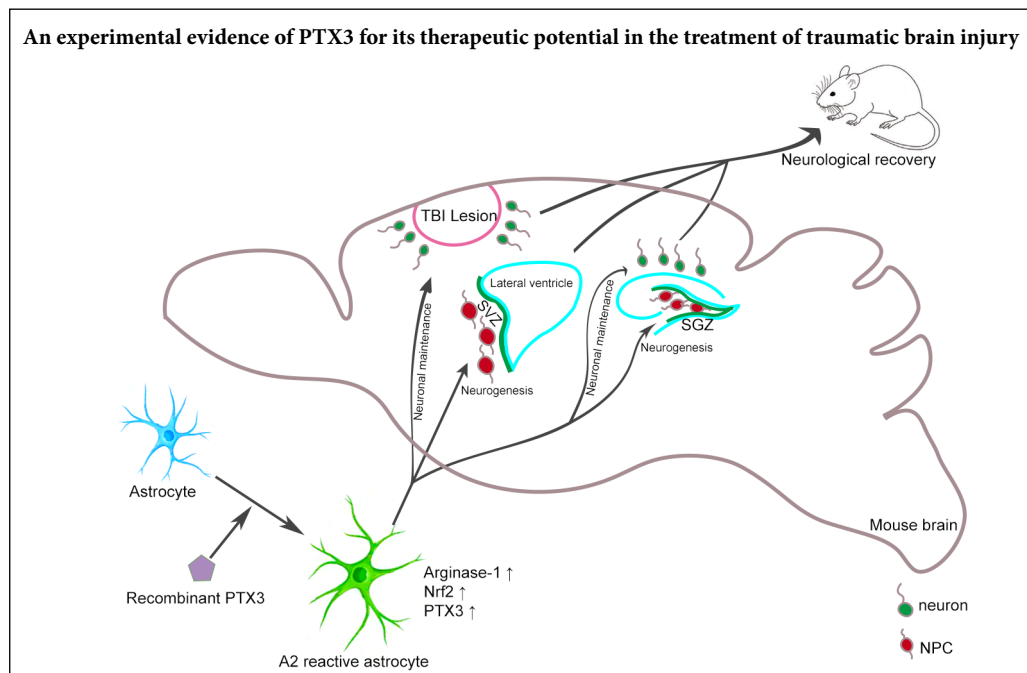● RESEARCH ARTICLE

Pentraxin 3 contributes to neurogenesis after traumatic brain injury in mice

Chao Zhou, Hong Chen, Jian-Feng Zheng, Zong-Duo Guo, Zhi-Jian Huang, Yue Wu, Jian-Jun Zhong, Xiao-Chuan Sun*, Chong-Jie Cheng*
Department of Neurosurgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

Graphical Abstract

An experimental evidence of PTX3 for its therapeutic potential in the treatment of traumatic brain injury

Correspondence to:
Xiao-Chuan Sun, MD, PhD,
sunxiaochuan@cqmu.edu.cn;
Chong-Jie Cheng, MD, PhD,
358187887@qq.com.

orcid:
0000-0001-6992-332X
(Xiao-Chuan Sun)
0000-0002-0680-2448
(Chong-Jie Cheng)

Abstract

Emerging evidence indicates that pentraxin 3 is an acute-phase protein that is linked with the immune response to inflammation. It is also a newly discovered marker of anti-inflammatory A2 reactive astrocytes, and potentially has multiple protective effects in stroke; however, its role in the adult brain after traumatic brain injury is unknown. In the present study, a moderate model of traumatic brain injury in mice was established using controlled cortical impact. The models were intraventricularly injected with recombinant pentraxin 3 (the recombinant pentraxin 3 group) or an equal volume of vehicle (the control group). The sham-operated mice underwent craniotomy, but did not undergo the controlled cortical impact. The potential neuroprotective and neuroregenerative roles of pentraxin 3 were investigated on days 14 and 21 after traumatic brain injury. Western blot assay showed that the expression of endogenous pentraxin 3 was increased after traumatic brain injury in mice. Furthermore, the neurological severity test and wire grip test revealed that recombinant pentraxin 3 treatment reduced the neurological severity score and increased the wire grip score, suggesting an improved recovery of sensory-motor functions. The Morris water maze results demonstrated that recombinant pentraxin 3 treatment reduced the latency to the platform, increased the time spent in the correct quadrant, and increased the number of times traveled across the platform, thus suggesting an improved recovery of cognitive function. In addition, to investigate the effects of pentraxin 3 on astrocytes, specific markers of A2 astrocytes were detected in primary astrocyte cultures *in vitro* using western blot assay. The results demonstrated that pentraxin 3 administration activates A2 astrocytes. To explore the protective mechanisms of pentraxin 3, immunofluorescence staining was used. Intraventricular injection of recombinant pentraxin 3 increased neuronal maintenance in the peri-injured cortex and ipsilateral hippocampus, increased the number of doublecortin-positive neural progenitor cells in the subventricular and subgranular zones, and increased the number of bromodeoxyuridine (proliferation) and neuronal nuclear antigen (mature neuron) double-labeled cells in the hippocampus and peri-injured cortex. Pentraxin 3 administration also increased the number of neurospheres and the number of bromodeoxyuridine and doublecortin double-labeled cells in neurospheres, and enhanced the proliferation of neural progenitor cells in primary neural progenitor cell cultures *in vitro*. In conclusion, recombinant pentraxin 3 administration activated A2 astrocytes, and consequently improved the recovery of neural function by increasing neuronal survival and enhancing neurogenesis. All experiments were approved by the Animal Ethics Committee of the First Affiliated Hospital of Chongqing Medical University, China on March 1, 2016.

Key Words: brain injury; brain trauma; cells; neurogenesis; plasticity; protein; recovery; regeneration

Chinese Library Classification No. R453; R363; R364

Introduction

Traumatic brain injury (TBI) is initiated by the application of mechanical force to the head and is a leading cause of death and disability across all ages worldwide (Sun, 2014; Xiong et al., 2015). In China, it is estimated that there are approximately 0.8–0.9 million new cases each year (Jiang et al., 2019). In addition to causing transient primary damage to brain structure and tissue, secondary brain injury can induce long-term and complex molecular cascades, for example by elevating the levels of the pro-inflammatory cytokines interleukin-1α, interleukin-6, and tumor necrosis factor α, which amplify neurotoxic injury and eventually increase neurodegeneration and neuronal loss (Xiong et al., 2015). Survivors of TBI experience chronic motor, cognitive, and neuropsychological disorders after injury and throughout life, causing a large burden to society and families. Unfortunately, effective treatments for TBI remain very limited (Grafman, 2016; Jiang et al., 2019).

Pentraxin 3 (PTX3) is an acute-phase protein that is linked to the immune response to inflammation (Erreni et al., 2017). The expression of PTX3 in the normal mouse brain is developmentally regulated; it reaches its highest levels in the embryonic stage, and then gradually decreases. PTX3 is eventually maintained at a low expression level after postnatal day 14 (Fossati et al., 2018). It has been reported that PTX3 can bind the complement cascade and prevent toxic inflammatory reactions in the epileptic brain (Fornai et al., 2015). It also has been demonstrated that PTX3 gene knockout causes a reduction in neural repair and regeneration following ischemic brain injury (Fornai et al., 2015; Rodriguez-Grande et al., 2015; Shindo et al., 2016). Moreover, recombinant PTX3 (rPTX3) administration increases neural stem cell proliferation *in vitro* (Rodriguez-Grande et al., 2015) and promotes synaptogenesis in hippocampal neuronal cultures (Fossati et al., 2018). Additionally, one study reported that higher serum PTX3 level is associated with higher hospital mortality in severe TBI patients; however, the mechanism underlying this association remains unclear (Gullo Jda et al., 2011).

Astrocytes can alter their gene expression, morphology, and function after TBI to maintain normal brain activities (Burda et al., 2016). Furthermore, emerging evidence has demonstrated that astrocytes can be transformed into the harmful, pro-inflammatory A1 phenotype or the beneficial, anti-inflammatory A2 phenotype. Moreover, activated A2 astrocytes can help to alleviate chronic neurodegeneration (Neal et al., 2018). PTX3 is a newly discovered marker of A2 reactive astrocytes (Liddelow et al., 2017). In our previous transcriptome analysis, PTX3 expression was significantly upregulated in the brain of an adult mouse model of TBI caused by controlled cortical injury (CCI) (Zhong et al., 2016). Thus, it is worth considering whether PTX3 can activate astrocytes to the A2 phenotype and play a protective role in TBI. To investigate this idea, rPTX3 protein was applied to investigate its potential role in astrocyte activation and TBI recovery. We hypothesized that PTX3 might have neuroprotective and neuroregenerative roles via A2 reactive astrocytes in a mouse model of TBI.

Materials and Methods

Animals

A total of 108 adult male C57BL/6 mice, aged 8–12 weeks and weighing 20–25 g, and 6 newborn C57BL/6 mice, aged 1–2 days old and weighing 1.5–2.0 g, were purchased from the Experimental Animal Center of Chongqing Medical University, China (license No. SYXK-(Yu)-2018-0003). The mice were used to establish CCI models of TBI as previously described (Martinez and Peplow, 2017; Zhong et al., 2017; He et al., 2018). In brief, mice were anesthetized with inhalation of 3% isoflurane (Yuyan Instruments, Shanghai, China) in 67% N$_2$O/30% O$_2$ (Yuyan Instruments) until they did not respond to a tail pinch. Thereafter, 1.5% isoflurane was used for anesthesia maintenance. Craniotomy was performed on the right side of the skull, 2.0 mm posterior to bregma and 1.0 mm lateral from the midline, using a 5 mm diameter drill. The following impact parameters were used to mimic moderate TBI: 5.0 m/s velocity, 2.0 mm depth, 100 ms dwell time, and 3.0 mm diameter impactor. The sham-operated mice underwent craniotomy only, and did not undergo CCI. The body temperatures of the mice were maintained at 37°C throughout the whole procedure, and the mice were then housed in a 12-hour light/dark cycle with free access to food and water.

One hundred and two mice were randomly divided into four groups. In the TBI group (CCI, *n* = 42), six mice were used for immunostaining on day 1 after craniotomy, while six mice were used for western blot assay at each of the six different time points after CCI (at 6 hours and on days 1, 3, 7, 14, and 21). In the sham group (craniotomy, *n* = 28), six mice were used for immunostaining and six were used for western blot assay on day 1 after craniotomy, while six were used for immunostaining on day 14. In addition, 10 mice underwent behavioral tests and were then used for immunostaining on day 21 after craniotomy. In the control group [CCI + vehicle (phosphate-buffered saline; PBS; containing 0.1% bovine serum albumin), *n* = 16], six mice were used for immunostaining on day 14 after CCI, and 10 mice underwent behavioral tests and were then used for immunostaining on day 21. In the rPTX3 group (CCI + rPTX3; *n* = 16), six mice were used for immunostaining on day 14 after CCI, and 10 mice underwent behavioral tests and were then used for immunostaining on day 21.

The rPTX3 (R&D Systems, Minneapolis, MN, USA) was reconstituted at 10 μg/mL in sterile PBS containing 0.1% bovine serum albumin. The rPTX3 was first intraventricularly injected on day 4 after CCI, at a dose of 1 μg/kg, and was then injected daily for 7 consecutive days. The injection site was anteroposterior −0.5 mm, mediolateral −1.5 mm, and dorsoventral −1.5 mm, starting from bregma. Bromodeoxyuridine (BrdU; Sigma-Aldrich, St. Louis, MO, USA) was dissolved in sterile PBS at 10 mg/mL. It was first injected intraperitoneally on day 4 after CCI, at a dose of 50 mg/kg, and was then injected daily for 7 consecutive days. In addition, six adult C57BL/6 mice were used to collect primary

neural progenitor cells (NPCs) and six newborn C57BL/6 mice were used to collect primary astrocytes, for cell culture experiments.

All experiments were approved by the Animal Ethics Committee of the First Affiliated Hospital of Chongqing Medical University, China on March 1, 2016. The experimental procedures followed the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

NPC cultures
NPCs were isolated and dissociated from the subventricular zone of adult male C57BL/6 mouse brains as previously described (Azari et al., 2010). These single cell suspensions were then plated at a density of 5×10^5 cells/T25 flask in 5 mL complete neural stem cell medium, which was prepared by mixing neural stem cell Basal Medium (Stemcell Technologies, Vancouver, BC, Canada) and neural stem cell Proliferation Supplements (Stemcell Technologies) at a 9:1 ratio. In addition, 20 ng/mL epidermal growth factor (R&D Systems), 10 ng/mL basic fibroblast growth factor (R&D Systems), and 1 μL/mL 0.2% heparin (Stemcell Technologies) were added. After incubation at 37°C and 5% CO_2 for 7 days, primary neurospheres were generated, passaged by dissociation with Accutase (Gibco Life Technologies, Rockville, MD, USA), and reseeded at a concentration of 5×10^5 cells/T25 flask in 5 mL complete neural stem cell medium (passage 1). Neurospheres were passaged every 5–7 days, and experiments were performed at passages 3–7.

Neurosphere formation and BrdU incorporation assays were performed to assess NPC proliferation. As previously described (Yu et al., 2018), a single NPC suspension was plated in each well of a 12-well plate (Corning, New York, NY, USA) at a concentration of 1×10^4 cells/mL. Next, rPTX3 protein (R&D Systems) was added to the culture medium at a concentration of 100 ng/mL. The relative neurosphere number was counted at 7 days using an inverted microscope (Leica, Wetzlar, Hesse, Germany). Additionally, BrdU incorporation was performed in neurospheres at 6 days of culture in normal culture medium after NPC passage 3–7, and the neurospheres were then treated with rPTX3 (100 ng/mL) for 24 hours and BrdU (20 μg/mL) for 6 hours. Neurospheres were collected and then plated on laminin-coated coverslips (50 μg/mL; Corning) for immunofluorescence staining.

Astrocyte cultures
Primary mouse astrocytes were prepared from the whole brains of neonatal mice as previously described (Cui et al., 2019). Briefly, single cell suspensions were obtained by gentle dissociation and trituration of the neonatal brain, and were then plated in T75 culture flasks precoated with poly-D-lysine (Sigma-Aldrich). Cells were cultured in Dulbecco's modified Eagle medium (Gibco Life Technologies) containing 4.5 g/L D-glucose, 110 mg/L sodium pyruvate, and 4 mM L-glutamine, supplemented with 10% heat-inactive fetal bovine serum (Gibco Life Technologies) and 1% penicillin-streptomycin (Gibco Life Technologies), and were

maintained in a cell incubator at 37°C and 5% CO_2. The purity of all astrocyte cultures was greater than 95%, and the cells were positive for glial fibrillary acidic protein (GFAP). Purified astrocytes were treated with rPTX3 (100 ng/mL) for 24 hours and then collected for western blot assay.

Behavioral tests
Wire grip scores were used to evaluate vestibulomotor function at 1 day before injury and on days 1, 3, 7, 14, and 21 after CCI. A 45 cm long metal wire with a 3 mm diameter was suspended 45 cm above the ground with two vertical wooden sticks. Each mouse was put on the middle of the wire and the latency (the time that the mouse remained on the wire within a 60-second period) was assessed. A five-point scale was used (Bermpohl et al., 2006). Five points represented the minimum deficit and zero points represented the maximum deficit, as follows. Zero points: falling off the wire within 30 seconds; one point: failed to hold onto the wire with fore paws and hind paws together; two points: holding onto the wire with fore paws and hind paws together, but without using the tail; three points: holding onto the wire with fore paws, hind paws, and tail together; four points: moving along the wire with fore paws, hind paws, and tail together; five points: moving along the wire with fore paws, hind paws, and tail together, and climbing to the bottom via either of the two sticks. Neurological severity scores (NSS) were evaluated 1 day before injury and on days 1, 3, 7, 14, and 21 after CCI. Ten different tasks, measuring general behavior, balance, alertness, and motor ability, were performed to evaluate neurological ability. One point was obtained for failing each of these tasks. Ten points represented the maximum deficit and zero points represented the minimum deficit. Cognitive function was detected using the Morris water maze test over 6 consecutive days. In brief, latency and the time spent in the correct quadrant were measured from days 16 to 20 after CCI using the navigation test. The time spent traveling across the platform was measured at day 21 after CCI using the probe trial test. Detailed protocols for the NSS, wire grip, and Morris water maze test have been described in previous studies (Cheng et al., 2017; He et al., 2018). The investigators were blinded to the experimental groups for all tests.

Western blot assay
Western blot assays were performed as previously described (Huang et al., 2016; Cheng et al., 2017). In brief, experimental mice were sacrificed at 6 hours and 1, 3, 7, 14, and 21 days after CCI, and the ipsilateral brains were collected for total protein extraction. Protein samples from the primary astrocyte cultures were also extracted using this procedure. In brief, the sample proteins were separated in 5–12% sodium dodecyl sulfate-polyacrylamide gels (Beyotime Biotechnology, Nanjing, China) and transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat milk at room temperature for 1 hour and incubated overnight at 4°C with the following primary antibodies: rabbit monoclonal anti-PTX3 (1:1000; Abcam, Cambridge, MA, USA), rabbit monoclonal

anti-arginase-1 (1:1000; Cell Signaling Technology, Danvers, MA, USA; a marker of A2 reactive astrocytes), rabbit monoclonal anti-nuclear factor-E2-related factor 2 (Nrf2; 1:500; Abcam; a marker of A2 reactive astrocytes), and rabbit monoclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:2000; Proteintech, Wuhan, Hubei, China; loading control). After washing with Tris-buffered saline/ Tween, the membranes were incubated for 1 hour at 37°C with horseradish peroxidase-conjugated AffiniPure goat anti-rabbit IgG (1:5000; Proteintech) secondary antibody. Proteins in the membranes were visualized using an enhanced chemiluminescence reagent (Millipore) and a Fusion Solo 6S image analysis system (Vilber Lourmat, Paris, France). Relative protein intensities were quantified using Gel-Pro Analyzer 4.0 (Media Cybernetics, Silver Spring, MD, USA).

Immunofluorescence staining
Immunofluorescence was performed following a previously described protocol (Redmond et al., 2019; Zhang et al., 2019). Briefly, mice were sacrificed on days 1, 14, and 21 after CCI and then perfused with PBS and 4% paraformaldehyde for fixation. The collected brains were postfixed overnight at 4°C in 4% paraformaldehyde, and were then cryoprotected in graded sucroses (10%, 20%, and 30%). Next, the brains were embedded in optimal cutting temperature compound and cut into 10 μm frozen coronal sections. Neurospheres were fixed on coverslips for 20 minutes in 4% paraformaldehyde and then immunostained as previously described (Rodriguez-Grande et al., 2015). In brief, slides were washed, treated with antigen retrieval, and blocked with 5% bovine serum albumin at room temperature for 30 minutes. The sections were then incubated overnight at 4°C with primary antibodies, including mouse monoclonal anti-GFAP (1:500; Proteintech; a marker of astrocytes), rabbit polyclonal anti-PTX3 (1:50; Proteintech), goat polyclonal anti-ionized calcium-binding adaptor molecule 1 (Iba1; 1:200; Abcam; a marker of microglia), mouse monoclonal anti-neuronal nuclear antigen (NeuN; 1:200; Novus Biologicals, Littleton, NH, USA; a marker of mature neurons), mouse monoclonal anti-doublecortin (DCX; 1:100; Abcam; a marker of newly generated immature neurons), and rabbit polyclonal anti-BrdU (1:100; Abcam; a marker of cell proliferation). Slides were then washed and incubated at room temperature for 2 hours with the appropriate secondary antibodies: donkey anti-goat Coralite 594 (1:100; Proteintech), donkey anti-rabbit Coralite 594 (1:100; Proteintech), donkey anti-rabbit Coralite 488 (1:100; Proteintech), donkey anti-mouse Coralite 594 (1:100; Proteintech), and donkey anti-mouse Coralite 488 (1:100; Proteintech). Cell nuclei were stained with 4′,6-di-amidino-2-phenylindole (DAPI; Sigma-Aldrich). Images were captured using a fluorescence microscope (Leica, Wetzlar, Hesse, Germany). Using ImageJ (NIH, Bethesda, MD, USA), we counted the number of DCX-positive cells per section in the subventricular zone and subgranular zone, the number of NeuN-positive cells per mm^2 in the hippocampal CA1 area and peri-injured cortex, the number of NeuN/ BrdU double-positive cells per section in the hippocampus

and cortex, and the percentage of BrdU-DCX double positive cells in neurospheres. Six coronal sections from each brain or six coverslips of neurospheres from each treatment were imaged.

Statistical analysis
The data are presented as the mean ± SEM. Neurological behavior was analyzed using two-way repeated measures analysis of variance. Immunoblotting and immunofluorescence were analyzed using Student's *t*-test or one-way analysis of variance followed by Tukey's *post hoc* test. All statistical analyses and figures were made using GraphPad Prism 8.0.1 software (GraphPad Software, San Diego, CA, USA). A value of $P < 0.05$ was considered statistically significant for all comparisons.

Results
PTX3 expression is upregulated after TBI
All of the experiments that were performed at different time points for the *in vivo* study are shown in **Figure 1A**. To investigate the endogenous expression of PTX3 in response to TBI, PTX3 protein levels were measured at different time points after TBI using western blot assay. Compared with the sham group, PTX3 expression was rapidly upregulated at 6 hours. This increase lasted for 3 days, and the expression then returned to baseline levels (Huang et al., 2016; Cheng et al., 2017; **Figure 1B**). The localization of PTX3 was also measured by immunofluorescence staining on day 1 after TBI. PTX3 was expressed in the cerebral cortex around the injury site and was colocalized with Iba1, GFAP, and NeuN (**Figure 1C**).

rPTX3 administration activates A2 astrocytes
To determine the phenotype of reactive astrocytes, the ideal approach is to perform gene transcriptome analysis. However, astrocytes with A2 phenotypes can also be identified simply by analyzing the expression of specific proteins that are associated with reactive astrocytes, as a previous study has reported (Neal et al., 2018). We therefore measured the protein levels of three different A2 reactive astrocyte markers after rPTX3 treatment: Nrf2, arginase-1, and PTX3. As shown in **Figure 2**, the expression of all of these markers was significantly upregulated compared with the normal control group ($P < 0.001$), and PTX3 was the most markedly upregulated. These findings indicate that PTX3 may be amplified in astrocytes and might polarize astrocytes toward the A2 phenotype.

rPTX3 treatment improves neuronal maintenance after TBI
Neuronal survival is the foundation of neurological function. We therefore measured the effect of rPTX3 injection on neuronal maintenance after TBI. NeuN-positive cells in the ipsilateral hippocampus (**Figure 3A**) and cortex (**Figure 3B**) were measured on day 14 after TBI. Compared with vehicle treatment, rPTX3 treatment significantly increased neuronal maintenance in the hippocampal CA1 and peri-injured cortex ($P < 0.01$ and $P < 0.001$, respectively).

rPTX3 treatment promotes neurogenesis after TBI

Immunostaining showed that, compared with vehicle treatment, long-term rPTX3 injection significantly increased the number of DCX-positive cells in both the ipsilateral subgranular zone (**Figure 4A**) and ipsilateral subventricular zone (**Figure 4B**) at 21 days post TBI. We also observed BrdU/NeuN double-positive cells in the dentate gyrus of the ipsilateral hippocampus (**Figure 4C**) and in the peri-injured cortex (**Figure 4D**) after rPTX3 treatment, although these were present in small numbers. In contrast, few double-positive cells were found in the sham and control groups. Together, these data indicate that long-term injection of rPTX3 promotes neurogenesis in mice after TBI.

rPTX3 administration promotes neurosphere proliferation

To investigate the roles of PTX3 in neurogenesis *in vitro*, we tested the effect of rPTX3 protein on proliferation in adult-derived NPCs in culture. The administration of rPTX3 protein significantly increased neurosphere formation compared with the control group ($P < 0.001$; **Figure 5A**). In addition, the number of BrdU/DCX double-positive cells was significantly increased after rPTX3 administration ($P < 0.001$; **Figure 5B**). Together, these data indicate that rPTX3 directly promotes neuroshpere proliferation *in vitro*.

rPTX3 treatment improves the recovery of neurological function after TBI

To evaluate the roles of PTX3 in the recovery of neurological function after TBI, we measured sensory-motor behavioral outcomes, including performance in the wire grip test and NSS test. CCI-injured mice had significantly impaired sensory-motor functions. Compared with vehicle treatment, rPTX3 treatment after CCI achieved an earlier return to baseline in wire grip test scores (**Figure 6A**) and significantly improved NSS scores (**Figure 6B**). These data indicate that rPTX3 administration induces significantly improved sensory-motor function recovery after TBI. Beginning on day 16 after TBI, the Morris water maze assay was also performed for 6 consecutive days to test post-injury cognitive function. As expected, CCI significantly increased learning latency compared with the sham group. However, rPTX3 treatment significantly decreased learning latency compared with vehicle treatment (**Figure 6C**). CCI-injured mice spent significantly less time in the correct quadrant than sham-operated mice. Compared with vehicle treatment, rPTX3 administration significantly increased the time spent in the correct quadrant (**Figure 6D**). The probe trial of the Morris water maze was performed at 21 days after CCI, and the vehicle-treated mice crossed the platform location significantly fewer times than the sham-operated mice. In contrast, rPTX3 treatment led to significantly increased numbers of crossings (**Figure 6E**). Collectively, these results indicate that rPTX3 treatment improves sensory-motor and cognitive functional recovery after TBI.

Discussion

Under normal circumstances, the expression of PTX3 in the adult brain is maintained at low levels (Fossati et al., 2018). In the present study, we demonstrated that PTX3 protein expression was rapidly upregulated 6 hours after TBI, and that this increase lasted for 3 days; the expression levels then returned to baseline. Additionally, PTX3 was found in neurons, astrocytes, and microglia. Next, we investigated the effect of intraventricular rPTX3 injection on TBI mice *in vivo*, as well as the effect of rPTX3 on astrocyte and NPC cultures *in vitro*. The major findings of our study were that rPTX3 administration activated A2 astrocytes, reduced neural damage, increased neurogenesis, and consequently improved neural functional recovery.

Astrocytes are the most abundant cell type in the cerebrum, and play important roles in maintaining physiological functions and supporting normal brain activities. After brain trauma, astrocytes can be activated by complex injury-induced multicellular responses that alter gene expression, morphology, and function, and both hinder and support recovery (Myer et al., 2006; Zamanian et al., 2012; Ding, 2014; Choudhury and Ding, 2016; Liddelow et al., 2017). Recently, transcriptome analysis demonstrated that astrocytes can adopt a harmful, pro-inflammatory A1 reactive phenotype or a protective, anti-inflammatory A2 reactive phenotype. PTX3 is considered a new biomarker of A2 reactive astrocytes (Liddelow et al., 2017; Neal et al., 2018). A1 astrocytes are classically activated by interleukin 1α, tumor necrosis factor α, and C1q, which are secreted from microglia. A1 astrocytes lose most normal astrocytic functions and exhibit neurotoxic functions; thus, they are considered harmful (Liddelow et al., 2017). For example, Liddelow et al. (2017) cocultured A1 astrocytes with purified retinal ganglion cells and found that retinal ganglion cells quickly died in A1 reactive astrocyte-conditioned media. In contrast, A2 astrocytes are activated by anti-inflammatory cytokines, such as interleukin-4 and prokineticin-2 (Neal et al., 2018). A2 astrocytes can upregulate many neurotrophic factors, such as brain-derived neurotrophic factor, glial cell-derived neurotrophic factor, and nerve growth factor. These factors facilitate neural survival, neuroregeneration, and synaptic repair; thus, A2 astrocytes are considered protective (Liddelow and Barres, 2017; Datta et al., 2018; Oliveira-Junior et al., 2019). Anderson et al. (2016) ablated scar-forming astrocytes by deleting State3, and this led to a worse injury outcome. Interestingly, no axonal regrowth occurred when scarring was prevented, but newly generated immature scar-forming astrocytes helped with axonal regeneration, which was augmented by brain-derived neurotrophic factor supplementation (Anderson et al., 2016). These scar-forming astrocytes were later demonstrated to be A2 reactive astrocytes (Liddelow and Barres, 2017). In the present study, we observed that, when primary mouse astrocytes were treated with rPTX3 protein *in vitro*, A2 astrocyte-specific markers, including arginase-1, Nrf2, and PTX3, were significantly increased. These findings indicate that PTX3 expression can be augmented in astrocytes, and that increased PTX3 may activate the A2 astrocyte phenotype.
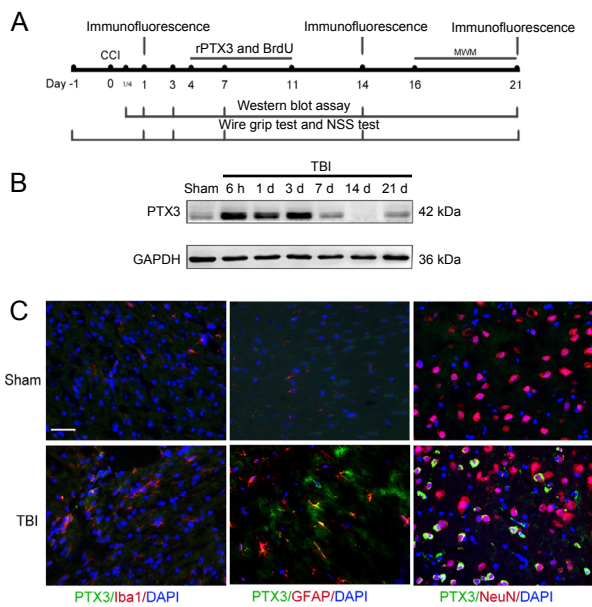
Figure 1 Experimental timeline and PTX3 expression in the adult mouse brain after TBI.
(A) Graphic experimental timeline of the *in vivo* studies. (B) Western blot image of PTX3 protein levels in the ipsilateral cortex at 6 hours and 1, 3, 7, 14, and 21 days following CCI; samples from the sham-operated group were used as controls. PTX3 expression in the perilesional cortex was upregulated at 6 hours; this increase lasted for 3 days and then the expression returned to baseline levels. GAPDH was used as the loading control. (C) Representative images of immunostaining for PTX3 on day 1 after CCI. PTX3 (green) was localized to Iba1-positive microglia (red, left image), GFAP-positive astrocytes (red, middle image), and neuronal nuclei antigen (NeuN)-positive neurons (red, right image) in the perilesional cortex. Cell nuclei were stained with DAPI (blue). However, there were no PTX3-positive cells in the sham-operated mice. *n* = 6 per group, scale bar: 40 μm. BrdU: Bromodeoxyuridine; CCI: controlled cortical impact; DAPI: 4′,6-di-amidino-2-phenylindole; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GFAP: glial fibrillary acidic protein; Iba1: ionized calcium-binding adaptor molecule 1; MWM: Morris water maze; NSS: neurological severity score; PTX3: pentraxin 3; rPTX3: recombinant pentraxin 3; TBI: traumatic brain injury.
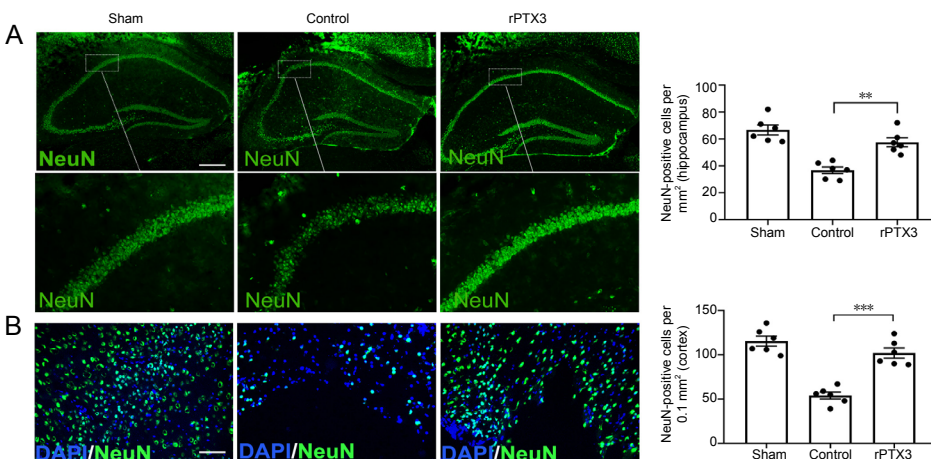

Figure 2 rPTX3 activates A2 astrocytes.
Western blot assay of the A2 reactive astrocyte markers PTX3, arginase-1, and Nrf2 after 100 ng/mL rPTX3 administration for 24 hours in primary astrocytes *in vitro*. GAPDH was used as an internal loading control. Representative images of PTX3, arginase-1, and Nrf2 protein expression (A) and the quantification data (B–D) reveal that these markers were significantly upregulated after rPTX3 treatment. ***$P < 0.001$. Data are expressed as the mean ± SEM (*n* = 6; Student's *t*-test). GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; Nrf2: nuclear factor-E2-related factor 2; PTX3: pentraxin 3; rPTX3: recombinant pentraxin 3.

Figure 3 rPTX3 increases neuronal maintenance after traumatic brain injury.
Neuronal survival in the ipsilateral hippocampus and cortex was measured by immunostaining on day 14 after controlled cortical impact. (A) Neurons (neuronal nuclear antigen, NeuN; green) in the hippocampus (left, upper) and an enlarged view of the hippocampal CA1 area (left, lower). The number of neurons was significantly higher after rPTX3 treatment compared with vehicle treatment (right). (B) Neurons (NeuN, green) in the peri-injured cortex. DAPI (blue) was used to stain the nuclei (left). The number of neurons was significantly higher after rPTX3 treatment compared with vehicle treatment (right). Scale bars: 200 μm (hippocampus); 40 μm (cortex). **$P < 0.01$, ***$P < 0.001$. Data are expressed as the mean ± SEM (*n* = 6; one-way analysis of variance followed by Tukey *post hoc* test). DAPI: 4′,6-Diamidino-2-phenylindole; rPTX3: recombinant pentraxin 3.

A previous study has reported that a lack of PTX3 significantly reduces neuronal survival by impairing cerebral blood flow at 14 days after middle cerebral artery occlusion (Rajkovic et al., 2018). Decreased vessel density and astrocyte proliferation impai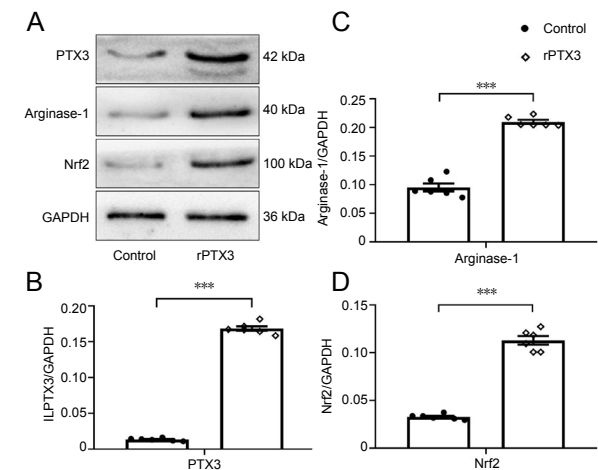red blood-brain barrier recovery and hindered neural nutrient supplement. Thus, PTX3 probably protects neurons in multiple ways. In response to secondary brain injury, a series of long-term and complex cascades that lead to neuronal death and aggravate neurological dysfunction are activated, including oxidative stress,

iron overload, glutamate excitotoxicity, apoptosis, and inflammation (Xiong et al., 2015). As we have described in the present study, PTX3 activated A2 astrocytes and increased Nrf2 expression. Previous investigations have demonstrated that astrocyte-derived Nrf2 can protect neurons against iron overload cytotoxicity and oxidative damage (Cui et al., 2016; Ishii et al., 2019). Moreover, PTX3 interacts with ficolin-1 to form a PTX3–ficolin-1 complex that can exert anti-inflammatory effects by inhibiting the secretion of interleukin-8 by macrophages (Ma et al., 2013). Furthermore, PTX3 can impair apoptotic cell antigen cross-presentation and promote the phagocytotic clearance of cell debris by microglia (Jeon et al., 2010; Fornai et al., 2015). In the current study, we intraventricularly injected low-dose rPTX3 protein (1 μg/kg) for 7 consecutive days. rPTX3 administration significantly increased neuronal density in both the ipsilateral hippocampus and the peri-injured cortex compared with the control group. Together, these results suggest that PTX3 enhances neuronal survival via A2 reactive astrocytes.

Recent research has suggested that enhancing neurogenesis is a promising strategy to repair the central nervous system following TBI (Ngwenya and Danzer, 2018). Emerging evidence suggests that NPCs exist in the subventricular zone and subgranular zone of the hippocampus in the adult brain in mammals, including humans (Boldrini et al., 2018; Moreno-Jiménez et al., 2019; Yörg Dillen, 2019). NPCs proliferate in these regions before migrating and eventually differentiating into newly generated neurons; this process is referred to as neurogenesis. Intriguingly, neurogenesis, which is considered to promote neural repair, is increased by TBI (Parent, 2003). However, this kind of endogenous neurogenesis is very limited in TBI patients, and a more effective approach for promoting neurogenesis needs to be identified. One previous study observed that neural stem cell proliferation is decreased in the subventricular and subgranular zones in PTX3-knockout mice after stroke (Rodriguez-Grande et al., 2015). However, the mechanisms for this decrease were not investigated. In our study, we demonstrated that rPTX3 injection increased DCX-positive NPC proliferation in both the subventricular zone and the subgranular zone; additionally, there were more newly generated mature neurons (BrdU/NeuN double-positive) in the rPTX3-treated mice. In the present study, PTX3 activated A2 astrocytes, and these can secrete high levels of neurotrophic factors and cytokines, such as nerve growth factor, cardiotrophin-like cytokine factor 1, leukemia inhibitory factor, and interleukin-6 (Zamanian et al., 2012; Lu et al., 2013). Lin et al. (2015) demonstrated that the overexpression of nerve growth factor increases neurite outgrowth and regeneration and consequently rescues impaired cognitive function following TBI in rats. Our data also showed that rPTX3 treatment directly promoted NPC proliferation *in vitro*, which indicates that PTX3 itself might also be an endogenous neurotrophic factor that mediates neuroregeneration, although the specific mechanisms need to be confirmed by further research. These findings indicate that rPTX3 treatment increases neurogenesis via A2

reactive astrocytes in TBI mice. Rescuing motor and cognitive function deficits is the major goal of TBI treatment. In our study, rPTX3 treatment improved sensory-motor and cognitive functional recovery after TBI; this suggests that increased neuronal maintenance and neurogenesis by A2 astrocytes might contribute to this process. Thus, PTX3 could be a promising therapeutic target for TBI recovery.

There are a few limitations to our study. First, PTX3 is a new marker of A2 astrocytes, and our understanding remains very limited. Further studies are needed to elucidate the molecular mechanisms of PTX3 in regulating neurogenesis and astrocyte reactivity. Second, the recovery of neural function is a complex pathophysiological process, and determining the effects of PTX3 on neurogenesis and neuronal survival alone may not sufficiently explain its effects on neural repair. Other aspects of neurorestoration, such as angiogenesis and axonal remodeling, could also be considered in the future. Third, a clinical study has reported that higher hospital mortality of patients with severe TBI is associated with increased serum PTX3 levels (Gullo Jda et al., 2011). This inconsistency with our findings may be because of the difference in trauma severity or because of differences between the central and peripheral nervous systems; however, the possible reason why our findings are inconsistent with Gullo Jda et al.'s study needs to be investigated in further studies.

In conclusion, endogenous PTX3 expression rapidly increases in the adult mouse brain following TBI, and this increase lasts for 3 days. rPTX3 administration activates the A2 astrocyte phenotype, which consequently improves the recovery of neural function by increasing neuronal maintenance and enhancing neurogenesis. These results suggest that rPTX3 treatment may be a promising therapeutic strategy for TBI. It would have been necessary to investigate angiogenesis in future study, because the SGZ and SVZ also have angiogenic activity.

Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*
Open access statement: *This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non-Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.*
Open peer reviewer: *Nafisa M. Jadavji, Carleton University, Canada.*
Additional file: *Open peer review report 1.*

References

Anderson MA, Burda JE, Ren Y, Ao Y, O'Shea TM, Kawaguchi R, Coppola G, Khakh BS, Deming TJ, Sofroniew MV (2016) Astrocyte scar formation aids central nervous system axon regeneration. Nature 532:195-200.

Azari H, Rahman M, Sharififar S, Reynolds BA (2010) Isolation and expansion of the adult mouse neural stem cells using the neurosphere assay. J Vis Exp doi:10.3791/2393.

Bermpohl D, You Z, Korsmeyer SJ, Moskowitz MA, Whalen MJ (2006) Traumatic brain injury in mice deficient in Bid: effects on histopathology and functional outcome. J Cereb Blood Flow Metab 26:625-633.

Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, Rosoklija GB, Stankov A, Arango V, Dwork AJ, Hen R, Mann JJ (2018) Human hippocampal neurogenesis persists throughout aging. Cell Stem Cell 22:589-599.

Burda JE, Bernstein AM, Sofroniew MV (2016) Astrocyte roles in traumatic brain injury. Exp Neurol 275 Pt 3:305-315.

Cheng C, Yu Z, Zhao S, Liao Z, Xing C, Jiang Y, Yang YG, Whalen MJ, Lo EH, Sun X, Wang X (2017) Thrombospondin-1 gene deficiency worsens the neurological outcomes of traumatic brain injury in mice. Int J Med Sci 14:927-936.

Choudhury GR, Ding S (2016) Reactive astrocytes and therapeutic potential in focal ischemic stroke. Neurobiol Dis 85:234-244.

Cui YQ, Zheng Y, Tan GL, Zhang DM, Wang JY, Wang XM (2019) (5R)-5-hydroxytriptolide inhibits the inflammatory cascade reaction in astrocytes. Neural Regen Res 14:913-920.

Cui Z, Zhong Z, Yang Y, Wang B, Sun Y, Sun Q, Yang GY, Bian L (2016) Ferrous iron induces Nrf2 expression in mouse brain astrocytes to prevent neurotoxicity. J Biochem Mol Toxicol 30:396-403.

Datta I, Ganapathy K, Razdan R, Bhonde R (2018) Location and number of astrocytes determine dopaminergic neuron survival and function under 6-OHDA stress mediated through differential BDNF release. Mol Neurobiol 55:5505-5525.

Ding S (2014) Dynamic reactive astrocytes after focal ischemia. Neural Regen Res 9:2048-2052.

Erreni M, Manfredi AA, Garlanda C, Mantovani A, Rovere-Querini P (2017) The long pentraxin PTX3: A prototypical sensor of tissue injury and a regulator of homeostasis. Immunol Rev 280:112-125.

Fornai F, Carrizzo A, Ferrucci M, Damato A, Biagioni F, Gaglione A, Puca AA, Vecchione C (2015) Brain diseases and tumorigenesis: the good and bad cops of pentraxin3. Int J Biochem Cell Biol 69:70-74.

Fossati G, Pozzi D, Canzi A, Mirabella F, Valentino S, Morini R, Ghirardini E, Filipello F, Moretti M, Gotti C, Annis DS, Mosher DF, Garlanda C, Bottazzi B, Taraboletti G, Mantovani A, Matteoli M, Menna E (2018) Pentraxin 3 regulates synaptic function by inducing AMPA receptor clustering via ECM remodeling and β1-integrin. EMBO J doi: 10.15252/embj.201899529.

Grafman J (2016) Long-Term Follow-up of TBI. N Engl J Med 375:1597.

Gullo Jda S, Bertotti MM, Silva CC, Schwarzbold M, Diaz AP, Soares FM, Freitas FC, Nunes J, Pinheiro JT, Morato EF, Prediger RD, Linhares MN, Walz R (2011) Hospital mortality of patients with severe traumatic brain injury is associated with serum PTX3 levels. Neurocrit Care 14:194-199.

He J, Liu H, Zhong J, Guo Z, Wu J, Zhang H, Huang Z, Jiang L, Li H, Zhang Z, Liu L, Wu Y, Qi L, Sun X, Cheng C (2018) Bexarotene protects against neurotoxicity partially through a PPARgamma-dependent mechanism in mice following traumatic brain injury. Neurobiol Dis 117:114-124.

Huang Z, Cheng C, Jiang L, Yu Z, Cao F, Zhong J, Guo Z, Sun X (2016) Intraventricular apolipoprotein ApoJ infusion acts protectively in traumatic brain injury. J Neurochem 136:1017-1025.

Ishii T, Warabi E, Mann GE (2019) Circadian control of BDNF-mediated Nrf2 activation in astrocytes protects dopaminergic neurons from ferroptosis. Free Radic Biol Med 133:169-178.

Jeon H, Lee S, Lee WH, Suk K (2010) Analysis of glial secretome: the long pentraxin PTX3 modulates phagocytic activity of microglia. J Neuroimmunol 229:63-72.

Jiang JY, Gao GY, Feng JF, Mao Q, Chen LG, Yang XF, Liu JF, Wang YH, Qiu BH, Huang XJ (2019) Traumatic brain injury in China. Lancet Neurol 18:286-295.

Liddelow SA, Barres BA (2017) Reactive astrocytes: production, function, and therapeutic potential. Immunity 46:957-967.

Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Munch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, et al. (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541:481-487.

Lin Y, Wan JQ, Gao GY, Pan YH, Ding SH, Fan YL, Wang Y, Jiang JY (2015) Direct hippocampal injection of pseudo lentivirus-delivered nerve growth factor gene rescues the damaged cognitive function after traumatic brain injury in the rat. Biomaterials 69:148-157.

Lu J, Frerich JM, Turtzo LC, Li S, Chiang J, Yang C, Wang X, Zhang C, Wu C, Sun Z, Niu G, Zhuang Z, Brady RO, Chen X (2013) Histone deacetylase inhibitors are neuroprotective and preserve NGF-mediated cell survival following traumatic brain injury. Proc Natl Acad Sci U S A 110:10747-10752.

Ma YJ, Doni A, Romani L, Jurgensen HJ, Behrendt N, Mantovani A, Garred P (2013) Ficolin-1-PTX3 complex formation promotes clearance of altered self-cells and modulates IL-8 production. J Immunol 191:1324-1333.

Martinez B, Peplow PV (2017) MicroRNAs as diagnostic markers and therapeutic targets for traumatic brain injury. Neural Regen Res 12:1749-1761.

Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N, Ávila J, Llorens-Martín M (2019) Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nat Med 25:554-560.

Myer DJ, Gurkoff GG, Lee SM, Hovda DA, Sofroniew MV (2006) Essential protective roles of reactive astrocytes in traumatic brain injury. Brain 129:2761-2772.

Neal M, Luo J, Harischandra DS, Gordon R, Sarkar S, Jin H, Anantharam V, Desaubry L, Kanthasamy A, Kanthasamy A (2018) Prokineticin-2 promotes chemotaxis and alternative A2 reactivity of astrocytes. Glia 66:2137-2157.

Ngwenya LB, Danzer SC (2018) Impact of traumatic brain injury on neurogenesis. Front Neurosci 12:1014.

Oliveira-Junior MS, Pereira EP, de Amorim VCM, Reis LTC, do Nascimento RP, da Silva VDA, Costa SL (2019) Lupeol inhibits LPS-induced neuroinflammation in cerebellar cultures and induces neuroprotection associated to the modulation of astrocyte response and expression of neurotrophic and inflammatory factors. Int Immunopharmacol 70:302-312.

Parent JM (2003) Injury-induced neurogenesis in the adult mammalian brain. Neuroscientist 9:261-272.

Rajkovic I, Wong R, Lemarchand E, Rivers-Auty J, Rajkovic O, Garlanda C, Allan SM, Pinteaux E (2018) Pentraxin 3 promotes long-term cerebral blood flow recovery, angiogenesis, and neuronal survival after stroke. J Mol Med (Berl) 96:1319-1332.

Redmond SA, Figueres-Onate M, Obernier K, Nascimento MA, Parraguez JI, Lopez-Mascaraque L, Fuentealba LC, Alvarez-Buylla A (2019) Development of ependymal and postnatal neural stem cells and their origin from a common embryonic progenitor. Cell Rep 27:429-441.

Rodriguez-Grande B, Varghese L, Molina-Holgado F, Rajkovic O, Garlanda C, Denes A, Pinteaux E (2015) Pentraxin 3 mediates neurogenesis and angiogenesis after cerebral ischaemia. J Neuroinflammation 12:15.

Shindo A, Maki T, Mandeville ET, Liang AC, Egawa N, Itoh K, Itoh N, Borlongan M, Holder JC, Chuang TT, McNeish JD, Tomimoto H, Lok J, Lo EH, Arai K (2016) Astrocyte-derived pentraxin 3 supports blood-brain barrier integrity under acute phase of stroke. Stroke 47:1094-1100.

Sun D (2014) The potential of endogenous neurogenesis for brain repair and regeneration following traumatic brain injury. Neural Regen Res 9:688-692.

Xiong Y, Zhang Y, Mahmood A, Chopp M (2015) Investigational agents for treatment of traumatic brain injury. Expert Opin Investig Drugs 24:743-760.

Yörg Dillen HK, Pascal Gervois (2020) Adult neurogenesis in the subventricular zone and its regulation after ischemic stroke: implications for therapeutic approaches. Transl Stroke Res 1:60-79.

Yu Z, Cheng C, Liu Y, Liu N, Lo EH, Wang X (2018) Neuroglobin promotes neurogenesis through Wnt signaling pathway. Cell Death Dis 9:945.

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA (2012) Genomic analysis of reactive astrogliosis. J Neurosci 32:6391-6410.
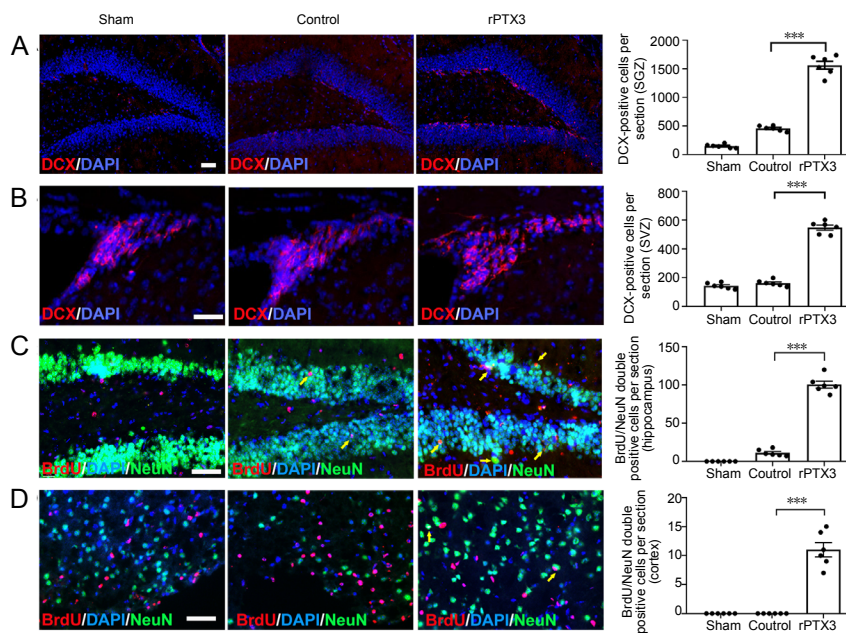
Figure 4 rPTX3 treatment increases neurogenesis after traumatic brain injury.
Neural progenitor cells and newly generated neurons were measured by immunostaining on day 21 after controlled cortical impact. (A) DCX (red) immunostaining in the ipsilateral hippocampus, with DAPI (blue) staining of the nuclei (left). The number of DCX-positive cells in the ipsilateral SGZ was significantly higher in the rPTX3 group compared with the control group (right). (B) DCX (red) and DAPI (blue) immunostaining in the ipsilateral SVZ (left). The number of DCX-positive cells in the ipsilateral SVZ was significantly higher in the rPTX3 group compared with the control group (right). (C) NeuN (green), BrdU (red), and DAPI (blue) immunostaining in the ipsilateral hippocampus (arrows; left). The number of NeuN/BrdU double-positive cells in the ipsilateral hippocampus was significantly higher in the rPTX3 group compared with the control group (right). (D) NeuN (green), BrdU (red), and DAPI (blue) immunostaining in the peri-injured cortex (arrow, left). The number of NeuN/BrdU double-positive cells in the peri-injured cortex was significantly higher in the rPTX3 group compared with the control group. Scale bars: 40 μm. ***$P < 0.001$. Data are expressed as the mean ± SEM ($n = 6$; one-way analysis of variance followed by Tukey *post hoc* test). BrdU: Bromodeoxyuridine; DAPI: 4′,6-diamidino-2-phenylindole; DCX: doublecortin; NeuN: neuronal nuclear antigen; rPTX3: recombinant pentraxin 3; SGZ: subgranular zone; SVZ: subventricular zone.
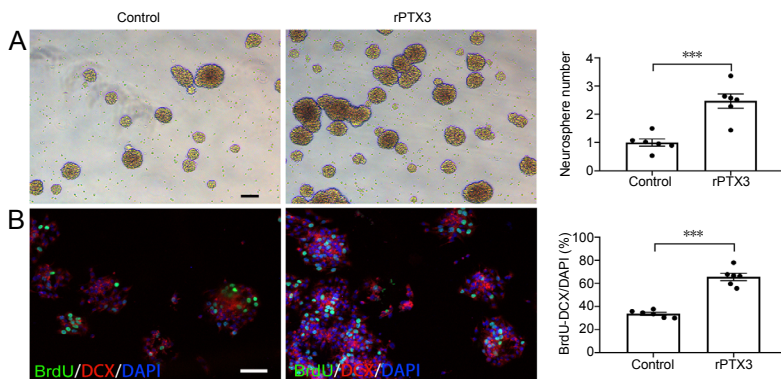

Figure 5 rPTX3 promotes neurogenesis *in vitro*.
Neurospheres were counted and immunostained at 7 days after passage. (A) Neurosphere formation (left). The number of neurospheres in neural progenitor cell cultures was significantly higher in the rPTX3 group compared with the control group (right). (B) BrdU (green) and DCX (red) immunostaining in neurospheres. DAPI (blue) represents the nucleus (left). The percentage of cells that were double-positive for DCX and BrdU was significantly higher in the rPTX3 group of neurospheres compared with the control group neurospheres (right). Scale bars: 100 μm. ***$P < 0.001$. Data are expressed as the mean ± SEM ($n = 6$; Student's *t*-test). BrdU: Bromodeoxyuridine; DAPI: 4′,6-diamidino-2-phenylindole; DCX: doublecortin; rPTX3: recombinant pentraxin 3.
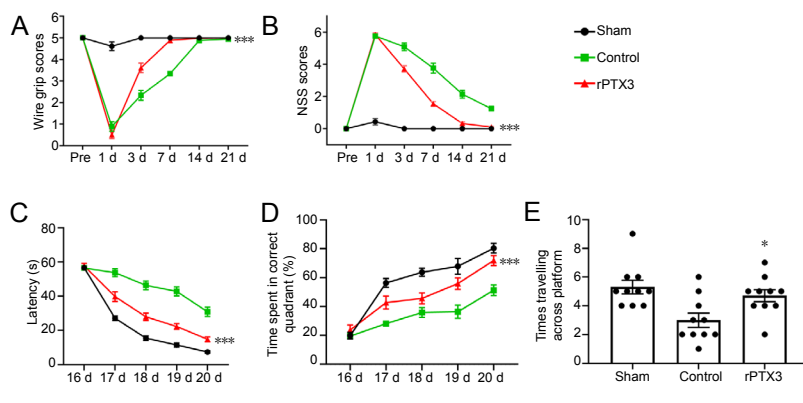

Figure 6 rPTX3 promotes functional recovery after traumatic brain injury.
Neurological function outcomes were measured after controlled cortical impact. (A, B) Motor-sensory functions, including NSS scores (A) and wire grip scores (B), were assessed 1 day before and on days 1, 3, 7, 14, and 21 after the controlled cortical impact. (C, D) Cognitive ability was assessed by the Morris water maze tests. In the navigation test, latency (C) and the time spent in the incorrect quadrant (D) were measured from days 16 to 20 after the controlled cortical impact. (E) The number of times the mice crossed the platform location in the probe trial test on day 21 after the controlled cortical impact. *$P < 0.05$, ***$P < 0.001$, *vs.* Control (Vehicle) group. Data are expressed as the mean ± SEM ($n = 10$; two-way repeated measures analysis of variance). NSS: Neurological severity scores; PTX3: pentraxin 3; rPTX3: recombinant pentraxin 3.

Zhang Z, Zhao G, Liu L, He J, Darwazeh R, Liu H, Chen H, Zhou C, Guo Z, Sun X (2019) Bexarotene exerts protective effects through modulation of the cerebral vascular smooth muscle cell phenotypic transformation by regulating PPARgamma/FLAP/LTB4 after subarachnoid hemorrhage in rats. Cell Transplant 28:1161-1172.

Zhong J, Jiang L, Cheng C, Huang Z, Zhang H, Liu H, He J, Cao F, Peng J, Jiang Y, Sun X (2016) Altered expression of long non-coding RNA and mRNA in mouse cortex after traumatic brain injury. Brain Res 1646:589-600.

Zhong J, Jiang L, Huang Z, Zhang H, Cheng C, Liu H, He J, Wu J, Darwazeh R, Wu Y, Sun X (2017) The long non-coding RNA Neat1 is an important mediator of the therapeutic effect of bexarotene on traumatic brain injury in mice. Brain Behav Immun 65:183-194.