Neural stem cell transplantation rescues cortical damage in rhesus monkey models of traumatic brain injury

Shuyi Liu
Kunming University of Science and Technology - Lianhua Campus: Kunming University of Science and Technology
Wenjing Dong
Kunming University of Science and Technology - Lianhua Campus: Kunming University of Science and Technology
Tianzhuang Huang
Kunming University Faculty of Life Sciences and Technology
Yuyi Luo
Kunming University of Science and Technology - Lianhua Campus: Kunming University of Science and Technology
Rui Zhu
Kunming University of Science and Technology - Lianhua Campus: Kunming University of Science and Technology
Liping Shi
Kunming University of Science and Technology - Lianhua Campus: Kunming University of Science and Technology
Yongchang Chen
Kunming University of Science and Technology - Lianhua Campus: Kunming University of Science and Technology
Shangang Li
Kunming University of Science and Technology - Lianhua Campus: Kunming University of Science and Technology
zhengbo wang (wangzb@lpbr.cn)
Kunming University of Science and Technology https://orcid.org/0000-0002-4717-5137

Research Article

Keywords: Traumatic brain injury, neural stem cells, transplantation, rhesus monkeys, cortical repair

Posted Date: May 16th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2894729/v1

Abstract

Background

Cortical traumatic brain injury (TBI) is a major cause of cognitive impairment accompanied by motor and behavioural deficits. Cell transplantation is the most promising treatment, and how to keep the transplanted cells are not removed via cerebrospinal fluid (CSF) is the key problem.

Methods

In this study, we transplanted neural stem cells (NSCs) and simultaneously injected bFGF/EGF into the cortex (visual and sensory cortices) of rhesus monkeys with superficial TBI.

Results

The results showed that the transplanted NSCs did not enter the CSF and were confined to the transplantation site for at least one year. The transplanted NSCs differentiated into mature neurons that formed synaptic connections with host neurons, but glial scar formation between the graft and the host tissue did not occur.

Conclusions

This study is the first to explore the efficacy of transplanting NSCs into the superficial cerebral cortex of rhesus monkeys after TBI, and the results show the ability of NSCs to survive long term and differentiate into neurons, demonstrating the clinical translation potential of NSC transplantation for cortical TBI.

Introduction

Traumatic brain injury (TBI) is mainly caused by an external mechanical force that induces neuronal death at the site of injury, resulting in diffuse axonal injury (DAI). TBI can lead to motor and cognitive impairment and even to long-term physical disability[1–6]. The main treatments for TBI include hyperbaric oxygen therapy, non-invasive brain stimulation, task-oriented functional electrical stimulation and physiotherapy. However, none of these treatments can effective reverse neurological deficits[7–9]. Therefore, a number of studies assessing the effectiveness of stem cells in treating TBI have been conducted[10–12].

Neural stem cells (NSCs) have self-renewal ability and can differentiate into mature neurons[13–14]. Studies on the effectiveness of NSCs in treating TBI in rodents have shown that transplanted NSCs can survive and differentiate into neurons and then partially replace the damaged neurons[15–18], resulting in

improved motor function after TBI. These results suggest that NSC transplantation has potential as a clinical treatment of TBI. Our previous study showed that transplanted NSCs could survive and differentiate into functional neurons in the inferior colliculus, a deep brain region, in injured rhesus monkeys[19]. Further, bFGF/EGF was found to improve the survival and differentiation of transplanted cells in the rat brain[20]. In treating cortical injury via transplantation of NSCs, it is essential that the transplanted cells are not removed via the cerebrospinal fluid (CSF) and that they survive and differentiate into neurons at the site of injury.

In the current study, a model of superficial cortical injury was established, and NSCs were transplanted and bFGF/EGF was injected into the injury site to determine whether the transplanted NSCs could survive in the cerebral cortex, differentiate into neurons *in situ* and alleviate TBI.

Materials and Methods

LYON embryonic stem cell culture

GFP-labelled rhesus ESCs were incubated in ES medium consisting of Knockout DMEM, 20% KO-SR, 1% nonessential amino acids, 2 mM L-glutamine, 0.1 mM β-mercaptoethanol, and 10 ng/ml basic fibroblast growth factor (BFGF). The medium was changed daily, and undifferentiated colonies were mechanically passaged every 5–7 days using flame-drawn Pasteur pipettes. Lyon-ESCs were cocultured with mitotically incapable mouse embryonic fibroblasts (CF-1-MEFs; ATCC). CF-1-MEFs were first grown in DMEM containing 2 mM L-glutamine and 15% FBS. All cells were grown at 37°C in 5% CO_2.

Induction of NSC differentiation

Lyon-ESC colonies were first digested with 1 mg/ml neutral protease, washed with ES medium to remove the protease, and then suspended in modified N/M medium (50% DMEM/F12, 50% neural medium, 1× N2 supplement, 1× B27, and 2 mM L-glutamine) for the induction of NSC differentiation. Then, cells were seeded in 15 mm × 30 mm agar-coated wells. The cells were allowed to aggregate for four days to form embryoid bodies (EBs). EBs of uniform size were selected, seeded in extracellular matrix (ECM)-coated four-well plates and cultured in NP medium for 10–14 days until rosette formation. The NP medium consisted of DMEM/F12, 1 3ITS-x, 2 ng/ml heparin and 2 mM L-glutamine. Rosettes were purified, and the purified NSCs were identified by immunofluorescence. Neural rosettes were digested with trypsin (0.05% in 0.1% EDTA) to form a cell suspension, and the cells were counted. NSCs were suspended in PBS at a concentration of 107 cells per microlitre and placed on ice for subsequent cell transplantation.

Immunofluorescence analysis of NSCs

Rosettes were stained with Nestin, Tuj-1, MAP2, TH, and LMX1A antibodies after purification to check the purity of the NSCs. For immunofluorescence analysis of NSCs, cells were cultured on chamber slides,

fixed with 4% paraformaldehyde for 20 min, and permeabilized with 0.1% Triton X-100 for 15 min. They were then blocked with PBS containing 5% BSA for 1 hour at room temperature. The samples were incubated overnight at 4°C in blocking buffer (1% BSA) containing primary antibody. The samples were washed three times with PBS overnight for 5 min each, and the cells were incubated with the corresponding secondary antibody (1:200) for 2 hours at 37°C. The samples were washed with the same wash protocol after secondary antibody incubation and stained with DAPI. Stained samples were examined with an FV1000 fluorescence microscope (OLYMPUS).

Brain damage surgery

Four rhesus monkeys (rhesus monkeys, 4–6 years old, weighing 6.5–7.5 kg) were used in this study. All animal procedures complied with the Animal Welfare Act. Our previously developed MRI-guided localization technique with an accuracy of 0.5 mm was used to pinpoint the visual and sensory cortices. The animals anaesthetized using ketamine hydrochloride (10 mg/kg) and sodium pentobarbital (20 mg/kg). Then, the monkey head was fixed to a stereoscope, dental cement was used to fix MRI-visible glass tubes (tubes filled with water) to the skull, and MRI scans were used to locate the visual cortex and sensory cortex. According to the relative coordinates obtained by MRI, the visual cortex and sensory cortex were mechanically damaged. Mechanical injury resulted in a cavity at the injury location. A week after injury, GFP^+ NSCs were transplanted into the cavity, and the state of each monkey was continuously observed after surgery.

Identification of cells by immunofluorescence

One year after transplantation, the animals were sacrificed, and their brains were collected, fixed at 4°C for 4 hours and then dehydrated in a sucrose gradient (15%, 20%, and 30%) for cryoprotection. After dehydration, frozen sections were cut at a thickness of 20 μm. The sections were permeabilized with 0.2% Triton X-100 for 40 minutes and blocked with 5% BSA for 1 hour at room temperature. The tissues were permeabilized with 0.2% Triton X-100 for 40 minutes and blocked with 5% BSA for 1 hour at room temperature. The tissue sections were then incubated with primary antibody at 4°C overnight. The following day, the tissue sections were washed three times and incubated with secondary antibodies for 2 hours at 37°C. The transplanted cells were identified by GFP fluorescence. GFP + cells were then double stained with antibodies against MAP2, NeuN, TUJ-1, NF, TH, c-fos and glial fibrillary acidic protein (GFAP) for different purposes, and the transplanted cells were stained with DAPI. Fluorescence was observed using a fluorescence microscope.

Statistics

The NeuN, NF, GFAP and c-Fos staining data were analysed using ImageJ. A t test was performed with GraphPad Prism 5.1 (GraphPad Software). Significance was set at $p<0.05$.

Results

Survival and differentiation of grafted NSCs in the site of TBI one year after transplantation

NSCs, which were positive for NSC marker (Nestin) and negative for neural markers (MAP2 and Tuj-1), were differentiated from ESCs (Figs. 1A-D). The differentiation capacity of NSCs was assessed by staining for neural markers (MAP2 and Tuj-1), including markers for dopaminergic (DA) neurons (TH and GirK2) (Figs. 2A-B).

A rhesus monkey TBI model was created by injuring the visual and sensory cortices using the technique described in a previous study[19]. Nestin^{+}/SOX1^{+} NSCs combined with bFGF/EGF were delivered to the injury site seven days after brain injury (Fig. 1A).

Transplanted NSCs stayed in the injury site one year and showed a "U"-shaped structure in the macaques (Fig. 1B). Immunohistochemical studies were performed after cell transplantation, and the results showed that the transplanted NSCs could survive for at least one year (Fig. 1C-E). The neuronal identity of the surviving transplanted GFP + cells was then confirmed by staining for the classic neural markers Tuj-1, neurofilament (NF) and NeuN. As shown in Figs. 1C and D, GFP + cells fused with Tuj-1 + and NF + nerve fibres, and the mature neuron marker NeuN was also expressed in the nuclei of the GFP + cells. The results showed that the transplanted NSCs could differentiate into neurons (Fig. 1E).

The same experiment was performed in the sensory cortex. The density of GFP + cells was similar to that in the visual cortex (Fig. 2A), and the GFP + cells expressed Tuj-1. The GFP + cells also expressed NF (Fig. 2B), suggesting that some NSCs had differentiated neurons that formed into mature nerve fibres. NeuN was also expressed in the nuclei of the GFP + cells, as in the visual cortex (Fig. 2A-C). Statistical analysis showed that there was no significant difference in the expression of NeuN or NF between the visual cortex and the sensory cortex (Fig. 2D-E). These results demonstrated that the transplanted GFP + NSCs could survive for at least one year and differentiate into neurons in the sensory cortex and the visual cortex.

Activity and migration of the grafted NSCs

Although the majority of transplanted cells in the visual cortex and sensory cortex remained at the transplant sites (damage sites), some of the transplanted cells migrated away from the damage sites as individual cells or clusters at the border between the host brain tissue and the graft. c-Fos is an immediate early gene and proto-oncogene that is highly expressed in active cells and is useful for studying cellular activity in the brain. c-Fos is coexpressed with neuronal markers (NF, NeuN), and staining for c-Fos is used to determine whether neurons differentiated from transplanted NSCs are active[21]. Figure 3A shows that c-Fos was expressed in both GFP + and GFP- cells (host cells), and as shown in Fig. 3B and 3C, NF/Tuj-1-positive nerve fibres comprised GFP + and GFP- cells. The staining

pattern of c-Fos, NF and Tuj-1 did not differ between host neurons and GFP + neurons. statistical analysis showed that there was no significant difference in the expression of c-Fos between the visual cortex and the sensory cortex (Fig. 5D). These results indicated that transplanted NSCs could differentiate into neurons with cellular activity in both the visual cortex and sensory cortex.

Synapsin I, a major phosphoprotein found in synaptic vesicles and located in the presynaptic membrane, was used as a marker to identify presynaptic terminals[22]. Some presynaptic terminals of host neurons (Synapsin I^+/GFP^-) were identified within the graft (Fig. 4A). This result, taken together with the postsynaptic density protein-95 (PSD-95)[23] staining data, showed that a large number of host neuron-derived synapses were formed on differentiated neurons in the transplantation site (Fig. 4B), possibly providing an anatomical basis for functional integration between host neurons and transplanted NSCs.

The results of immunohistochemical staining in the sensory cortex were similar results to those of immunohistochemical staining in the visual cortex. The transplanted GFP + NSCs expressed c-Fos, Tuj-1, and NF (Fig. 5A-C). There was no clear border at the graft edge. The GFP + fibres were partially fused with the host tissue.

Overall, these immunohistochemical data showed that NSCs survived after transplantation, that the cells differentiated into mature neurons, and that there was potential functional integration between differentiated and host neurons.

Differentiation of grafted NSCs into astrocytes

In vitro, NSCs can differentiate into glial cells, which express specific markers of astrocytes (GFAP). Therefore, GFAP staining was also performed on graft tissue sections. Immunohistology showed that in both the visual cortex and sensory cortex, some GFP + cells expressed GFAP, indicating that some of the grafted NSCs differentiated into glial cells. However, no glial scar was observed at the edge of the injury site (Fig. 6A-B), and statistical analysis showed that there was no significant difference in the expression of GFAP between the visual cortex and the sensory cortex, as shown in Fig. 6C. These results suggested that the formation of connections between newborn neurons and host neurons was not impeded and demonstrate the feasibility of NSC therapy for TBI.

Differentiation of grafted NSCs into dopaminergic neurons

Our in vitro experiment and previous animal studies have shown that transplanted NSCs can differentiate into specific neurons, including DA neurons (Figs. 2A-B). Therefore, the distribution of DA fibres in the visual cortex was analysed by staining for tyrosine hydroxylase (TH). The results showed that GFP + cells partially differentiated into DA neurons, and fibres extending from host tissue could be observed in the graft (Fig. 7). There was no clear boundary between GFP+/TH + cells and GFP-/TH + cells. This result

indicated widespread differentiation of transplanted NSCs not only into mature neurons but also into special types of neurons.

Discussion

TBI is a global health issue for which effective therapies are lacking. In recent years, extensive research on the effectiveness of NSCs in the treatment of TBI has been conducted. Cortical TBI usually results in a larger lesion cavity, and transplanted cells can easily enter the CSF[32]. Therefore, a previously developed mechanical injury method[19] was used to damage the visual and sensory cortices, and then NSCs were transplanted into the injury site. The results showed that the transplanted NSCs did not enter the CSF, were confined to the transplanted site, filled in the injury site and survived for at least one year. The surviving engrafted cells differentiated into mature neurons, including DA neurons. There was no clear boundary between the periphery of the graft and the host tissue, and there was no glial scar at the border. Furthermore, the transplanted NSCs differentiated into mature neurons with cellular activity and formed synaptic connections with host neurons.

In a previous study, transplantation of human foetal neural progenitor cells (hfNPCs) into lesions were found to reduce reactive astrogliosis, but only a few of the transplanted hfNPCs differentiated into neurons[24]. Similarly, Haus et al. transplanted hNSCs into the lesion in an immunodeficient athymic nude rat model of controlled cortical impact and found that the transplanted hNSCs could differentiate into neurons, astrocytes, and oligodendrocytes, but a proportion of transplanted cells still expressed NSC markers, such as nestin[25]. Regarding survival and differentiation after transplantation, neurotrophic factors such as BDNF and B-cell lymphoma-extra-large (Bcl-xL) have been shown to play a key role in differentiation after transplantation[26–28]. Our previous studies in rats have also shown that the administration of bFGF/EGF in combination with transplanted NSCs improves cell survival and the rate of neuronal differentiation[20]. In this study, NSCs transplanted into the injured cortex (including the visual cortex and sensory cortices) survived for at least one year and differentiated into mature neurons. There was no significant difference in neuronal or glial cell differentiation between the visual cortex and sensory cortex. Unlike in previous studies, NSCs transplanted in rhesus monkeys survived and differentiated into neurons in large numbers in this study. These findings in rhesus monkeys demonstrate the feasibility of NSC transplantation for the treatment of TBI[29] and demonstrate the advantages of NSC transplantation combined with bFGF/EGF injection in brain injury treatment. In addition, this is the first time that NSCs were transplanted into the superficial cortex of rhesus monkeys with TBI, and the results showed that NSCs were confined to the transplantation site and fill the injury site, which provides a new reference for the treatment of superficial cortical TBI.

Previous studies in rats have shown that integration of transplanted NSCs into the host neural circuitry is important for improving the efficacy of NSC transplantation[30–31]. Recent studies have also shown that transplanted human brain organoids can structurally and functionally integrate into the visual system after cortical injury, demonstrating the importance of functional integration in the treatment of TBI by cell

transplantation[32]. In this study, the transplanted NSCs not only showed cellular activity but also expressed PSD-95 and Synapsin I, indicating that there were synaptic connections between transplanted NSCs and host neurons; this suggests that transplanted NSCs could repair damage tissue in the lesion site *in situ* and reduce secondary apoptosis in nearby brain areas. Although the injured areas were small and no significant behavioural changes were observed, these results still suggest a long-term beneficial effect of NSC transplantation in the treatment of TBI.

Previous studies have shown that TBI triggers secondary neuroinflammation, reactive gliosis, and even glial scar formation *in situ* and that the transplantation of NSCs can reduce the inflammatory response[33–35]. In this study, transplantation was performed seven days after mechanical injury. The results showed that only a small number of $GFAP^+$ cells were present one year after transplantation and that there was no glial scar, which indicated that NSC transplantation could protect against the secondary inflammatory response at the injury site, suggesting the safety and neuroprotective effect of NSC transplantation.

Previous studies using NSC transplantation for the treatment of TBI have shown the effectiveness of this treatment strategy, but most of these studies were conducted in rodent TBI models. In our previous study, NSCs transplanted into deep brain areas in rhesus monkeys after injury survived and differentiated into neurons, but the effect of transplanting NSCs into superficial brain areas in monkeys has not been explored. This study was the first to transplant NSCs into the superficial cortex of rhesus monkeys after TBI, and the results showed that the transplanted NSCs remained in the lesion site. Moreover, the simultaneous application of bFGF/EGF allowed the transplanted NSCs to survive long term and differentiate into neurons without inducing glial scar formation, which can hinder recover after TBI. At present, the clinical potential of NSC transplantation in the treatment of TBI needs to be better understood[36]. This study provides reliable results in rhesus monkeys supporting the clinical translation of NSC transplantation.

Conclusions

Grafted NSCs did not enter the CSF, were confined to the transplanted site, filled in the injury site and survived for at least one year. The surviving engrafted cells differentiated into mature neurons, including DA neurons. There was no clear boundary between the periphery of the graft and the host tissue, and there was no glial scar at the border. Furthermore, the transplanted NSCs differentiated into mature neurons with cellular activity and formed synaptic connections with host neurons.

Abbreviations

TBI	Traumatic brain injury
CSF	Cerebrospinal fluid
NSCs	Neural stem cells
bFGF	Basic fibroblast growth factor
EGF	Epidermal Growth Factor
DAI	Diffuse axonal injury
ESCs	Embryonic stem cells
KOSR	Knock out serum replacement
MEFs	Mouse embryonic fibroblasts
FBS	Fetal bovine serum
PBS	Phosphate-buffered saline
ITS-X	Insulin-transferrin-selenium-X
BSA	Bovine serum albumin
MRI	Magnetic resonance imaging
DA	Dopaminergic
NF	Neurofilament
PSD-95	Postsynaptic density protein-95
TH	Tyrosine hydroxylase
hfNPSs	Human foetal neural progenitor cells
BDNF	Brain-derived neurotrophic factor
Bcl-xL	B-cell lymphoma-extra-large
DMEM	Dulbecco's modified eagle medium
DMEM/F12	Dulbecco's modified eagle media: nutrient mixture F-12
EDTA	Ethylene diamine tetraacetie acid
EBs	Embryoid bodies
ECM	Extracellular matrix
GFP	Green fluorescent protein

Declarations

Acknowledgements

The authors would like to thank the veterinarians for taking care of experimental animals.

Author contributions:

WZ designed research; WZ, LS, DW, and HT performed the research; LS, LY, ZR and SL analyzed data; and LS, WZ, LS and CY wrote the paper.

Funding

This study was supported by grants from the Natural Science Foundation of China (No. 31960120), the National Key Research and Development Program of China (No.2018YFA0801403) and the Natural Science Foundation of Yunnan Province (No. 202001BC070001 & 202102AA100053, 202105AC160041).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The experimental plan of monkey model generation and NSCs injection was approved by the Institutional Animal Care and Use Committee of Kunming University of Science and Technology in advance (approval number: LPBR202201007). Date of approval is Aug 15, 2022. All procedures involving animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals (the 8th edition, NIH). All cynomolgus monkeys were housed and raised at the facility of the State Key Laboratory of Primate Biomedical Research.

Author contributions:

WZ designed research; WZ, LS, DW, and HT performed the research; LS, LY, ZR and SL analyzed data; and LS, WZ, LSG and CY wrote the paper.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Author details

[1]State Key Laboratory of Primate Biomedical Research; Institute of Primate Translational Medicine, Kunming University of Science and Technology, Kunming, Yunnan,650500, China. [2]Yunnan Key Laboratory of Primate Biomedical Research, Kunming, Yunnan, 650500, China.

References

1. Iaccarino, C.; Carretta, A.; Nicolosi, F.; Morselli, C. Epidemiology of severe traumatic brain injury. J. Neurosurg. Sci. 2018, 62, 535–541.
2. Dewan, M.C.; Rattani, A.; Gupta, S.; Baticulon, R.E.; Hung, Y.-C.; Punchak, M.; Agrawal, A.; Adeleye, A.O.; Shrime, M.G.; Rubiano, A.M.; et al. Estimating the global incidence of traumatic brain injury. J. Neurosurg. 2019, 130, 1080–1097.
3. Walker, W.C.; Stromberg, K.A.; Marwitz, J.H.; Sima, A.P.; Agyemang, A.A.; Graham, K.M.; Harrison-Felix, C.L.; Hoffman, J.M.; Brown, A.W.; Kreutzer, J.S.; et al. Predicting Long-Term Global Outcome after Traumatic Brain Injury: Development of a Practical Prognostic Tool Using the Traumatic Brain Injury Model Systems National Database. J. Neurotrauma 2018, 35, 1587–1595.
4. Van Eijck, M.M.; Schoonman, G.G.; van der Naalt, J.; de Vries, J.; Roks, G. Diffuse axonal injury after traumatic brain injury is a prognostic factor for functional outcome: A systematic review and meta-analysis. Brain Inj. 2018, 32, 395–402.
5. Königs, M.; Beurskens, E.A.; Snoep, L.; Scherder, E.J.; Oosterlaan, J. Effects of Timing and Intensity of Neurorehabilitation on Functional Outcome After Traumatic Brain Injury: A Systematic Review and Meta-Analysis. Arch. Phys. Med. Rehabil. 2018, 99, 1149–1159.e1.
6. Hay, J.R.; Johnson, V.E.; Young, A.M.; Smith, D.H.; Stewart, W. Blood-Brain Barrier Disruption Is an Early Event That May Persist for Many Years After Traumatic Brain Injury in Humans. J. Neuropathol. Exp. Neurol. 2015, 74, 1147–1157.
7. Lozano, D.; Gonzales-Portillo, G.S.; Acosta, S.; de la Pena, I.; Tajiri, N.; Kaneko, Y.; Borlongan, C. Neuroinflammatory responses to traumatic brain injury: Etiology, clinical consequences, and therapeutic opportunities. Neuropsychiatr. Dis. Treat. 2015, 11, 97–106.
8. Dang, B.; Chen, W.; He, W.; Chen, G. Rehabilitation Treatment and Progress of Traumatic Brain Injury Dysfunction. Neural Plast. 2017, 2017, 1582182.
9. Hylin, M.J.; Kerr, A.L.; Holden, R. Understanding the Mechanisms of Recovery and/or Compensation following Injury. Neural Plast. 2017, 2017, 7125057.
10. Zhou, Y.; Shao, A.; Xu, W.; Wu, H.; Deng, Y. Advance of Stem Cell Treatment for Traumatic Brain Injury. Front. Cell. Neurosci. 2019, 13, 301.
11. Weston, N.M.; Sun, D. The Potential of Stem Cells in Treatment of Traumatic Brain Injury. Curr. Neurol. Neurosci. Rep. 2018, 18, 299–307.

12. Chrostek, M.R.; Fellows, E.G.; Guo, W.L.; Swanson, W.J.; Crane, A.T.; Cheeran, M.C.; Low, W.C.; Grande, A.W. Efficacy of Cell-Based Therapies for Traumatic Brain Injuries. Brain Sci. 2019, 9, 270.
13. De Gioia R, Biella F, Citterio G, Rizzo F, Abati E, Nizzardo M, Bresolin N, Comi GP, Corti S. Neural Stem Cell Transplantation for Neurodegenerative Diseases. Int J Mol Sci. 2020 Apr 28;21(9):3103.
14. Velloso FJ, Shankar S, Parpura V, Rakic P, Levison SW. Neural Stem Cells in Adult Mammals are not Astrocytes. ASN Neuro. 2022 Jan-Dec; 14:17590914221134739.
15. Haus, D.L.; López-Velázquez, L.; Gold, E.M.; Cunningham, K.M.; Perez, H.; Anderson, A.J.; Cummings, B.J. Transplantation of human neural stem cells restores cognition in an immunodeficient rodent model of traumatic brain injury. Exp. Neurol. 2016, 281, 1–16.
16. Yan, Z.-J.; Zhang, P.; Hu, Y.-Q.; Zhang, H.-T.; Hong, S.-Q.; Zhou, H.-L.; Zhang, M.-Y.; Xu, R.-X. Neural Stem-Like Cells Derived from Human Amnion Tissue are Effective in Treating Traumatic Brain Injury in Rat. Neurochem. Res. 2013, 38, 1022–1033.
17. Xiong, L.-L.; Hu, Y.; Zhang, P.; Zhang, Z.; Li, L.-H.; Gao, G.-D.; Zhou, X.-F.; Wang, T.-H. Neural Stem Cell Transplantation Promotes Functional Recovery from Traumatic Brain Injury via Brain Derived Neurotrophic Factor-Mediated Neuroplasticity. Mol. Neurobiol. 2018, 55, 2696–2711.
18. Marklund N. Rodent Models of Traumatic Brain Injury: Methods and Challenges. Methods Mol Biol. 2016; 1462:29-46.
19. Wei JK, Wang WC, Zhai RW, Zhang YH, Yang SC, Rizak J, Li L, Xu LQ, Liu L, Pan MK, Hu YZ, Ghanemi A, Wu J, Yang LC, Li H, Lv LB, Li JL, Yao YG, Xu L, Feng XL, Yin Y, Qin DD, Hu XT, Wang ZB. Neurons Differentiated from Transplanted Stem Cells Respond Functionally to Acoustic Stimuli in the Awake Monkey Brain. Cell Rep. 2016 Jul 26;16(4):1016-1025.
20. Li H, Gan X, Pan L, Zhang Y, Hu X, Wang Z. EGF/bFGF promotes survival, migration and differentiation into neurons of GFP-labeled rhesus monkey neural stem cells xenografted into the rat brain. Biochem Biophys Res Commun. 2022; 620:76-82.
21. Gutman, A., and Wasylyk, B. (1991). Nuclear targets for transcription regulation by oncogenes. Trends Genet. 7, 49–54.
22. Ferreira, A., and Rapoport, M. (2002). The synapsins: beyond the regulation of neurotransmitter release. Cell. Mol. Life Sci. 59, 589–595.
23. Hunt, C.A., Schenker, L.J., and Kennedy, M.B. (1996). PSD-95 is associated with the postsynaptic density and not with the presynaptic membrane at forebrain synapses. J. Neurosci. 16, 1380–1388.
24. Skardelly M, Gaber K, Burdack S, Scheidt F, Hilbig H, Boltze J, Forschler A, Schwarz S, Schwarz J, Meixensberger J, Schuhmann MU. Long-term benefit of human fetal neuronal progenitor cell transplantation in a clinically adapted model after traumatic brain injury. J Neurotrauma 2011; 28: 401-414.
25. Haus DL, López-Velázquez L, Gold EM, Cunningham KM, Perez H, Anderson AJ, Cummings BJ. Transplantation of human neural stem cells restores cognition in an immunodeficient rodent model of traumatic brain injury. Exp Neurol 2016; 281: 1-16.

26. Chen T, Wu Y, Wang Y, Zhu J, Chu H, Kong L, Yin L, Ma H. Brain-derived neurotrophic factor increases synaptic protein levels via the MAPK/Erk signaling Pathway and Nrf2/Trx axis following the transplantation of neural stem cells in a rat model of traumatic brain injury. Neurochem Res 2017; 42: 3073-3083.
27. Fang AL, Xiong LL, Xia QJ, Liu F, Wang YC, Liu F, Zhang P, Meng BL, Tan S, Wang TH. Neural stem cell transplantation is associated with inhibition of apoptosis, Bcl-xL upregulation, and recovery of neurological function in a rat model of traumatic brain injury. Cell Transplant 2017; 26: 1262-1275.
28. Xiong LL, Hu Y, Zhang P, Zhang Z, Li LH, Gao GD, Zhou XF, Wang TH. Neural stem cell transplantation promotes functional recovery from traumatic brain injury via brain derived neurotrophic factor-mediated neuroplasticity. Mol Neurobiol 2018; 55: 2696-2711.
29. Daadi MM, Barberi T, Shi Q, Lanford RE. Nonhuman primate models in translational regenerative medicine. Stem Cells Dev. 2014 Dec;23 Suppl 1(Suppl 1):83-7.
30. Yan ZJ, Zhang P, Hu YQ, Zhang HT, Hong SQ, Zhou HL, Zhang MY, Xu RX. Neural stem-like cells derived from human amnion tissue are effective in treating traumatic brain injury in rat. Neurochem Res 2013; 38: 1022-1033.
31. Lin GQ, He XF, Liang FY, Guo Y, Sunnassee G, Chen J, Cao XM, Chen YY, Pan GJ, Pei Z, Tan S. Transplanted human neural precursor cells integrate into the host neural circuit and ameliorate neurological deficits in a mouse model of traumatic brain injury. Neurosci Lett 2018; 674: 11-17.
32. Jgamadze D, Lim JT, Zhang Z, et al. Structural and functional integration of human forebrain organoids with the injured adult rat visual system. Cell Stem Cell. 2023;30(2):137-152.e7.
33. Gao J, Grill RJ, Dunn TJ, Bedi S, Labastida JA, Hetz RA, Xue H, Thonhoff JR, DeWitt DS, Prough DS, Cox CS, Jr., Wu P. Human neural stem cell transplantation-mediated alteration of microglial/macrophage phenotypes after traumatic brain injury. Cell Transplant 2016; 25: 1863-1877.
34. Lee JY, Acosta S, Tuazon JP, Xu K, Nguyen H, Lippert T, Liska MG, Semechkin A, Garitaonandia I, Gonzalez R, Kern R, Borlongan CV. Human parthenogenetic neural stem cell grafts promote multiple regenerative processes in a traumatic brain injury model. Theranostics 2019; 9: 1029-1046.
35. Nasser M, Ballout N, Mantash S, Bejjani F, Najdi F, Ramadan N, Soueid J, Zibara K, Kobeissy F. Transplantation of embryonic neural stem cells and differentiated cells in a controlled cortical impact (CCI) model of adult mouse somatosensory cortex. Front Neurol 2018; 9: 895.
36. Alizada M, Lin S, Gao H. Recent advances in the treatment of traumatic brain injury with autologous and non-autologous multipotent stem and progenitor cells: preclinical models and clinical trials. Folia Neuropathol. 2021;59(3):298-316.

Figures

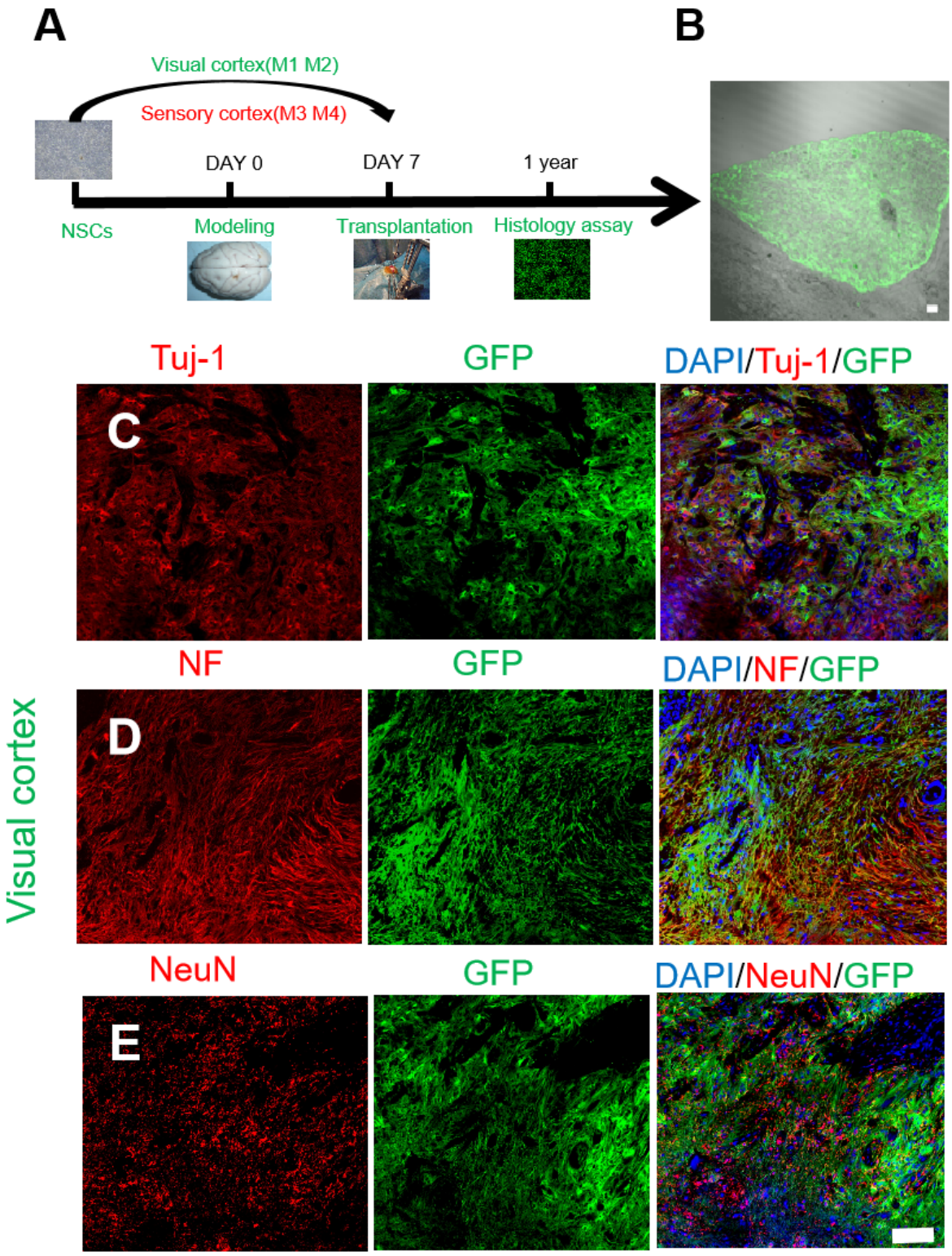

Figure 1

Schematic diagrams showing the mechanical injury protocol and histological images of transplanted GFP-labelled NSCs in the visual cortex.

(A) Schematic of the mechanical injury protocol and cell transplantation into the macaque sensory/visual cortex.

(B) GFG-marked NSCs transplantation into the mechanical damage of the macaque visual cortex after 1 year. (C-E) Transplanted NSCs (GFP) differentiated into mature neurons in the injured area of the macaque visual cortex (Tuj-1/NF/NeuN, red). Scale bars, 100 μm.

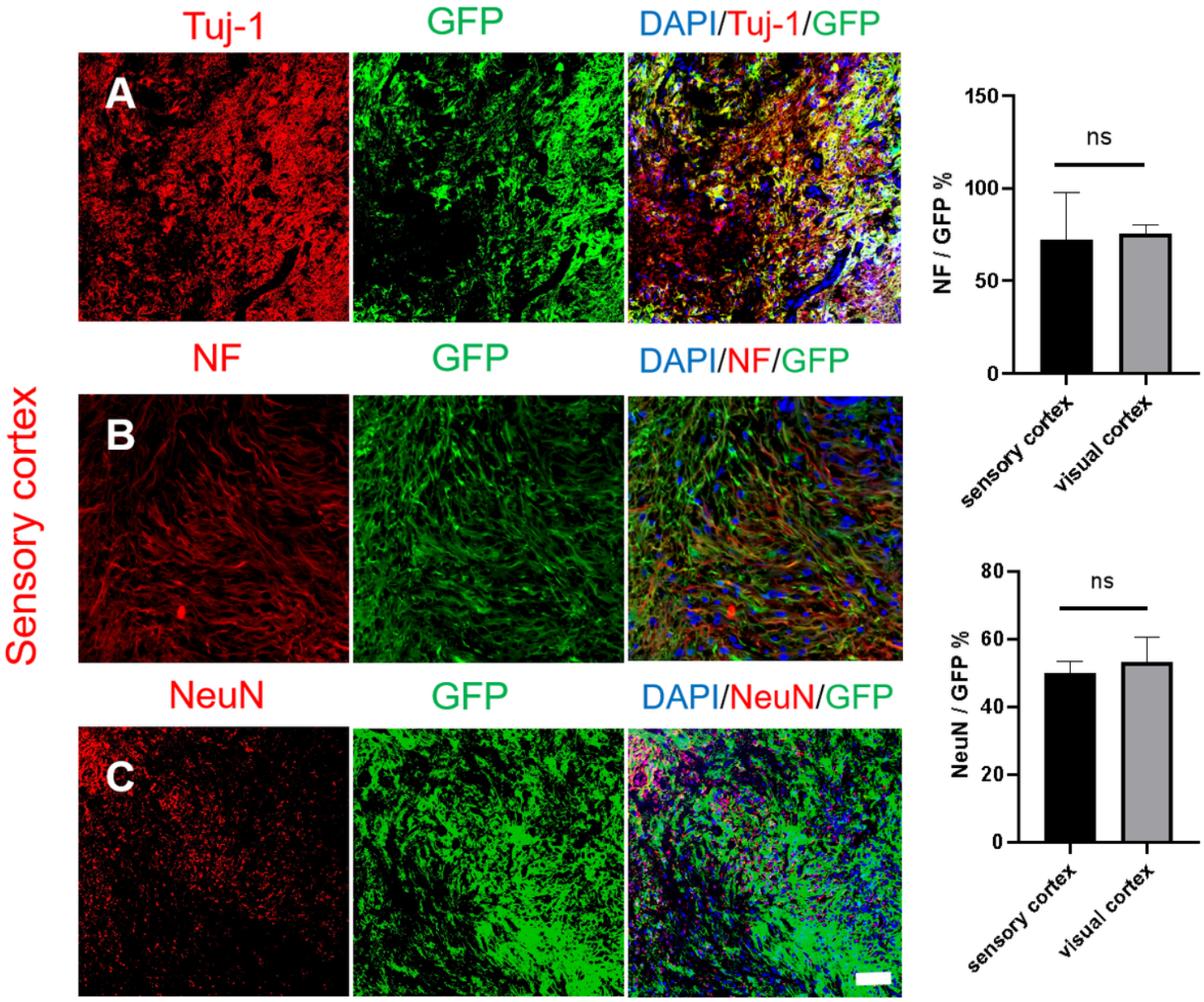

Figure 2

Histological images of transplanted GFP-labelled NSCs in the injured macaque sensory cortex.

(A-C) Transplanted NSCs (GFP) differentiated into mature neurons in the injured area of the macaque sensory cortex (Tuj-1/NF/NeuN, red). Scale bars, 100 μm.

(D) NeuN expression was not different between the sensory cortex and visual cortex.

(E) NF expression was not different between the sensory cortex and visual cortex. p 0.05.

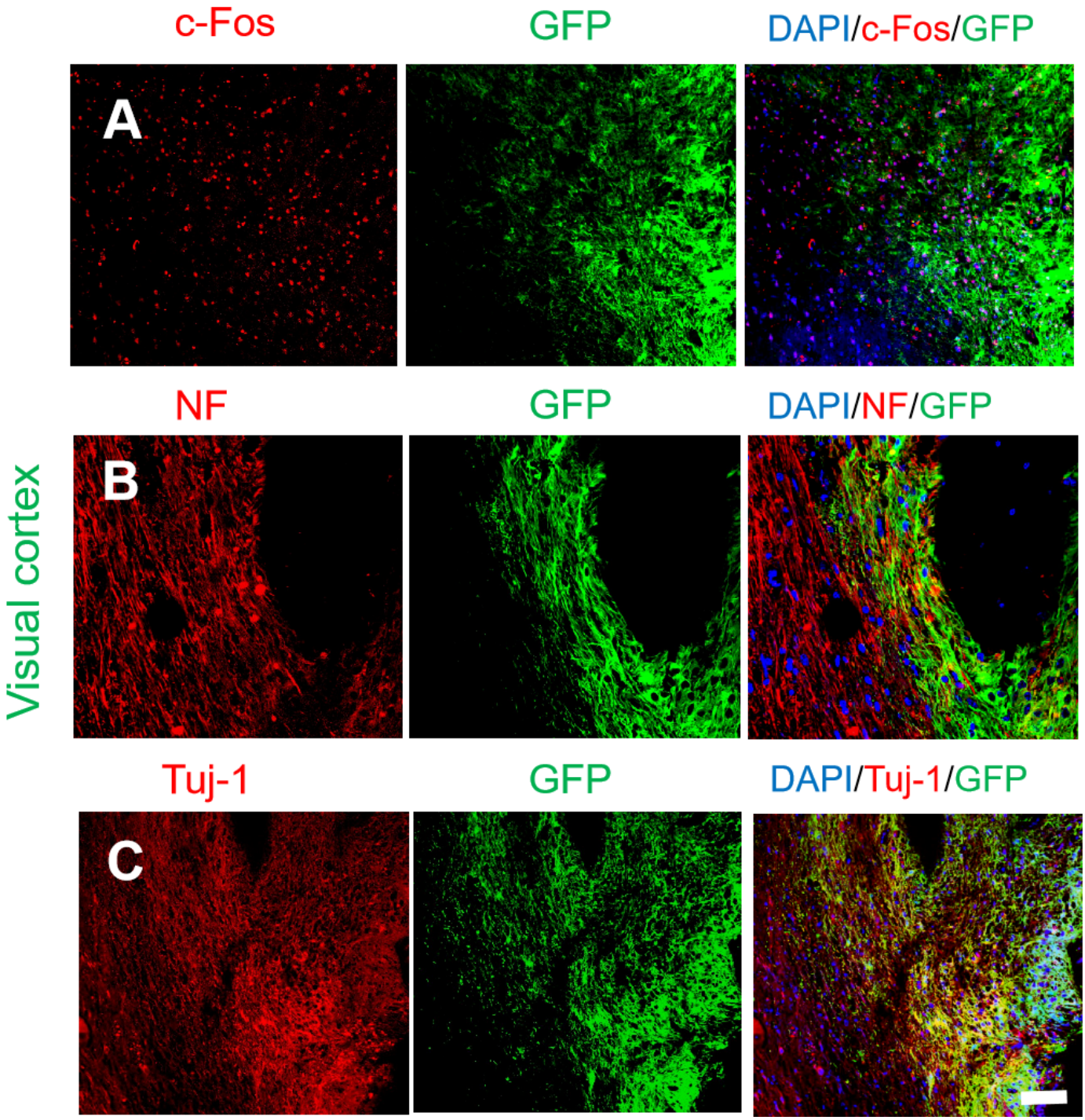

Figure 3

Survival and differentiation of transplanted cells in marginal areas of the damaged visual cortex.

(A) Some of the transplanted cells were c-Fos+ (DAPI, blue; GFP, green; c-Fos, red).

(B-C) Transplanted NSCs differentiated into mature neurons at the edge of the injury site (DAPI, blue; GFP, green; Tuj-1, red; NF, red). Scale bars, 100 μm.

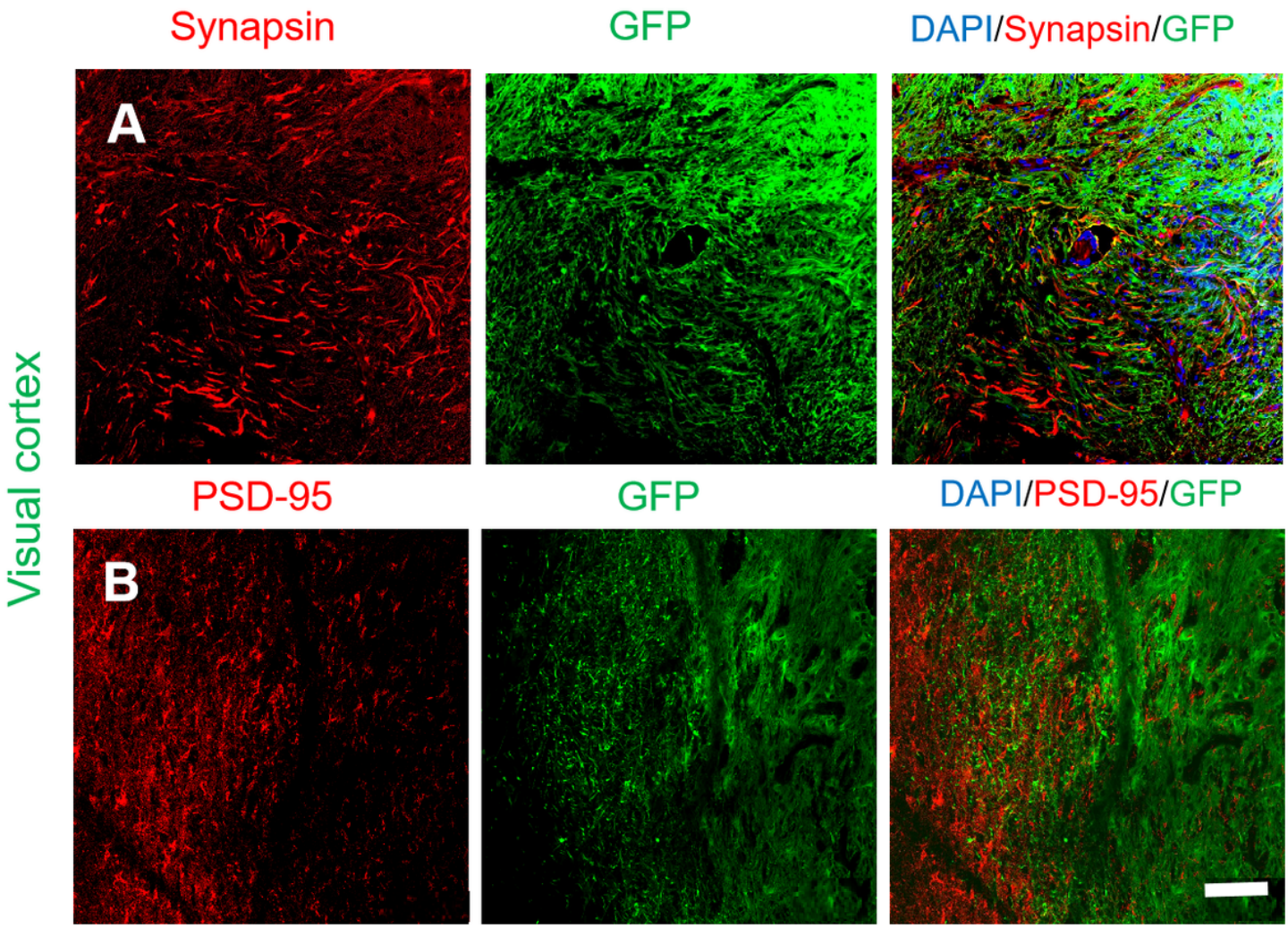

Figure 4

Neurons differentiated from GFP-labelled transplanted NSCs formed synapses with neighbouring host neurons.

(A) Presynaptic terminals of hose and differentiated neurons in the transplantation site (DAPI, blue; GFP, green; Synapsin I, red).

(B) Postsynaptic terminals of differentiated neurons (DAPI, blue; GFP, green; PSD-95, red). Scale bars, 100 μm

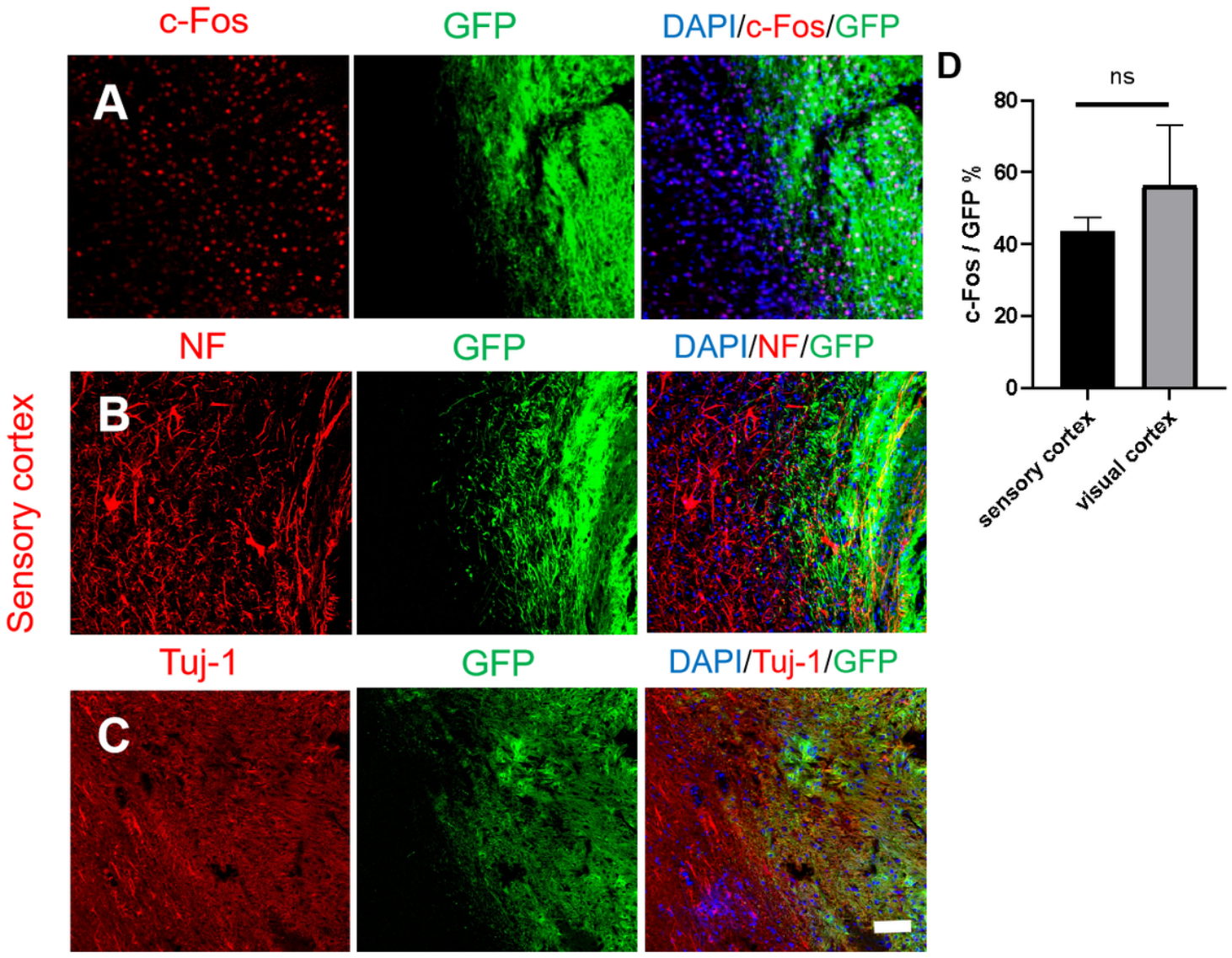

Figure 5

Histological images of transplanted GFP-labelled NSCs in the border between the graft and host tissue in the sensory cortex.

(A) The transplanted NSCs (GFP) differentiated into active cells (DAPI, blue; GFP, green; c-Fos, red).

(B-C) The transplanted NSCs (GFP) differentiated into mature neurons atthe graft edge (DAPI, blue; GFP, green; Tuj-1, red; NF, red). Scale bars, 100 μm.

(D)c-Fos expression was not different between the sensory cortex and visual cortex. p 0.05.

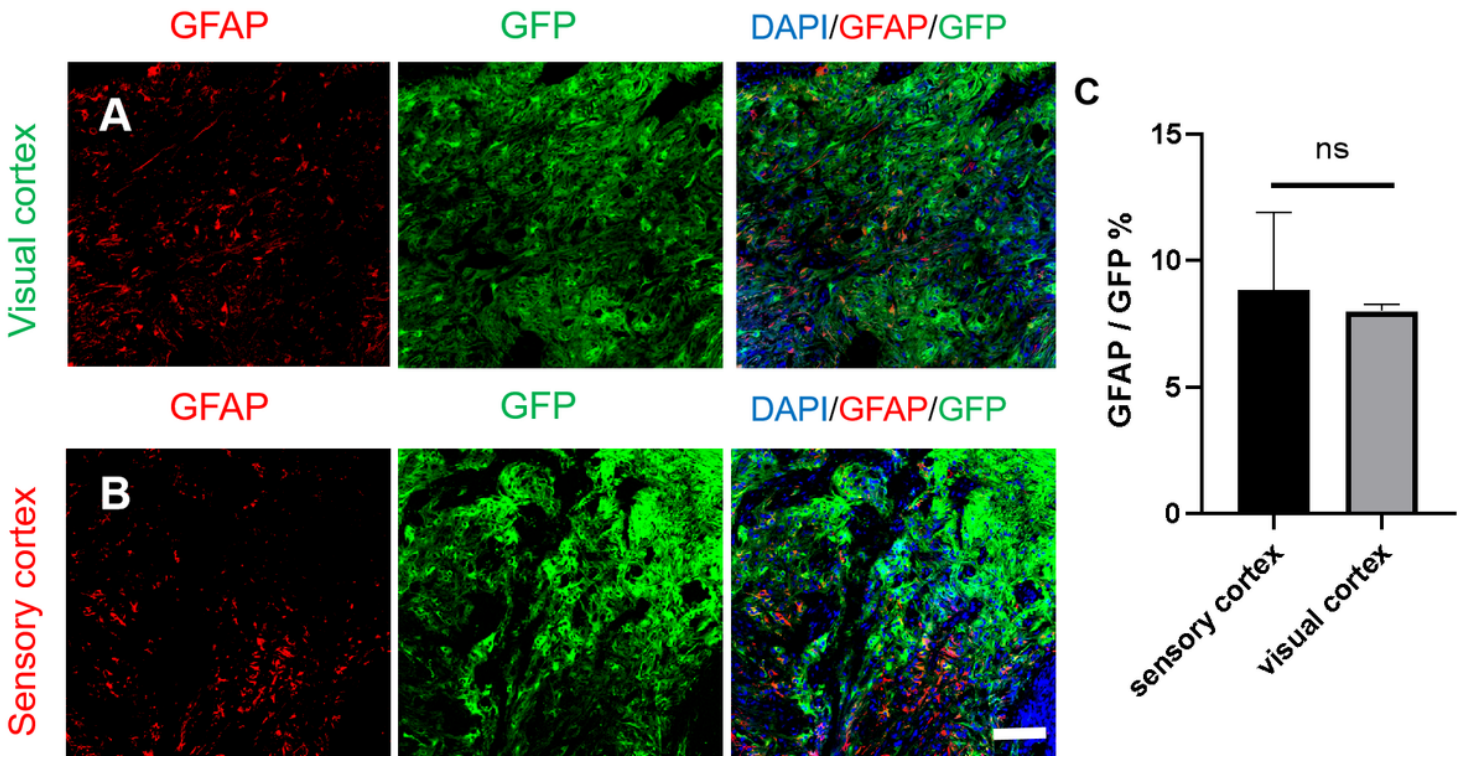

Figure 6

The transplanted NSCs differentiated into astrocytes in the visual cortex and the sensory cortex.

(A) The transplanted NSCs (GFP) differentiated into astrocytes in the visual cortex (DAPI, blue; GFP, green; GFAP, red).

(B) The transplanted NSCs (GFP) differentiated into astrocytes in the sensory cortex (DAPI, blue; GFP, green; GFAP, red). Scale bars, 100 μm.

(C) GFAP expression was not different between the sensory cortex and visual cortex. p 0.05.

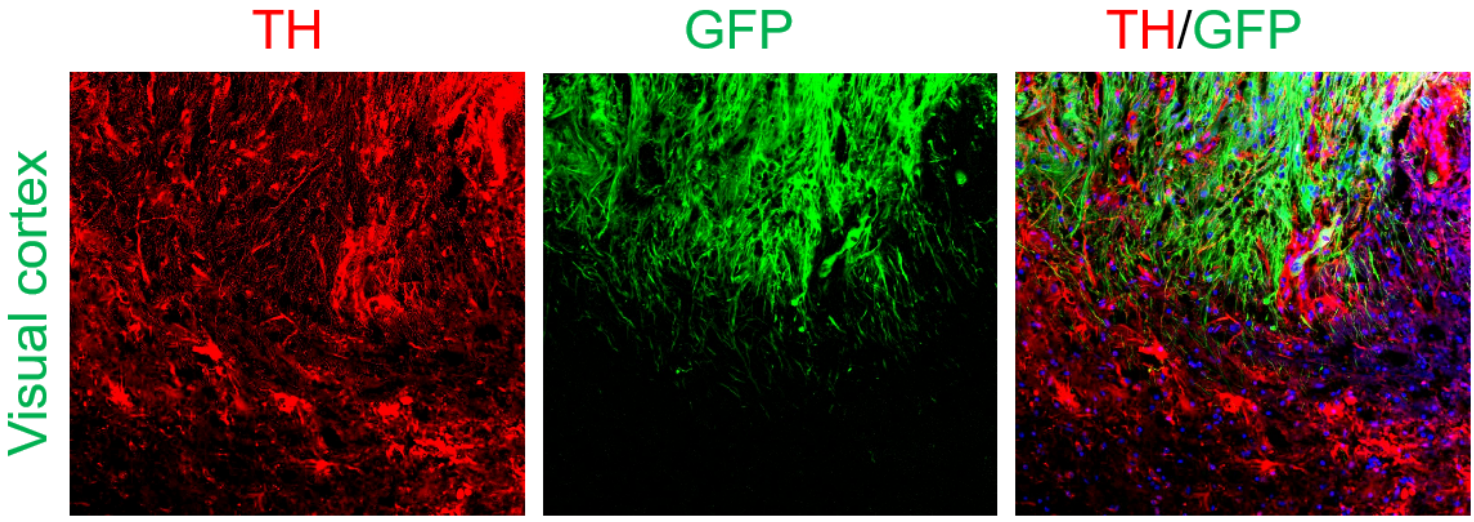

Figure 7

The transplanted NSCs differentiated into DA neurons in the visual cortex.

The transplanted NSCs (GFP) differentiated into DA neurons in the visual cortex (DAPI, blue; GFP, green; TH, red). Scale bars, 100 μm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement8.docx