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Estradiol attenuates spinal cord injury-induced pain by suppressing microglial activation in thalamic VPL nuclei of rats

Elham Saghaei[a], Fatemeh Abbaszadeh[b], Kobra Naseri[a], Samar Ghorbanpoor[b], Mina Afhami[b], Ali Haeri[a], Farzaneh Rahimi[c], Masoumeh Jorjani[a,b,*]

[a] Department of Pharmacology, Shahid Beheshti University of Medical Sciences, Evin, Tehran, Iran
[b] Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Evin, Tehran, Iran
[c] Department of Pathology, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Evin, Tehran, Iran

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ABSTRACT

In our previous study we showed that central pain syndrome (CPS) induced by electrolytic injury caused in the unilateral spinothalamic tract (STT) is a concomitant of glial alteration at the site of injury. Here, we investigated the activity of glial cells in thalamic ventral posterolateral nuclei (VPL) and their contribution to CPS. We also examined whether post-injury administration of a pharmacological dose of estradiol can attenuate CPS and associated molecular changes. Based on the results,in the ipsilateral VPL the microglial phenotype switched o hyperactive mode and Iba1 expression was increased significantly on days 21 and 28 post-injury. The same feature was observed in contralateral VPL on day 28 ($P < .05$). These changes were strongly correlated with the onset of CPS ($r^2 = 0.670$). STT injury did not induce significant astroglial response in both ipsilateral and contralateral VPL. Estradiol attenuated bilateral mechanical hypersensitivity 14 days after STT lesion ($P < .05$). Estradiol also suppressed microglial activation in the VPL. Taken together, these findings indicate that selective STT lesion induces bilateral microglia activation in VPL which might contribute to mechanical hypersensitivity. Furthermore, a pharmacological dose of estradiol reduces central pain possibly via suppression of glial activity in VPL region.

1. Introduction

Central pain syndrome (CPS) is defined as "pain initiated or caused by a primary lesion or dysfunction in the CNS". CPS results from a variety of conditions and spinal cord injuries (SCI), multiple sclerosis (MS), and cerebrovascular lesions (stroke) are most common of all (Masri et al., 2009).

In response to neuronal injury, the nervous system undergoes extensive pathological changes which result in pain signal generation along this pathway. Emerging evidence indicate that remote signaling after neuronal injury contributes to the active modulation of nociceptive network activity in real time and at multiple locations along the sensory neuraxis through a variety of cell types with unique intracellular and cell–cell signaling mechanisms (Yezierski, 2000; Bryce et al., 2012).

Thalamus is an important brain structure which receives pain signals through spinothalamic tract (STT), a major pathway of pain (Davis et al., 1998; Thompson and Bushnell, 2012).

The role of thalamus in chronic pain has been reported in both clinical and experimental studies. Weng et al. (2003) showed that, primate CPS model increased the incidence of spike-bursts in cells of deafferented thalamus (Weng et al., 2003). A significant increase in spontaneous burst activity of neurons as well as up regulation of sodium channels (Na(v)1.3 type) in VPL region has been also demonstrated in a rodent model of CPS (Hains et al., 2006; Zhao et al., 2006). Increased thalamic activity in imaging studies and biochemical changes have also been reported in patients with pain after spinal cord injury (Kupers et al., 2000; Anderson et al., 2006; Gustin et al., 2011).

On the other hand, extensive studies have shown that glial cells have pivotal role in neuronal sensitization and pain behavior enhanced by different nerve injury models (Coyle, 1998; Chadi et al., 2001; Coull et al., 2005). Thalamic glial alterations occur in central pain models such as spinal cord injury pain or post stroke

Abbreviations: CPS, central pain syndrome; VPL, ventral posterolateral; Est, estradiol; Contra, contralateral; Ipsi, ipsilateral; Les, lesion; STT, spinothalamic tract; MBP, myelin basic protein.

* Corresponding author at: Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Evin, Tehran, Iran. Tel.: +98 21 22429768; fax: +98 21 22431624.
E-mail addresses: Saghaei999@yahoo.com (E. Saghaei), f.abbaszadehm@gamail.com (F. Abbaszadeh), naseri5674@yahoo.com (K. Naseri), Samar_ghorbanpoor@gmail.com (S. Ghorbanpoor), Mina.afhammi@gmail.com (M. Afhami), haeria@sbmu.ac.ir (A. Haeri), farzirahimi@yahoo.com (F. Rahimi), msjorjani@sbmu.ac.ir, msjorjani@yahoo.com (M. Jorjani).

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pain (Zhao et al., 2007; Wasserman and Koeberle, 2009) as well as in peripheral neuropathic and inflammatory pain (Huber et al., 2006; Toth et al., 2010).

CPS is resistant to conventional therapies, for a variety of factors including anatomical, neurochemical, excitotoxic, inflammatory, ion channel expression, and neuroimmune responses which are believed to be involved in causing such pain (Hains et al., 2001, 2003; Finnerup and Jensen, 2004; Hulsebosch, 2005). Indeed, currently available drugs for neuropathic pain provide transient relief in only a fraction of patients while causing severe CNS side effects. It seems that these drugs do not target all underlying causes of central pain syndrome (Sandhir et al., 2011).

Recently, Estrogen (17β-estradiol) has received particular attention as a potential therapy because it targets many of the pathways contributing to pain. Estrogen protects the nervous system against the noxious consequences of nerve injury. Both neurons and glia have been suggested as important components of the protective mechanisms of estrogen (Gyenes et al., 2010; Samantaray et al., 2011).

In our previous study, we demonstrated the involvement of spinal microglia in pain development stage whereas astrocytes were involved in the late phase or maintained chronic pain following unilateral electrolytic lesion on STT (Naseri et al., 2012a). Estradiol improved the functional recovery and decreased the extent of demyelination at the site of lesion and restored the MBP (myelin basic protein) reduction which was concomitant of microglial reactivity (Afhami et al., 2012). Our findings led us to think that the molecular events and glial reactivity at the spine makes remote glial alterations at the supra spinal level leading to allodynic pain. Considering functional improvement by estradiol, it was reasonable to examine the administration of 17-Beta estradiol after neuronal injury and study the possibility of the steroid role in alleviating CPS and the suppression of thalamic glial activity.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats ($n = 84$; weight 250–300 g) were housed in individual cages with food and water ad lib. All procedures were approved by ethical committee of Shahid Beheshti University of Medical Sciences.

2.2. Spinal lesion

Rats were anaesthetized using Ketamine/Xylazine (60/20 mg/kg, I.P.). CPS was induced according to the method described by Wang and Thompson (2008) with a little modification. Briefly, a dorsal laminectomy was performed at spinal segments T8–T9. After exposure of the spinal cord, the dura was incised by fine tip iris scissor. Then, a tungsten electrode (5 μm tip, 1 MΩ) was targeted to the right spinothalamic tract (STT), based on stereotaxic coordinates (laterality to midline: 0.5–0.7 mm and depth: 1.6–1.9 mm). Lesion was made by a brief current pulse (300 μA, 90 s) passed through the electrode. Electrical current was raised gradually up to 300 μA in 10 s to reduce animal reflex and more consistent outcome. After surgery, all animals received 1 mL of saline (S.C.) to balance electrolytes as well as Penicillin G (I.M.) to prevent infection. Besides, during the surgery and recovery from anesthesia, the rats were covered with warm sterilized towels. The rats were maintained in single cage in a temperature-controlled room at 25 °C.

2.3. Experimental groups

Rats were divided into three main groups. Sham-group received just a laminectomy, without electrolytic lesion. Estradiol-group received electrolytic lesion and a single dose of 4 mg/kg 17 β-estradiol (Sigma–Aldrich, St. Louis, MO). Lesion-group, rats were subjected to the electrical lesion and equal volume of sesame oil as a vehicle of estradiol. All compounds were administered by intra peritoneal injection at 30 min post-injury.

2.4. Mechanical allodynia

Mechanical allodynia was assessed as originally described by Ren (1999). Mechanical hind paw thresholds were measured bilaterally using calibrated von Frey filaments (Stoelting, IL, U.S.A). Filaments were applied in ascending order to the dorsal surface of hind paw (Ren demonstrated that dorsal approach is more reliably and consistently detects threshold changes). Paw withdrawal threshold was defined as the force at which the animal withdrew the paw to three of the five stimuli delivered.

2.5. Western blot analysis

Both right and left thalamus nuclei were homogenized separately and Whole-cell lysates were prepared in accordance to the methods described previously (Lee et al., 2012). 60 μg protein was subjected to a 12% polyacrylamide gel electrophoresis (Bio-Rad), and transferred electrophoretically to polyvinylidenedifluoride (PVDF) membranes. After blocking, the membranes were incubated with anti-Iba1 antibody (1:1000, WAKO Pure Chemical Industries, Osaka, Japan), anti-GFAP (1:1000 Cell Signaling Technology) and anti-β actin (1:10000, Cell Signaling Technology), then incubated with HRP-conjugated secondary antibody (1:10000 Cell Signaling Technology). The blots were detected using ECL western blotting detection system (Amersham, Piscataway, NJ) and exposed to X-ray film (Kodak, Rochester, NY). Bands were quantified using the software NIH Image J.

2.6. Immunohistochemistry

The reactivity of astrocytes and microglia in sham, lesion, and estradiol groups were measured by analyzing the expression of GFAP (Glial Fibrilary Acidic Protein) and Iba1 (Ionized calcium binding adaptor molecule 1) respectively by immunohisthochemistry at different time points. Rats were deeply anesthetized with ketamine (65 mg/kg I.P.) and transcardially perfused with 0.1 M phosphate buffer saline (PBS), followed by 4% paraformaldehyde/0.1 M phosphate buffer. Whole brain were removed and fixed in the same fixative overnight. Tissues were embedded in paraffin, and 4-μm-thick serial sections were cut with a sliding microtome. Sections on slides were deparaffinized in xylene and rehydrated through descending grades of ethanol, treated with EDTA buffer (pH 8.5) at 90–100 °C for antigen retrieval, and soaked in 1% hydrogen peroxide (30%)/methanol for blocking endogenous peroxidase activity. The unspecific background reactions were blocked with 10% bovine serum albumin/DAKO-Buffer. Slides were then incubated with primary antibody (rabbit anti-GFAP, 1:2000, Cell Signaling Technology; and rabbit anti-Iba1, 1:3000, WAKO Pure Chemical Industries, Osaka, Japan) at 4 °C overnight in humid chamber. After several washing steps in TBS (pH 7.5), sections were incubated with biotin-conjugated secondary antibody (donkey-anti rabbit, 1:2000, Jackson Immunoresearch) at 37 °C for 1 h, and subsequently with a biotin-avidin-enzyme complex (1:100,Vectastain ABC kit, Vector Laboratories, Burlingame, USA).Antibody binding was visualized by diaminobenzidine (DAB). Then, sections were counterstained

in Mayer acidic hemalum for 10 min, rinsed for 5 min in running tap water, dehydrated in graded ethanol as well as xylene, and finally mounted with mounting media (Enthelan, Merck) and cover slipped. Images were captured using Olympus microscope and attached camera and then analyzed according to the method described by Halushka et al. (2010). Briefly, The VPL of thalamus at each side of brain section was selected as region of interest (ROI) and distribution of DAB stain intensity was calculated using Image-J software.

2.7. Statistical analysis

Statistical analysis was performed using two-way ANOVA and/or repeated measure ANOVA comparisons tests followed by Bonferroni's post hoc analysis. Pearson's correlation and logistic regression were measured to evaluate the relationship between IHC staining and mechanical threshold. An alpha level of significance was set at 0.05 for all statistical tests and data were expressed as mean ± SEM.

3. Results

3.1. Mechanical allodynia

In our previous study we showed that CPS was established bilaterally on day 14 and proceeded to day 28 after STT lesion. The present investigation showed that in spinal cord injured rats, the administration of estradiol led to the elevation of mechanical pain threshold bilaterally (Fig. 1A and B). Data analysis revealed significant benefit for estradiol therapy on pain threshold specially those days in which pain was established ($P < 0.05$).

3.2. Western blot analysis

The expression of Iba1 in homogenates of ipsi- and contra lateral thalamus was investigated by Western blot at several time points in injured groups with and without treatment ($n = 4$/group/time point) (Fig. 2A and B). A basal level of Iba1 was detected in thalamus from sham-operated animals (Fig. 2D and E). Western blotting of contralateral thalamus revealed lower level of Iba1 in comparison with that of ipsilateral one throughout the experiment especially at day 28 (Fig. 2C, $P < .05$). In contralateral thalamus there were no significant changes in Iba1 protein expression at several time points except day 28 in which Iba1 increased significantly in comparison with sham group (Fig. 2D, $P < .001$). A steady increase of Iba1 level in ipsilateral thalamus was observed from day 14, but significant differences were obtained at days 21 and 28 post injury in comparison with sham group as well as earlier days post injury (Fig. 2E, $P < .001$). Estradiol therapy reduced Iba1 protein expression in both thalamus nuclei and maintained its level as much as sham group all the time (Fig. 2D and E). To detect GFAP protein expression, whole extracts from spinal cord of each rat were also analyzed by western blot analysis. A similar level of GFAP expression in thalamus samples was detected in sham, lesion, and estradiol treated groups.

3.3. Immunohistochemistry

After immunohistological staining in brain sections (obtained from sham, injured, and injured estradiol treated groups) which had specifically VPL nucleus of thalamus complex, the number as well as intensity of Iba1 positive cells were investigated. The location of VPL in a representative section overlaid by standard rat brain atlas has been shown in Fig. 3A. Fig. 3B and H shows resting phenotype of microglia in contralateral and ipsilateral VPLs in sham-operated animals. After unilateral electrolytic lesion on

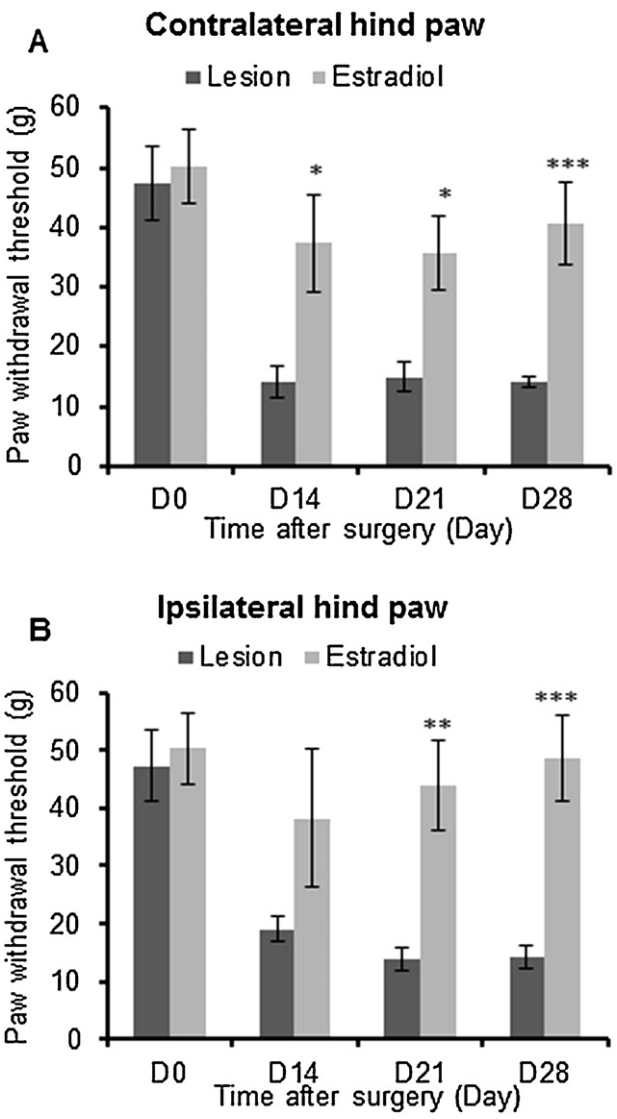

Fig. 1. Effect of estradiol on pain threshold of hind paws, contralateral (A) and ipsilateral (B) to STT electrolytic lesion. Withdrawal thresholds were significantly decreased bilaterally in lesion group and reversed by single dose of estradiol. Analysis was done by two-way ANOVA followed by Bonferroni *posttest*: $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ lesion *vs.* estradiol.

STT, microglia exhibited marked morphological changes including larger cell body and thicker processes (hypertrophic mode) which became more pronounced by 28 days. Microglial alterations were observed since day 21 in contralateral VPL (Fig. 3C and D) and day 14 in ipsilateral one (Fig. 3I–K). The microglia cell counts were relatively constant throughout the experiment in all groups; whereas intensity value altered and two way ANOVA analysis has shown that significant Iba1 expression was observed starting in contralateral VPL at day 28 and in ipsilateral VPL at days 21 and 28 post injury in comparison with sham group (Fig. 3G and O respectively, $P < .05$). Estradiol therapy in injured animals restored microglia at resting mode of which was displayed in sham animals (Fig. 3E, F and L–N); related intensity had no significant difference compared to sham group one bilaterally throughout the experiment (Fig. 3G and O). Immunostaining was done for asteroglia cells as well; astrocytic response either as intensity value or as cell counts had no change in both ipsi- and contra lateral VPL after injury. Additionally, the onset of microglia activation in ipsilateral VPL was strongly correlated with the severity of contralateral allodynia after unilateral spinal injury (Fig. 4A; $r^2 = 0.670$, $P < .001$),

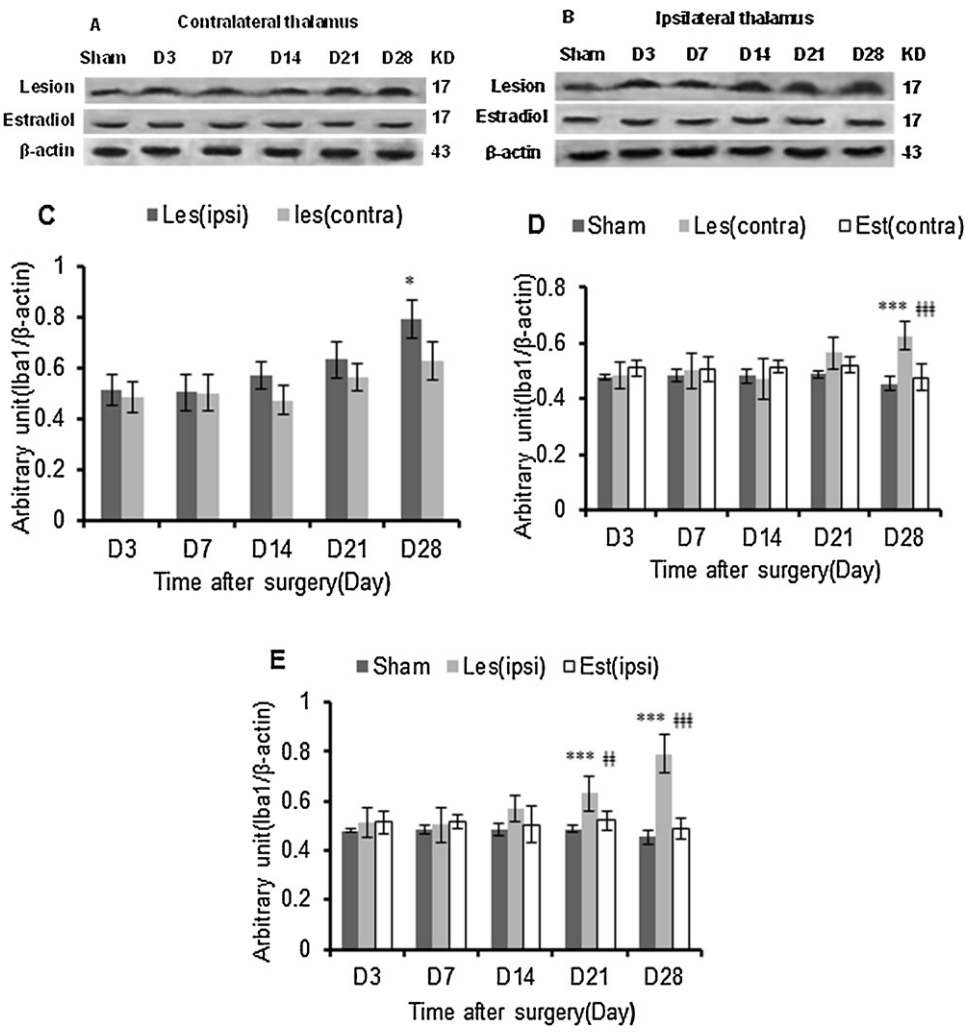

Fig. 2. The effect of unilateral electrolytic lesion followed by estradiol therapy on microglial marker in thalamus nuclei. Representative of western blotting analysis of Iba1 protein in contralateral (A) and ipsilateral (B) thalamus nuclei in both lesion and estradiol treated groups ($n = 4$ in each group). (C) Bilateral comparison of thalamic Iba1 level after unilateral electrolytic lesion, plot indicates that Iba1 protein expression in contralateral thalamus is greater from day 14 post injury, significant difference was seen at day 28 in comparison with that of ipsilateral thalamus (*$P < .05$). (D) Iba1 expression in thalamus nuclei contralateral to lesion site, significant difference was at day 28 between lesion and sham (***$P < .001$) as well as estradiol groups (***$P < .001$). (E) Observed Iba1 expression in thalamus nuclei ipsilateral to injury site. Iba1 level increased progressively, significant differences were at days 21 and 28 in comparison with sham group (***$P < .001$) as well as with estradiol treated group (**$P < .01$, ***$P < .001$). Est: estradiol, Les: lesion, Thal: thalamus, ipsi: ipsilateral, Contra: contralateral.

whereas there was no strong relationship between contralateral microglial reactivity and ipsilateral pain threshold (Fig. 4B; $r^2 = 0.184$, $P < .055$).

4. Discussion

This study was designed to investigate the impact of unilateral spinal lesion on thalamic glial activation as well as its correlation with the onset of injury-induced central pain syndrome.

Altered pain sensation and thalamic neural excitability have been demonstrated in rodent models of spinal injury and peripheral neuropathic pain previously. Although several thalamic molecular changes such as hyperactivity of T type calcium channel CaV3.1, upregulation of sodium channel NaV3.1, increased number of nicotinic acetylcholine receptors, and reduced mu-opioid receptor-mediated-G protein activity have been suggested to beinvolved in altered processing of somatosensory information and the establishment of chronic pain (Hains and Waxman, 2006; Wang and Thompson, 2008; Hoot et al., 2011; Ueda et al., 2011), but a considerable amount of evidence has demonstrated that remote glial activation signaling from the site of injured neurons is accompanied with pain. For instance, Hains and Waxman (2006) showed that after T9 contused spinal injury, microglia in lumbar dorsal horn became active and played a pivotal role in neuronal hyper-responsiveness and establishment of below level neuropathic pain via activation of p38 MAPK and ERK1/2. So far, the main cause of glial activation at locations distant to the site of injury has not been well established. Remote activation of microglia as well as proinflmmatory cytokines such as TNF-α, IL-1β and IL-6 in lumbar dorsal horn are likely to be the sensitive biomarkers of neuropathic pain (Detloff et al., 2008). In a peripheral neuropathic pain model (CCI), thermal hyperalgesia was correlated with increased expression of OX-42 which predominantly colocalized with P-p38 in the VPL (Leblanc et al., 2011). Here, we demonstrate time dependent microglial activation (as hypertrophic mode) in VPL, ipsilateral to injury site, which is concomitant with the severity of mechanical allodynia. Microglia activation was more limited in contralateral VPL. Time dependent changes in microglia and astrocyte activity at the site of lesion after STT injury (Naseri et al., 2012a,b) lead us to think that maladaptive plasticity at the site of spinal injury mediated by glial cell activation may be involved in supraspinal changes. Indeed, spinal microglia and astrocyte reactivity in the initial and

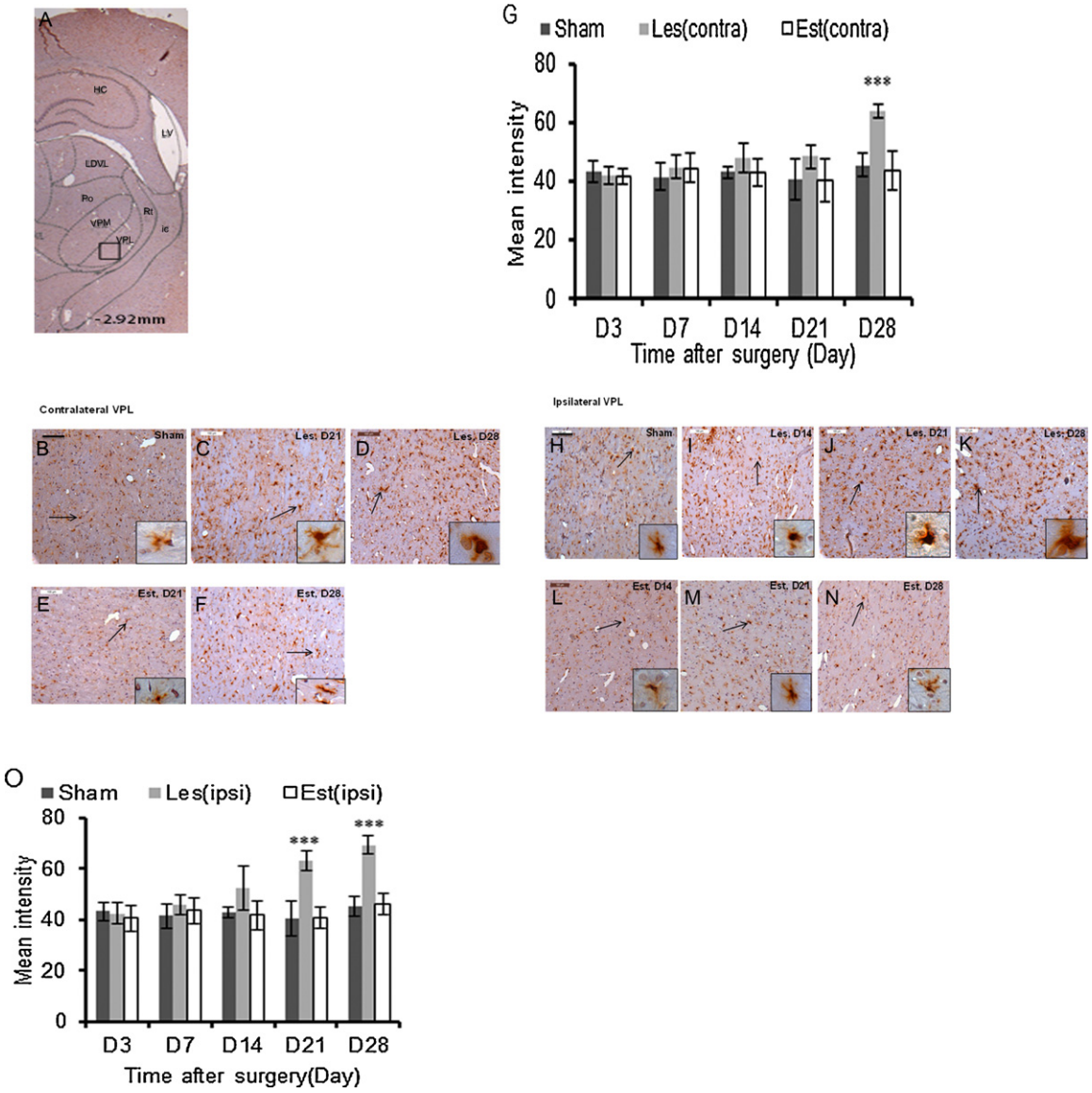

Fig. 3. Immunolabeling and quantification of microglia using Iba1a in VPL bilaterally after unilateral STT lesion. (A) Schema of ventral posteriolatral (VPL) in brain sections. (B and H) Resting microglia was observed in bilateral VPLs in sham groups. Resting modes of microglia with small somata and narrow processes were displayed by higher magnification (insets of B and H). (C, D and I–K) Activated microglia, characterized by retracted processes and large cell bodies (high magnification insets of C, D, I–K) have been found at days 21 (C) and 28 (D) in contralateral VPL as well as at days 14 (I) 21 (J) and 28 (K) in ipsilateral VPL. (E, F and L–N) The positive effect of estradiol on microglial activity was displayed; microglia cells were in resting mode throughout the experiment. (G and O) Bar diagrams quantifying Iba1 immunoreactivity in contralateral (G) and ipsilateral (O) VPLs. DAB intensity was measured in region of interest within VPL. [Significance in G and O: repeated ANOVA $^{***}P < .001$ vs. sham as well as Est]. Les: lesion, Est: estradiol, contra: contralateral, ipsi: ipsilateral, VPL: ventral posterolateral. Scale bar: B–F and H–N = 200 μm; inset scale bar = 20 μm.

chronic phase respectively after SST lesion, possibly account for abnormal activity of STT pathway and indirectly alter glial activity in the VPL in the second half of the study. As we know, activated glial cells produce abnormally secreted products (e.g. proinflammatory cytokines) contributed to neuroanatomical and neurochemical changes leading to maintained hyperecxitability throughout neural axis (STT) and then central sensitization (Hulsebosch, 2008; Austin and Moalem-Taylor, 2010). It should be noted that Iba1 reactivity does not reflect the exact level of pro-inflammatory and/or anti-inflammatory cytokines and chemokines produced by activated microglia such as IL-1 beta, IL-6, IL-10, TNF-alpha and etc. On the other hand, several microglial factors have protective roles which are dependent on the time of factor release possibly on the severity of CNS injury. Anti-inflammatory factors facilitate the clearance of apoptotic cells and tissue debris and increase the expression of self-associated proteins to dampen continued pro-inflammatory actions. IL-10 is well described anti-inflammatory cytokine that has been shown to prevent and reverse pathological pain (Milligan and Watkins, 2009). Therefore, the precise role of microglia would be clarified if we determine the level of different cytokines or mediators produced by activated microglia in VPL. Electrolytic lesion in the unilateral STT did not induce astroglial response in both ipsilateral and contralateral VPL. Several studies have shown that the contribution of spinal astrocytes in neuropathic pain is somewhat delayed compared to microglia (Graeber and Kreutzberg, 1988; Tetzlaff et al., 1988; Hald, 2009; Naseri et al., 2012a). Perhaps astrocyte reactivity would be visible after 4 weeks. To address it, we need to follow the glial changes in longer time.

Zhao et al. (2007) suggested that chemokine CCl21 production in STT neurons potently activates microglia within VPL after thoracic spinal cord injury and inhibition of either CCl21 or microglia can attenuate below level allodynia (Zhao et al., 2007).

The other interesting finding in our study was microglial activation in contralateral VPL. Wang and Thompson (2008) showed

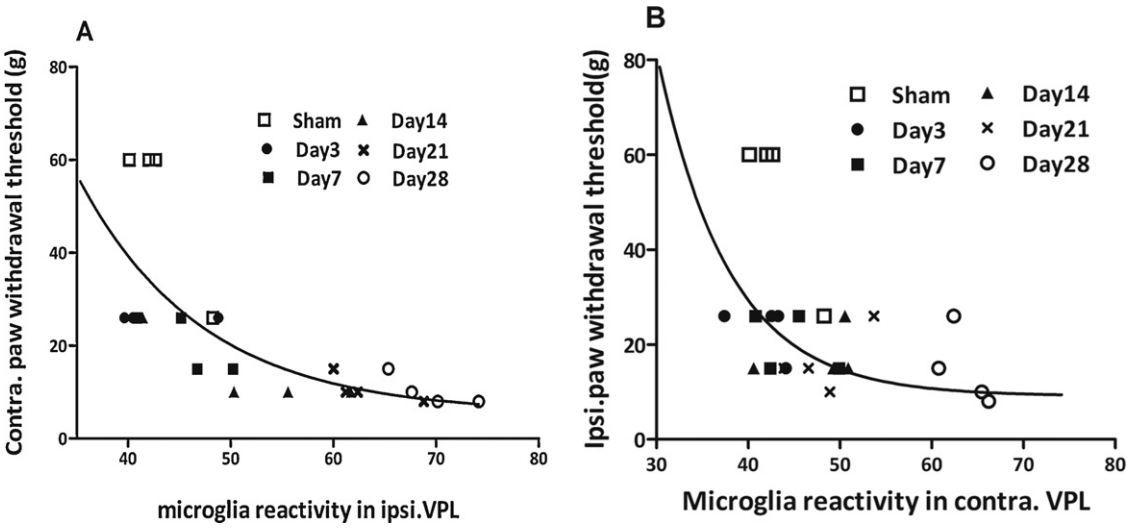

Fig. 4. Correlation analysis between pain behavior and microglial activation in VPL in injured animals.

that unilateral spinal injury produces bilaterally CPS and increased excitability of thalamic VPL neurons (Wang and Thompson, 2008). The main mechanisms which contribute to changes of contralateral side of injury have not been well documented. However, there are some speculations such as existence of bilateral projections from zona incerta (a nucleus that normally sends GABAergic projection to the thalamus nuclei and CPS suppresses its activity) to thalamic nuclei in both hemispheres, and bilateral interactions between zonaincerta and its counterpart in the other hemisphere (Masri et al., 2009; Quiton et al., 2010). This is similar to "mirror-image pain", in which glial activation, the release of inflammatory mediators and cytokines in dorsal root ganglion as well as in the spinal cord have algesic effects on contralateral neurons (Milligan et al., 2003; Yang et al., 2005). Focusing on astrocytes, it has been suggested that activated astrocytes spread excitation from one side to other side of spinal cord through gap junctions and finally upregulate cytokines (Hatashita et al., 2008). In our previous study, we demonstrated contralateral astrogliosis in spinal cord at the first week post-injury (Naseri et al., 2012a). We suggest that the extension of inflammation to the other side being mediated by astrocytes may lead to neural hyperecxitability, microglial activation in VPL, and the decrease of pain threshold in contralateral side.

Central pain has often been more difficult to treat than peripheral neuropathic pain, and based on randomized controlled trials, there is no good evidence for this claim. Available drugs targeted neuronal transmission and hyperexcitability have limited success with high frequency of side effects. It's time to focus on a new line of treatments which do not directly target neurotransmission but may target non neuronal cells including glia while offering lower adverse effects (Gwak et al., 2012).

Previously, we observed that estradiol attenuated the increase in microglia at the site of injury in the induction phase of pain as well as related astrogliosis in chronic phase of pain (Naseri et al., 2012b). Interestingly, in the present study post-lesion estradiol therapy decreased microglia activation in VPL nucleus and restored bilateral nociceptive mechanical threshold.

Although these findings do not clarify the exact mechanisms underlying estradiol-mediated suppression of microglial activation or pain reduction, but there are several reports on beneficial effects of estradiol on neuronal damage that provide some explanation.

17β-estradiol affects all cell types in the spinal cord via several signaling mechanisms to reduce secondary damage after SCI. This agent acts as an anti-oxidant, anti-inflammatory, and anti-apoptotic steroid hormone leading to the reduction of calpain expression and activity and to the increase of Bcl2 gene expression, resulting in reduced axon degeneration and neuronal apoptosis in SCI (Sribnick et al., 2006; Yune et al., 2008). Supraphysiologic dose of estrogen (4 mg/kg) attenuated infiltration of macrophages and microglia, myelin loss, and translocation of the inflammatory transcription factor (NF-κB) from the cytosol to the nucleus and restored locomotor function in rats following chronic SCI in vivo (Sribnick et al., 2005). Treatment of male SCI mice with 17β-estradiol reduced neutrophil infiltration, inflammatory cytokines or chemokines including TNF-α, IL-1β, IL-6, expression of iNOS, and cyclooxygenase-2 (Cuzzocrea et al., 2008). In our lab, we have demonstrated that single dose of estradiol is able to prevent neuronal STT demyelination, restore MBP expression, and decrease apoptotic cell death at the site of injury during first week after SCI (Afhami et al., 2012). These findings suggest that estradiol promote axonal regeneration at the site of injury and improves functional recovery after SCI.

Regarding pain control, estrogens trigger neurochemical changes that modulate pain responses. Estrogen modulates GABAergic neurons by activating estrogen receptor beta bearing inhibitory neurons (Blurton-Jones and Tuszynski, 2006), and decreases pain sensitivity via opioid receptors in the spinal cord and periaqueductal gray (Ceccarelli et al., 2004). Furthermore, estradiol participates in control of peripheral pain signal transduction by modulating P2X3 receptor (Ma et al., 2011).

Taken together, estradiol is able to attenuate abnormal neural hyperexcitabiliy in sensory spinothalamic tract resulted from mechanical damage and inhibit glial activity in VPL region. Subsequently this agent might attenuate cytokine and chemokine secretion underlying neural damage implicating remote microglial and neuronal activation in VPL. However other possible effects of estradiol such as modulation of antinociceptive corticospinal descending pathways and/or the likely its direct effect on neuronal and glial function in the thalamus complex which was not investigated in this study should not be excluded. Indeed, to determine the precise mechanism of estradiol on microgila activity, the subtypes of estrogen receptors, (ERalpha and ERbeta) on microglia should be characterized. Further studies on the inhibitory effects of estradiol on cytokine production in cultured rat microglia followed by determining the effects of estrogen antagonists to reverse the estradiol-mediated inhibitory effects on microglial activation will more clarify more the role of estrogen in this regard. Moreover, the administration of the drug at later time points or local administration in VPL in order to examine the effects of estradiol on both

glia activity and mechanical hypersensitivity will be a worthwhile experiment to follow.

5. Conclusions

Central pain syndrome resulted from specific damage to afferent pathway, STT, was followed by glial alterations in bilateral VPL nuclei. Estradiol as a multiplicative therapeutic agent could attenuate pain sensation and related glial changes properly. However, more investigations are needed to reveal the exact role of thalamic glial cells in this condition and to determine the exact mechanisms of estradiol on nociception and glial activity following STT damage.

Conflict of interest

The authors declare that there are no conflicts of interest in the publication of this manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neures.2013.01.010.

References

Afhami, M., Abbaszadeh, F., Saghaei, E., Naseri, K., Moradi, F., Javan, M., Jorjani, M., 2012. Effect of estradiol on locomotor function and myelin following lesion in the ventrolateral funiculus of spinal cord in male rats. FENS Abstract. 6, p. 012.02, 201. 8th FENS forum of neuroscience, Madrid, Spain. Available at http://fens.ekonnect.co/FENS

Anderson, W.S., O'hara, S., Lawson, H.C., Treede, R.D., Lenz, F.A., 2006. Plasticity of pain-related neuronal activity in the human thalamus. Prog. Brain Res. 157, 353–364.

Austin, P.J., Moalem-Taylor, G., 2010. The neuro-immune balance in neuropathic pain: Involvement of inflammatory immune cells, immune-like glial cells and cytokines. J. Neuroimmunol. 229, 26–50.

Blurton-Jones, M., Tuszynski, M.H., 2006. Estradiol-induced modulation of estrogen receptor-beta and GABA within the adult neocortex: a potential transsynaptic mechanism for estrogen modulation of BDNF. J. Comp. Neurol. 499, 603–612.

Bryce, T.N., Biering-Sorensen, F., Finnerup, N.B., Cardenas, D.D., Defrin, R., Ivan, E., Lundeberg, T., Norrbrink, C., Richards, J.S., Siddall, P., Stripling, T., Treede, R.D., Waxman, S.G., Widerstrom-Noga, E., Yezierskl, R.P., Dijkers, M., 2012. International spinal cord injury pain (ISCIP) classification: Part 2. Initial validation using vignettes. Spinal Cord 50, 404–412.

Ceccarelli, I., Fiorenzani, P., Grasso, G., Lariviere, W.R., Massafra, C., Massai, L., Muscettola, M., Aloisi, A.M., 2004. Estrogen and mu-opioid receptor antagonists counteract the 17 beta-estradiol-induced licking increase and interferon-gamma reduction occurring during the formalin test in male rats. Pain 111, 181–190.

Chadi, G., Andrade, M.S., Leme, R.J., Gomide, V.C., 2001. Experimental Models of partial lesion of rat spinal cord to investigate neurodegeneration, glial activation, and behavior impairments. Int. J. Neurosci. 111, 137–165.

Coull, J.A.M., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., Gravel, C., Salter, M.W., De koninck, Y., 2005. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 438, 1017–1021.

Coyle, D.E., 1998. Partial peripheral nerve injury leads to activation of astroglia and microglia which parallels the development of allodynic behavior. Glia 23, 75–83.

Cuzzocrea, S., Genovese, T., Mazzon, E., Esposito, E., Di paola, R., Muia, C., Crisafulli, C., Peli, A., Bramanti, P., Chaudry, I.H., 2008. Effect of 17 beta-estradiol on signal transduction pathways and secondary damage in experimental spinal cord trauma. Shock 29, 362–371.

Davis, K.D., Kwan, C.L., Crawley, A.P., Mikulis, D.J., 1998. Functional MRI study of thalamic and cortical activations evoked by cutaneous heat, cold, and tactile stimuli. J. Neurophysiol. 80, 1533–1546.

Detloff, M.R., Fisher, L.C., Mcgaughy, V., Longbrake, E.E., Popovich, P.G., Basso, D.M., 2008. Remote activation of microglia and pro-inflammatory cytokines predict the onset and severity of below-level neuropathic pain after spinal cord injury in rats. Exp. Neurol. 212, 337–347.

Finnerup, N.B., Jensen, T.S., 2004. Spinal cord injury pain – mechanisms and treatment. Eur. J. Neurol. 11, 73–82.

Graeber, M.B., Kreutzberg, G.W., 1988. Delayed astrocyte reaction following facial nerve axotomy. J. Neurocytol. 17, 209–220.

Gustin, S.M., Peck, C.C., Wilcox, S.L., Nash, P.G., Murray, G.M., Henderson, L.A., 2011. Different pain, different brain: thalamic anatomy in neuropathic and non-neuropathic chronic pain syndromes. J. Neurosci. 31, 5956–5964.

Gwak, Y.S., Kang, J., Unabia, G.C., Hulsebosch, C.E., 2012. Spatial and temporal activation of spinal glial cells: role of gliopathy in central neuropathic pain following spinal cord injury in rats. Exp. Neurol. 234, 362–372.

Gyenes, A., Hoyk, Z., Csakvari, E., Siklos, L., Parducz, A., 2010. 17beta-estradiol attenuates injury-induced microglia activation in the oculomotor nucleus. Neuroscience 171, 677–682.

Hains, B.C., Johnson, K.M., Eaton, M.J., Willis, W.D., Hulsebosch, C.E., 2003. Serotonergic neural precursor cell grafts attenuate bilateral hyperexcitability of dorsal horn neurons after spinal hemisection in rat. Neuroscience 116, 1097–1110.

Hains, B.C., Saab, C.Y., Waxman, S.G., 2006. Alterations in burst firing of thalamic VPL neurons and reversal by Na(v)1.3 antisense after spinal cord injury. J Neurophysiol. 95, 3343–3352.

Hains, B.C., Waxman, S.G., 2006. Activated microglia contribute to the maintenance of chronic pain after spinal cord injury. J. Neurosci. 26, 4308–4317.

Hains, B.C., Yucra, J.A., Hulsebosch, C.E., 2001. Reduction of pathological and behavioral deficits following spinal cord contusion injury with the selective cyclooxygenase-2 inhibitor NS-398. J. Neurotrauma. 18, 409–423.

Hald, A., 2009. Spinal astrogliosis in pain models: cause and effects. Cell. Mol. Neurobiol. 29, 609–619.

Halushka, M.K., Cornish, T.C., Lu, J., Selvin, S., Selvin, E., 2010. Creation, validation, and quantitative analysis of protein expression in vascular tissue microarrays. Cardiovasc. Pathol. 19, 136–146.

Hatashita, S., Sekiguchi, M., Kobayashi, H., Konno, S.-I., Kikuchi, S.-I., 2008. Contralateral neuropathic pain and neuropathology in dorsal root ganglion and spinal cord following hemilateral nerve injury in rats. Spine 33, 1344–1351.

Hoot, M.R., Sim-Selley, L.J., Selley, D.E., Scoggins, K.L., Dewey, W.L., 2011. Chronic neuropathic pain in mice reduces mu-opioid receptor-mediated G-protein activity in the thalamus. Brain Res. 1406, 1–7.

Huber, J.D., Campos, C.R., Mark, K.S., Davis, T.P., 2006. Alterations in blood-brain barrier ICAM-1 expression and brain microglial activation after lambda-carrageenan-induced inflammatory pain. Am. J. Physiol. Heart Circ. Physiol. 290, 732–740.

Hulsebosch, C.E., 2005. From discovery to clinical trials: treatment strategies for central neuropathic pain after spinal cord injury. Curr. Pharm. Des. 11, 1411–1420.

Hulsebosch, C.E., 2008. Gliopathy ensures persistent inflammation and chronic pain after spinal cord injury. Exp. Neurol. 214, 6–9.

Kupers, R.C., Gybels, J.M., Gjedde, A., 2000. Positron emission tomography study of a chronic pain patient successfully treated with somatosensory thalamic stimulation. Pain 87, 295–302.

Leblanc, B.W., Zerah, M.L., Kadasi, L.M., Chai, N., Saab, C.Y., 2011. Minocycline injection in the ventral posterolateral thalamus reverses microglial reactivity and thermal hyperalgesia secondary to sciatic neuropathy. Neurosci. Lett. 498, 138–142.

Lee, J.Y., Choi, S.Y., Oh, T.H., Yune, T.Y., 2012. 17beta-estradiol inhibits apoptotic cell death of oligodendrocytes by inhibiting RhoA-JNK3 activation after spinal cord injury. Endocrinology 153, 3815–3827.

Ma, B., Yu, L.H., Fan, J., Cong, B., He, P., Ni, X., Burnstock, G., 2011. Estrogen modulation of peripheral pain signal transduction: involvement of P2X(3) receptors. Purinergic Signal. 7, 73–83.

Masri, R., Quiton, R.L., Lucas, J.M., Murray, P.D., M. Thompson, S., Keller, A., 2009. Zona incerta: a role in central pain. J. Neurophysiol. 102, 181–191.

Milligan, E.D., Twining, C., Chacur, M., Biedenkapp, J., O'connor, K., Poole, S., Tracey, K., Martin, D., Maier, S.F., Watkins, L.R., 2003. Spinal glia and proinflammatory cytokines mediate mirror-image neuropathic pain in rats. J. Neurosci. 23, 1026–1040.

Milligan, E.D., Watkins, L.R., 2009. Pathological and protective roles of glia in chronic pain. Nat. Rev. Neurosci. 10, 23–36.

Naseri, K., Saghaei, E., Abbaszadeh, F., Afhami, M., Haeri, A., Rahimi, F., Jorjani, M., 2012a. Role of microglia and astrocyte in central pain syndrome following electrolytic lesion at the spinothalamic tract in rats. J. Mol. Neurosci., June 22.

Naseri, K., Saghaei, E., Abbaszadeh, F., Afhami, M., Haeri, A., Jorjani, M., 2012b. The effect of estradiol on astrogliosis related to central pain syndrome after spinal cord injury in male rat. 14th World Congress on Pain, Milan, Italy, Aug 27–31. Avaliable at http://www.iasp-pain.org

Quiton, R.L., Masri, R., Thompson, S.M., Keller, A., 2010. Abnormal activity of primary somatosensory cortex in central pain syndrome. J. Neurophysiol. 104, 1717–1725.

Ren, K., 1999. An improved method for assessing mechanical allodynia in the rat. Physiol. Behav. 67, 711–716.

Samantaray, S., Smith, J.A., Das, A., Matzelle, D.D., Varma, A.K., Ray, S.K., Banik, N.L., 2011. Low dose estrogen prevents neuronal degeneration and microglial reactivity in an acute model of spinal cord injury: effect of dosing, route of administration, and therapy delay. Neurochem. Res. 36, 1809–1816.

Sandhir, R., Gregory, E., He, Y.Y., Berman, N.E., 2011. Upregulation of inflammatory mediators in a model of chronic pain after spinal cord injury. Neurochem. Res. 36, 856–862.
Sribnick, E.A., Matzelle, D.D., Ray, S.K., Banik, N.L., 2006. Estrogen treatment of spinal cord injury attenuates calpain activation and apoptosis. J. Neurosci. Res. 84, 1064–1075.
Sribnick, E.A., Wingrave, J.M., Matzelle, D.D., Wilford, G.G., Ray, S.K., Banik, N.L., 2005. Estrogen attenuated markers of inflammation and decreased lesion volume in acute spinal cord injury in rats. J. Neurosci. Res. 82, 283–293.
Tetzlaff, W., Graeber, M.B., Bisby, M.A., Kreutzberg, G.W., 1988. Increased glial fibrillary acidic protein synthesis in astrocytes during retrograde reaction of the rat facial nucleus. Glia 1, 90–95.
Thompson, S.J., Bushnell, M.C., 2012. Rodent functional and anatomical imaging of pain. Neurosci. Lett. 520, 131–139.
Toth, C.C., Jedrzejewski, N.M., Ellis, C.L., Frey, W.H., 2010. Cannabinoid-mediated modulation of neuropathic pain and microglial accumulation in a model of murine type I diabetic peripheral neuropathic pain. Mol. Pain 6, 16.
Ueda, M., Iida, Y., Yoneyama, T., Kawal, T., Ogawa, M., Magata, Y., Saji, H., 2011. In vivo relationship between thalamic nicotinic acetylcholine receptor occupancy rates and antiallodynic effects in a rat model of neuropathic pain: persistent agonist binding inhibits the expression of antiallodynic effects. Synapse 65, 77–83.
Wang, G., Thompson, S.M., 2008. Maladaptive homeostatic plasticity in a rodent model of central pain syndrome: thalamic hyperexcitability after spinothalamic tract lesions. J. Neurosci. 28, 11959–11969.
Wasserman, J.K., Koeberle, P.D., 2009. Development and characterization of a hemorrhagic rat model of central post-stroke pain. Neuroscience 161, 173–183.
Weng, H.R., Lenz, F.A., Vierck, C., Dougherty, P.M., 2003. Physiological changes in primate somatosensory thalamus induced by deafferentation are dependent on the spinal funiculi that are sectioned and time following injury. Neuroscience 116, 1149–1160.
Yang, C.S., Jung, C.Y., Ju, J.S., Lee, M.K., Ahn, D.K., 2005. Intracisternal administration of mitogen-activated protein kinase inhibitors reduced IL-1β-induced mirror-image mechanical allodynia in the orofacial area of rats. Neurosci. Lett. 387, 32–37.
Yezierski, R.P., 2000. Pain following spinal cord injury: pathophysiology and central mechanisms. Prog. Brain Res. 129, 429–449.
Yune, T.Y., Park, H.G., Lee, J.Y., Oh, T.H., 2008. Estrogen-induced Bcl-2 expression after spinal cord injury is mediated through phosphoinositide-3-kinase/Akt-dependent CREB activation. J. Neurotrauma. 25, 1121–1131.
Zhao, P., Waxman, S.G., Hains, B.C., 2006. Sodium channel expression in the ventral posterolateral nucleus of the thalamus after peripheral nerve injury. Mol. Pain 2, 27.
Zhao, P., Waxman, S.G., Hains, B.C., 2007. Modulation of thalamic nociceptive processing after spinal cord injury through remote activation of thalamic microglia by cysteine cysteine chemokine ligand 21. J. Neurosci. 27, 8893–8902.