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ORIGINAL PAPER

Glial Plasticity in the Trigeminal Root Entry Zone of a Rat Trigeminal Neuralgia Animal Model

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Abstract

The trigeminal root entry zone (TREZ) is the transitional zone of central and peripheral tissue compartments in the trigeminal root. Microvascular compression on the TREZ is the main etiology of most idiopathic trigeminal neuralgia (TN) patients. However, the pathogenesis of TN is still uncertain. To investigate the glial plasticity changes in oligodendrocytes, Schwann cells, astrocytes and microglia/macrophages in the TREZ in TN, immunohistochemical staining and Western blot methods were performed in rats with TN induced by compression injury. The results showed that mechanical compression injury in the trigeminal nerve of the TN rats induced glial plasticity in the TREZ, which dynamically changed the glial interface of the CNS–PNS transitional zone. Additionally, glial fibrillary acidic protein (GFAP)-immunoreactive astrocyte processes significantly proliferated and extended distally from the central region to the peripheral side of the TREZ after nerve compression injury in the TN group. Moreover, the expression of p75 in Schwann cells was upregulated on the peripheral side of the TREZ, and activated Iba-1-immunoreactive microglia/macrophages were observed on both sides of the TREZ. A significantly higher number of Schwann cells, astrocytes and microglia/macrophages were found in the TN group than in the sham operation group ($p < 0.05$). In conclusion, mechanical compression injury in the TN rats activated various glial cells, including oligodendrocytes, astrocytes, Schwann cells and microglia/macrophages, in the CNS–PNS transitional zone of TREZ. Changes in glial cell plasticity in the TREZ after compression injury might be involved in TN pathogenesis.

Keywords Glial plasticity · Trigeminal root entry zone · Trigeminal neuralgia · Animal model · Mechanical compression

Introduction

Glial cells mainly include oligodendrocytes, Schwann cells, astrocytes and microglia and play important roles in forming myelin, providing support and protection for neurons, and maintaining nervous system homeostasis [1, 2]. In recent years, glial cells have received increasing attention in the pathogenesis of neurological diseases. Oligodendrocytes and Schwann cells are the myelinating glia of the central nervous system (CNS) and peripheral nervous system (PNS), respectively. Astrocytes and microglia also have different complex functions in the nervous system. Dysregulation of glial functions in the nervous system might play a key role in chronic pain mechanisms [3].

Trigeminal neuralgia (TN) is a severe orofacial neuropathic pain. Accumulating neuroimaging evidence has indicated that microvascular decompression surgery provides the most effective treatment for TN patients with orofacial pain disorder associated with aberrant neurovascular compression on the trigeminal root entry zone (TREZ) [4]. Therefore, the TREZ was considered to be associated with the pathogenesis of TN.

The TREZ is a transitional zone that is adjacent to the brainstem for several millimeters and contains both CNS and PNS tissues [5]. The proximal myelin sheaths of rootlets are produced by oligodendrocytes in the central TREZ region, and the distal portions are produced by Schwann cells on the

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peripheral TREZ end. Astrocytes are arranged concentrically around individual myelinated axons or unmyelinated axon bundles to form the bulk of the CNS–PNS transitional zone tissue of the TREZ [6]. The CNS–PNS transitional zone of the TREZ is more than just a fascinating biological interface; it may possess unique features due to its solid glial dome formed by a variety of tissue types [7].

Therefore, glial plasticity in the TREZ junction area may be involved in the pathogenesis of TN. However, there are few reports on the study of glial cells in the TREZ during TN pathology; in particular, it remains unknown whether the glial plasticity changes under compression injury in the TREZ of TN animal models. Thus, using a TN animal model induced by trigeminal nerve compression, we studied the glial plasticity changes of oligodendrocytes, Schwann cells, astrocytes and microglia in the TREZ after mechanical compression injury.

Materials and Methods

Adult male Sprague–Dawley rats weighing 150 ± 20 g were obtained from Fujian Medical University Laboratory Animal Center. Rats were housed in a temperature- and humidity-controlled room under a 12–12 h light–dark cycle. Water and food were available ad libitum. Rats were randomly assigned to the trigeminal nerve root compression operation or sham operation group. The experimental procedures were followed by the guidelines of Fujian Medical University Institutional Animal Care and Use Committee, and the studies have been approved by the research ethics committee of Fujian Medical University. A randomized, double-blind, controlled animal trial was designed in this study.

TN Animal Model of Trigeminal Nerve Root Compression

The TN animal model was established as previously described [8]. Briefly, rats were anesthetized with pentobarbital (40 mg/kg, *i.p.*), and then a small curved anterior–posterior skin incision was made above the right eye. To expose the right infraorbital nerve, the orbital fascia was gently stripped laterally to reveal the infraorbital groove of the maxillary bone. Then, a small plastic filament (1 mm in diameter) was slowly and carefully inserted 1.2 cm into the intracalvarium through the inferior orbital fissure to compress the trigeminal nerve root. Animals in the sham operation group underwent the same procedure, except the right infraorbital nerve was exposed without inserting the filament to the trigeminal nerve root. The incision was closed using silk sutures (6–0). The orofacial mechanical stimulation threshold of rats were test before operation for baseline and after operation for mechanical allodynia by Von Frey monofilaments (Stoelting, Kiel, WI, USA).

Immunohistochemistry

Animals from the sham group and TN group on postoperation day (POD) 7, 14, 21 and 28 (n = 24 for each group) were transcardially perfused through the left ventricle with 4% paraformaldehyde phosphate buffer (pH 7.4) after they were deeply anesthetized with sodium pentobarbital (200 mg/kg). The trigeminal nerve segment from the junction of the trigeminal nerve root with the brainstem to the trigeminal ganglion was dissected and then cryoprotected with 30% (W/V) sucrose in 0.1 M PB overnight at 4 °C. A series of 10 μm sections of trigeminal nerve roots were longitudinally sectioned with a Cryostat Microtome (Leica CM1950, Heidelberger, Germany).

For immunohistochemical staining, sections were washed three times in 0.1 M PBS for 10 min and then blocked in 3% bovine serum albumin (BSA) for 30 min. The sections were subsequently incubated with primary antibodies after removal of the blocking solution without washing. The following primary antibodies were used in this study: rabbit polyclonal antibody against MBP (1:500, Abcam, MA, USA), mouse monoclonal antibody against GFAP (1:1500, Proteintech, IL, USA), rabbit polyclonal antibody against Iba-1 (1:1000, Wako, Osaka, Japan), mouse monoclonal antibody against $p75^{NTR}$ (1:200, Abcam), rabbit polyclonal antibody against $p75^{NTR}$ (1:200, Merck Millipore, Carolina, USA), mouse monoclonal antibody against $p75^{NGF}$ (1:200, Abcam) and rabbit monoclonal antibody against Ki67 (1:150, Abcam). Biotinylated donkey anti-Rabbit IgG (1:200, Vector, CA, USA), biotinylated donkey anti-mouse IgG (1:200; Vector), FITC-avidin (1:1000, Vector) and DAPI (1:1000, Invitrogen, CA, USA) were also used.

The ratio of Iba-1 immunoreactive microglia/macrophages in the TREZ was calculated by independent observers who quantified and compared the Iba-1-positive cells and DAPI-stained cells using Image-Pro Plus software (version 6.0; Media Cybernetics, MD, USA).

Western Blot

TN group (n = 24) and sham group (n = 24) rats were sacrificed at different postoperation time point under anesthesia, and the trigeminal nerve root between the trigeminal root junction and trigeminal ganglion (not including the ganglion) was quickly removed and frozen rapidly in liquid nitrogen. The samples from different groups were homogenized in an extraction buffer (100 mM Tris, pH 7.4) with 2 mM phenylmethanesulfonyl fluoride (PMSF) and 10 mg/mL aprotinin. The solutions were centrifuged at 12,000×*g* for 10 min at 4 °C. The protein concentration of

the supernatants was determining with the Pierce BCA Protein Assay Kit. Protein samples (10–20 μg) were loaded for electrophoresis on 10% or 12% polyacrylamide SDS-PAGE gels and transferred onto polyvinylidene difluoride (PVDF) membranes. Then, the membranes were blocked with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween 20 for 1 h at room temperature. Next, the membranes were incubated with the following primary antibodies overnight at 4 °C: rabbit-monoclonal antibody against MBP (1:1000, CST, MA, USA), mouse monoclonal antibody against GFAP (1:50,000, Proteintech), rabbit-antibody against Iba-1 polyclonal antibody (1:1000, Wako), rabbit polyclonal antibody against $p75^{NTR}$ (1:1000, Merck Millipore), mouse anti-β-actin (1:1000, TransGen, Beijing, China) and GAPDH (1:1000, Cell Signaling, MA, USA). The blots were incubated with goat anti-mouse IgG-HRP (1:1000, EarthOX, CA, USA) or goat anti-rabbit IgG (H + L)-HRP (1:1000, Bioworld, OH, USA) secondary antibody for 2 h at room temperature. They were detected using Immobilon Western Chemiluminescent reagent (Merck Millipore P90720).

Statistical Analysis

Data were analyzed with two-way ANOVA and Sidak's multiple comparisons test and are presented as the mean ± SEM. $p < 0.05$ was considered statistically significant. Significant differences are noted by asterisks, with single asterisks representing $p < 0.05$, two asterisks representing $p < 0.01$, three asterisks representing $p < 0.001$, and four asterisks representing $p < 0.0001$.

Results

Mechanical Compression in the TREZ of Rats Induced Orofacial Allodynia

Most TN group rats were insensitive to the mechanical stimulation on the orofacial vibrissa pad after TREZ compression injury operation to POD 7 by Von Frey filaments, but they displayed a significant increase in the mechanical allodynia according to the withdrawal response of TN rats from POD 14 to 28 (Fig. 1). Sham group rats showed no obvious changes in the orofacial mechanical stimulation threshold from POD 7 to 28 by comparing with TN group (Fig. 1).

The CNS–PNS Myelinating Glial Interface in the TREZ

The TREZ is a transitional zone containing both central and peripheral nervous tissues, with oligodendrocyte myelin sheaths in the central TREZ region and Schwann cell myelin sheaths in the peripheral TREZ end. There is a dome-shaped CNS–PNS glial interface in the TREZ, as shown in Fig. 2i.

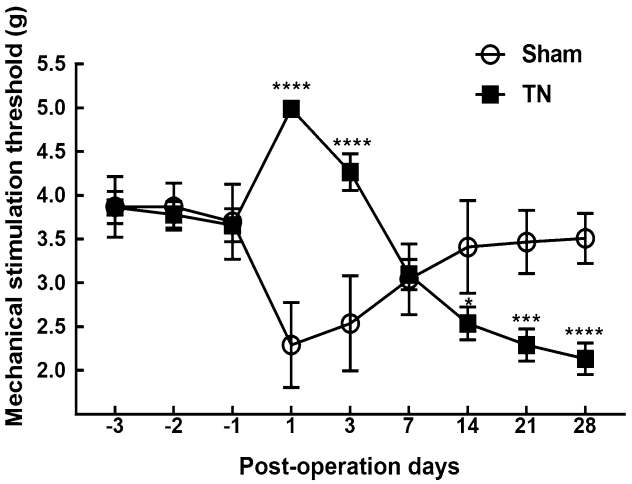

Fig. 1 Mechanical compression in the TREZ of rats induced orofacial allodynia. Sham group and TN group rats showed a similar baseline of orofacial mechanical stimulation threshold before operation by Von Frey filaments test. Compared with sham group, TN group rats were insensitive to the mechanical stimulation after TREZ compression injury surgery to postoperation day (POD) 7, but they displayed a significant increase in the mechanical allodynia according to the withdrawal response of TN rats from POD 14 to 28, especially on POD 21 and 28. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$

With immunohistochemical staining, we observed the expressions of all examined markers of the glial cells in the TREZ in both TN and sham groups. The results of immunofluorescence histochemical double-staining for MBP and $p75^{NGF}$ showed that MBP was highly expressed in the proximal CNS–PNS transitional zone of the TREZ. In the TREZ region, MBP-immunoreactive oligodendrocytes showed a distinct and dome-shaped central glial interface in both the TN and sham groups, whereas the $p75^{NGF}$-immunoreactive cells were observed only on the peripheral side of the TREZ in the TN group (Fig. 2a–h).

The central part of the CNS–PNS transitional zone marked by MBP immunopositive staining products did not have obvious changes in the immunoreactive-oligodendrocyte glial interface from POD 7 to 28 in either the sham group (Fig. 2a–d) or the TN group (Fig. 2e–h). The $p75^{NGF}$-immunoreactive cells, which were more obvious in the TN group, were almost all located on the peripheral side of the CNS–PNS transitional zone of the TREZ. The amount of $p75^{NGF}$-immunoreactive cells significantly increased from POD 7 to 14 (Fig. 2e, f), and then gradually decreased from POD 21 to 28 after chronic compression injury in the TN group (Fig. 2g–h).

The MBP-immunoreactive oligodendrocytes on central side and $p75^{NGF}$-immunoreactive Schwann cells on peripheral side of TREZ arranged relatively disorganized in the TN group (Fig. 2e–h).

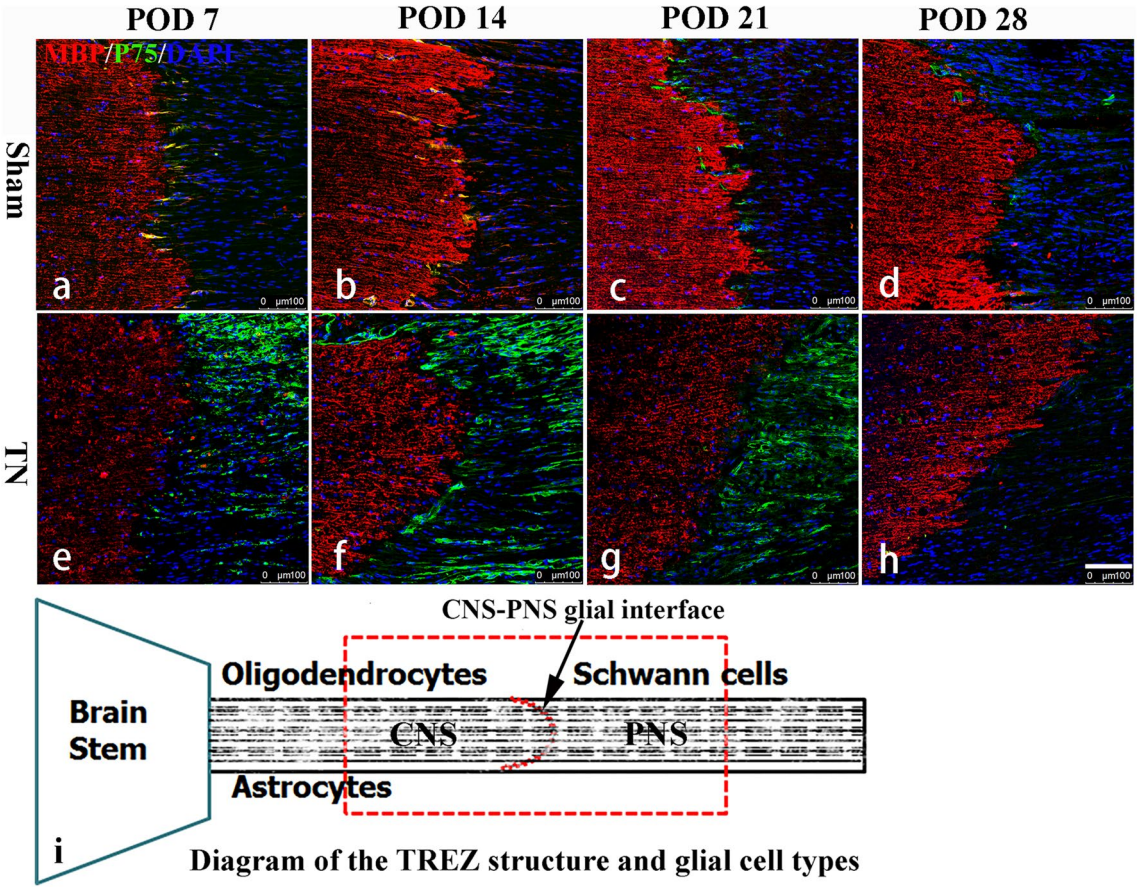

Fig. 2 Immunohistochemical staining of myelin basic protein (MBP) and $p75^{NGF}$ in the trigeminal root entry zone (TREZ). **a**–**h** Representative images of the TREZ obtained from the sham (n = 24) and TN (n = 24) groups on POD 7, 14, 21 and 28, respectively. MBP-immunoreactive oligodendrocytes (red) on the central side of the TREZ in both TN and sham groups. $p75^{NGF}$-immunoreactive Schwann cells (green) mainly existed on the peripheral side of the TREZ in the TN group and gradually increased from POD 7 to 28. The nuclei of these cells were stained with DAPI (blue). Bar = 100 μm. **i** Diagrammatic representation of the TREZ anatomical structure, indicated by the red broken-line rectangle, showing the central tissues comprising oligodendrocytes and astrocytes in the proximal TREZ and peripheral tissues comprising Schwann cells in the distal TREZ. The CNS–PNS glial interface is shown by the red curved line (Color figure online)

Western blot analysis of proteins extracted from the TREZ revealed three major bands for the MBP protein (14 kDa, 18 kDa and 23 kDa); there was a significantly lower level of MBP protein in the TN animal model from POD 7 to 21 than in the sham group, and then it was upregulated and recovered to a similar level as the sham group on POD 28 (Fig. 3a–b). Compared with the sham group, the expression of $p75^{NTR}$ in the TN group increased from POD 7 to 21 and decreased to a lower level on POD 28 (Fig. 3c–d).

Iba-1 Immunoreactive Microglia/Macrophages are Activated After Compression Injury

Iba-1-immunoreactive microglia/macrophages were widely activated in the TREZ in both sham and TN groups (Fig. 4a–h). On POD 7, the activation of Iba-1-immunoreactive microglia/macrophages was detected at a relatively moderate level, and there were no significant differences in the number of Iba-1-immunoreactive microglia/macrophages between the groups (Fig. 4a, e). The Iba-1-immunoreactive microglia/macrophages number peaked in the TN group on POD 14 and then decreased from POD 21 to 28 after mechanical compression injury on the trigeminal nerve (Fig. 4f–h). According to immunofluorescence staining and cell counting, there were fewer Iba-1-immunoreactive cells in the sham group (Fig. 4b–d) than in the TN group from POD 21 to 28 (Fig. 4g–h). In addition, most of the Iba-1-immunoreactive cells had a small cell body with highly ramified processes in the sham group (Fig. 4a–d, a'–d'), but they had hypertrophied cell bodies with shortened and swollen processes in the TN group (Fig. 4e–h, e'–h').

The Iba-1-immunoreactive cell count demonstrated that microglia/macrophages were activated on POD 7 in both groups. Activation peaked on POD 14 and then decreased in the TN group. The amount of activated microglia/

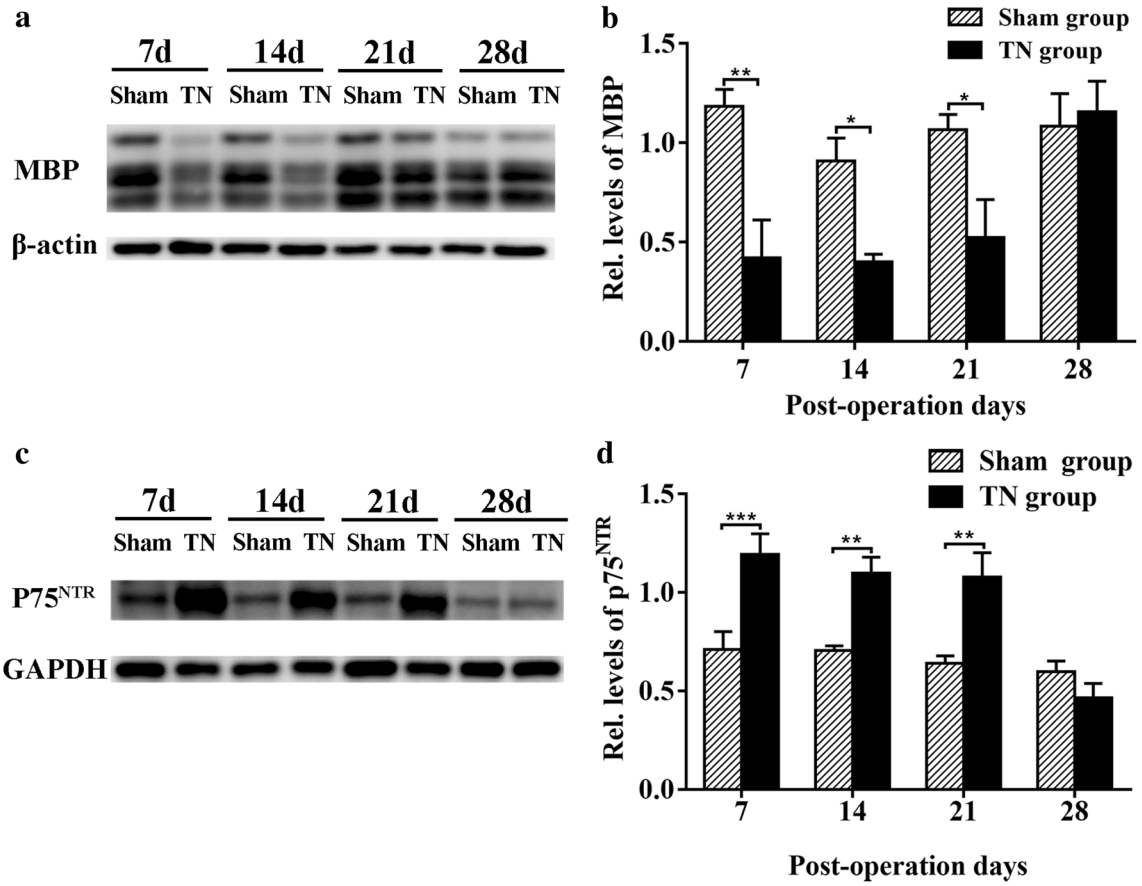

Fig. 3 The changes in the expression of oligodendrocytes and Schwann cells in the TREZ were analyzed by Western blot and cell counting. **a**, **b** Western blot analysis of MBP extracted from the TREZ. Representative images show a significant decrease in MBP expression in the TN animal model from POD 7 to 28. MBP isoforms at 14, 18 and 23 kDa are indicated. **c**, **d** Representative Western blot show that the expression of $p75^{NTR}$ increased on POD 7 and decreased from POD 14 to 21 in the TN group. Western blot values represent the mean + SEM normalized ratios of the MBP and $p75^{NTR}$ bands normalized to the GAPDH or β-actin internal standard, respectively

macrophages in the sham group was lower than that in the TN group from POD 14 to 28 (Fig. 4i).

GFAP-Immunoreactive Astrocytes are Activated in the Central Border of TREZ After Compression Injury

According to immunofluorescence double-staining, there was also a glial interface marked by GFAP-immunoreactive astrocytes in the CNS–PNS transitional zone of the TREZ (Figs. 4, 5). Interestingly, the GFAP-immunoreactive glial interface in the TREZ of the TN group changed dynamically after trigeminal nerve compression injury. The GFAP-immunoreactive cells in the TN group projected filamentous processes from the central to the peripheral side of the TREZ from POD 7 to 28 (Figs. 4e–h, 5e–h). The processes of GFAP-immunoreactive astrocytes extended to the $p75^{NTR}$-immunoreactive Schwann cells on the peripheral side of the CNS–PNS transitional zone in the TREZ. Some of these astrocytes appeared to intersperse with each other along the glial interface and peripheral side of the TREZ (Fig. 5e–h). The processes near the glial interface of the TREZ were more disorganized and obvious than those in other regions; even the nuclei near the glial interface were more disordered, as observed from the DAPI staining results.

Western blot analysis of proteins extracted from the TREZ revealed that the band for the GFAP in the TN group consistently exhibited higher protein expression from POD 7 to 28 than that in the sham group (Fig. 5i, j). Western blot values represent the mean ± SEM normalized ratios of the protein of interest relative to GAPDH, which were internal standards, at each time point in both groups.

Double-labelling for proliferation marker Ki-67 and astrocytes marker GFAP showed that only a few sporadic Ki-67-immunoreactive cells were observed in the TREZ in the TN group rats from POD 7 to 28 (Fig. 6e–h, e'–h'), and it seemed that only on POD 14 of the TN group rats was a little

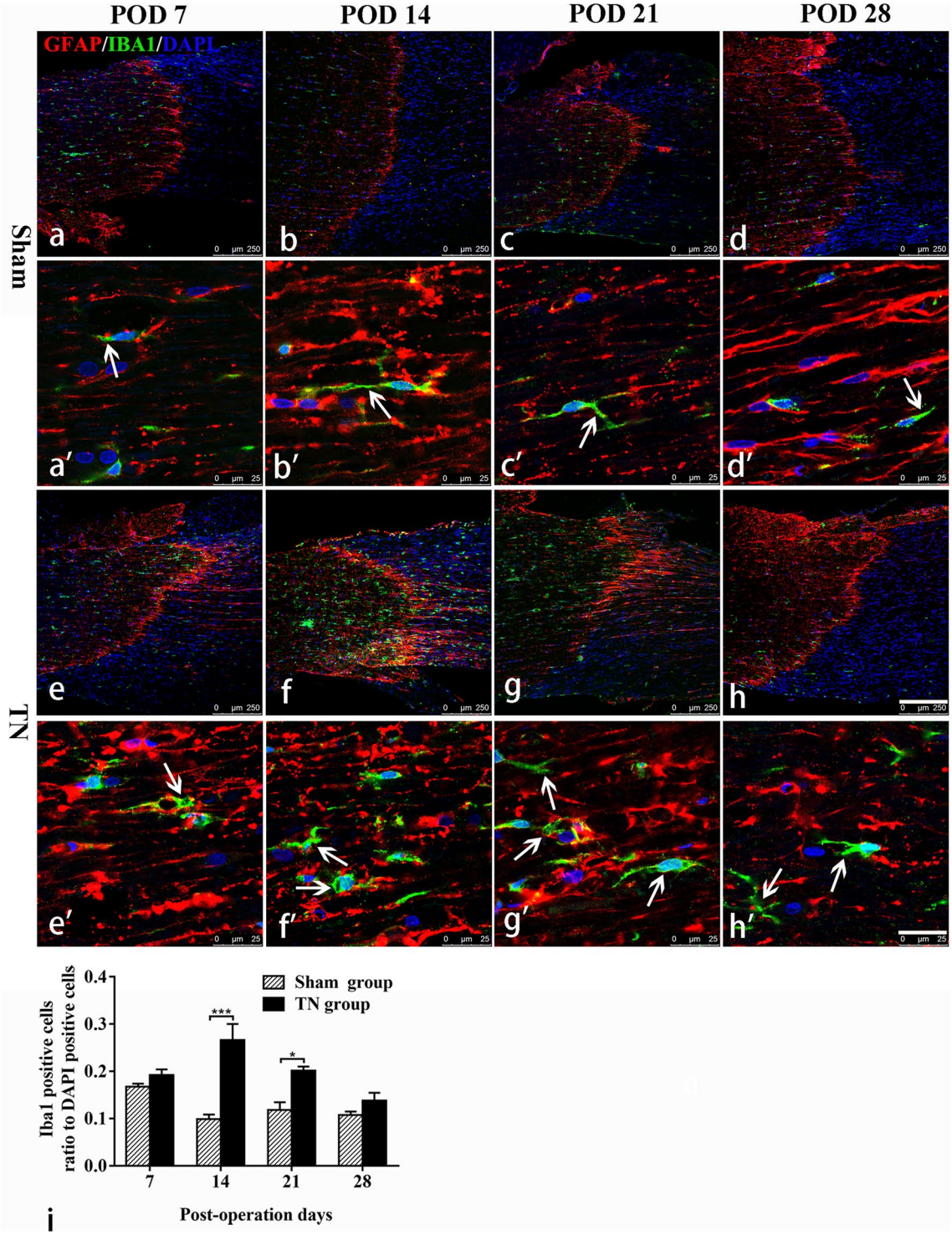

Fig. 4 Mechanical compression injury of the TREZ induced activation of microglia/macrophages (green) and astrocytes (red) immunofluorescence. **a**–**h** The immunoreactive astrocytes labeled by GFAP (red) delimited a glial interface in the central part of the CNS–PNS transition zone of the TREZ. **a**–**d** The sham group (n = 24) showed relatively low expression of Iba-1 after sham operation on the rats; there was no obvious GFAP-immunoreactive glial interface change in the sham group. **e**–**h** The expression of Iba-1 in the TN group (n = 24) increased from POD 7 and peaked on POD 14, after which it decreased from POD 21 and recovered to a lower level on POD 28; large quantities of GFAP-positive astrocyte processes (red) sprouted from the central region to the peripheral side of the CNS–PNS transitional zone. The nuclei are stained with DAPI (Blue). **a**–**h** Bar = 250 μm; **a'**–**h'**, Bar = 25 μm. **i** Quantitative analysis of Iba-1-immunoreactive microglia/macrophages in the TREZ by cell counting. $*p < 0.05$, $***p < 0.001$ (Color figure online)

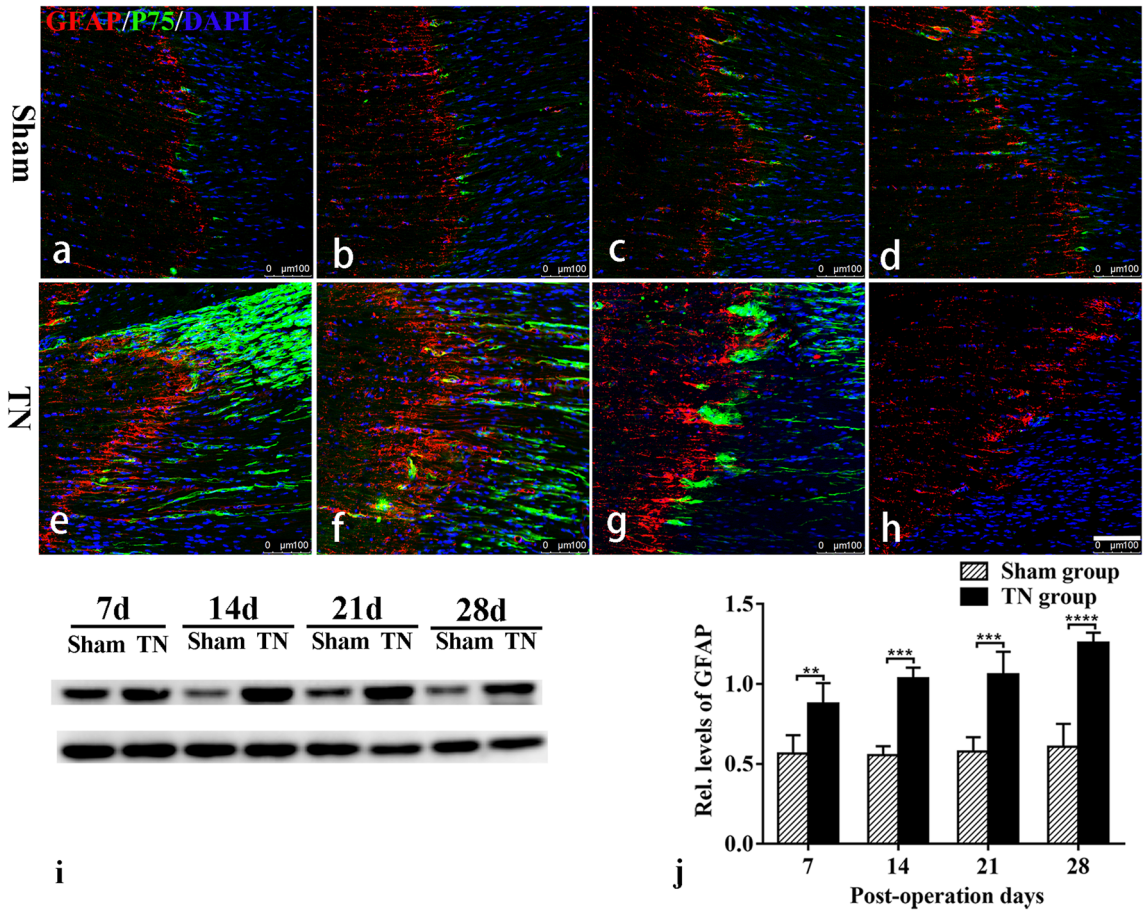

Fig. 5 Immunoreactive astrocytes and Schwann cells in the TREZ. **a**–**d** Compared with the sham group (n = 24), **e**–**h** large quantities of GFAP-positive astrocyte processes (red) sprouted from the central region to the peripheral side of the CNS–PNS transitional zone on POD 7, 14, 21 and 28 in the TN group (n = 24), respectively. $p75^{NTR}$ (green)-positive Schwann cells were also observed after mechanical compression injury in the TN group. The nuclei in the TREZ were stained with DAPI (blue). Bar = 100 μm. **i** Representative images showed the Western blot analysis of GFAP extracted from the TREZ. High GFAP expression was observed in the TN group from POD 7 to 28 compared to the sham group. **j** GFAP bands normalized to the GAPDH internal standard. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ (Color figure online)

more Ki-67-immunoreactive cells (Fig. 6f), but it is also not so significant than others.

Discussion

The present study demonstrated that mechanical compression injury in the trigeminal nerve of the TN animal model induced glial plasticity in the TREZ, which dynamically changed the glial interface of the CNS–PNS transitional zone. Additionally, GFAP-immunoreactive astrocyte processes significantly proliferated and extended distally from the central side to the peripheral side of the TREZ after nerve compression injury in the TN group. The expression of p75 in Schwann cells was upregulated on the peripheral side of the TREZ, and activated Iba-1-immunoreactive microglia/macrophages were distributed on both sides of the TREZ. These plasticity changes induced by mechanical compression injury in the trigeminal nerve could impact the microenvironment and neurophysiology in the TREZ of the TN rats.

Accumulating evidence has shown that glial cells play a key role in the pathogenesis of neuropathic pain and that glial dysfunction is involved in the initiation and maintenance of pathologic pain states [9–11]. Injury of the nervous system may activate glial cells, and then various neural and immune mediators are released into the microenvironment. This process leads to an immune response near the site of injury, further sensitizing the neurons and resulting in central sensitization to induce neuropathic pain [3, 10]. Microvascular chronic compression injury of the TREZ is the main etiology for most TN patients [12]. There is also a distinct CNS–PNS glial transitional zone in the TREZ

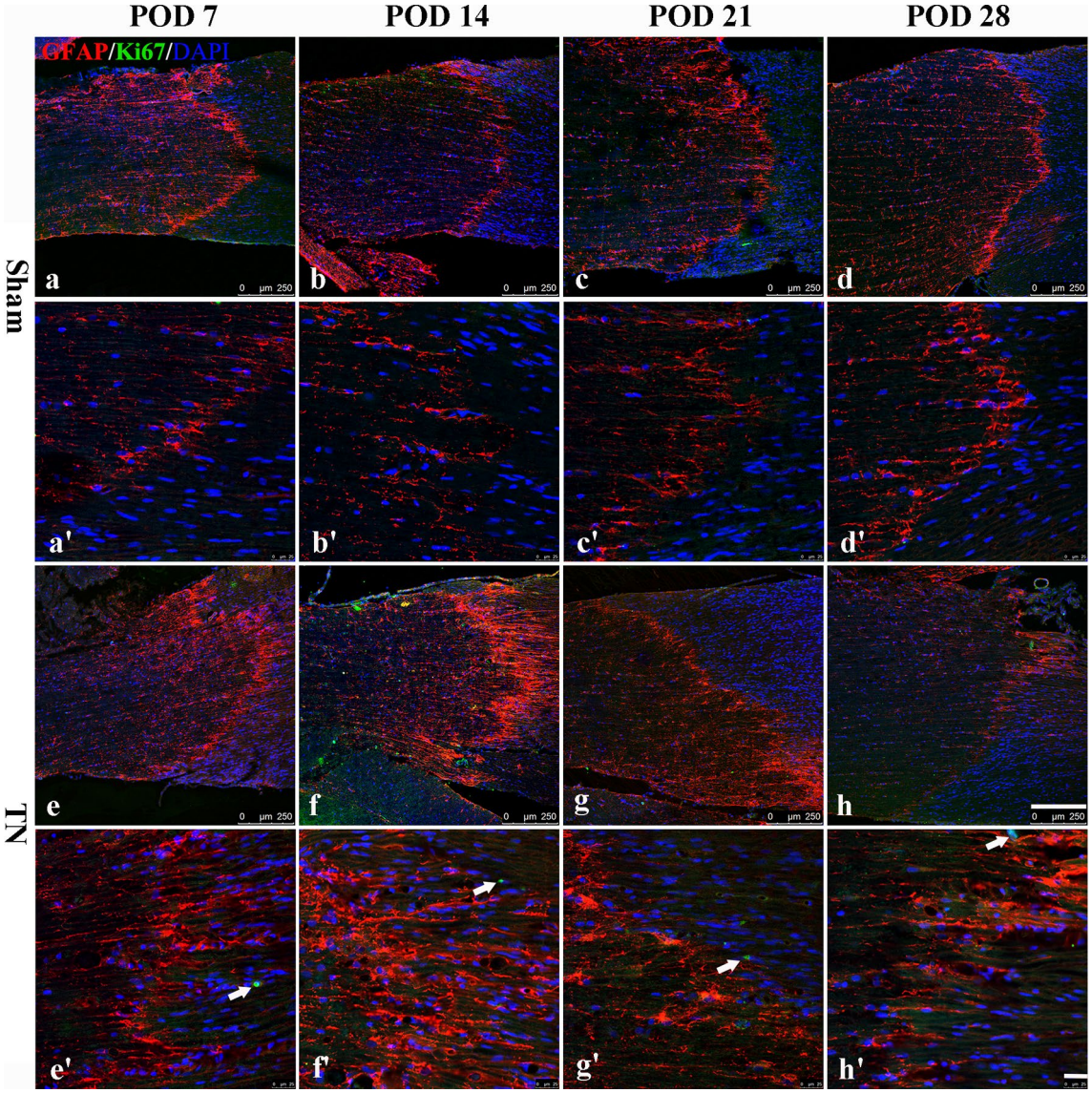

Fig. 6 Double-labelling for proliferation marker Ki-67 and astrocytes marker GFAP in the TREZ. **a**–**h** Immunofluorescence staining showed that only a few sporadic Ki-67-immunoreactive cells (green) were observed in the TREZ on both sham group and TN group rats from POD 7 to 28. **f** It seemed that only on POD 14 of TN group rats was a little more than others. Ki-67-immunoreactive cells overlaid with GFAP-immunoreactive astrocytes (red), as indicated by the arrow head. **a**–**h** Bar = 250 μm; **a'**–**h'** Bar = 25 μm (Color figure online)

[6], similar to what we described in the trigeminal root of rats in the present and previous studies [8, 13].

Homeostasis of both CNS and PNS myelination in the CNS–PNS transitional zone of the TREZ depends on the crosstalk between oligodendrocytes, Schwann cells, astrocytes and microglia. The results of previous studies have shown that perturbation of oligodendrocyte functions by genetic ablation induces central neuropathic pain [14]. This effect may activate the receptors in astrocytes and microglia to release pro-inflammatory cytokines to contribute to neuropathic pain [15]. Oligodendrocyte precursor cells (OPCs) in the CNS maintain a certain proliferation ability to generate oligodendrocytes after CNS injury and promote tissue repair [16]. Schwann cells exhibit plastic changes for PNS myelination when peripheral nerves are injured, and nonmyelinating Schwann cells reprogram to de-differentiate and drive the regeneration process [17, 18]. The neurotrophin receptor $p75^{NTR}$ is a positive modulator that is widely expressed in Schwann cells during myelination and remyelination and in peripheral nerve regeneration after peripheral nerve injury [19, 20]. Schwann cell demyelination, apoptosis and proliferation may also be induced by chronic mechanical compression from the microenvironment even without axonal injury [21, 22].

Astrocyte activation is critical to maintaining the balance between oligodendrocyte and Schwann cell remyelination in the nervous system [23]. Astrocytes may promote oligodendrogenesis by secreting brain-derived neurotrophic factor (BDNF) to support OPC maturation [24], proliferation, differentiation, oligodendrocyte-axon contact and myelination [11, 25–27]. The absence of astrocytes decreases oligodendrocyte-mediated remyelination and increases Schwann cell-mediated remyelination [23].

The results of previous studies have demonstrated that astrocytes provide a signal to the environment to clear myelin debris through recruitment of microglia during regeneration in demyelinating diseases [28]; astrocytes also contribute to oligodendrocyte and myelin damage during inflammation [29, 30]. It was inferred that microglia were more efficient at promoting OPC proliferation and differentiation than astrocytes [31–33].

We found that the homeostasis of glial cells in the TREZ was impaired, and astrocytes on the central side and microglia/macrophages on both sides of the CNS–PNS transitional zone were obviously activated in the early postoperative period after trigeminal nerve compression injury in the TN animal model. From the results of double-labelling for proliferation marker Ki-67 and astrocytes marker GFAP, there only a few sporadic Ki-67-immunoreactive cells overlaid with astrocytes, which suggested that the increase expression of GFAP is not because of astrocytes proliferation but astrocyte activation. We considered that astrocyte activation in the TREZ may be an endogenic reaction to maintain the balance between oligodendrocyte and Schwann cell remyelination after mechanical compression injury. Microglia/macrophages activation may be induced by mechanical stimulation and inflammatory response to clear myelin debris and promote oligodendrocyte and Schwann cell remyelination. Based on the $p75^{NTR}$-immunoreactive Schwann cells on the peripheral side of the CNS–PNS transitional zone in the TN group after trigeminal nerve compression injury, we believe that some Schwann cells regenerated or remyelinated; this is because $p75^{NTR}$ is always expressed or upregulated in developing Schwann cells and remyelination during regeneration [20]. As the TREZ is in the hybrid zone of the central and peripheral tissue compartments, it may possess a special microenvironment and complex glial-glial or glial-axonal crosstalk. The unique anatomical features and neurophysiological features of the TREZ make it more susceptible to exogenous stimulation or injury. Therefore, after suffering decades of chronic compression injury on the TREZ, the morbid and dysfunctional trigeminal nerve system becomes hypersensitive to noxious and nonnoxious stimulation, resulting in the grievous disease of TN.

The present study also has limitations. For example, we only investigated the glial cells in the TREZ under trigeminal nerve mechanical compression injury in the TN animal model, but there are a few clinical cases of TN in which microvascular compression was not detected with imaging [34]. We have not developed methods to simulate nonvascular compression TN cases in the animal model or to study the pathogenesis of TN. Additionally, another small fraction of patients were found to have trigeminal nerve compression but not TN disease [35]; this result could be attributed to the insufficient duration of TREZ compression injury based on individual differences. Experimental animals are different from humans; the optimal duration in TN animal experiments that will reflect decades of trigeminal nerve compression injury in TN patients remains unknown. We hope to further this research in the future to address these limitations and answer these questions.

In summary, mechanical compression injury in the TN animal model actives various glial cells, including oligodendrocytes, astrocytes, Schwann cells and microglia/macrophages, in the CNS–PNS transitional zone of the TREZ, and these changes in glial cell plasticity might be involved in the pathogenesis of TN.

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Compliance with Ethical Standards

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

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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