Neurochemical Research (2019) 44:2765–2775
https://doi.org/10.1007/s11064-019-02897-z

ORIGINAL PAPER

Transplantation of NSCs Promotes the Recovery of Cognitive Functions by Regulating Neurotransmitters in Rats with Traumatic Brain Injury

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Received: 19 February 2019 / Revised: 11 October 2019 / Accepted: 19 October 2019 / Published online: 7 November 2019

Abstract

Transplantation of neural stem cells (NSCs) may be a potential strategy for traumatic brain injury treatment (TBI) due to their intrinsic advantages, such as cell replacement, secretion of neurotrophins and formation of functional synapses with host. However the underlying effects of transplanted NSCs on host micro-environment still need to be further elucidated. In this manuscript the effects of NSCs on release of neurotransmitter, survival of hippocampal neurons, reactivity of astrocytes and recovery of cognitive function after TBI were observed. The NSCs were isolated from cortex of neonatal Sprague–Dawley rat and then transplanted into injured brain regions caused by free-weight drop. The proliferation of astrocytes around injured sites were examined by GFAP immunofluorescent staining on 3, 7, 14 days after injury. The survival of neurons at CA1 regions of hippocampus toward contused regions was observed by HE staining on 3 and 14 days post-injury. The content of glutamic acid (Glu) and GABA in hippocampal tissues was examined on 1, 3, 7, 14, 28 days after injury by ELISA. On third day post-injury, hippocampal-dependent spatial memory was measured for 5 days without intermittent. NSCs in culture have the ability to proliferate and differentiate into different phenotypes of neural cells. After transplantation of NSCs, the proliferation of astrocytes around injured site was significantly inhibited compared to the injured group. At the same time the survival of neurons in hippocampal CA1 region were much more than those in injured group on 14 days post-injury. Meanwhile, the cognitive functions in NSC transplanted group was remarkably improved compared with injured group ($p < 0.05$). Furthermore, NSCs transplantation dramatically inhibited the release of Glu and maintained the content of GABA in injured hippocampal tissues on 1, 3, 7, 14, 28 days post-injury, which was of difference in statistics ($p < 0.05$). NSCs transplantation can effectively alleviate the formation of glial scar, enhance the survival of hippocampal neurons and improve cognitive function defects in rats with TBI. The underlying mechanism may be related to their effects on inhibiting the release of Glu and maintaining the content of GABA, so as to down-regulate excitotoxicity of neurotransmitter and improve the micro-environment in injured sites.

Keywords Neural stem cells · Rats · Transplantation · Behavior · Traumatic brain injury

Introduction

Traumatic brain injury (TBI) is one of the major cause of death and permanent disability in traumatic patient [1, 2]. Neuronal degeneration following TBI is believed to involve in primary mechanical injury and progressive secondary injury [1]. The primary brain damage that occurs at the time of injury is characterized by diffused degeneration, neuronal death and interruption of axons. The secondary brain damage occurs subsequently, leading to additional pathological features such as cellular apoptosis or necrosis and demyelination of nerve fibers. These damages result in serious and irreversible sensory-motor and cognition-memory dysfunctions [2]. Currently, therapeutic strategies for

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11064-019-02897-z) contains supplementary material, which is available to authorized users.

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TBI mainly focus on rescuing damaged neurons, promoting regeneration of axons and rehabilitation training after injury. However, these therapeutic effects were limited. Cellular or tissue transplantation has been considered as one of the most promising strategies for TBI treatment. The characterization of neural stem cells (NSCs) have led to new possibilities for direct transplantation of these cells into the injured brain, due to the multi-potential ability to self-renew and differentiate into different types of neural cells. Moreover their inherent ability to adapt to signals from the host cells and the extracellular environment is crucial for the donor cells to interact with the host tissue [3, 4].

In recently, many experiments have shown that the transplanted NSCs had stronger migratory features and were better integrated with host tissue [5]. Ma et al. indicated that transplanted NSCs could differentiate into neuronsand promote the motor function of forelimb and hindlimb in TBI rats. Engrafted NSCs increase the expression of synaptophysin (SYP) and regeneration-associated protein (GAP43) in the injured brain, which may be one of the mechanisms underlying the improved functional recovery of motor behavior [6]. However, the other mechanisms underlying transplantation-mediated recovery following TBI have yet to be determined.

It was indicated that neurotransmitters played important roles in maintaining the homeostasis of micro-environment in normal conditions. In the progression of TBI excessive amounts of glutamate in extracellular space may lead to elevated levels of intracellular calcium and excessive influx of sodium ions, which can cause edema and eventually cell death. At the same time, the less release of GABA after TBI may alleviate the antagonism on excitotoxicity of glutamate. But whether transplantation of NSCs can improve the microenvironment after TBI by regulating the release of Glu/GABA and contribute to the recovery of cognitive functions remains to be elucidated.

In this study, the effects of NSCs transplantation on structural and cognitive function in the acute stage of TBI rats were examined and further explored the changes of Glu and GABA in brain tissues post-injury, through which provided additional important evidence for the therapy of TBI by NSCs transplantation.

Methods

Culture and Purification of NSCs

All experiments were approved by the animal care and experimental committee of the Army Medical Military University. Primary cultures of NSCs were derived from embryonic 13–5 days Sprague–Dawley rats as described previously [4, 7]. Briefly, the telencephalon was rapidly dissected and placed into 1.5 ml tubes containing 0.25% trypsin. The tissues were mechanically dissociated into single cell suspensions. Cell numbers and viability were assessed by staining a small volume of cell suspension with 0.4% trypan blue. Single-cell suspensions were then transferred to medium consisting of NB + 2% B27 supplemented with 20 ng/ml human recombinant basic fibroblast growth factor (bFGF, Gibico Invitrogen, USA), 20 ng/ml of epidermal growth factor (EGF, Gibico Invitrogen, USA) at 4×10^5 cells/ml. The cells were then planted into culture flasks and maintained under a humidified atmosphere of 5% CO2 in air at 37 °C. After 5–6 day in vitro, the neurospheres were dissociated into single cell suspensions and seeded into 96-well plates at 1–2 cells per well. The neurosphere subcultures were digested and another passage was performed as before.

The cell passage protocol was performed every 6 days to obtain neurospheres originating from a single primary cell. Secondary or tertiary neurospheres were used for subsequent experiments. For Brd-U labeling, NSCs were incubated in medium containing 10 μM Brd-U for 18 h prior staining [7].

Immunocytochemistry Staining of NSCs and Brain Tissues After TBI

After 30-min fixation with 4% paraformaldehyde, NSCs were washed three times with PBS. 0.5% Triton-X-100 and 1% BSA prior to an overnight incubation with the primary mouse anti-rat Brd-U antibody (1:800, Sigma-Aldrich, USA), mouse anti-rat Nestin (1:200, Chemicon, USA). In order to identify the multiple differentiation of NSC, the differentiated cells from NSCs were double stained with mouse anti-rat β-tublin III (1:800, orbeyt Inc, Britain) and rabbit anti-rat glial fibrillary acidic protein (GFAP 1:400, orbeyt Inc, Britain) antibodies. After 3 × 5 min washed, fluorescein isothiocyanate (FITC)-conjugated donkey anti-mouse secondary antibody (1:1000, Jackson, USA) was used for staining β-tublin III and tetramethylrhodamine isothio-cyanate (TRITC)-conjugated donkey anti-rabbit secondary antibody (1:1000, Jackson, USA) was staining for GFAP respectively. Subsequently the samples were counterstained with 300 nM of 4,6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma–Aldrich) for 3 min and then washed three times in PBS prior to scanning with laser confocal microscope (Leica, SP-2, Germany) [8].

The harvested brain tissues were processed as described. Horizontal cryostat sections (20 μm in thickness) were sliced and then stained for rabbit monoclonal anti- GFAP body. Rhodamine conjugated goat anti-rabbit secondary antibody was used for detection.

The slides were mounted with fluorescence mounting medium and analyzed under fluorescent microscopy and confocal microscopy (Leica, Germany) [9].

Preparation of Rat TBI Models

Forty-eight healthy adult female Sprague–Dawley rats (Research Institute of Surgery, Daping Hospital, the Army Military Medical University, Chongqing, China) weighing 220–250 g were randomly divided into three groups: sham-injured group (n = 16); TBI group: rats underwent injury at the cortical motor area (n = 16);NSC transplanted group: NSCs were transplanted into the injured sites (n = 16) respectively. The TBI models were prepared for twice according to the same procedure in order to examine different data. The total number of rat was ninety-six. All experimental protocols and animal handling procedures were approved by the Animal Care and Use Committee of Research Institute of Surgery, Daping Hospital and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Briefly, male Sprague–Dawley rats at the age of 12 weeks were anesthetized with pentobarbital sodium (45 mg/kg). A piece of skull over the right frontal cortex was excised with a drill. Then, dura incision was performed and forebrain was exposed. One 5-mm diameter craniotomy was performed over the right parietal cortex (midway between bregma and lambda sutures and adjacent to the central suture) (Fig. 1a, b). The right cortex was impacted with a 10 g piston containing a 5-mm diameter tip at a rate of 4 m/s and 2.5 mm of compression (Fig. 1c). Sham injury was performed by anesthesia, midline incision, separation the skin and connective tissues before closing the incision. The body temperature was maintained at 37 °C by heating pads [9, 10].

Transplantation of NSCs

After culturing for 14 days, the NSCs for transplantation were obtained by direct digestion and then cultured with 20 ng/ml DAPI for 20 min at 37 °C to label the nuclei. After labeling, the cells were washed twice with 0.01 M PBS. The cell density was adjusted to 1×10^7/ml before transplantation. After TBI, NSCs were immediately transplanted into the brain of rats. A Hamilton 5 µl-microinjection needle was used to stereotactically guide the placement of the cells in injured and the penumbral areas. 5 µl cellular suspensions were injected at four sites: 3 mm to rostral, caudal, left and right from the epicenter of the injured site, respectively. For each site, the cell suspensions were delivered at three depth points: 2 mm, 1.5 mm and 1 mm from the surface of the cortex. The velocity of injection was controlled at 1 µl/min. The given volume of cell suspension was 1.5 µl, 1 µl, 1 µl and 1.5 µl, respectively. After each injection, the needle was held in location for 5 min before withdraw. Rats of control group underwent identical procedures injected with 0.01 M PBS without NSCs. After this treatment, the wounds were sutured with 4-0 surgical suture and the rats were received extensive post-operation care and intra-peritoneal injection of penicillin (50,000 U/kg per day) Bid for 3 days [11, 12].

HE Staining of Hippocampal Tissues

The rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with 4% paraformaldehyde on 3 and 14 days post-injury. The thoracic cavity was opened and normal saline at room temperature was infused for 5 min through the left ventricle. The heart was spontaneously beating on initiation of infusion, and the rat was simultaneously allowed to exsanguinate through right atrium puncture. Cold 0.1 M phosphate buffered saline and 4% para-formaldehyde were then perfused for 15 min through left ventricle. The brain tissues were extracted and placed in 4% para-formaldehyde at 4 °C for 24–48 h; 5 µm thick coronal sections including entire hippocampal CA1 region, were stained with hematoxylin and eosin. The extent of neuronal death in CA1 region of hippocampus was quantitatively assessed

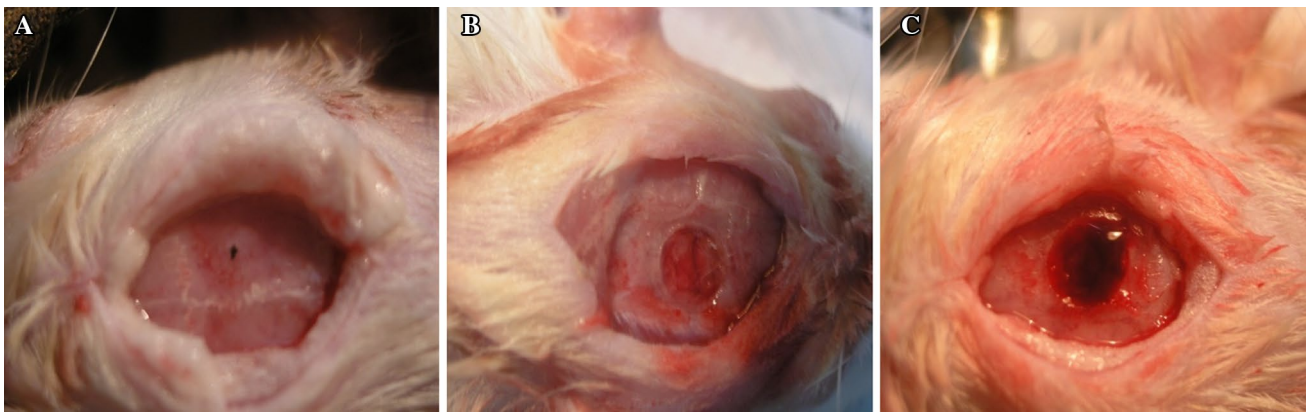


Fig. 1 Surgery procedure of traumatic brain injury. **a** Anatomy location of injury site; **b** 5-mm diameter and round shape of bony fenestra; **c** hemorrhagic focus immediate post-injury

by counting neuronal density in every 1 mm square of CA1 region. Only large intact cells with clearly stained cytoplasm and a distinct nucleus, usually with evident nucleoli, were counted as healthy neurons. Cells with shrunken bodies and nuclear condensation or round cell bodies with pale cytoplasmic staining and signs of cytoplasmic vacuolation and fragmentation (necrotic cells) were counted as dead or dying cells [13].

ELISA Examination of Neurotransmitters

The content of Glu and GABA in hippocampal tissues was examined on 1, 3, 7, 14, 28 days after TBI by ELISA kits. The injured hippocampus collected from rats in the three groups at different time-point. And then the tissues were homogenized in ice-cold lysis buffer containing 20 mM Tris–HCl (pH 7.5), 1 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM phenyl-methylsulfonyl fluoride, and a protease inhibitor cocktail (Pierce, Rockford, USA). The tissue homogenates were centrifuged at 12,000 rpm for 15 min at 4 °C. The proteins were added into the plate. The plates were washed 3 in PBS and added with the bound antibody that reacted with HRP-conjugated goat anti-rat IgG (1:10,000; SABC, state) for 1 h at 37 °C. Optical density (OD) at 450 nm was measured with an ELISA reader [14, 15].

Cognitive Behavioral Examination

Learning and spatial memory performance was evaluated via the Morris water maze (MWM), which consisted of a circular black-colored pool (160 cm diameter, 55 cm deep) filled with water stained with dark ink. Submerged heaters maintained the water temperature between 23 and 25 °C. A round 10 cm diameter platform was submerged 5 cm beneath the water surface. The pool was divided into 4 equal-sized quadrants, one of which included the hidden platform. Three extra-maze cues were set on the wall surrounding the pool. A digital video camera was attached to a computer-controlled system and positioned directly above the pool, enabling full recording of the swimming activities in different quadrants (Smart video-tracking system, Panlab, Spain). MWM evaluated parameters included escape latency (time to locate platform) and swim speed. Before injury the rats were tested for three consecutive days, each day consisting of 4 trials at 5-min intervals. For each trial, animals were released from the edge of the pool into a randomly selected quadrant. Release point order was identical for all animal groups. During each trial, the animals were allowed 60 s to find the hidden platform. If they failed to locate it, they were guided to the platform. Animals were required to remain on the platform for an additional 30 s for memory acquisition training [16–18]. Cognitive behavioral testing was performed on the third day after injury, continuing for 5 days. The assessment was conducted at approximately the same time during the light phase of the cycle. All tests were performed by an observer blinded to the experimental groups.

Statistics

In all quantification and analysis procedures, observers were blinded in the experimental procedure. SPSS 9.0 software was used to analyze the data. Data are expressed as mean ± standard error of the mean (SEM). Survival of neurons and quantification of GFAP staining were analyzed by one way analysis of variance (ANOVA). ELISA analysis and significance of MWN outcomes was determined by two way repeated measures ANOVA or Student's t test depending on the outcome. Significance level was $p < 0.05$ using two tailed testing.

Results

Characterization of NSC In Vitro Culture

On the second day after primary culture, the cells grew as spheres in suspension and expressed NSC specific marker Nestin (Fig. 2a, b). Detection of DNA replication by Brd-U immune-staining in spheres confirmed that these cells underwent proliferation (Fig. 2c). At the same time, the NSCs have a multi-differentiation potential which can differentiate into neurons and astrocytes after withdraw of bFGF (Fig. 2d).

NSCs Transplantation Inhibited the Proliferation of Astrocytes After Injury

Compared with the sham and NSC transplanted groups, the GFAP positive cells around the injured sites in injured group exhibited large bodies with more branches, meanwhile the number of which was also increased 3 days after injury. On 7 days post-injury, the number of reactive astrocytes didn't decreased significantly both in injury and NSC transplanted groups, but the body of astrocytes and branches in NSCs transplanted group were smaller and shorter. Furthermore, on 14 days after injury, glial scar were formed in injured group (shown by arrows), but in NSCs transplanted group the barrier of astrocytes was not obvious. It was indicated that traumatic stimulation can increase the activity of astrocyte to repair the injured tissues, but over proliferation would inhibited the regeneration of injured axons and dendrites. However, in NSC transplantation group, the expression of GAFP around injured sites was less than those in injured group, It was indicated that NSCs transplantation after TBI can inhibit the excessive proliferation of astroyctes, which may be beneficial to the regeneration of axons (Fig. 3a, b).

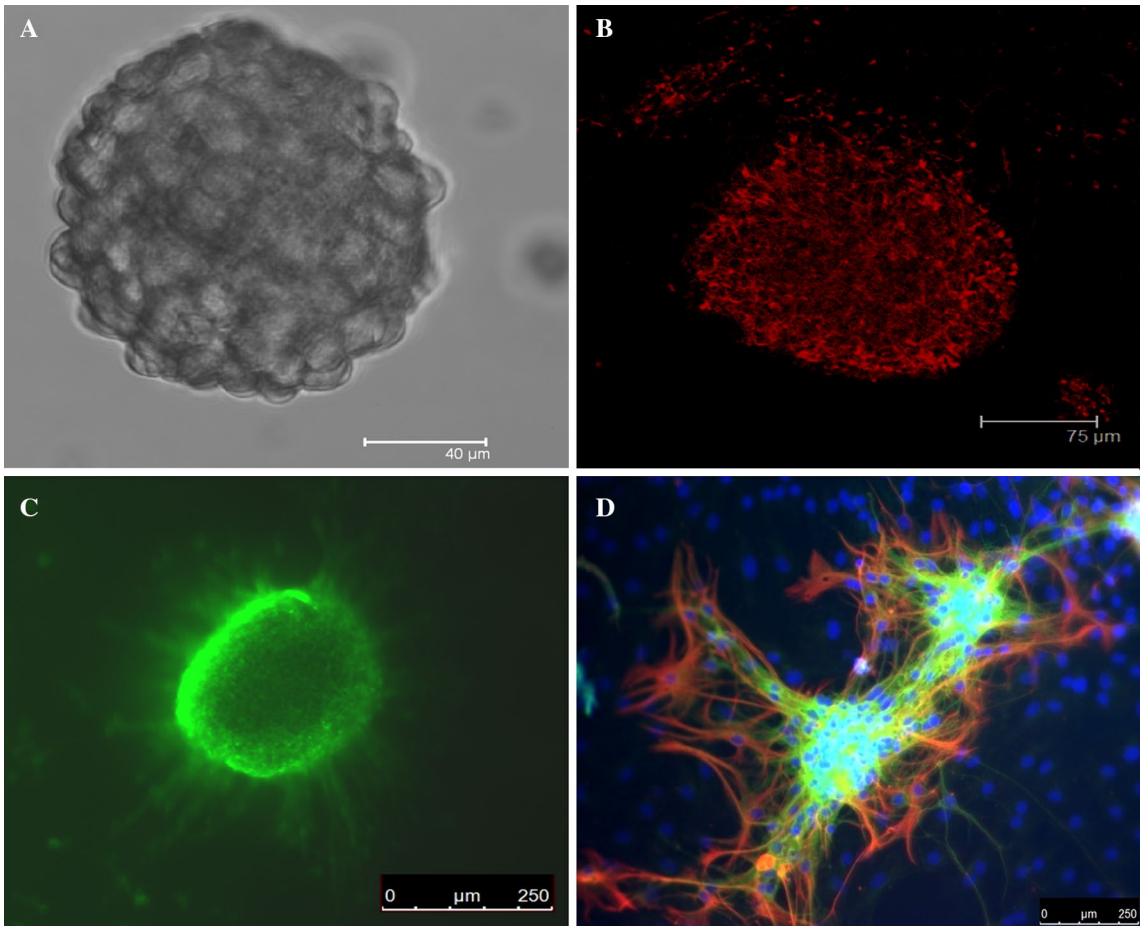


Fig. 2 Culture and identification of NSCs in vitro. **a** Representative neurospheres in culture. **b** Immunocytochemical staining of purified NSCs with Nestin (bar = 100 μm). **c** Immunocytochemical staining of NSCs with anti-Brd-U antibody (bar = 75 μm). **d** Immunocytochemical staining of differentiated cells from NSCs (green indicates neuron-specific marker β-tubulin III; red indicates the astrocyte-specific marker GFAP; blue indicates DAPI labeled nucleus; bar = 37.5 μm) (Color figure online)

NSCs Transplantation Protects Host Hippocampal Neurons

HE staining was used to observe neurons in brain tissues. Our data showed that in NSCs transplanted and injured groups, intact neurons were significantly decreased 3 days after TBI compared with the sham group (Fig. 4a), indicating significant neuron death in the present TBI model. Our data further showed that NSCs transplantation remarkably reduced TBI induced neuronal death on 14 days after injury compared with the injured group (Fig. 4b). Taken together, this study demonstrates that NSCs transplantation protects against neuronal death in TBI rats.

Content of Glutamate and GABA in Hippocampal Tissue After TBI

To reveal the potential mechanism of neurotransmitter on neuronal death in TBI, Content of Glu and GABA in hippocampal tissues were examined. Compared with the sham group, the concentration of Glu in injured group was dramatically increased at 1 day post injury, lasting to 7 days, then maintained at higher levels till 28 days. But in NSC transplantation group, the content of Glu also increased at 1 day post injury, and began to decrease on the third days, which was lower than that in injured group till 28 days after injury ($p < 0.05$) (as shown in Fig. 5a, Supplementary Table 1). Meanwhile, compared with sham group, GABA started to decrease on the first day after injury in injury group. Significant down-regulation occurred on the third and seventh day then maintained at relative lower level until 28 days post-injury, but in NSC transplantation group there was no significant difference in GABA release at different time-point after injury (as shown in Fig. 5b, Supplementary Table 2). It was indicated that transplantation of NSCs after TBI can inhibit the increasing of Glu and decreasing of GABA in hippocampus of TBI rats.

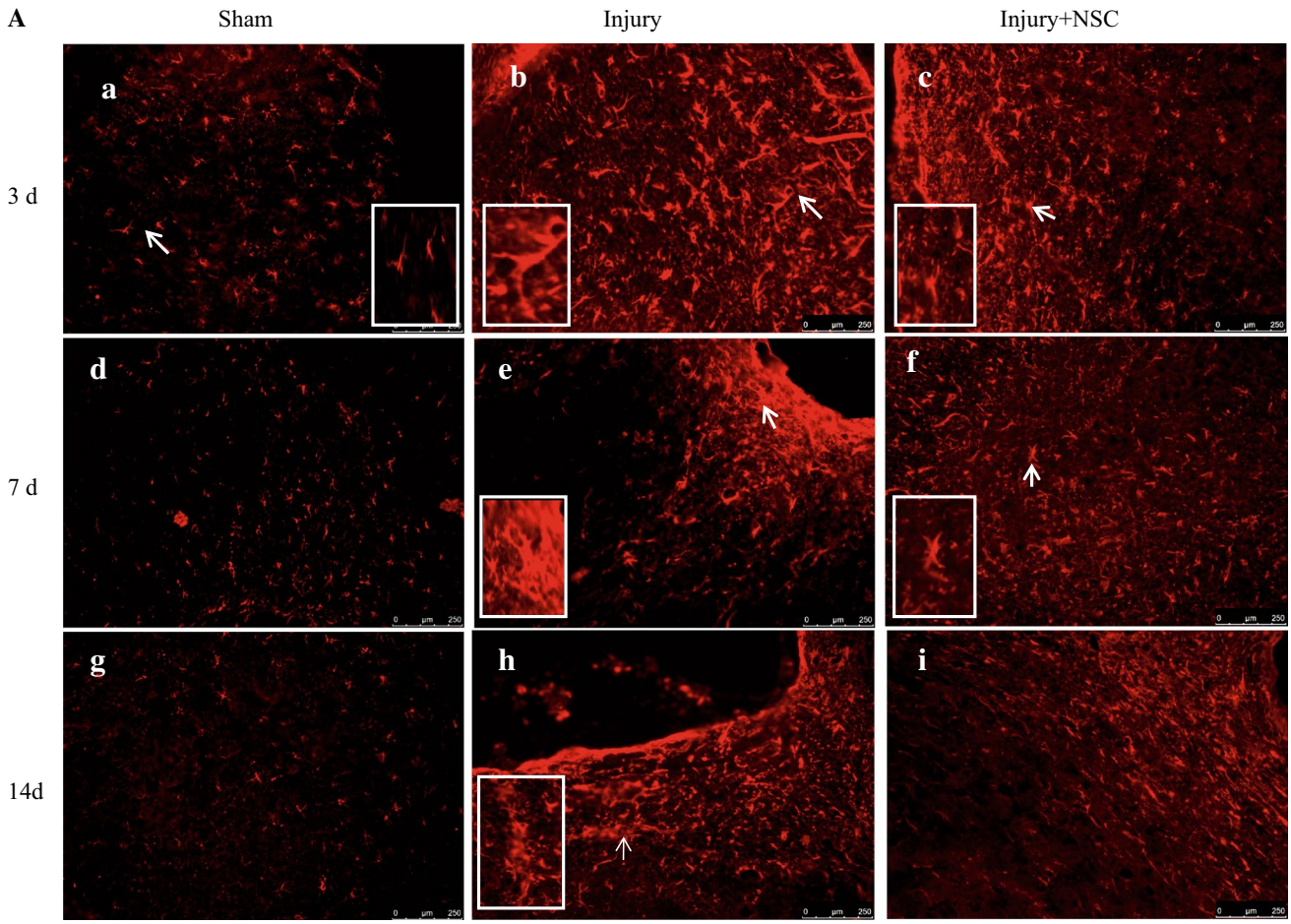


Fig. 3 NSCs transplantation inhibited the activity of astrocytes. **a** GFAP immunohistochemical staining of brain tissues in three groups on 3, 7 and 14 days after injury. Reactive astrocytes in injury group exhibited large and long branches on 3 and 7 days after injury (shown by arrows in **a**, **b**, **e**), but the body of astrocytes and branches in NSCs transplanted group were smaller and shorter (shown by arrows in **a**–**c**, **f**). Furthermore, on 14 days after injury, glial scar were formed in injured group (shown by arrows in **a**–**h**). **b** Quantification of GFAP immunofluorescent intensity in different groups at different time after injury. (bar = 250 μm)

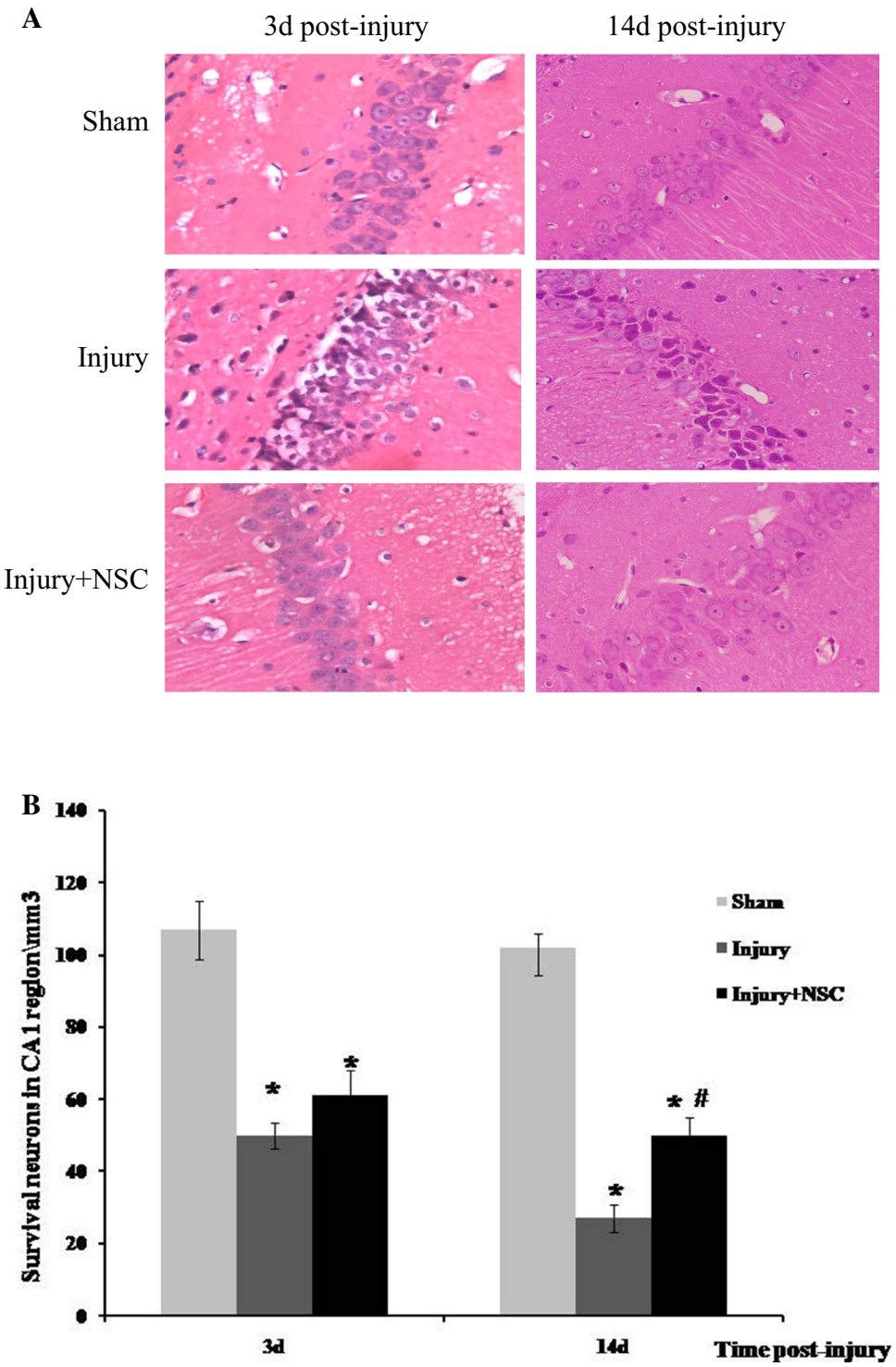


Fig. 4 Transplanted NSCs protected hippocampal neurons. **a** Representative image of hippocampal neurons in CA1 region on 3 and 14 days post-TBI; Neurons exhibited swelling body and lightly staining with HE both in injury and NSCs transplanted groups. Intact neurons were increased 3 days after TBI compared with the sham group ($^{*}p < 0.05$); **b** Quantification of neuronal survival in the hippocampal CA1 region showed that rats receiving NSCs transplantation had significantly increased levels of survival neurons on 14 days post injury compared with injury group ($^{\#}p < 0.05$)

Transplanted NSCs Ameliorated Cognitive Deficits After TBI

Whether these histological changes resulted in any functional differences was evaluated by behavioral examination. Hippocampal-dependent spatial memory capacity was evaluated with MWM paradigm 3 days after surgery. It was not of significantly difference between injury and NSC-transplanted groups on the first and second day. On the third day, the memory and learning deficits of NSCs-transplanted group were significantly better than those in injured group on MWM hidden platform task (as shown in Fig. 6a). Meanwhile, escape latency was significantly reduced in NSCs-transplanted group relative to injury group (as shown in

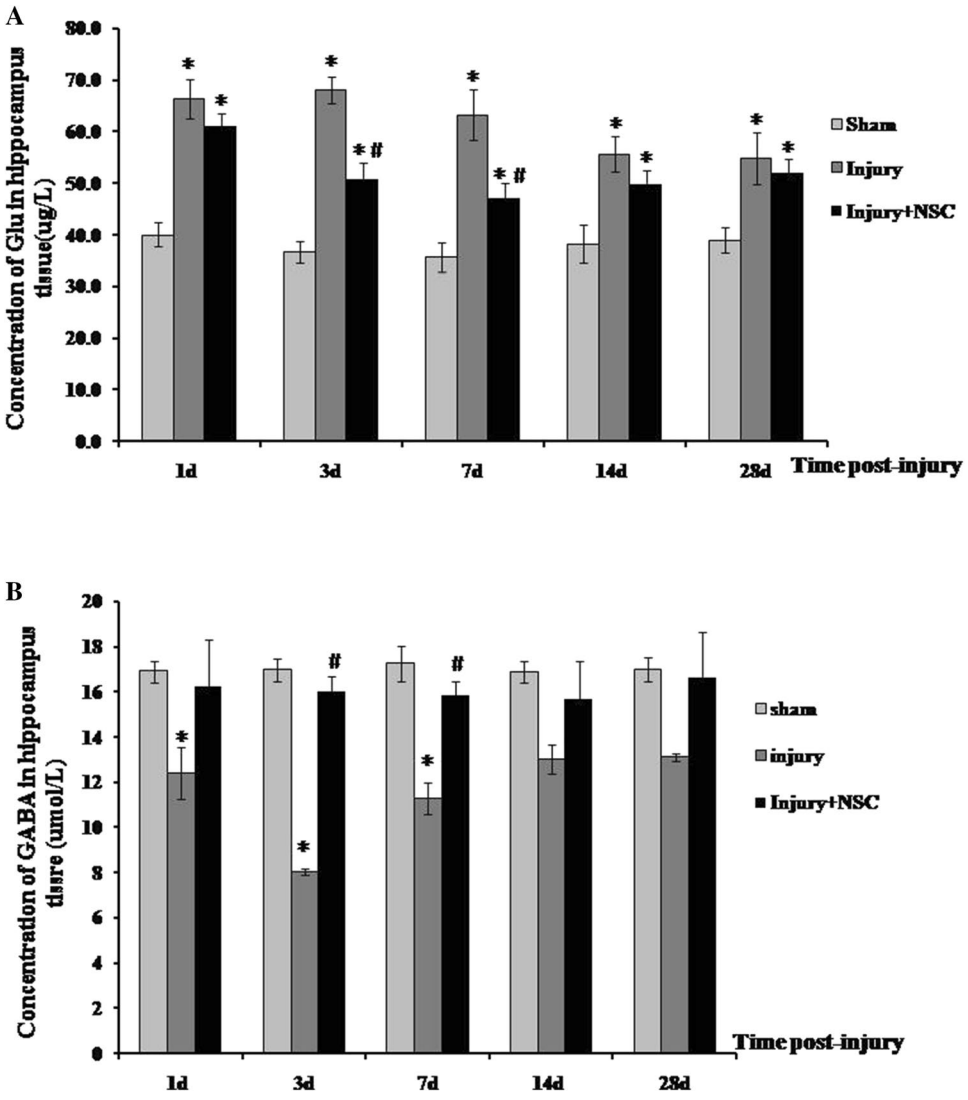


Fig. 5 Content of neurotransmitter in hippocampal tissue after brain injury. **a** The concentration of Glu in injury and NSCs transplanted group both increased after injury compared with sham group ($^{*}p < 0.05$). The concentration of Glu in injury group was evidently higher than that in NSCs transplantation group on 3 and 7 days after injury, which had significant difference ($^{\#}p < 0.05$). **b** In injury group GABA significantly down-regulated on 3 and 7 days after injury compared with sham and NSCs transplanted group, then maintained at relative lower level until 28 days (compared with sham group:$^{*}p < 0.05$, compared with NSCs transplanted group:$^{\#}p < 0.05$). In NSC transplantation group there was no significant difference in GABA release at different time-point after injury compared with sham group

Fig. 6b, Supplementary Table 3). But swimming speed was not significantly different among three groups (as shown in Supplementary Table 4), which indicated that the observed differences in memory and learning functions were not a result of an in-ability to execute the swim task (as shown in Fig. 6c).

Discussion

Traumatic brain injury (TBI) is a global problem that often results in long-lasting function impairment caused by primary injury and progressive secondary injury. Usually, functional defects results from a combination of pathological events which include neuronal death, axonal injury, the formation of glial scar, imperfect microenvironment for regeneration and so on. Based on these, many measures were applied to repair the deficit in structure and rehabilitate motor and sensory functions after TBI, which included rescuing the survival of injured neurons, inducing lateral sprouting of injured axons by neurotrophins, decreasing the proliferation of astrocytes through transfect special genes, even reducing microglia inflammatory response and inhibiting the release of Glutamine by anti-inflammatory drugs or antagonist against exciting amino acid. But these effects are limited for the improvement of functions after TBI. During this process, more and more attentions were paid to replace the loss of neuronal cells [1, 7, 9].

In order to repair this damage, suitable neural cell types were required in injured areas. Because NSCs have the ability to self-renew and differentiate into neurons, astrocytes and oligodendrocytes, transplantation of NSC to repair the CNS injury become an attractive strategy. A great deal of researches have indicated transplanted NSCs into injured brain tissue can improve the structural and functional repair through replacing the lost neural cells,

secreting neurotrophins such as BDNF, NT-3, VEGF. Furthermore, NSCs transplanted directly into the injured brain can increase the expression of some special proteins, such as SYP and GAP43, which can induce the regeneration of injured axons and formation of synapses between transplanted NSCs and host [2, 4, 6, 19, 20].

Glutamate is the primary excitatory neurotransmitter in brain, which is in concert with GABA to control the balance of excitation and inhibition in many brain circuits. The Glutamate plays important roles in the progression of injury following TBI due to increased synaptic release of it. Excessive amounts of glutamate in extracellular space may lead to elevated levels of intracellular calcium hyperactivity, and excessive influx of sodium ions that can cause edema and eventually cell death. At the same time, the release of GABA in neuronal synapses decreased after TBI may alleviate the antagonism for glutamate, which aggravated the secondary injury of TBI. In our experiment, it was indicated that transplantation of NSCs in TBI rats can inhibit the release of Glu, which may alleviate the excitotoxicity to neurons in some extent. Meanwhile, the level of GABA always maintained at relatively normal levels compared to the injury and sham group, which may have effective antagonism on the excitotoxicity of Glu [6, 16, 18].

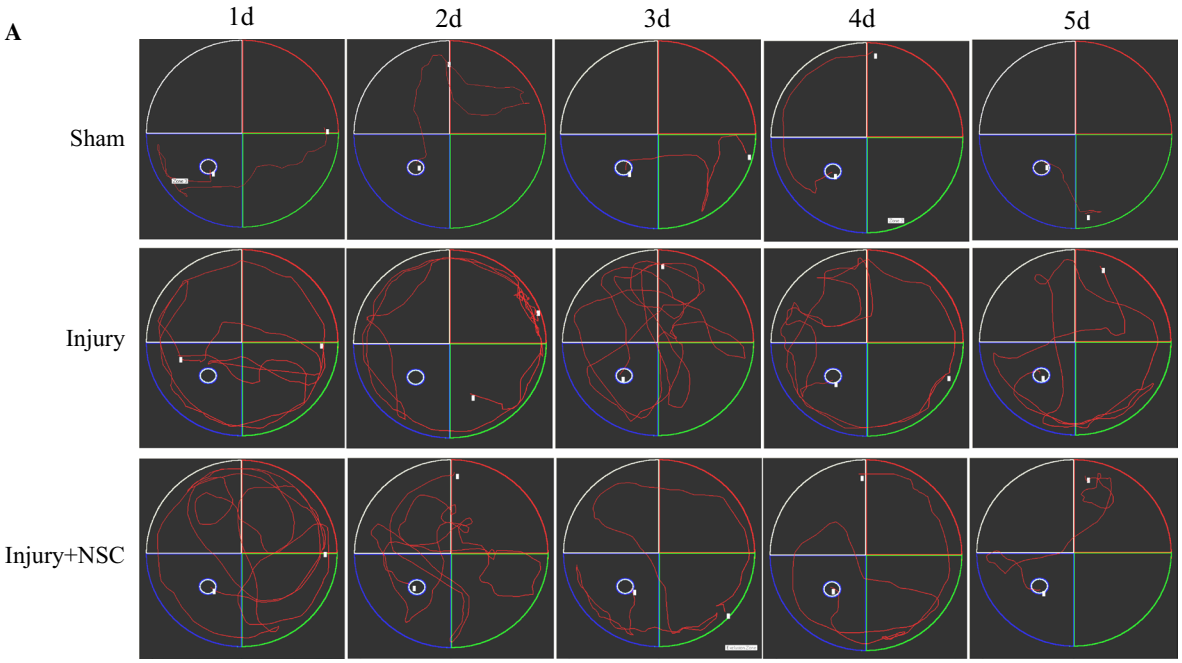
On these conditions, GFAP-positive cells around the injured site in injured group exhibited large bodies with more branches, meanwhile the number of which was also increased significantly after TBI. However, in NSCs transplantation group, the expression of GAFP around injured sites was less than those in injured group. Although proliferation of astrocytes can repair the defects of neural tissues after injury in some degree, the barrier of glial scar may obstruct the regeneration of injured axons and dendrites. So inhibiting proliferation of astrocytes becomes a potential strategy to treat TBI [21–23]. In our study, it was indicated that transplantation of NSCs after TBI can inhibit the activity of astroyctes around the injured site. These findings provided critical evidence supporting that NSCs are beneficial cells in reducing the formation of glial bars after TBI.

Further, the HE staining showed obvious cellular death in the TBI group, whereas the number of necrotic cells was significantly decreased in NSCs-transplanted rats compared with the injured group. It is suggested that NSCs transplantation can inhibit cellular death in rats with TBI. A previous study has reported that implanted NSCs in rats with brain ischemia could ameliorate neurological defects, enhance glycometabolism and improve the expression of growth factors and anti-apoptosis proteins in host brain. Therefore, NSCs transplantation plays a critical neuro-protective role by inhibiting neuronal death after TBI [4, 17, 20, 24]. Our study supported that transplantation of NSCs in TBI rats could inhibit neuronal degeneration and cellular death which may be related to the improvement of neurotransmitter balance between Glu and GABA.

Whether these histological changes resulted in any functional differences was also evaluated by hippocampal-dependent spatial memory capacity with the MWM paradigm. Both injury and NSCs transplanted groups exhibited significant spatial memory defects compared to sham group after injury. But there was not obvious difference between injury and NSC transplanted groups on the first and second day. On the third day of testing, escape latency was significantly reduced in NSCs transplanted groups relative to injury group which had difference in statistics ($p < 0.05$). On the fourth and fifth day, the escape latency of NSCs group was continuously reduced compared with injury group. This trend continued to 14 and 28 days post injury (as shown in S1). Furthermore, NSCs transplanted group spent a relatively greater percentage of total swim time in the target quadrant relative to injury group counterparts on day 3, 4 and 5. Swimming speed was not different among the three groups, indicating that the observed differences were not a result of an in-ability to execute the swimming task. Improvement in hippocampal-dependent spatial memory as assessed by MWM performance in NSCs transplanted animals is consistent with the histological improvement and better balance of Glu/GABA levels relative to injury group.

In this study, we established the weight drop model to determine the therapeutic effects of NSCs transplantation on TBI and its possible underlying mechanism. It was indicated that the rats with TBI exhibited severe neurological functional defects that could be improved by NSCs transplantation. Morphological examination was indicated that NSCs transplantation in TBI rats could promote the survival of neurons and inhibit the proliferation of astrocytes. The possible mechanism is related to the down-regulation of Glu and up-regulation of GABA by NSCs transplantation after TBI.

In summary, NSCs transplantation could effectively alleviate the formation of glial scar, enhance the survival of hippocampal neurons and improve cognitive functional defects in TBI rats. The underlying mechanism may be related to the effects of transplanted NSC on inhibiting the release of Glu and maintaining the content of GABA, so as to down-regulate excitotoxicity of neurotransmitter and improve the micro-environment in injured site.


A
1d
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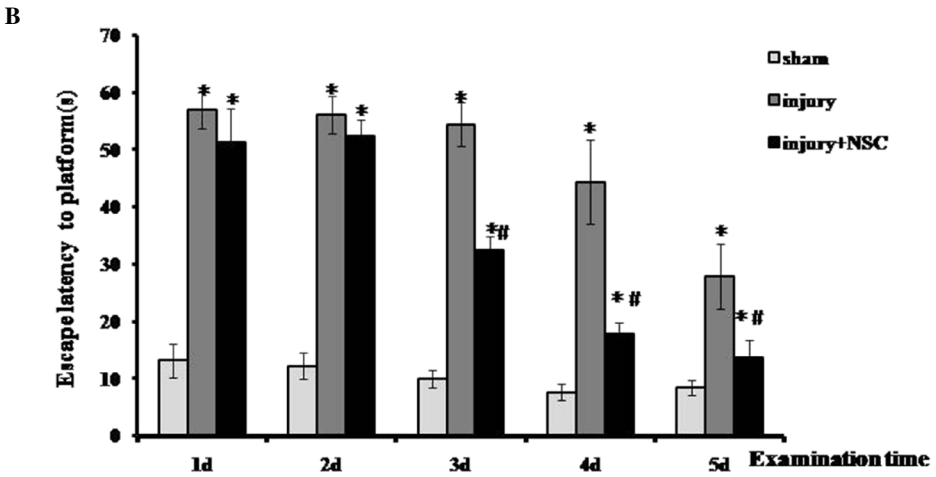
Injury+NSC


B
Escape latency to platform(s)
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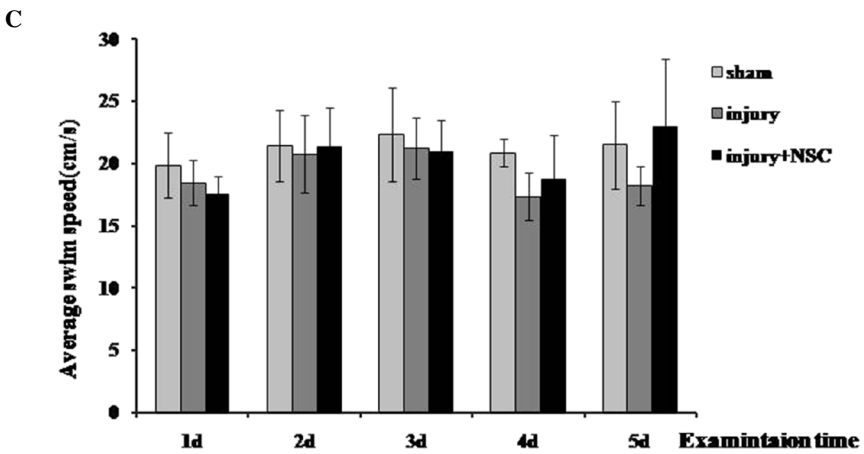
C
Average swim speed(cm/s)
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injury
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Examintaion time
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2d
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4d
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◂**Fig. 6** NSCs transplantation improved hippocampal-dependent cognitive outcomes. **a** The path track after platform removal for the aminals in spatial probe test. **b** On the first and second day, escape latencies in injury and NSCs transplanted groups were evidently longer than that in sham group ($^{*}p < 0.05$). On 3, 4, 5 days of testing, NSCs-transplanted TBI groups had significantly reduced escape latencies on the hidden platform task relative to injury group ($^{\#}p < 0.05$). **c** There were no significant differences in swim speeds among three groups, suggesting that the injury did not affect swimming skills

Funding Funding was provided by Qinnian Peiyu project of Military (Grant No. 16QNP105) and national natural scientific fund of China (Grant No. 81772066).

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