe TBI. The initial results outlined, and the ease of drug delivery justify further exploration, especially in patients with TBI with initially more favorable neurological conditions, to better understand the advantages of NGF in cerebral function recovery. While further controlled, randomized, double-blind studies are needed to clarify the neuroprotective mechanisms of this neurotrophin, intranasal NGF administration emerges

as a promising and safe therapeutic approach for children with neurological sequelae resulting from severe head injury.

Our report signifies the first documented case of a non-invasive treatment method with positive impacts on cognitive cerebral functions in a child with DAI.

Conflict of Interest
The authors declare that they have no conflict of interest.

Acknowledgments
The authors thank Dompè Farmaceutici Spa (Milan, Italy) for the technical support provided during all stages of this study.

Funding
This work has been funded by the Italian Ministry of Health, Grant RF-2018-12366594, "Nerve Growth Factor in pediatric severe traumatic brain injury: translational and clinical studies on a candidate biomarker and therapeutic drug." Clinical grade hr-NGF was freely provided by Dompé Farmaceutici Spa, Milan (Italy).

Ethics Approval
The study was approved by the Ethics Committee (approval No. 5169/20, ID 2989) of the Fondazione Policlinico Universitario Agostino Gemelli, IRCCS, Rome (Italy).

Data Availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Informed Consent
Written informed consent was obtained from the patient's parents.

Consent for Publication
Written informed consent was obtained from the parents of the patient for his anonymized information to be published in this article.

ORCID ID
Lavinia Capossela: 0000-0001-9771-1949
Benedetta Graglia: 0009-0004-1441-7584
Serena Ferretti: 0000-0001-6479-1540
Lorenzo Di Sarno: 0000-0002-4837-9297
Antonio Gatto: 0000-0002-8778-9328
Maria Lucia Calcagni: 0000-0002-0805-8245
Daniela Di Giuda: 0000-0002-5758-3986
Fabrizio Cocciolillo: 0000-0002-5655-1448
Domenico Marco Romeo: 0000-0002-6229-1208
Luigi Manni: 0000-0001-7844-6836
Marzia Soligo: 0000-0002-1420-0914
Susanna Staccioli: 0000-0001-6327-770X
Eleonora Napoli: 0000-0002-1436-2825
Antonio Chiaretti: 0000-0002-9971-1640

Authors' Contributions
LC, BG, SF and LDS researched the literature and wrote the initial draft. AG, MLC, DDG, FC, DMM, LM and MS revised the manuscript. SS and EN supplemented the materials. AC developed the study design and conceptualization. All authors reviewed and agreed on the final manuscript.

AI Disclosure
The authors declare that they used AI software only for English editing and grammar checks in the preparation of this manuscript.

References

1) Olsen M, Vik A, Lund Nilsen TI, Uleberg O, Moen KG, Fredriksli O, Lien E, Finnanger TG, Skandsen T. Incidence and mortality of moderate and severe traumatic brain injury in children: A ten year population-based cohort study in Norway. Eur J Paediatr Neurol 2019; 23: 500-506.
2) Lumba-Brown A, Yeates KO, Sarmiento K, Breiding MJ, Haegerich TM, Gioia GA, Turner M, Benzel EC, Suskauer SJ, Giza CC, Joseph M, Broomand C, Weissman B, Gordon W, Wright DW, Moser RS, McAvoy K, Ewing-Cobbs L, Duhaime AC, Putukian M, Holshouser B, Paulk D, Wade SL, Herring SA, Halstead M, Keenan HT, Choe M, Christian CW, Guskiewicz K, Raksin PB, Gregory A, Mucha A, Taylor HG, Callahan JM, DeWitt J, Collins MW, Kirkwood MW, Ragheb J, Ellenbogen RG, Spinks TJ, Ganiats TG, Sabelhaus LJ, Altenhofen K, Hoffman R, Getchius T, Gronseth G, Donnell Z, O'Connor RE, Timmons SD. Centers for Disease Control and Prevention Guideline on the Diagnosis and Management of Mild Traumatic Brain Injury Among Children. JAMA Pediatr 2018; 172: e182853.
3) Mohamadpour M, Whitney K, Bergold PJ. The Importance of Therapeutic Time Window in the Treatment of Traumatic Brain Injury. Front Neurosci 2019; 13: 07.
4) Mattson MP, Scheff SW. Endogenous neuroprotection factors and traumatic brain injury: mechanisms of action and implications for therapy. J Neurotrauma 1994; 11: 3-33.

5) Treble-Barna A, Zang H, Zhang N, Taylor HG, Yeates KO, Wade S. Long-Term Neuropsychological Profiles and Their Role as Mediators of Adaptive Functioning after Traumatic Brain Injury in Early Childhood. J Neurotrauma 2017; 34: 353-362.
6) Di Sarno L, Curatola A, Cammisa I, Capossela L, Eftimiadi G, Gatto A, Chiaretti A. Non-pharmacologic approaches to neurological stimulation in patients with severe brain injuries: a systematic review. Eur Rev Med Pharmacol Sci 2022; 26: 6856-6870.
7) Lin PH, Kuo LT, Luh HT. The Roles of Neurotrophins in Traumatic Brain Injury. Life (Basel) 2021; 12: 26.
8) da Silva Meirelles L, Simon D, Regner A. Neurotrauma: The Crosstalk between Neurotrophins and Inflammation in the Acutely Injured Brain. Int J Mol Sci 2017; 18: 1082.
9) Manni L, Conti G, Chiaretti A, Soligo M. Intranasal nerve growth factor for prevention and recovery of the outcomes of traumatic brain injury. Neural Regen Res 2023; 18: 773-778.
10) Chiaretti A, Eftimiadi G, Buonsenso D, Rendeli C, Staccioli S, Conti G. Intranasal nerve growth factor administration improves neurological outcome after GBS meningitis. Childs Nerv Syst 2020; 36: 2083-2088.
11) Gatto A, Capossela L, Conti G, Eftimiadi G, Ferretti S, Manni L, Curatola A, Graglia B, Di Sarno L, Calcagni ML, Di Giuda D, Cecere S, Romeo DM, Soligo M, Picconi E, Piastra M, Della Marca G, Staccioli S, Ruggiero A, Cocciolillo F, Pulitanò S, Chiaretti A. Intranasal human-recombinant NGF administration improves outcome in children with post-traumatic unresponsive wakefulness syndrome. Biol Direct 2023; 18: 61.
12) Curatola A, Graglia B, Granata G, Conti G, Capossela L, Manni L, Ferretti S, Di Giuda D, Romeo DM, Calcagni ML, Soligo M, Castelli E, Piastra M, Mantelli F, Della Marca G, Staccioli S, Romeo T, Pani M, Cocciolillo F, Mancino A, Gatto A, Chiaretti A. Combined treatment of nerve growth factor and transcranical direct current stimulations to improve outcome in children with vegetative state after out-of-hospital cardiac arrest. Biol Direct 2023; 18: 24.
13) Chiaretti A, Conti G, Falsini B, Buonsenso D, Crasti M, Manni L, Soligo M, Fantacci C, Genovese O, Calcagni ML, Di Giuda D, Mattoli MV, Cocciolillo F, Ferrara P, Ruggiero A, Staccioli S, Colafati GS, Riccardi R. Intranasal Nerve Growth Factor administration improves cerebral functions in a child with severe traumatic brain injury: A case report. Brain Inj 2017; 31: 1538-1547.
14) Chen XQ, Fawcett JR, Rahman YE, Ala TA, Frey WH. Delivery of Nerve Growth Factor to the Brain via the Olfactory Pathway. J Alzheimers Dis 1998; 1: 35-44.
15) Jönhagen ME, Nordberg A, Amberla K, Backman L, Ebendal T, Meyerson B, Olson L, Shigeta M, Theodorsoon E, Viitanen M, Winblad B, Wahlund LO. Intracerebroventricular infusion of nerve growth factor in three patients with Alzheimer's disease. Dement Geriatr Cogn Disord 1998; 9: 246-257.
16) Chiaretti A, Antonelli A, Genovese O, Pezzotti P, Di Rocco C, Viola L, Riccardi R. Nerve growth factor and doublecortin expression correlates with improved outcome in children with severe traumatic brain injury. J Trauma 2008; 65: 80-85.
17) Wechsler D, Psychological Corporation. WISC-IV Integrated: Administration and Scoring Manual. 4th ed. Pearson Education, Inc; Psychological Corporation, 2004.
18) Heaton R, Chelune C, Talley J, Kay G, Curtiss G. Wisconsin Card Sorting Test Manual – Revised and Expanded. Psychological Assessment Resources, 1993.
19) Keith Conners C, Sitarenios G, Ayearst LE. Conners' Continuous Performance Test Third Edition. Encycl Clin Neuropsychol 2018; 929-933.
20) Kemp SL. NEPSY-II. Encycl Clin Neuropsychol 2011; 1732-1736.
21) Cornoldi C, Pra Baldi A, Friso G, Giacomin A, Giofrè D, Zaccaria S. MT avanzate - 2 : prove MT avanzate di lettura e matematica 2 per il biennio della scuola secondaria di 2. grado : manuale e guide all'uso. Giunti, 2010.
22) Sparrow SS, Cicchetti D V. Diagnostic uses of the Vineland Adaptive Behavior Scales. J Pediatr Psychol 1985; 10: 215-225.
23) Preda C. Test of visual-motor integration: construct validity in a comparison with the Beery-Buktenica Developmental Test of Visual-Motor Integration. Percept Mot Skills 1997; 84: 1439-1443.
24) Johnson VE, Stewart W, Smith DH. Axonal pathology in traumatic brain injury. Exp Neurol 2013; 246: 35-43.
25) Galgano M, Toshkezi G, Qiu X, Russell T, Chin L, Zhao LR. Traumatic Brain Injury: Current Treatment Strategies and Future Endeavors. Cell Transplant 2017; 26: 1118-1130.
26) Trevino JT, Quispe RC, Khan F, Novak V. Non-Invasive Strategies for Nose-to-Brain Drug Delivery. J Clin Trials 2020; 10: 439.
27) Rizzi C, Tiberi A, Giustizieri M, Marrone MC, Gobbo F, Carucci NM, Meli G, Arisi I, D'Onofrio M, Marinelli S, Capsoni S, Cattaneo A. NGF steers microglia toward a neuroprotective phenotype. Glia 2018; 66: 1395-1416.
28) Race E, Keane MM, Verfaellie M. Medial temporal lobe damage causes deficits in episodic memory and episodic future thinking not attributable to deficits in narrative construction. J Neurosci 2011; 31: 10262-10269.
29) Verfaellie M, Keane MM. Neuropsychological Investigations of Human Amnesia: Insights Into the Role of the Medial Temporal Lobes in Cognition. J Int Neuropsychol Soc 2017; 23: 732-740.
30) Gabrieli JDE, Brewer JB, Poldrack RA. Images of medial temporal lobe functions in human learning

and memory. Neurobiol Learn Mem 1998; 70: 275-283.

31) Strick PL, Dum RP, Fiez JA. Cerebellum and nonmotor function. Annu Rev Neurosci 2009; 32: 413-434.

32) Buckner RL. The cerebellum and cognitive function: 25 years of insight from anatomy and neuroimaging. Neuron 2013; 80: 807-815.

33) Nudo RJ. Recovery after brain injury: mechanisms and principles. Front Hum Neurosci 2013; 7: 887.

34) Wake H, Ortiz FC, Woo DH, Lee PR, Angulo MC, Fields RD. Nonsynaptic junctions on myelinating glia promote preferential myelination of electrically active axons. Nat Commun 2015; 6: 7844.

35) Takase H, Washida K, Hayakawa K, Arai K, Wang X, Lo EH, Lok J. Oligodendrogenesis after traumatic brain injury. Behav Brain Res 2018; 340: 205-211.