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Role of Microglia and Astrocyte in Central Pain Syndrome Following Electrolytic Lesion at the Spinothalamic Tract in Rats

Kobra Naseri · Elham Saghaei · Fatemeh Abbaszadeh · Mina Afhami · Ali Haeri · Farzaneh Rahimi · Masoumeh Jorjani

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Abstract Central pain syndrome (CPS) is a debilitating state and one of the consequences of spinal cord injury in patients. Many pathophysiological aspects of CPS are not well documented. Spinal glia activation has been identified as a key factor in the sensory component of chronic pain. In this study, the role of glial subtypes in the process of CPS induced by unilateral electrolytic lesion of spinothalamic tract (STT) is investigated. Male rats received a laminectomy at T8–T9 and then unilateral electrolytic lesion centered on the STT. Thermal and mechanical thresholds as well as locomotor function were measured on days 0, 3, 7, 14, 21, and 28 post-injuries by tail flick, von Frey filament, and open field tests, respectively. To investigate the spinal glial activation following denervation in STT-lesioned groups, Iba1 and GFAP were detected by immunohistochemistry and Western blotting at the same time points. Data showed that STT lesion significantly decreased thermal pain at day 3 in comparison with sham groups. Significant bilateral allodynia appeared in hind paws at day 14 after spinal cord injury and continued to day 28 ($P<0.05$). Additionally, electrolytic spinal lesion attenuated locomotor function of injured animals after 7 days ($P<0.05$). In both histological assessments and Western blotting, Iba1 increased at days 3 and 7 while increased GFAP occurred from day 14 to 28 after lesion. It appears that microglial activation is important in the early stages of pain development and astrocytic activation occurs later. These events may lead to behavioral outcomes especially central neuropathic pain.

Keywords Central pain syndrome · Spinal cord injury · Astrocyte · Microglia

K. Naseri · E. Saghaei · A. Haeri · M. Jorjani
Department of Pharmacology, Faculty of Medicine,
Shahid Beheshti University of Medical Sciences,
Chamran Exp. way,
Evin, Tehran, Iran

K. Naseri
e-mail: naseri5674@yahoo.com

E. Saghaei
e-mail: elhams77@yahoo.com

A. Haeri
e-mail: haeria@pasteur.ac.ir

K. Naseri · E. Saghaei · F. Abbaszadeh · M. Afhami · M. Jorjani (✉)
Neuroscience Research Center,
Shahid Beheshti University of Medical Sciences,
Tehran, Iran
e-mail: msjorjani@sbmu.ac.ir

F. Abbaszadeh
e-mail: f.abbaszadehm@gmail.com

M. Afhami
e-mail: mina.afhammi@gmail.com

F. Rahimi
Department of Pathology, Taleghani Hospital,
Shahid Beheshti University of Medical Sciences,
Tehran, Iran

F. Rahimi
e-mail: farzirahimi@yahoo.com

Introduction

Neuropathic pain as a pathological pain has many aspects such as central pain syndrome. Central pain syndrome (CPS) is defined as pain resulting from a lesion or pathology of the CNS (Arevalo et al. 2010; Gao and Ji 2010). CPS is the main complain of spinal cord-injured patients (Masri et

al. 2009) and a significant complication for almost half of patients with multiple sclerosis, particularly those with spinal involvement (Wang and Thompson 2008). These patients experience severe and excruciating pain originating from areas in and around the primary sensory loss produced by the lesion (Tasker et al. 1992) and there is no satisfying effective treatment. Many patients also suffer from allodynia and hyperalgesia, an increased sensitivity to weakly painful stimuli (Greenspan et al. 2004). The initiation of CPS is usually delayed weeks or months from the original injury (Tasker et al. 1991). In spite of these findings, the cause of CPS remains unclear, and most of experimental models introduced for CPS studies may have not been comprehensive enough.

Wang and Thompson (2008) established a model of CPS by making an electrolytic lesion on the STT tract in rats. This model was consistent with previous studies in which chordotomy tract lesions being used to relieve chronic pain were not successful, since pain relapse and even severer was often experienced. They reported that CPS results from hyperexcitability of thalamic neurons and that it is unlikely that STT axons interruptions alter pain information from the spinal levels (Wang and Thompson 2008).

Initial injury or mechanical lesion on spinal cord tissue is followed by secondary damages that cause long-term dysfunction. These secondary reactions were reported to be the cause behind demyelination, axonal degeneration, neuronal death, cavitations, glial scar formation, glial activation, and pain perception (Fitch et al. 1999; Loane and Byrnes 2010).

Various cellular and molecular changes including activated glia, induction of selected intracellular signaling pathways, and robust increases in cytokine and chemokine synthesis have all been associated with hind paw allodynia after nerve lesion (Okada et al. 2004; Ritz and Hausmann 2008). Although these cellular and molecular changes are observed in the secondary phase after spinal cord injury (SCI), it remains unknown whether they contribute to or are responsible for the onset and maintenance of CPS induced by unilateral electrolytic lesion on spinothalamic tract.

Spinal glia are believed to be involved in pathological pain (Cao and Zhang 2008). Two types of glial cells: microglia and astrocytes are highlighted as modulators of chronic pain following SCI. These cells have been identified as key factors in the sensory component of chronic pain and in the response to direct injuries of the central nervous system and pain development (Ji et al. 2006; McMahon and Malcangio 2009; Scholz and Woolf 2007).

Therefore, glial activity may be responsible for the development and maintenance of pain in CPS condition following STT lesion. In this study, we investigated the role of microglia and astrocytes in the processing of altered pain sensation in CPS model related to spinothalamic tract lesion in rats.

Materials and Methods

Animals

Experiments were performed on adult male Sprague Dawley rats weighing 200–290 g. Rats were kept on a 12-h light/dark cycle, housed two to three/cage, and received food and water ad libitum. All efforts were made to minimize animals suffering. All experimental procedures were approved by the ethical committee of the Neuroscience Research Center of Shahid Beheshti University of Medical Sciences (Tehran, Iran).

Spinothalamic Tract Lesion

Spinothalamic lesion (STT) was induced by the method described by Wang and Thompson with a modification (Wang and Thompson 2008). Animals were anesthetized with ketamine/xylazine (60:5 mg/kg, i.p.). A laminectomy was performed on either the T8 or T9 vertebrae. The dura was lifted by a fine forceps and opened with iris scissors. The right STT pathway was lesioned using a tungsten microelectrode (1 MΩ) positioned 0.5–0.7 mm lateral to midline and 1.6–1.9 mm deep. A brief current pulse (300 μA, 90 s) was passed between the electrode and a ground electrode placed in the muscle beside the spine in order to lesion the nearby axons. It is necessary to note that during the primary 10 s of lesion induction, the current pulse was increased from 0 to 300 μA and this current passed for 80 s (total time=90 s). These modifications in the protocol prevented severe spinal reflexes and produced features of mechanical allodynia. All animals received physiological saline (S.C.) for electrolytic balance and Penicillin G (I.M.) for prevention of infection. Animals only laminectomized were considered as sham group and rats lesioned into STT described as STT-injured group.

Behavioral Assessment

Behavioral assessments including motor activity and nociceptive responses were investigated in separate groups pre- and post-surgery. To determine the effects of STT lesion over longer time after central pain syndrome, we applied all behavioral tests on days 3, 7, 14, 21, and 28 after injury in all groups.

Rota Rod Test

This test was used to evaluate the effects of spinothalamic tract lesion on motor coordination. The rate of animal rotation on the rotating rod (Ugo Basile, Model 7750, Italy) was adjusted to allow the normal rats to stay on it for 2 min (7 rpm). Each rat was given five trials before the actual reading was taken. Those animals staying on revolving rod for a period of 2 min before surgery were selected and

followed for 4 weeks after surgery. Each animal was tested on the rota rod and the time they remained on the bar for up to 120 s was recorded.

Open Field Test

Motor activity was also assessed using a 60×60 cm arena and 40 cm height black wooden box. Animals were habituated in the room for 30 min and then put in one corner of the box and allowed to explore freely for 5 min. For each trial, the open field box was thoroughly cleaned with 70 % ethanol solution and afterwards by a dry cloth. The experiments were conducted under artificial laboratory illumination (fluorescent lamps, above level of box).

The sessions were recorded by a camera positioned right above the box hanging from the ceiling. Data were obtained using Ethovision software (version 7), a video tracking system for automation of behavioral experiments (Noldus Information Technology, Netherlands). During the 5-min trial, the behavior of each rat was recorded as distance moved by rats in open field box.

Thermal Pain

The tail flick method of D'Amour and Smith was used to determine the sensitivity to thermal nociceptive stimulation (D'Amour and Smith 1941). An analgesimeter (model 33a, Life Science Instruments, USA) was used. The tail flick response was elicited by applying radiant heat to the dorsal surface of the tail and the duration of time (in seconds) taken for animal to move (flick) its tail away from the heat is recorded as tail flick latency. Each animal was tested three times at baseline and at the stated times, and the average of the three values was taken. The cutoff time was considered 10 s in order to avoid tissue damage.

Mechanical Pain

Mechanical nociception was obtained using the method described by Ren (1999) with calibrated von Frey filaments (Stoelting, Wood Dale, IL) that was applied in ascending order to the hind paw. Filaments were applied to the dorsal surface of the paws based on studies demonstrating that the dorsal approach more reliably and consistently detects threshold changes. Stimulus–response function curves were prepared by plotting the paw withdrawal threshold (PWT) vs. days post-surgery.

Paw withdrawal threshold was defined as the force at which the animal withdrew to three of the five stimuli delivered in grams. PWT were assessed by blinded observer to the surgery. Each animal was used as its own control then pre- and post-lesion responses were measured.

Histology

Histological study was performed to verify the spinothalamic tract lesion in experimental rats. A segment of spinal cord at level of injury removed, perfused, and fixed with paraformaldehyde 4 %, and then tissues were paraffin embedded. Sections of injured tissue were stained with Luxol fast blue (LFB), tissue injury was observed, and lesion placement was investigated.

Immunohistochemistry

The activation of astrocytes and microglia after STT lesion were examined by analyzing the expression of glial fibrillary acidic protein (GFAP) and Iba1 (ionized calcium binding adaptor molecule 1), respectively, at different time intervals by immunohistochemistry. Animals were terminally anesthetized with ketamine and perfused through the ascending aorta with phosphate buffer saline (PBS) followed by 4 % paraformaldehyde. After the perfusion, the spinal cord segments (T8–10) were removed and post fixed in the same fixative overnight. After tissue dehydration/rehydration, spinal cord segments were paraffin embedded. Spinal sections were cut by microtome and 5-μm sections were prepared for immunohistochemical staining with antibodies. For immunostaining of GFAP and Iba1, at first sections were deparaffinized in xylene, hydrated through a graded series of ethanol, and then immersed in 3 % hydrogen peroxide in 100 % methanol for 15 min to inhibit endogenous peroxidase activity. Antigen retrieval was carried out by microwaving in 10 mM citrate buffer (pH=6) to activate the antigens, for 10–30 min.

After being rinsed in PBS, the sections were incubated with normal goat serum for 1 h at room temperature and then incubated overnight at 4 °C in humid chambers with the primary antibody (anti-GFAP, 1:100, mouse, Cell signaling Co; anti-Iba1, 1:50, mouse, Santa Cruz Co). The sections were washed and then incubated with a ready-to-use anti-rabbit secondary antibody from Dako (EnVision Plus®), and color reaction was developed by immersion in 0.01 % H_2O_2 and 0.05 % diaminobenzidine for 20 min as the chromogen. Light counterstaining with hematoxylin was performed. After dehydration in graded alcohol and xylene, the sections were coverslipped with Permount mounting medium (Entellan®, Merk). Slides were examined with light microscope (Labomed, USA) and images were captured and studied by Digi3 software. Negative controls were incubated with the primary antibody diluents instead of the primary antiserum. The density of GFAP and Iba1 immunostaining was assessed semiquantitatively by blinded pathologist using a four-point scale that 0=no staining and 3=maximal staining within the experiment (1=mild, 2=moderate, and 3=severe expression).

Western Blot Analysis

Western Blotting was performed on T8–10 spinal segments at lesion site. Immediately after tissue removal, spinal tissues were snap frozen and then homogenized with lysis buffer (Tris–HCL 50 mM, NaCl 150 mM, Triton X-100 0.1 %, sodium deoxycholate 0.25 %, SDS 0.1 %, EDTA 1 mM, and protease inhibitor cocktail 1 %) by a tissue homogenizer. Total protein extract was obtained after centrifugation for 45 min at 13,000 rpm, 4 °C. After Bradford assay, equivalent protein sample was run on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membrane. The membranes were blocked with 2 % nonfat dry milk (Amersham, Ecl AdvanceTM) for 75 min and incubated with anti-Iba1 (mouse,1:700, Santa Cruz Co.), anti-GFAP (mouse,1:1,000, Cell signaling Co.), and anti-β actin (mouse, 1:10,000, Cell signaling) overnight at 4 °C.

These membranes were further incubated with HRP-conjugated secondary antibody, developed with a chemiluminescence kit (ECL solution) and exposed onto X-ray films for 1–10 min. The intensity of specific bands was quantified by densitometry with Image J software. To confirm equal loading of protein anti-β actin was used.

Statistical Analysis

Data were analyzed by two-way ANOVA with Bonferroni posttest. A value of $P<0.05$ was considered as statistically significant. All data are expressed as mean values±SEM.

Results

Histological Verification

Localization and the extent of lesion were verified with LFB staining. Myelin loss was significant 1 week after injury. Behavioral and histological data were excluded if there was evidence that the lesion site was extending into the gray matter or not on the ventrolateral quadrant of the white matter.

Behavioral Results

Behavioral assessments including motor activity and nociceptive responses were investigated in the separated groups pre- and post-surgery. Only animals that were in the normal range for behavioral tests were selected for experiments. Generally, we did not observe paralysis on STT-injured rats.

Locomotor Function

Electrolytic STT lesion affected the motor coordination of animals. As shown in Fig. 1, endurance time in STT-injured group was decreased significantly with maximum reduction on day 3 after injury. Decrease in motor performance was also observed in sham group but it tends to return to the basal level from day 3. Generally, there were differences in the motor coordination outcome between sham and STTs-injured groups which was significant on day 7 post-surgery ($P<0.01$, $F=1.844$). Although there was a slight decrease in the distance moved by animals in STT-injured group, we did not observe any significant difference in locomotor function between injured animals and sham control group using open field test.

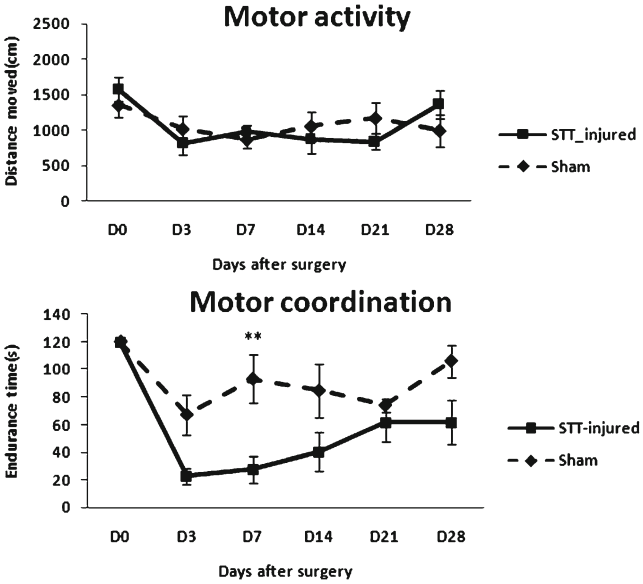

Fig. 1 Effect of electrolytic lesion of spinothalamic tract on motor performance of animals. Data show that STT lesion has no significant effects on motor activity of animals but decreases the times that rats could remain on rotating rod. This effect is significant at day 7 post-lesion (two-way ANOVA followed by Bonferroni posttest, $^{**}P<0.01$, $F=1.844$), $n=8$

Thermal and Mechanical Pain

Data showed that tail flick latency in injured animals significantly increased 3 days after lesion ($P<0.001$, $F=3.446$ compared to sham control group) but the latency reduced after that and reached to sham control value at day 14 (Fig. 2). Regarding mechanical pain, electrolytic lesion caused a significant decrease in paw withdrawal threshold in both paws of rats. The difference in mechanical pain was significant between STT-injured animals and sham control group 7 days after surgery ($P<0.05$ on days 7–14 and $P<0.001$ on days 21–28). Allodynia was observed in the contralateral paw to the lesion side at day 7 post-injury and persisted for >28 days. In the ipsilateral paw, allodynia was significant at day 21 which was persistent to day 28 after surgery (Fig. 3a, b).

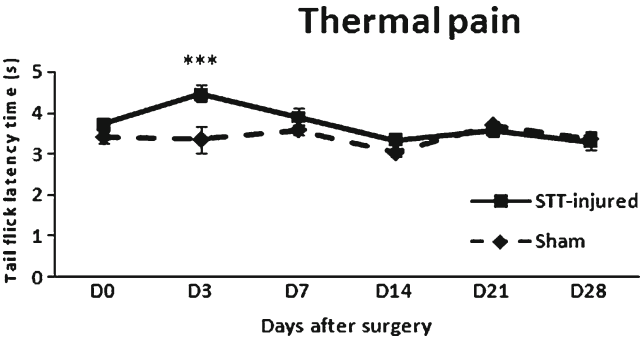

Fig. 2 Effect of electrolytic spinothalamic tract lesion on thermal pain. Three days after surgery, tail flick latency significantly increased compared to the sham group. This effect disappeared after that (two-way ANOVA followed by Bonferroni posttest, ***$P<0.001$), $n=8$

Glial Activation Markers

The expression of the glial cell markers Iba1 and GFAP was detected in order to find the type and time of glial cell activation following STT injury. The lesion site was characterized by evidences of gliosis extending around the lesion site. The immunostained sections showed gliosis 3 days after electrolytic lesion in ventrolateral spinal cord at T8–T9 vertebral level. Generally, GFAP immunostaining was much more than Iba1. A substantial increase in Iba1 expression at lesion site was observed following spinal cord injury. Microglia activation showed a significant peak on day 3 post-lesion and then was gradually decreased (Fig. 4a). More Iba1 expression was scored in STT-injured animals than sham group with maximum differences on days 3–7 ($P<0.01$, Fig. 4b).

Number and density of astrocytes were higher in contralateral paw during the first week after SCI with maximum difference at day 3. This lateral specificity disappeared gradually and the GFAP expression was increased in the right side being centered on the lesion site. More significant astroglias are shown ipsilateral to lesion side at day 21 (Figs. 5 and 6a). Increased GFAP expression was observed for 4 weeks (Fig. 6).

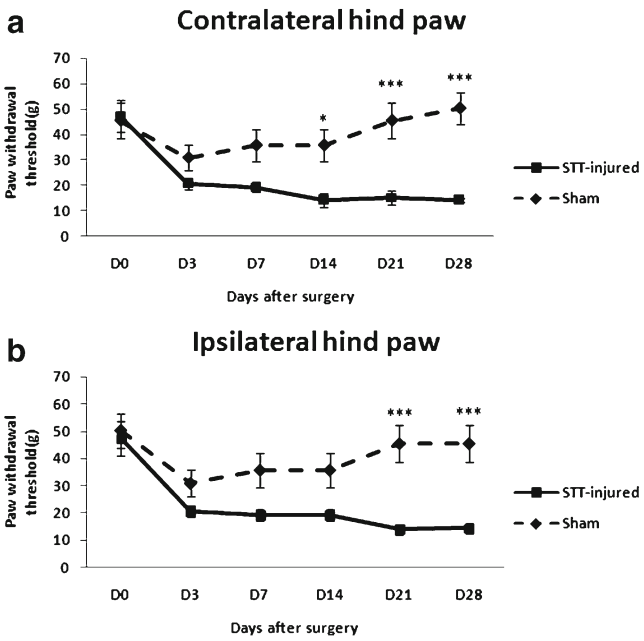

Fig. 3 Effect of unilateral electrolytic lesion of spinothalamic tract on mechanical pain. Paw withdrawal threshold significantly decreased 7 days after surgery in comparison to control sham groups in the left (**a**) hind paw. The right paw (**b**) showed significant difference with control value at days 21–28 (two-way ANOVA followed by Bonferroni posttest, *$P<0.05$, $F=2.839$; ***$P<0.001$, $F=4.122$), $n=8$

Western Blot Analysis

According to the time courses of surgery, microglia and astrocytes activation were examined in the spinal cord tissue (T8–T10 segments) at 3, 7, 14, 21, and 28 days after STT lesion. Western blot analysis in sham group showed a low level of basal expression of GFAP and Iba1 in all time intervals examined. Notably, the basal GFAP levels were much higher than basal Iba1 levels. Electrolytic spinal cord injury induced a rapid increase in Iba1 at days 3 and 7 in spinal cord tissue at lesion site. Increase in GFAP levels was significant at day 14 and remained high till 28 days post-injury (Fig. 7).

Discussion

Research on specific pathways that interact with central pain syndrome following spinal cord injury faces many problems such as widespread degeneration in the tissue which makes it difficult to explain the underlying or cellular mechanisms after a selective tract lesion. After processing of sensory inputs in the spinal dorsal horn, pain information is carried by several pathways to distinct projection sites in the brain. The lateral spinothalamic tract projects multimodal sensory inputs to the lateral thalamus.

By making selective lesion on spinothalamic tract, the pain sensation is significantly altered. The same finding has been reported by several investigators previously (Lucas et al. 2011; Masri et al. 2009; Wang and Thompson 2008). It is not clear whether interruption of STTs axons transmit altered pain information from spinal levels or pain is mainly caused by hyperexcitability of thalamic neurons as described by Wang and Thompson (2008). Reduced motor function after spinal cord damage may be also associated with pain sensation. Ventral and lateral white matters contain interspersed descending pathways that are necessary in the initiation of locomotor function (Shefchyk 2006). Damage to these descending tracts may produce motor deficits due to myelin destruction as it is observed in this

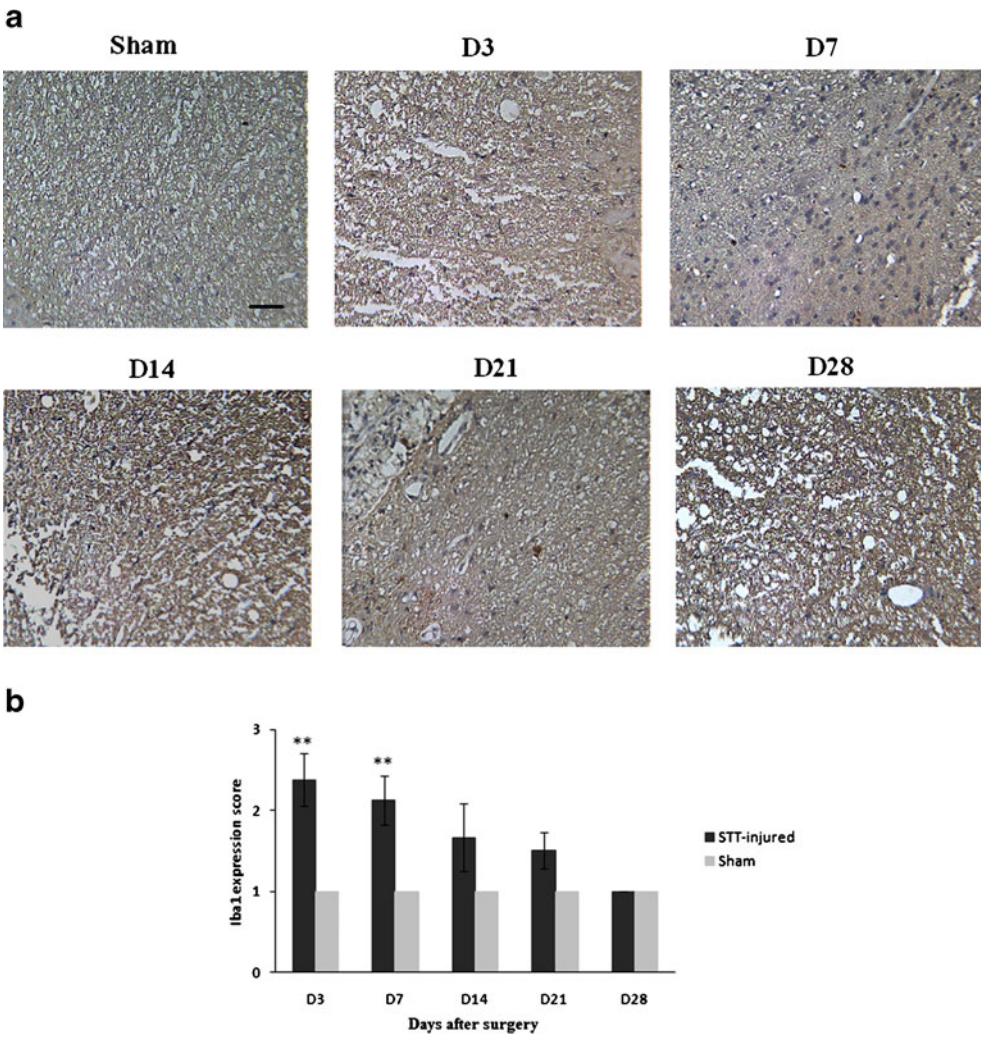

Fig. 4 Expression of Iba1 in spinal cord sections after STT lesion. **a** IHC images of spinal cord tissue at lesion site in the sham and STT-injured animals (D3–D28), scale bars, 20 μm. **b** Iba1 expression scores in the spinal cord sections STT lesion. Electrical lesion significantly increased expression of Iba1 in the spinal cord 3 and 7 days after injury (scores: 1=mild, 2=moderate, and 3=severe expression), $^{**}P<0.01$, $n=4$

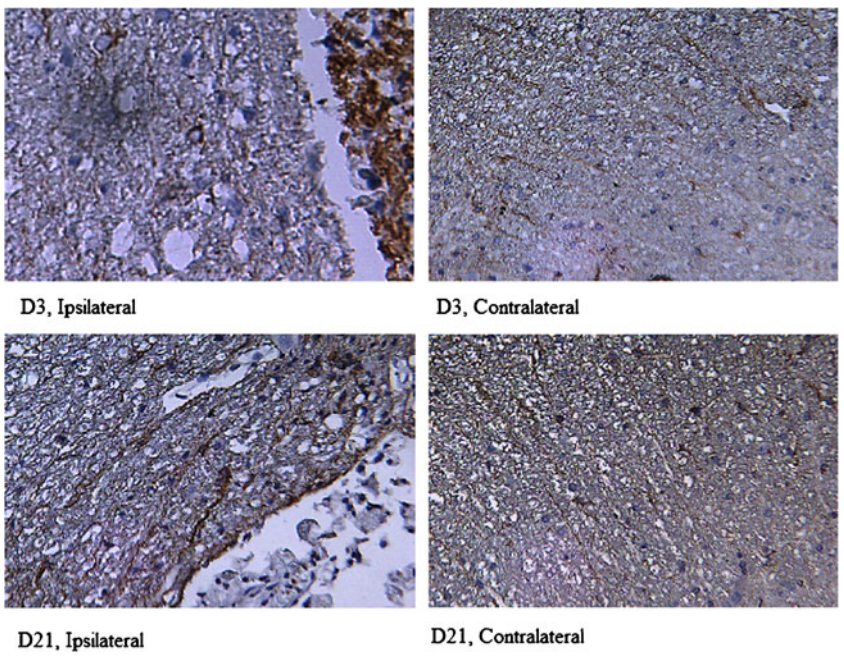

Fig. 5 Laterality of GFAP expression in the spinal cord following electrical STT lesion. At day 3 post-lesion, the number and density of astroglia are higher in contralateral side to lesion site but at day 21 the astrocytes are more expressed ipsilaterally and centered at the lesion site

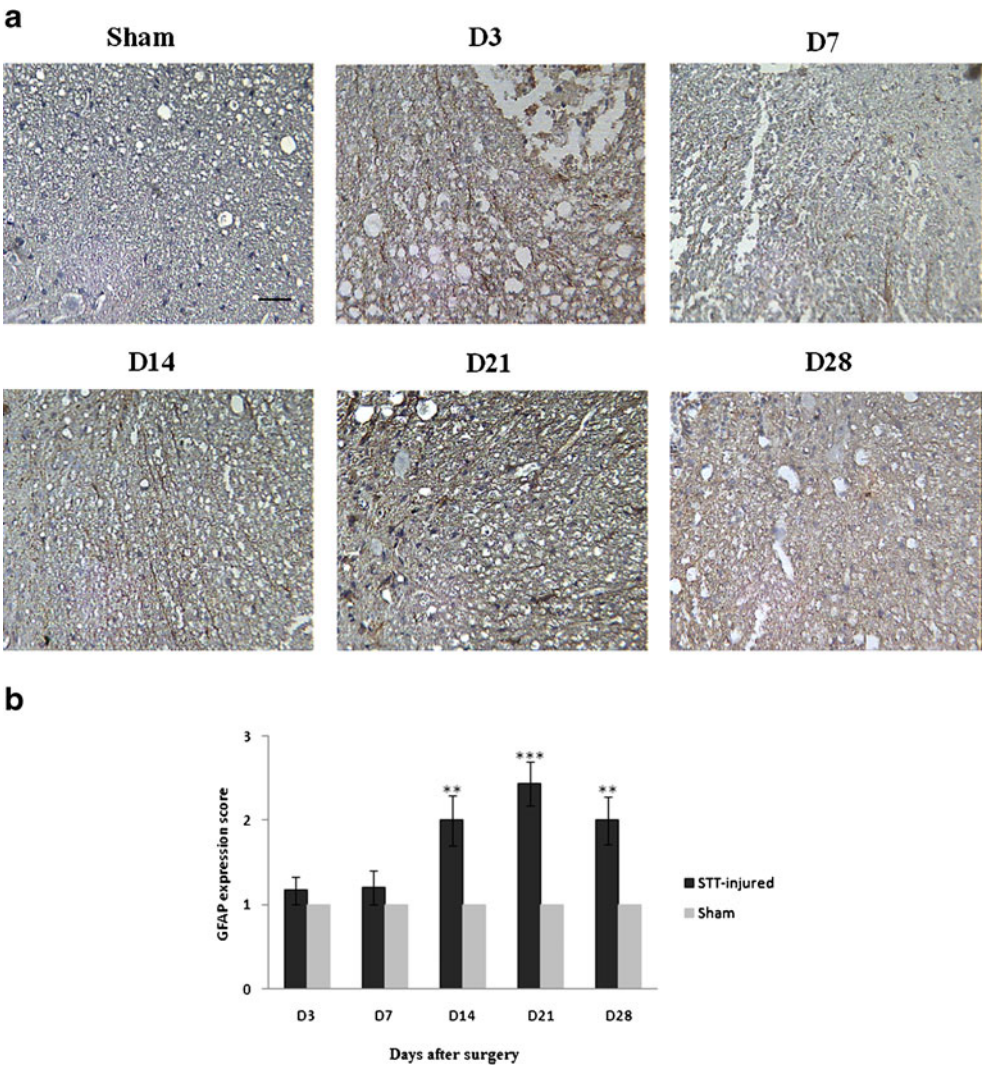

Fig. 6 Expression of GFAP in spinal cord sections after STT lesion. **a** IHC images of spinal cord tissue at lesion site in the sham and STT-injured animals (D3–D28), scale bars, 20 μm. **b** Immunohistochemistry revealed an increase in GFAP immunoreactivity in the spinal cord after STT lesion. GFAP expressions were significantly increased 14 days post-injury and remained till day 28. n=4, $^{**}P<0.01$ and $^{***}P<0.001$

study. Although, the motor function of animals was not significantly decreased, the endurance time was reduced on rotating rod. This effect is also shown in the sham group, but it does not seem to be solely related to laminectomy because no myelin damage was observed in the sham group while an extensive myelin destruction was clearly observed in SCI group (data in press). The other interpretation is that the injury on the back of animals during the first days following surgery makes it difficult for them to stay on rotating rod at day 3. Furthermore, the significant difference between the control and experimental group on day 7 can be contributed to the increased result values obtained in the sham group rather those obtained from the SCI ones.

Interestingly, electrical lesion on STT increased the tail flick latency at day 3. The transient thermal hypoalgesia following electrolytic lesion has been also reported by other investigators (Masri et al. 2009). It is possible that STT lesion interrupt the transmission of information from the periphery to the cortex which perceives pain. At this time, the cellular events leading to secondary inflammatory phase are not fully developed. Involvement of descending pain inhibitory systems to compensate the injury-induced pain cannot be excluded. It is also speculated that modulation of spinal nerve morphology and density in the spinal cord refractory to lesion may also lead to amelioration of injury-induced hyperalgesia (Kuner 2010).

In a rodent model for CPS, introduced by Wang and Thompson (2008), electrical lesion of STT caused significant allodynia and hyperalgesia. These findings were consistent with previous reports, reflecting the use of spinothalamic tract lesion in the treatment and the relief of chronic pain, but this procedure failed to provide treatment for a wider coverage of sufferers from allodynia and CPS (Vierck et al. 1990). Based on the results of the present study, the allodynia and hyperalgesia were observed bilaterally in the rat paws yet occurring in the left paw earlier than in the right paw. In several animal models of neuropathic pain, allodynia is produced in both the contralateral and ipsilateral hind paws, accompanied by bilateral spinal expression of proinflammatory cytokines

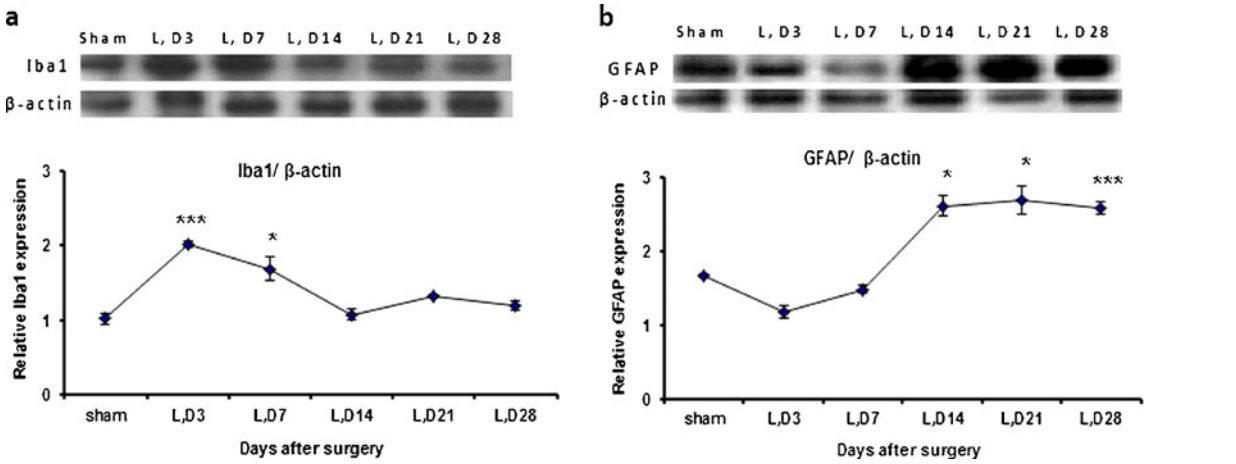

Fig. 7 Effects of STT lesion on the spinal levels of Iba1 and GFAP proteins. **a** Western blot analysis showing time course of STT lesion-induced expression of Iba1 in the spinal cord of rats at lesion site. Increase in Iba1 expression band is significant 3 days after injury up to 7 days. Low panel shows densities of Iba1 bands after being normalized to β-actin appropriately. *L* lesion, *D* days post-lesion. **P*<0.05, ****P*<0.001 compared to sham control animals, *n*=4. **b** Time course of STT lesion-induced expression of GFAP in the spinal cord of rats at lesion site. GFAP expression is highly increased from day 14. Low panel shows densities of GFAP bands after being normalized to β-actin. *L* lesion, *D* days post-lesion. **P*<0.05; ****P*<0.001 compared to sham group, *n*=4

and glial activation. As reported by other investigators, neuropathic pain may extend beyond the initially injured nerves and occurred bilaterally in mirror image sites (Zhang et al. 2011). Spinal cord lesions make CPS bilaterally, in accordance to Quiton' study (Quiton et al. 2010). Cerebral cortex has an important role in pain perception, so abnormal cortical activity after CPS from this supraspinal projection and various thalamus nucleuses could be responsible for bilateral pain (Masri et al. 2009; Wang and Thompson 2008).

The other point is that, for a long time, it was believed that only neurons and their neuronal circuits were responsible for the development of chronic pain. In the last decade, non-neuronal cells especially glial cells have been studied in the chronic pain conditions (Vierck et al. 1990). Although most of reports have been directed to the association of spinal glial activation with chronic pain, it was felt needed to investigate the molecular events after STTs lesion that could correlate between behavioral changes and interruption of ascending spinothalamic afferent pathways (Vierck et al. 1990). It is also necessary to define when glial cells are activated in association with pain.

Spinal glial activation which is shown by increased expression of GFAP and Iba1 in lesion-targeted areas suggests that this kind of gliosis may be directly produced by local tissue damage after electrolytic stimulation, different with subsequent glial activation in the spinal dorsal horn after peripheral injury. Based on our results, following injury, Iba1 expression was increased early (from day 3 to 7) but GFAP expression was increased later (from day 4 to 28). During the first 2 weeks after injury, paw withdrawal threshold was declining and Iba1 expression was increasing being the reason behind naming this early phase as "pain induction" while the next period was called by other investigators as "microglial activation period." We also observed that from day 14 to 28 after STT lesion, increasing GFAP expression and pain perception was evident. This late phase can be referred to as delayed pain perception and astrocyte activation period. Similar to our findings, other studies using different models of injury have shown that microglia were activated in an earlier phase after injury and were involved in the onset of neuropathic pain, while astrocytes were activated in a later phase after the injury (Colton and Wilcock 2010).

It seems that both ipsilateral and contralateral glial proliferation associated with nerve injury which is observed in Figs. 4 and 5 resulting in an extension of pain to the contralateral side which is known as mirror image pain. Electrical lesion induces a substantial increase in GFAP expression in astrocytes in the lesion site. During the first week after injury, the density of astrocytes is higher in the contralateral side than the ipsilateral, with maximum difference at day 3. After 2 weeks, the number and density of astroglia are increased in the right side especially around the lesion site, and at day 21, we can see more significant astrocytes ipsilateral to the lesion. Upregulation of the microglial marker (Iba1) in the acute phase is parallel to the onset of pain whereas the high expression of astrocyte marker (GFAP) in the late phase is accompanied by pain continuation. Indeed astroglial reaction after injury is more persistent than microglial reaction and displays a better correlation with chronic pain behavior.

Hulsebosch et al. showed cysteine–cysteine chemokine ligand 21 to be upregulated in the cell bodies of STT neurons, the axons of which had been damaged after SCI (Hulsebosch et al. 2009). Therefore, any damage on STT neurons could develop chronic pain syndrome and motor dysfunction; such results we also observed in this study (Song et al. 2006). STT carries nociceptive information supraspinally and involved in neuropathic pain (Ren 1999). The activation of microglia and astrocytes and downstream signaling after injury have shown to play an important role in the process of pain (Gao and Ji 2010; Graeber 2010; Gwak et al. 2008; Hulsebosch 2008; Scholz and Woolf 2007; Verkhratskiĭ and Butt 2007; Wu et al. 2005; Zhuang et al. 2005), but the involvement of this pathway in STT injury-induced CPS is not known. Very low threshold of activation is one of the microglia properties while they can respond to tiny pathological alterations that affect CNS directly or indirectly (Kreutzberg 1996), and microglia activation occurs very fast but can remain for a long time (Colton and Wilcock 2010). Similarly, in the STT-injured animals, the microglia was activated fast, although this activation disappeared after 7 days.

Further studies indicate that microglial reaction precedes astrocytic reaction (Tanga et al. 2004) and likelihood results to astrocyte reaction (Svensson et al. 1993). Increasing evidence suggest that activated astrocytes are required for the induction of chronic pain hallmark, mechanical allodynia (Gao and Ji 2010; Meller et al. 1994) and astrocyte reaction that may remain for more than 5 months after nerve injury (Zhang and De Koninck 2006). There are some reports noting that intrathecal injection of general glial inhibitors like fluorocitrate or fluoroacetate has been shown to attenuate pain behaviors in neuropathic rats (Meller et al. 1994; Okada-Ogawa et al. 2009).

Although, there are also reports that Iba1 is expressed specifically in microglia, both in cultured brain cells and in the brain, but Wu et al. showed that Iba1 in resting and activated microglia is present (Ito et al. 1998; Wu et al. 2005). In addition, GFAP is the main hallmark of astrocyte cells that its upregulation indicates astrocyte activation (Gao and Ji 2010).

In the present study, STT lesion caused transient hypoalgesia response to thermal pain at day 3 post-lesion, but there was no significant difference after that. Comparing with previous studies (Gao and Ji 2010), different response to thermal stimuli after day 3 may be related to the different mechanisms in pain processing when the stimulus is applied on paw compared to tail of animals. Bilateral mechanical allodynia in hind paw occurred significantly on day 14 post-injury that was later than the other previous report (Christensen and Hulsebosch 1997). Hyperalgesia and allodynia are the most typical symptoms that were reported by CPS patients (Finnerup et al. 2004; Wang and Thompson 2008). Taken together, these findings suggest that this model could be considered as an appropriate experimental animal model for studies on CPS. Decrease in locomotor function after STT lesion has been reported by other investigators previously (Webb and Muir 2004), but one of the advantages of this model is that there is no paralysis interfering with measuring pain threshold owing investigation on pain pathways specifically.

As discussed in recent studies, the delayed expression of CPS and the diffuse localization of painful symptoms suggest that the pathophysiology does not reflect only direct effects at the denervated spinal segments. So it could imply the appearance of maladaptive plasticity in supraspinal structures and/or the existence of non-neuronal structure such as glial cells (Masri et al. 2009), and glia as non-neuronal elements in pain is suggested as novel therapeutic target for SCI patients (O'Callaghan and Miller 2010). In this study, we showed the involvement of spinal microglia and astrocytes in the development of CPS. Activation of microglia and astrocytes following STT injury may be related to the onset and maintenance of pain perception, respectively. These findings are all in agreement with previous studies about glia activation and pain threshold. Our findings of glial cell's marker levels imply that microglia is the predominant form of glial cell in the spinal cord for starting of pain process and astrocytes have critical role in pain preservation in this CPS animal model.

Conclusion

Selective loss of certain ascending input from the STT develops persistent pain states and motor coordination disturbances. An early involvement of microglial activation and a later delay of astrocytic activation are responsible for the induction and maintenance of hyperalgesia and allodynia after STT injury. More investigations are needed to clarify the precise role of these cells in this pathological event.

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