



Transcriptional expression of inflammatory mediators in various somatosensory relay centers in the brain of rat models of peripheral mononeuropathy and local inflammation



Farah Chamaa^{a,1}, Maya Chebaro^{a,1}, Bared Safieh-Garabedian^b, Ryan Saadeh^a, Suhayl J. Jabbur^a, Nayef E. Saadé^{a,*}

^a Department of Anatomy, Cell Biology and Physiology, Faculty of Medicine, American University of Beirut, Lebanon

^b College of Medicine, Qatar University, Doha, Qatar

ARTICLE INFO

Article history:

Received 20 January 2016

Received in revised form 19 April 2016

Accepted 6 May 2016

Keywords:

Cytokines

NGF

Plasticity

Neuropathic pain

Inflammation

ABSTRACT

Contradictory results have been reported regarding the role of inflammatory mediators in the central nervous system in mediating neuropathic pain and inflammatory hyperalgesia following peripheral nerve injury or localized inflammation. The present study aims to correlate between the mRNA expression and protein secretion of proinflammatory cytokines and nerve growth factor (NGF), in the dorsal root ganglia (DRGs), spinal cord, brainstem and thalamus, and pain-related behavior in animal models of peripheral mononeuropathy and localized inflammation.

Different groups of rats ($n = 8$, each) were subjected to either lesion of the nerves of their hindpaws to induce mononeuropathy or intraplantar injection of endotoxin (ET) and were sacrificed at various time intervals. TNF- α , IL-1 β and NGF mRNA expression and protein levels in the various centers involved in processing nociceptive information were determined, by RT-PCR and ELISA. Control groups were either subjected to sham surgery or to saline injection.

Mononeuropathy and ET injection produced significant and sustained increases in the mRNA expression and protein levels of TNF- α , IL-1 β and NGF in the ipsilateral and contralateral DRGs, spinal cord, and brainstem. No significant and consistent changes in the mRNA expression of cytokines were noticed in the thalamus, while a downregulation of the NGF-mRNA level was observed.

The temporal and spatial patterns of the observed changes in mRNA expression of cytokines and NGF are not closely in phase with the observed allodynia and hyperalgesia in the different models, suggesting that the role of these mediators may not be reduced exclusively to the production and maintenance of pain.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The idea of the involvement of proinflammatory mediators in the generation of pain derives from the traditional definition of the cardinal signs of inflammation listing pain as a main component or determinant of the vital reaction to invasion and/or injury. Studies during the last two decades have contributed to the elucidation of the causal role of these mediators in the initiation and development of acute and chronic pains. As illustration, several studies have reported a close link between

the increase of inflammatory mediators in the peripheral tissues and the induction and permanence of pain. These observations were made following either intraplantar injections of TNF- α , IL-1 β or other irritants (Kanaan et al., 1996; Saadé et al., 2002; Safieh-Garabedian et al., 1995) or systemic administration of IL-1 β (Ferreira et al., 1988), NGF (Lewin et al., 1993) or endotoxin (Maier et al., 1993). In animal models of mononeuropathy, on the other hand, injury to the sciatic nerve induced bilateral increase of TNF- α and IL-1 β levels in the spinal cord (DeLeo et al., 1997) and in various brain centers (Al-Amin et al., 2011; Covey et al., 2002). Furthermore, intracerebroventricular microinfusion of TNF- α (Ignatowski et al., 1999), IL-1 β (Hori et al., 1998), or NGF (Hao et al., 2000) has been reported to induce transient neuropathic manifestations (allodynia and hyperalgesia). It is worth noting, however, that contradictory results have been reported following the administration of various cytokines or NGF in the CNS (Cirillo et al., 2010; Hori et al., 1998; Souter and Garry, 2000; Yabuuchi et al., 1996).

Despite the numerous reports correlating pain with increased expression/secretion of cytokines and growth factors in the nervous

Abbreviations: NGF, nerve growth factor; DRG, dorsal root ganglia; ET, endotoxin; ELISA, enzyme-linked immunosorbent assay; DH, dorsal horn; SNI, spared nerve injury; CCI, chronic constriction injury; ET, endotoxin; LPS, lipopolysaccharide; VF, Von Frey; PW, paw withdrawal; PCR, Polymerase Chain Reaction.

* Corresponding author at: Department of Anatomy, Cell Biology and Physiology, Faculty of Medicine, American University of Beirut, Riad El Solh, Beirut 1107-2020, Lebanon.

E-mail address: nesaade@aub.edu.lb (N.E. Saadé).

¹ authors with equal contribution.

system, several studies have highlighted temporal and spatial discrepancies between the regulation pattern of these molecules and the spatio-temporal progression of behavioral manifestations of pain (Al-Amin et al., 2011; Dubovy et al., 2013; Jančálek et al., 2010; Saab et al., 2009). In addition, most of the studies describing the relationship between neuropathic pain and cytokines and NGF have addressed this relationship at the level of the peripheral nervous system and the spinal cord, whereas only few have investigated regulation of these proteins at supra-spinal levels.

This study aims to reinvestigate the mRNA expression of TNF- α , IL-1 β and NGF at different neural levels involved in the processing of the nociceptive information, including dorsal root ganglia (DRG), spinal cord dorsal horn (DH), brainstem and thalamus. This investigation was carried on two rat models of mononeuropathy, the spared nerve injury (SNI) and the chronic constriction injury (CCI) and on one model of local inflammation with endotoxin (ET). It will attempt to answer the following questions: first, is there a direct correlation between the expression of cytokines and NGF with the behavioral manifestations of pain?; second, does the increase of these proteins explain the appearance and maintenance of pain?; third, what would be an alternative explanation of the role of these mediators in the brain following peripheral insults and/or injuries?

2. Methods

All experiments were performed on adult Sprague-Dawley rats (200–250 g) with strict adherence to the ethical guidelines for experimental work on animals (Zimmermann, 1983) and following approval by the Institutional Animal Care and Use Committee. Surgical procedures were carried under deep anesthesia using a mixture of atropine (atropine sulfate, Laboratoire Aguettant, dilution 1:10 in saline 0.05 mg/kg) and chlorpromazine (Largactil®, 8 mg/kg) injected intraperitoneally (i.p.) as pre-anesthetics, followed by i.p. injection of ketamine (Ketalar®, 50 mg/kg) 10 min later.

2.1. Induction of mononeuropathy

Nerve injury is induced either following the chronic constriction injury (CCI) or the spared nerve injury (SNI) (Decosterd and Woolf, 2000) model. For the CCI (4 groups, $n = 6$ each), the left sciatic nerve is exposed proximal to its trifurcation and four ligatures, with a chronic gut, were loosely tied around the nerve, with a distance of about 1 mm between adjacent ligatures. For the SNI (4 groups, $n = 6$ each), the tibial and common peroneal branches of the left sciatic nerve were ligated, with silk sutures and cut distal to the ligatures, while leaving the sural nerve intact. For the sham/control groups (2 groups, $n = 6$, each), animals were subjected to the same surgical procedure to expose the sciatic nerve, but the nerve was left intact. Following surgery, muscles and skin are sutured and baneocin (Biochemie, Austria) was applied topically on the skin and each rat received an i.p. injection of penicillin (1 million IU).

2.2. Inflammation

Localized inflammation was induced by intra-plantar (ipl) injection (1.25 μg in 50 μl saline) of endotoxin (ET) or lipopolysaccharide (LPS) (Sigma, from *Salmonella typhosa*) in the left hind paw (3 groups, $n = 6$ each). Control rats (2 groups, $n = 8$ each) received similar left hind paw injections with an equal volume of sterile saline.

2.3. Behavioral tests for nociception

Two behavioral tests were used to assess tactile and heat reactivity, which are considered as indicators of neuropathic and inflammatory manifestations (Saab et al., 2009). Briefly for mechanical reactivity, two Von Frey (VF) filaments, thin and thick, corresponding to 2.041 g

(18.5 mN) and 11.749 g (106.7 mN), respectively, are applied vertically on the lateral aspect of the plantar hind paw for 10 consecutive times with a force just enough to bend the filament. This stimulation is repeated 10 consecutive times at a minimum interval of 10 s on each paw while the number of paw withdrawal reactions is recorded for both intact and injured paws. Thermal reactivity was measured using the paw withdrawal (PW) for the assessment of heat hyperalgesia; a heating beam is applied on the lateral aspect of the plantar surface of the hindpaws and the duration, in seconds, of paw withdrawal (PWD) is recorded. Two trials separated by 3 min, are performed alternately on each paw. A maximum duration of 10 s and a minimum of 0.5 s are assigned to sustained and mild or brisk paw withdrawals, respectively.

2.4. Surgery and perfusion

To enhance efficiency in collecting fresh tissues, without its blood content, the lumbosacral enlargement of the spinal cord and the brain of deeply anesthetized animals were exposed prior to perfusion for 5–10 min using 0.9% sterile cold saline solution. The exposed spinal cord is first extracted (L2- to S1 segments) to be followed by isolation and extraction of the corresponding DRG's with parts of their spinal nerves. The removed lumbar spinal cord was cut midsagittally and the dorsal quadrant, containing the main components of the dorsal horn (DH), of each half was isolated. Simultaneously, tissues are sampled from various brain areas and divided into ipsilateral (left) and contralateral (right) parts, relative to the side of neuropathy or inflammation. This involved the removal of the area of the rostral ventro-medial medulla by dissecting and removing the median and paramedian sectors of the ventral aspect of the brainstem, at the level of transition from rostral medulla to pontine swelling. It involved also the removal of the caudo-ventral thalamus from both sides of the brain.

2.5. Tissue sampling and processing

Under deep anesthesia, animals were euthanized at various time intervals to extract their lumbar dorsal root ganglia, lumbar spinal cord, brainstem and thalamus. Tissue sampling from neuropathic rats was made at 1, 3, 7 and 21 days, as one group per time interval; while the sham animals were sacrificed 3 and 7 days post-surgery. For local inflammation with ET, rats were euthanized at 4, 24, and 72 h, while the sham animals were sacrificed 4 and 24 h, post-surgery.

All the tissues removed included ipsilateral and contralateral parts relative to nerve injury or paw inflammation. Removed tissues were immediately weighed and stored at -80°C .

Collected tissues were homogenized for 45 s on ice at 21,000 rpm using a homogenization probe (Tissue Tearor, Polytron, Biospec Products, Inc.) along with freshly prepared ice-cold extraction buffer (Tris 100 mM, NaCl 150 mM, EGTA 1 mM, EDTA 1 mM, Triton X-100 1%, Sodium deoxycholate 0.5%; pH = 7.4; 1000 μl /tissue) and protease inhibitor cocktail tablets (Roche Diagnostics, Mannheim, Germany; 2 tablets/100 ml). The homogenates were then centrifuged at 4°C for 1 h at a speed of 11,000 rpm ($15,000 \times g$) and the supernatants were collected in sterilized test tubes and stored at -80°C .

2.6. Enzyme-linked immunosorbent assay (ELISA)

Supernatants of the homogenized tissues are used for the detection of TNF- α , IL-1 β , and NGF. The protein concentration of each sample is first determined using the BCA protein assay according to the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA). The concentrations of NGF were detected using the four-day ELISA kit (R&D Systems, Minneapolis, MN) and following the protocol provided by the manufacturer. The concentrations of each cytokine; however, were determined using a modified two-site sandwich ELISA as previously described (Saadé et al., 2002; Safieh-Garabedian et al., 1995). First, the ELISA plates were coated with immunoaffinity-purified

polyclonal sheep anti-rat TNF- α or IL-1 β antibodies diluted in coating buffer and incubated overnight at 4 °C (100 μ l/well; NISBC, England). The plates were washed the second day with wash/dilution buffer, blocked for non-specific binding with a blocking buffer at 37 °C (3% BSA, 0.1% Tween, 20 in PBS) and then incubated with the samples overnight at 4 °C. On the third day, the plates were washed and respective biotin-conjugated immunoaffinity-purified polyclonal antibody diluted (1:4000) in 1% Normal Sheep Serum (NSS; abcam, Cambridge, UK) were added and incubated at 4 °C overnight. On the last day, the plates were washed, then loaded with the enzyme streptavidin horse-radish peroxidase (Amersham; diluted 1:8000 in wash buffer containing 1% BSA; 100 μ l/well) to be followed with a tetramethylbenzidine-containing substrate solution. Finally, the reaction was stopped by an acidic stop solution (1 M H₂SO₄; 100 μ l/well) and the optical density was measured using a 450 nm filter. Data are then analyzed using a four-parameter logistics curve-fit by Ascent Software for iEMS Reader. Cytokine levels are expressed as picograms per milligram protein.

2.7. mRNA extraction and reverse transcription

Ribonucleic acid was extracted using the Trizol reagent (Amresco, Solon, Ohio) and quantified by spectrophotometry using the NanoDrop ND-1000 3.2.1 software, where the absorbance is recorded at 260 nm wavelength and the absorbance ratio was set at 260/280 nm. RNA is reversely transcribed to cDNA using the Revert Aid first strand cDNA synthesis kit (Fermentas Life Sciences, EU); both steps are done according to the manufacturer's protocols.

Polymerase Chain Reaction (PCR) was performed with specific primers to monitor the expression of IL-1 β , TNF- α and NGF (Thermo Scientific primers) using the Fermentas PCR kit (Fermentas Life Sciences, EU) according to the manufacturer's directions. PCR was completed with a thermal cycler (Px2 thermal cycler, Thermo Electron; protocol: 2 min at 94 °C, 35 cycles of 20 s at 94 °C and 30 s at 55 °C). The products are run on 1.5% agarose gels and bands are developed using a UV transilluminator and then photographed. Subsequent analysis involved densitometric measurements of the mean gray levels and intensities of each band using Image J (NIH imaging software). Values are normalized against the housekeeping gene β -actin. Primer sequences for PCR are listed in Table 1.

2.8. Data analysis

All measurements made on individual rats in an experimental group were averaged and expressed as mean \pm SEM for each time point. Values are recorded separately for left and right tissues (with reference to the injected or lesioned paw) within the same experimental group. The data are analyzed using one way analysis of variance (ANOVA) followed by Tukey's post hoc test. The analyzed variables were irritant injection or nerve lesion (CCI or SNI) versus sham or control at different time intervals (1, 3, 7, 21 days for mononeuropathy, or at 4, 24 h for ET injection). Student *t*-test was also used for comparisons between left and right sides at the same level and time interval. The GraphPad Instat

3 and Prism 6 softwares were used for statistics and graphics (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Nociceptive manifestations

Nerve lesions produced tactile hyper reactivity (allodynia) and increased nociceptive heat reactivity (hyperalgesia) restricted to the injured paw (Fig. 1, A and B). These neuropathic manifestations reached a maximum within one to two weeks and were maintained in the form of a plateau for several weeks after surgery in the CCI and SNI models (Fig. 1, A and B).

Intraplantar injection of ET produced tactile hyperreactivity in addition to heat hyperalgesia starting at 1–3 h, reaching a peak at 6–9 h and then followed by a recovery to baseline values at 24 h post-injection (Fig. 1, C and D).

3.2. Effect of peripheral nerve lesions and inflammation on cytokines and NGF mRNA expression

3.2.1. TNF- α

3.2.1.1. DRGs. In the ET model, no significant changes in TNF- α mRNA levels in the DRGs were observed except at 24 h after injection of ET, whereby an increase was evident ($P < 0.001$) in the DRGs supplying the contralateral (right) non-injected paw (Fig. 2). It is worth noting here, that the changes in TNF- α level were observed at the time of recovery of tactile and heat sensitivity as shown in Fig. 1, C and D.

In the CCI model, a significant increase in TNF- α mRNA levels was observed in the ipsilateral ($P < 0.001$) and contralateral ($P < 0.01$) DRGs 1 day after the surgery (Fig. 2). A bilateral return to baseline values was observed at day 3 which was followed by a significant increase observed in the DRGs supplying the neuropathic paw, at day 7 ($P < 0.01$) and day 21 ($P < 0.001$) after surgery (Fig. 2).

In the SNI model, TNF- α mRNA levels were found to be significantly higher ($P < 0.001$) in the DRGs bilaterally at day 1 but only in those supplying the neuropathic hind limb at day 21, as compared to sham/control (Fig. 2). Values comparable to control, however, were observed bilaterally at days 3 and 7 and in the DRGs contralateral to the neuropathic paw at day 21.

3.2.1.2. Dorsal horn (DH). In the ET model, a significant ($P < 0.001$) bilateral increase in TNF- α mRNA levels was detected in the lumbosacral DH, 24 h post-injection, which remained significantly higher than control values ($P < 0.001$) at 72 h (Fig. 2).

In the CCI model, no significant fluctuations were observed in TNF- α mRNA expression except at 7 days post-surgery, where a bilateral increase was detected but was more significant in the ipsilateral ($P < 0.001$) than in the contralateral ($P < 0.05$) DHs (Fig. 2). At 21 days, values in both sides returned to control level (Fig. 2).

In the SNI model, TNF- α mRNA levels increased significantly at 1 day after surgery ($P < 0.001$) in the DH ipsilateral to the injured nerve (Fig. 2). At day 3, TNF- α expression returned back to baseline levels, before eliciting bilateral increase at day 7 ($P < 0.001$) and unilateral changes at day 21 ($P < 0.01$, contralateral DH) as shown in Fig. 2.

3.2.1.3. Brainstem. Despite minor fluctuations, no significant changes were observed in brainstem TNF- α mRNA level, in rats receiving intraplantar injections of ET (Fig. 2).

In the CCI model, bilateral significant increases ($P < 0.001$) in TNF- α expression levels were detected, peaking at 7 days and remaining significantly elevated at 21 days post-surgery, ($P < 0.001$) (Fig. 2).

In the SNI model, a bilateral significant ($P < 0.001$) increase was observed in the brainstem TNF- α mRNA levels starting at 3 days after surgery and was still maintained at day 7, but was then followed by a

Table 1
Primers used for the analysis of cytokine expression on mRNA level.

Gene	Primers	Size (bp)
TNF- α	F 5'-GTA GCC CAC GTC GTA GCA AA-3'	347
	R 5'-CCC TTC TCC AGC TGG AAG AC-3'	
IL-1 β	F 5'-ATG AGA GCA TCC AGC TTC AAA TC-3'	578
	R 5'-CAC ACT AGC AGG TCG TCA TCA TC-3'	
NGF	F 5'-CCA AAG GAT GAC AGC GTC CC-3'	408
	R 5'-TCT GCT GCC GCC GTC TCT TG-3'	
β -actin	F 5'-CGC TGC GCT GGT CGT CGA CA-3'	600
	R 5'-GTC ACG CAC GAT TTC CCG CT-3'	

F: forward primer; R: reverse primer.

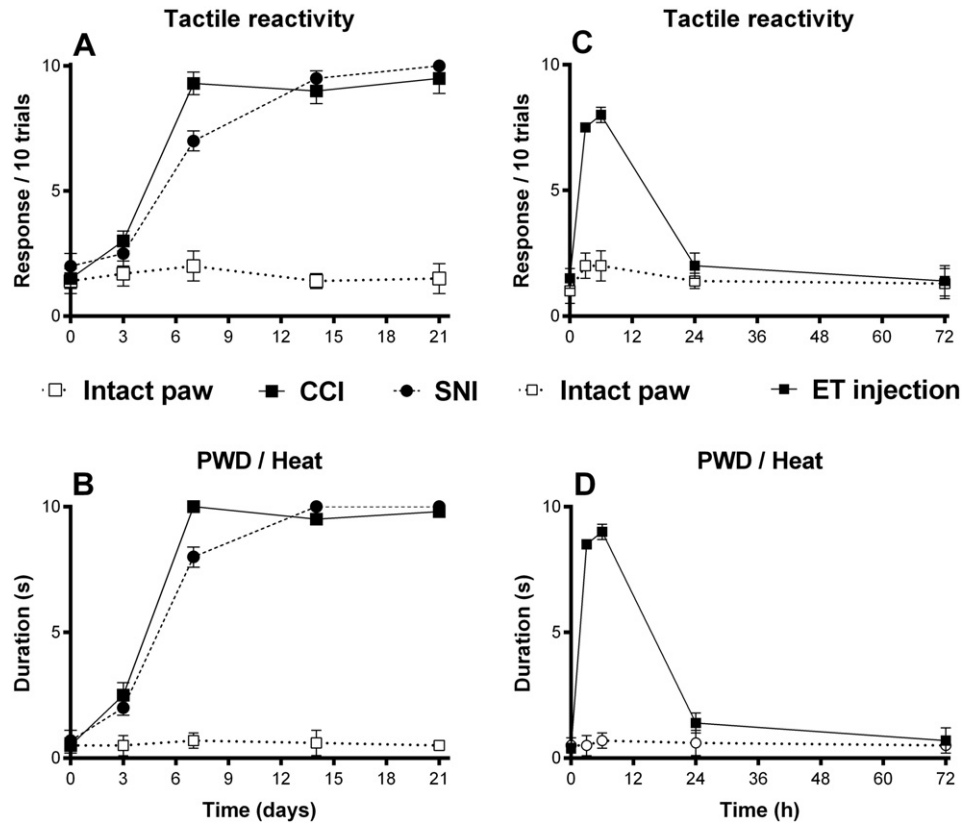


Fig. 1. Time courses of the tactile hyper reactivity (allodynia) and heat hyperalgesia induced by either nerve lesions (A and B) or intraplantar injection (C and D) of endotoxin (ET). Each point in each panel represents the average \pm SEM of measurements made on a separate group of 5 rats in each experimental model. Note the sustained allodynia and hyperalgesia induced in the two models of nerve injury as compared to the short-lived changes observed in the ET model. Pain manifestations reached a plateau within one to two weeks in the CCI and SNI models (A and B). Abbreviations: CCI, chronic constriction injury; ET, endotoxin; PWD, paw withdrawal duration; SNI, spared nerve injury.

sharp bilateral decrease ($P < 0.01$) to below-baseline levels at 21 days after surgery as depicted in Fig. 2.

3.2.1.4. Thalamus. No significant changes were elicited in mRNA levels, at different time intervals, in thalami of rats receiving intraplantar injections of ET (Fig. 2).

A transient but significant decrease of mRNA expression was observed at day 3 in the ipsilateral thalamus of rats subjected to CCI (Fig. 2). mRNA expression was maintained at control levels at days 7 and 21 post nerve injury.

In the SNI model, an early decrease ($P < 0.001$) of mRNA expression was observed in the contralateral thalamus at day 1 post injury, that was followed by a return to control level at days 3 and 7; however, a significant bilateral increase ($P < 0.001$) was noticed at day 21 post surgery (Fig. 2).

3.2.2. IL-1 β

3.2.2.1. DRGs. In the ET model, a significant ($P < 0.001$) bilateral increase in IL-1 β mRNA levels was observed at 4 h post-injection of ET, which was followed by a return to baseline levels after 24 h (Fig. 3).

In the CCI model, moderate increases in IL-1 β mRNA levels were observed, 1 day after surgery in the contralateral DRGs ($P < 0.05$) and 3 days post-surgery in the ipsilateral DRGs as shown in Fig. 3. More significant increases ($P < 0.01$), however, were detected 21 days after surgery in the DRGs supplying the neuropathic paws.

In the SNI model, a bilateral increase in IL-1 β mRNA levels ($P < 0.01$) was observed 1 day after the surgery. This increase became more pronounced ($P < 0.001$) in the DRGs supplying the neuropathic hind limbs at days 3 and 7 and declined at day 21 ($P < 0.05$). A less significant increase ($P < 0.05$) was observed in the contralateral paw at day 3 and

was followed by a return to control level at days 7 and 21 after surgery (Fig. 3).

3.2.2.2. Dorsal horn. In the ET model, a significant bilateral increase ($P < 0.001$) was observed in IL-1 β expression starting at 24 h and was still present 72 h post injection (Fig. 3).

In the CCI model, no significant changes in IL-1 β mRNA levels were observed during the first week post nerve lesion; a moderate increase, however, was apparent ($P < 0.05$) in the ipsilateral DH, at 7 and 21 days post-surgery (Fig. 3).

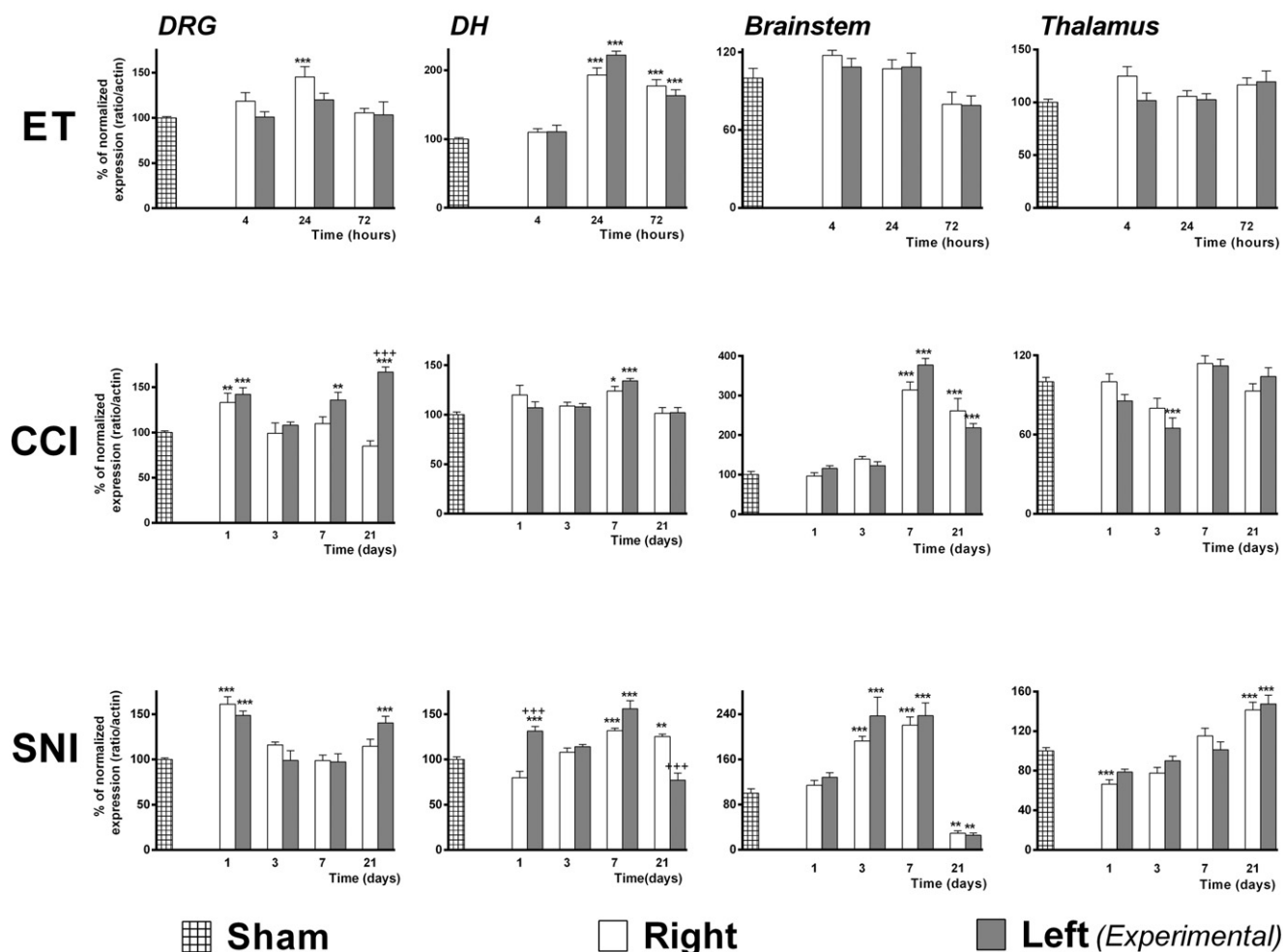
An opposite trend was observed in the SNI model, with a bilateral increase in IL-1 β mRNA levels at 1 day after nerve lesion, which was more pronounced in the ipsilateral side ($P < 0.001$) as compared to the ipsilateral DH ($P < 0.05$) (Fig. 3). On days 3, 7 and 21 post-lesion, IL-1 β expression levels fluctuated around the control values, with a recurrent moderate increase seen at day 21 in the ipsilateral DH (Fig. 3).

3.2.2.3. Brainstem. In the ET model, a significant bilateral decrease ($P < 0.001$) was observed in IL-1 β expression levels starting 24 h and was still maintained at 72 h post-injection (Fig. 3).

In the CCI model, a significant bilateral upregulation ($P < 0.001$) of IL-1 β mRNA expression was observed at days 1 and 3, which was followed by a return to control level during the remaining period of observation (Fig. 3).

In the SNI model, a significant bilateral increase ($P < 0.001$) in IL-1 β expression was detected 1 day after the surgery, as illustrated in Fig. 3; which was followed by some oscillations at days 3 ($P < 0.05$, ipsilateral; $P < 0.01$, contralateral) and 7 ($P < 0.001$, both sides) and then returned to baseline levels at day 21 (Fig. 3).

TNF- α



* P < 0.05; ** P < 0.01; *** P < 0.001; + P < 0.05; +++ P < 0.001

Fig. 2. Time courses of the mRNA expression of TNF- α in the DRGs and at different areas of the CNS, ipsilateral and contralateral to intraplantar injection of ET or to sciatic nerve lesion, as compared to control (sham). Each bar in each panel represents the average \pm SEM of measurements made on a separate group of rats (n = 6) at the indicated time interval and in each experimental model. The determination of significance of each value was made with reference to the sham group* (n = 6) or to the opposite side +, measured at the same time interval. Abbreviations: DH, spinal dorsal horn; DRG, dorsal root ganglia.

3.2.2.4. Thalamus. Moderate, but not significant, fluctuations of mRNA expression levels were observed in the thalami of rats injected with ET, at 4 and 24 h, post injection (Fig. 3). This was followed, however, by a significant decrease in mRNA expression in the contralateral ($P < 0.001$) and less significant in the ipsilateral ($P < 0.05$) thalami at 72 h post injection (Fig. 3).

In the CCI model, consistent and sustained decrease of mRNA expression was observed on both sides during the observation period (Fig. 3).

In the SNI model, significant ($P < 0.001$) increase in mRNA expression was observed at day 1 in the ipsilateral thalamus, which was followed by a return to baseline levels during the remaining period of observation (Fig. 3).

3.2.3. NGF

3.2.3.1. DRGs. In the ET model, a significant bilateral decrease ($P < 0.001$) in NGF mRNA levels was detected 4 h after the injection and this was reversed to a bilateral significant increases ($P < 0.001$) at 24 and 72 h post-injection (Fig. 4).

In the CCI model, a significant ($P < 0.01$ to $P < 0.001$) bilateral increase in NGF mRNA expression levels was observed at 3 days and continued as ipsilateral on days 7 and 21 after nerve lesion; while values returned to baseline levels in the contralateral DRGs (Fig. 4).

In the SNI model, moderate bilateral increase ($P < 0.05$) in NGF mRNA levels was observed in the DRGs on day 1 after the surgery (Fig. 4). On day 3, this increase became more pronounced in both the contralateral ($P < 0.01$) and ipsilateral ($P < 0.001$) DRGs. This increase was maintained throughout the observation period in the ganglia supplying the neuropathic limb, while it showed a return to basal level in the contralateral ganglia (Fig. 4).

3.2.3.2. Dorsal horn. In the ET model, a significant decrease ($P < 0.001$) in NGF mRNA expression was observed bilaterally at 4 h after the injection of ET and was maintained at 24 ($P < 0.01$) in the ipsilateral DH and in both sides at 72 h ($P < 0.001$) after the injection (Fig. 4).

In the CCI model, changes in NGF mRNA levels were detected starting day 7 after nerve lesion, where a significant bilateral decrease

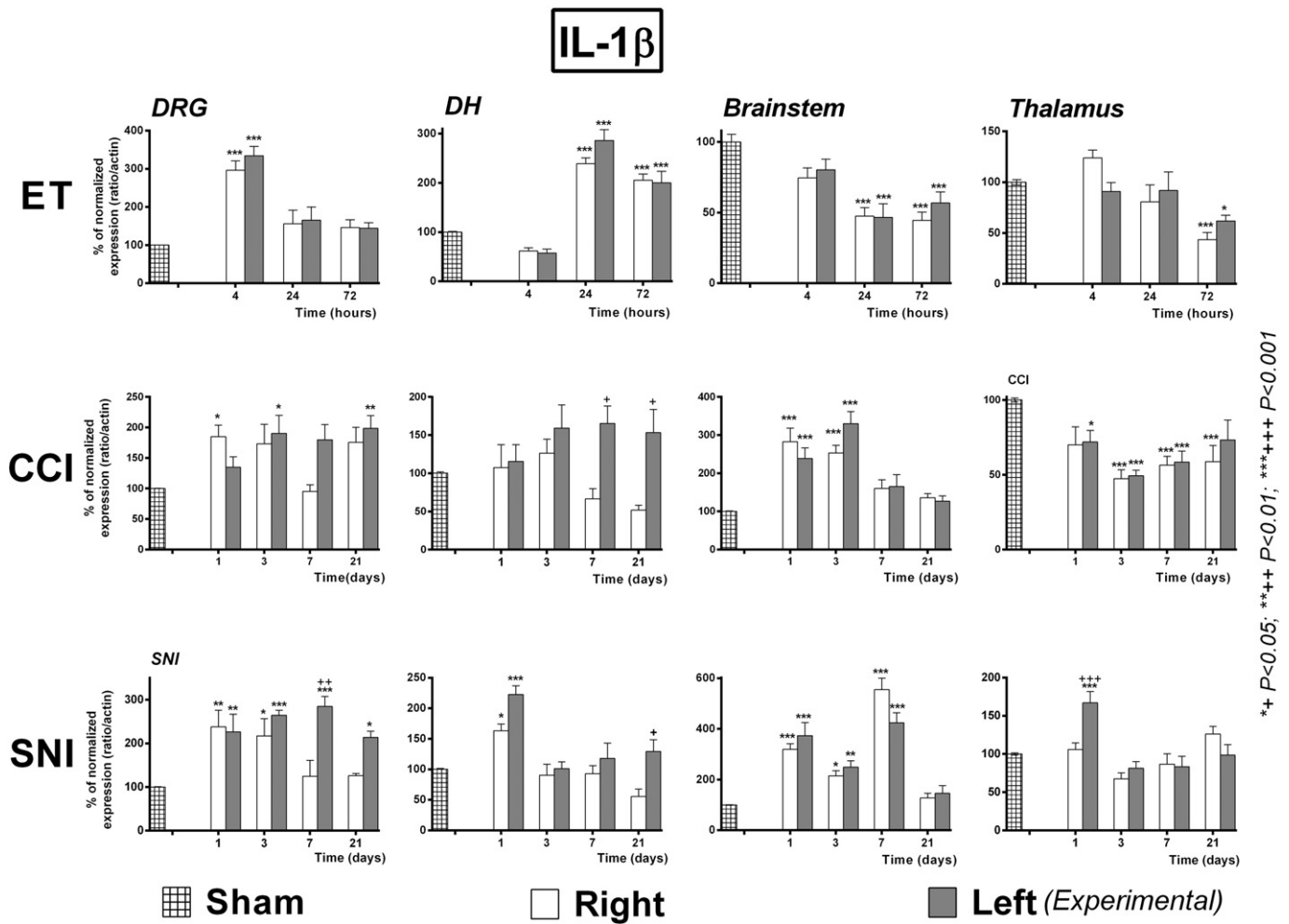


Fig. 3. Time courses of the mRNA expression of IL-1 β in the DRGs and at different areas of the CNS ipsilateral and contralateral to intraplantar injection of ET or to sciatic nerve lesion, as compared to control (sham). Each bar in each panel represents the average \pm SEM of measurements made on a separate group of rats ($n = 6$) at the indicated time interval and in each experimental model. The determination of significance of each value was made with reference to the sham group ($n = 6$) or to the opposite side +, measured at the same time interval. For abbreviation refer to Figs. 1 and 2.

($P < 0.01$) was observed and was maintained at 21 days post-lesion (Fig. 4).

In the SNI model, NGF expression levels did not vary from the control values except on day 7 after the lesion, where a transient significant decrease ($P < 0.01$) was observed in the contralateral spinal cord as illustrated in Fig. 4.

3.2.3.3. Brainstem. In the ET model, a significant bilateral decrease ($P < 0.001$) was observed starting 4 h after ET injection and was maintained at 24 and 72 h post-injection (Fig. 4).

In the CCI model, a significant bilateral increase ($P < 0.001$) in NGF mRNA levels was initiated on day 1 after the nerve lesion, that was maintained ($P < 0.001$) only in the contralateral brainstem at day 3, but then became bilaterally more pronounced ($P < 0.001$) on day 7, before returning to control values on day 21 (Fig. 4).

In the SNI model, bilateral increase in NGF expression was observed on day 1 after the lesion (Fig. 4). At day 3, NGF mRNA levels returned back to baseline values after which they displayed a bilateral upregulation ($P < 0.001$) on day 7, before decreasing again on day 21 to approach the baseline levels in the ipsilateral side, while remaining moderately higher in the contralateral brainstem ($P < 0.01$).

3.2.3.4. Thalamus. In the ET model, significant ($P < 0.001$) decrease of NGF mRNA expression started at 4 h in the ipsilateral thalamus and

became bilateral at 24 h. This was followed by a trend to recover the control level at 72 h after the injection (Fig. 4).

Significant and sustained decrease ($P < 0.001$) of the NGF mRNA expression was observed on days 1, 3 and 7, with a partial recovery at day 21 after nerve injury (Fig. 4).

In the SNI model, a bilateral and significant decrease of NGF mRNA expression started at day 1 ($P < 0.001$ contralateral and $P < 0.05$ ipsilateral) and was maintained ($P < 0.001$) throughout the remaining observation period (Fig. 4).

3.3. Effect of peripheral nerve lesions and inflammation on Cytokines and NGF protein secretion

3.3.1. IL-1 β

3.3.1.1. Dorsal horn. In the ET model, IL-1 β protein level showed significant bilateral increase at 4 h ($P < 0.001$) and 24 h ($P < 0.05$) and returned to basal level at 72 h after the injection (Fig. 5).

In the CCI model, bilateral increase was also observed at 1 ($P < 0.001$) and 3 days ($P < 0.001$ ipsilateral and $P < 0.05$ contralateral) after nerve injury; a return to basal level was observed at 7 days post-surgery (Fig. 5).

In the SNI model mild, but not significant, bilateral fluctuations of the IL-1 β protein level was noticed during the observation period (Fig. 5).

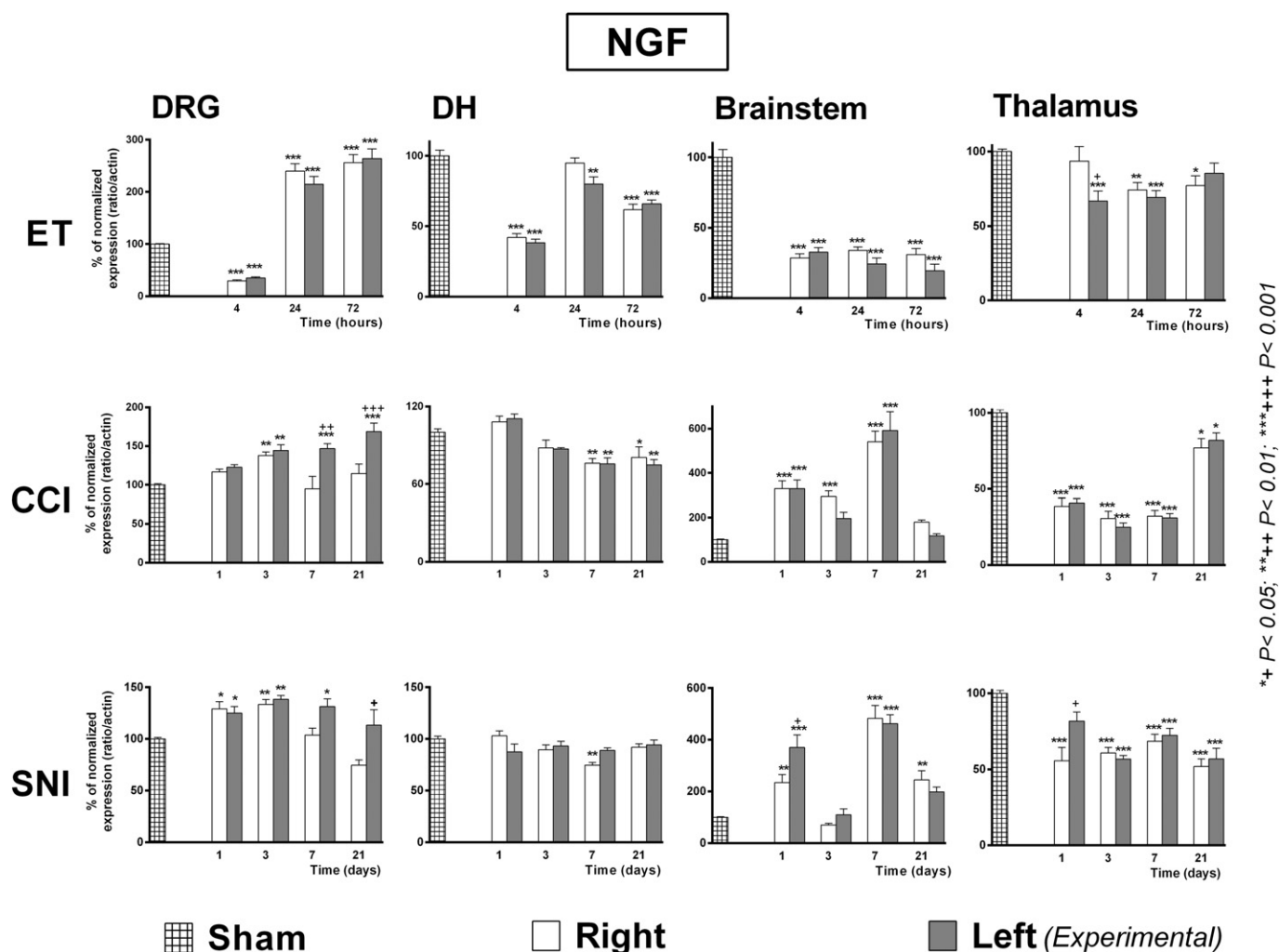


Fig. 4. Time courses of the mRNA expression of NGF in the DRGs and at different areas of the CNS ipsilateral and contralateral to intraplantar injection of ET or to sciatic nerve lesion as compared to control (sham). Each bar in each panel represents the average \pm SEM of measurements made on a separate group of rats ($n = 6$) at the indicated time interval and in each experimental model. The determination of significance of each value was made with reference to the sham group ($n = 6$) or to the opposite side +, measured at the same time interval. For abbreviation refer to Figs. 1 and 2.

3.3.1.2. Brainstem. In the ET model a delayed bilateral increase of IL-1 β protein level was observed at 24 h ($P < 0.001$) and 72 h ($P < 0.05$ ipsilateral and $P < 0.01$ contralateral) following intraplantar ET injection (Fig. 5).

In the CCI model, sustained bilateral increase of IL-1 β level was observed throughout the observation period of 7 days. Although highly significant, the observed increase was more pronounced in the ipsilateral brainstem, than a reversed pattern was noticed at 7 days post nerve injury (Fig. 5).

Sustained bilateral elevation of IL-1 β protein level was also observed in the SNI model throughout the observation period (Fig. 5).

3.3.2. NGF

3.3.2.1. Dorsal horn. In the ET model, a bilateral increase of the NGF protein level was observed at 4 h and 1 day ($P < 0.001$) after the injection; this was followed by a recovery of the basal level at 3 days after ET injection (Fig. 6).

In the CCI model, bilateral increase of NGF level was observed at one ($P < 0.01$) and 3 days ($P < 0.05$, ipsilateral) and this was followed by a return to basal level at 7 days post nerve injury (Fig. 6).

Similar temporal pattern of bilateral increase of NGF level was observed in the SNI model (Fig. 6).

3.3.2.2. Brainstem. In the ET model, a sustained and significant bilateral increase ($P < 0.001$) of NGF protein level was observed throughout the observation period of 3 days after intraplantar injection of ET (Fig. 6).

In the CCI model a significant increase of NGF level was observed, but alternating between the ipsilateral (days 1 and 3, $P < 0.001$) and the contralateral (days 3, $P < 0.01$ and 7, $P < 0.001$) sides of the brainstem (Fig. 6).

In the SNI model, significant ($P < 0.001$) increase in NGF level was noticed in days 1 and 3 post nerve injury (Fig. 6). A milder increase in NGF protein was also observed in the ipsilateral brainstem, and it reached significance on day 1 ($P < 0.05$) following nerve injury (Fig. 6).

4. Discussion

The present study aimed at providing evidence about de novo synthesis of proinflammatory mediators at different centers involved in the processing of nociceptive signaling and in the same rat subjected to localized peripheral inflammation or nerve injury. The study design was based on the use of three animal models of experimental pain characterized by different etiologies and different temporal patterns of their nociceptive manifestations. Furthermore, to insure that the observed changes in mediator's levels, in each area of the nervous system, is not the result of a humoral spreading, we choose to correlate

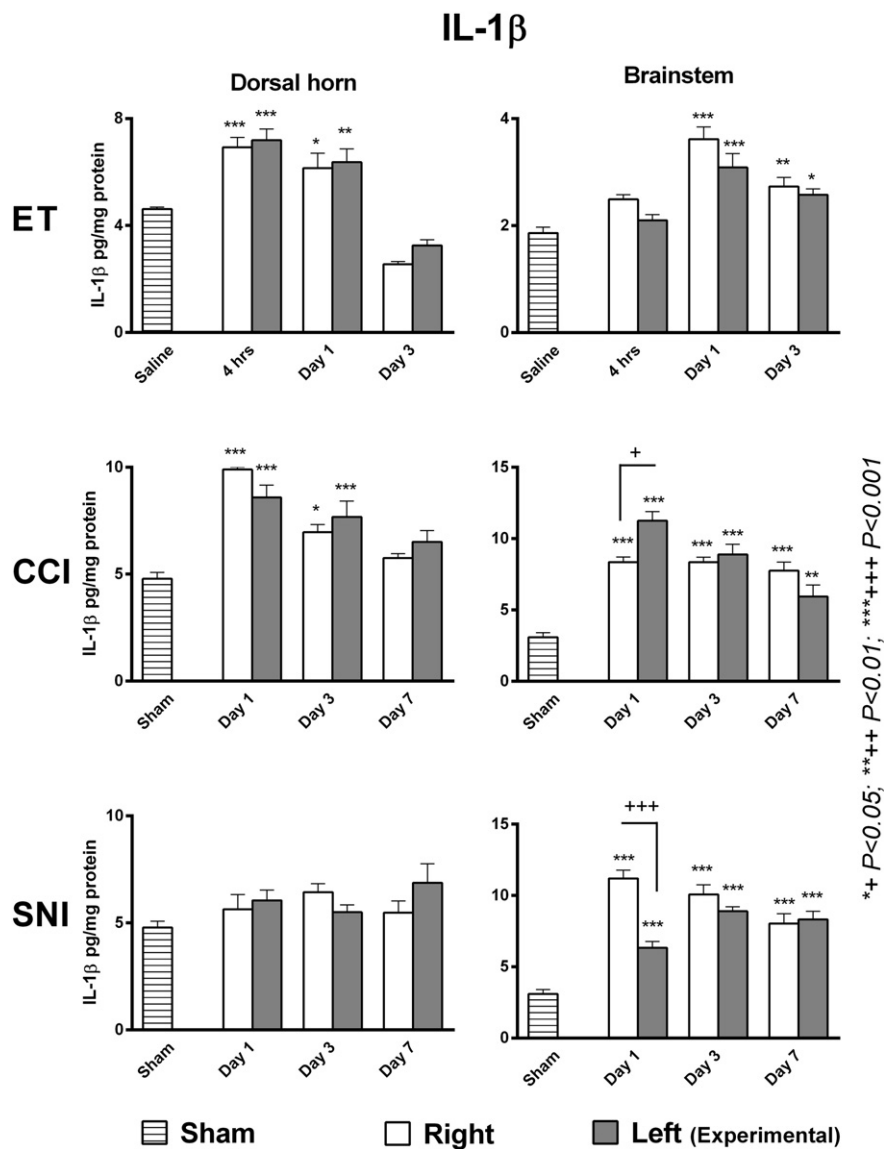


Fig. 5. Time courses of the IL-1 β protein secretion in the dorsal horn and brainstem ipsilateral and contralateral to intraplantar injection of ET or to sciatic nerve lesion, as compared to control (sham). Each bar in each panel represents the average \pm SEM of measurements made on a separate group of rats ($n = 6$) at the indicated time interval and in each experimental model. The determination of significance of each value was made with reference to the sham group * ($n = 8$) or to the opposite side +, measured at the same time interval. For abbreviation refer to Figs. 1 and 2.

between their mRNA expression and protein secretion in each area. Mapping of mRNA expression was made from tissues extracted from all areas and from the same animal; protein secretion, however, was restricted to the spinal cord and brain stem since their secretion was determined in the remaining areas in a previous study from our group (Saab et al., 2009) and due to limitations induced by the small amount of extracted tissue. The reported results allowed the observation, on the same figure, of the temporal variations of each mediator in the different brain areas in the three animal models of pain. The main features of these results can be summarized as follows:

- 1- A consistent bilateral alteration of mediator's expression in all centers and in the three different models, by contrast with the established unilateral manifestation of allodynia and hyperalgesia in all 3 models as illustrated in Fig. 1.
- 2- The intensity of changes and their timing could not directly be correlated to the laterality and the temporal evolution of nociceptive manifestations in each model.
- 3- The observed changes were not consistent throughout the nervous centers in the same model and also in the different models. In

other words a mediator such as NGF could elicit an increase in DRG or DH and a decrease in brainstem or thalamus.

- 4- The alteration of protein secretion in the dorsal horn and brainstem elicited comparable features to what we have reported in the DRG and limbic areas in previous studies (Al-Amin et al., 2011; Saab et al., 2009).
- 5- These changes were triggered, without any doubt, by the induced localized inflammation or nerve injury in the different models, since sham-operated rats did not elicit significant alteration of mRNA expression or protein levels comparable to the experimental (injured) animals.

Several studies, by us and others, have characterized the secretion of cytokines and NGF, in various animal models of inflammatory and neuropathic pain, in an attempt to correlate this secretion with nociceptive behavior and chronicity of pain. Despite some discrepancies, related to models or methods used, upregulation of cytokine and NGF levels has been reported in the dorsal root ganglia (Dubovy et al., 2013; Jančálek et al., 2010; Saab et al., 2009), spinal dorsal horn (DeLeo et al., 1997; Raghavendra et al., 2003), brainstem (Covey et al., 2000; Raghavendra

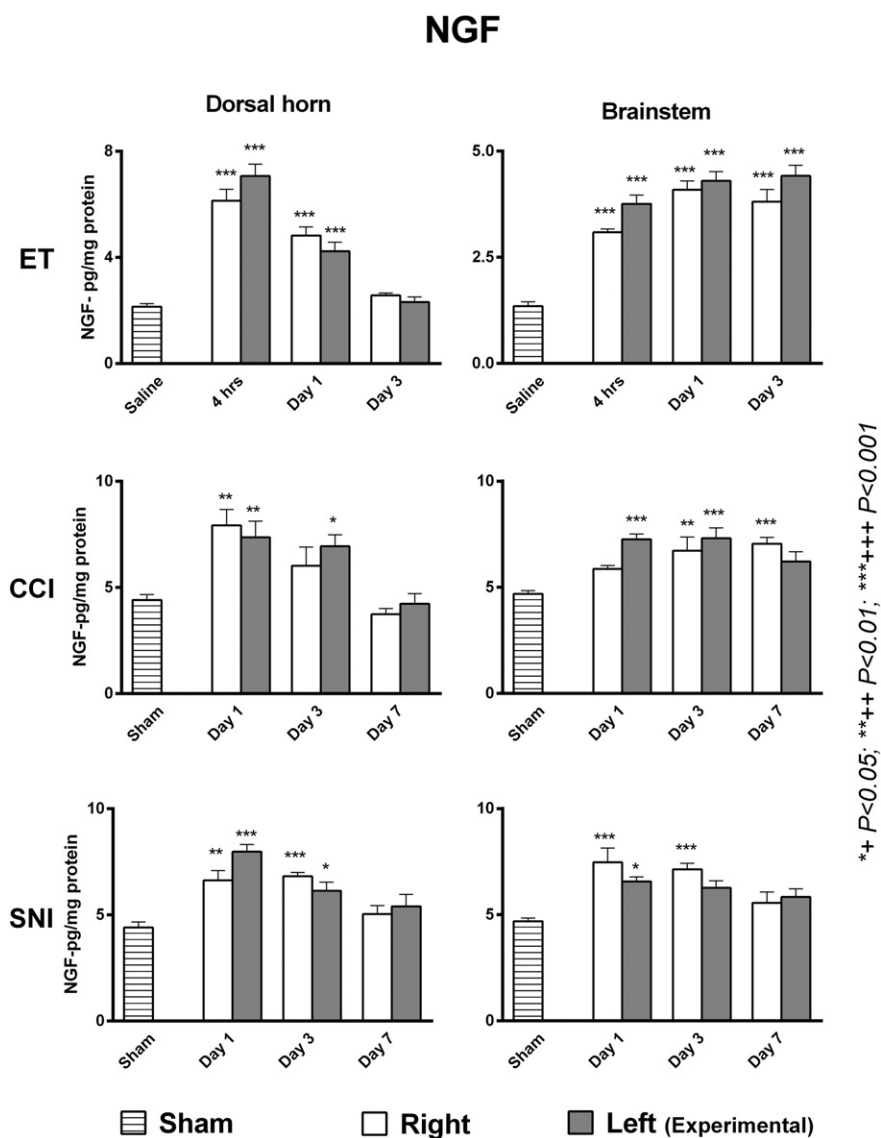


Fig. 6. Time courses of the NGF protein secretion in the dorsal horn and brainstem ipsilateral and contralateral to intraplantar injection of ET or to sciatic nerve lesion, as compared to control (sham). Each bar in each panel represents the average \pm SEM of measurements made on a separate group of rats ($n = 6$) at the indicated time interval and in each experimental model. The determination of significance of each value was made with reference to the sham group ($n = 8$) or to the opposite side +, measured at the same time interval. For abbreviation refer to Figs. 1 and 2.

et al., 2003), diencephalon and several limbic areas (Al-Amin et al., 2011; Apkarian et al., 2006; Del Rey et al., 2013). Most of these studies emphasized the potential link between these mediators and pain and their role in pain chronicity (for review, see Alexander et al., 2007; Calvo et al., 2012; Deleo and Yezierski, 2001; Grace et al., 2014; Milligan and Watkins, 2009; Moalem and Tracey, 2006).

Our results are in line with several studies by other authors showing bilateral alteration of cytokines and/or NGF levels in the DRG (Dubovy et al., 2013; Jančálek et al., 2010; Ruohonen et al., 2002), dorsal horn (DeLeo et al., 1997), brainstem (Apkarian et al., 2006; Covey et al., 2000) and diencephalon and cerebral cortex (Al-Amin et al., 2011; Apkarian et al., 2006; Del Rey et al., 2011). Furthermore, the observed changes in mRNA expression at different relay stations, involved in the processing of somatosensory information, provides clear evidence about the potential contribution of each center to the production of NGF and cytokines. This finding constitutes a positive answer to our question about the causal link between changes in mediator's levels and peripheral damage or injury leading to pain. This confirmation, however, is partial since the timing and laterality of these changes cannot be aligned with the observed behavioral manifestations of pain

in the used models. As illustration, changes can outlast the duration of hyperalgesia in the ET model and are bilateral, while data about neuronal changes in the three models follow in general the principle of unilaterality of projected information (i.e. ipsilateral spinal and contralateral supraspinal transmission).

The above mentioned observations can lead to the second question about the relationship between the increased expression and/or secretion of these mediators and the maintenance of pain. The increased expression of mRNA levels and protein secretion over days or weeks and in various centers can be considered as an indication of the relationship between these mediators and neuropathic manifestations. This temporal link has been emphasized by several authors (Apkarian et al., 2006; Del Rey et al., 2011; Leung and Cahill, 2010; Loggia et al., 2015; Raghavendra et al., 2003; Watkins and Maier, 2002). Evidence, however, about the missing link between these mediators and chronicity of pain resides in the bilaterality of their changes in contrast to the unilaterality of neuropathic manifestations. In addition their differential changes (increase or decrease) in time, among models and in different centers within the same model, are not in favour of a direct link between these mediators and persistence of pain manifestations. Such

mismatching between temporal patterns of cytokine expression and pain manifestations was reported by several authors. As illustration, a bilateral increase of cytokines was observed in lumbosacral area (DeLeo et al., 1997) and cervical DRGs in various animal models of pain (Dubovy et al., 2013; Jančálek et al., 2010; Saab et al., 2009). It is well established that cytokine upregulation is due to the activation of glia and to infiltrated resident immune cells in the nervous system (Aguzzi et al., 2013; Alexander et al., 2007; Calvo et al., 2012; Grace et al., 2014; Loggia et al., 2015; McMahon et al., 2005; Milligan and Watkins, 2009; Obata et al., 2010; Raghavendra et al., 2003; Watkins and Maier, 2002). Several studies, however, reported mismatching between glial activation and neuropathic manifestations. This consisted in, (1) differences related to timing (Colburn et al., 1997), (2) the animal model used (Hald et al., 2009), (3) the contribution of glial activation to mirror pain (Obata et al., 2010) and (4) microglial activation restricted only to the spinal cord with no signs of activation at supraspinal centers, including brainstem, limbic areas and somatosensory cortex (Zhang et al., 2008). On the other hand, studies stressing the contribution of neuroimmune mechanisms in chronic pain reported limitations related to targeting these mechanisms for the treatment of pain. As illustration, treatment with glial blocking agents did not always reverse neuropathic manifestations (Raghavendra et al., 2003), intrathecal injection of IL-1 β or NGF can result in antinociception (Cirillo et al., 2010; Souter and Garry, 2000) and finally the observed biphasic effects of intracerebroventricular injections of cytokines (Hori et al., 1998; Yabuuchi et al., 1996). Moreover data from clinical trials reported contradictory information about the glial involvement in chronic pain by showing negative correlation between the levels of protein marker of glial activation in the thalamus with clinical pain and circulating levels of the proinflammatory cytokine IL-6 (Loggia et al., 2015); in addition, disappointing results were reported from clinical trials targeting different cytokines for the treatment of chronic pain [reviewed by Calvo et al., 2012; Leung and Cahill, 2010].

Finally, the above-discussed issues lead to the question of the functional role of the observed changes in mediator's levels at different brain centers in response to peripheral inflammation or nerve injuries?. Without denying the causal links between nerve injury - glial activation - cytokine secretion and pain manifestations, an emerging more elaborate concept of the role of cytokines and neurotrophins is brought by several recent studies. This concept expands the role of these mediators beyond pain manifestations to involve more global functions related to brain plasticity. These include modulation of neuronal circuits and transmitter release that might lead ultimately to behavioral alterations (Felger and Lotrich, 2013; Lewitus et al., 2014; Singhal et al., 2014; Vezzani et al., 2008; Vitkovic et al., 2000), their contribution to sickness behavior (Dantzer et al., 2008) and persistent anxiety-like behavior (Wohleb et al., 2015), their role in emotional learning and memory formation (Del Rey et al., 2011, 2013) and finally their involvement in plastic changes and repair mechanisms in the brain (Covey et al., 2000; Michell-Robinson et al., 2015; Prieto et al., 2015; Vitkovic et al., 2000).

In conclusion, the present study demonstrates sustained endogenous changes in mRNA expression of cytokines and NGF in different peripheral and central components of the nervous system involved in pain signaling and modulation. These changes are triggered, in general, by peripheral inflammation and/or nerve injury. Their different spatio-temporal patterns suggest that the function of these mediators is not restricted to maintaining pain-related behavior, but rather may reflect complex and discrete homeostatic and plastic changes induced by peripheral challenges and injuries.

Conflict of interest

The authors declare that they have no conflicts of interest with the content of this article.

Author contribution

Chamaa, Chebaro and Saadeh R performed the experimental work and contributed to the writing of the final manuscript;

Jabbur and Safieh-Garabedian, contributed to the design and the discussion of the results;

Chamaa and Saadé N contributed to all steps of the study.

All authors have approved the final version of the manuscript.

This study has not been published previously and it is not under consideration for publication elsewhere.

Acknowledgements

The authors thank Sawsan Sharrouf and Bassem Najm for their technical assistance.

This work was supported by a grant from the Lebanese National Council for Scientific Research (2012–2014). Formal approval to conduct the experiments described has been obtained from the Animal Care Facility at the American University of Beirut and could be provided upon request. All efforts were made to minimize the number of animals used and their suffering.

There are no conflicts of interests.

References

- Aguzzi, A., Barres, B.A., Bennett, M.L., 2013. Microglia: scapegoat, saboteur, or something else? *Science* 339, 156–161.
- Al-Amin, H., Sarkis, R., Atweh, S., Jabbur, S., Saadé, N., 2011. Chronic dizocilpine or apomorphine and development of neuropathy in two animal models II: effects on brain cytokines and neurotrophins. *Exp. Neurol.* 228 (1), 30–40.
- Alexander, G.M., Perreault, M.J., Reichenberger, E.R., Schwartzman, R.J., 2007. Changes in immune and glial markers in the CSF of patients with Complex Regional Pain Syndrome. *Brain Behav. Immun.* 2, 668–676.
- Apkarian, A.V., Lavarello, S., Randolph, A., Berra, H.H., Chialvo, D.R., Besedovsky, H.O., Del Rey, A., 2006. Expression of IL-1 β in supraspinal brain regions in rats with neuropathic pain. *Neurosci. Lett.* 407, 176–181.
- Calvo, M., Dawes, J.M., Bennett, D.L.H., 2012. The role of the immune system in the generation of neuropathic pain. *Lancet Neurol.* 11, 629–642.
- Cirillo, G., Cavaliere, C., Bianco, M.R., De Simone, A., Colangelo, A.M., Sellitti, S., Alberghina, L., Papa, M., 2010. Intrathecal NGF administration reduces reactive astrogliosis and changes neurotrophin receptors expression pattern in a rat model of neuropathic pain. *Cell. Mol. Neurobiol.* 30 (1), 51–62.
- Colburn, R.W., DeLeo, J.A., Rickman, A.J., Yeager, M.P., Kwon, P., Hickey, W.F., 1997. Dissociation of microglial activation and neuropathic pain behaviors following peripheral nerve injury in the rat. *J. Neuroimmunol.* 79 (2), 163–175.
- Covey, W.C., Ignatowski, T.A., Knight, P.R., Spengler, R.N., 2000. Brain-derived TNF α : Involvement in neuroplastic changes implicated in the conscious perception of persistent pain. *Brain Res.* 859 (1), 113–122.
- Covey, W.C., Ignatowski, T.A., Renauld, A.E., Knight, P.R., Nader, N.D., Spengler, R.N., 2002. Expression of neuron-associated tumor necrosis factor alpha in the brain is increased during persistent pain. *Reg. Anesth. Pain Med.* 27 (4), 357–366.
- Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46–56.
- Decosterd, I., Woolf, C.J., 2000. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain* 87 (2), 149–158.
- Del Rey, A., Yau, H.J., Randolph, A., Centeno, M.V., Wildmann, J., Martina, M., Besedovsky, H.O., Apkarian, A.V., 2011. Chronic neuropathic pain-like behavior correlates with IL-1 β expression and disrupts cytokine interactions in the hippocampus. *Pain* 152, 2827–2835.
- Del Rey, A., Balschun, D., Wetzel, W., Randolph, A., Besedovsky, H.O., 2013. A cytokine network involving brain-borne IL-1 β , IL-1ra, IL-18, IL-6, and TNF α operates during long-term potentiation and learning. *Brain Behav. Immun.* 33, 15–23.
- DeLeo, J.A., Yezierski, R.P., 2001. The role of neuroinflammation and neuroimmune activation in persistent pain. *Pain* 90, 1–6.
- DeLeo, J.A., Colburn, R.W., Rickman, A.J., 1997. Cytokine and growth factor immunohistochemical spinal profiles in two animal models of mononeuropathy. *Brain Res.* 759 (1), 50–57.
- Dubovy, P., Brazda, V., Klusakova, I., Hradilova-Svizenska, I., 2013. Bilateral elevation of interleukin-6 protein and mRNA in both lumbar and cervical dorsal root ganglia following unilateral chronic compression injury of the sciatic nerve. *J. Neuroinflammation* 10, 55.
- Felger, J.C., Lotrich, F.E., 2013. Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. *Neuroscience* 246, 199–229.
- Ferreira, S.H., Lorenzetti, B.B., Bristow, A.F., Poole, S., 1988. Interleukin-1 β as a potent hyperalgesic agent antagonized by a tripeptide analogue. *Nature* 334 (6184), 698–700.
- Grace, P.M., Hutchinson, M.R., Maier, S.F., Watkins, L.R., 2014. Pathological pain and the neuroimmune interface. *Nat. Rev. Immunol.* 14, 217–231.

- Hald, A., Nedergaard, S., Hansen, R.R., Ding, M., Heegaard, A., 2009. Differential activation of spinal cord glial cells in murine models of neuropathic and cancer pain. *Eur. J. Pain* 13 (2), 138–145.
- Hao, J., Ebendal, T., Xu, X., Wiesenfeld-Hallin, Z., Eriksson, J., Jönhagen, M., 2000. Intracerebroventricular infusion of nerve growth factor induces pain-like response in rats. *Neurosci. Lett.* 286 (3), 208–212.
- Hori, T., Oka, T., Hosoi, M., Aou, S., 1998. Pain modulatory actions of cytokines and prostaglandin E₂ in the brain. *Ann. N. Y. Acad. Sci.* 840, 269–281.
- Ignatowski, T.A., Covey, W.C., Knight, P.R., Severin, C.M., 1999. Brain-derived TNF α mediates neuropathic pain. *Brain Res.* 841 (1–2), 70–77.
- Jančálek, R., Dubový, P., Sviženská, I., Klusáková, I., 2010. Bilateral changes of TNF- α and IL-10 protein in the lumbar and cervical dorsal root ganglia following a unilateral chronic constriction injury of the sciatic nerve. *J. Neuroinflammation* 7 (1), 11.
- Kanaan, S.A., Saadé, N.E., Haddad, J.J., Abdelnoor, A.M., Atweh, S.F., Jabbur, S.J., Safieh-Garabedian, B., 1996. Endotoxin-induced local inflammation and hyperalgesia in rats and mice: a new model for inflammatory pain. *Pain* 66 (2–3), 373–379.
- Leung, L., Cahill, C., 2010. TNF- α and neuropathic pain – a review. *J. Neuroinflammation* 7 (1), 27.
- Lewin, G.R., Ritter, A.M., Mendell, L.M., 1993. Nerve growth factor-induced hyperalgesia in the neonatal and adult rat. *J. Neurosci.* 13 (5), 2136–2148.
- Lewitus, G.M., Pribiag, H., Duseja, R., St-Hilaire, M., Stellwagen, D., 2014. An adaptive role of TNF- α in the regulation of striatal synapses. *J. Neurosci.* 34, 6146–6155.
- Loggia, M.L., Chonde, D.B., Akeju, O., Arabasz, G., Catana, C., Edwards, R.R., Hill, E., Hsu, S., Izquierdo-Garcia, D., Ji, R.R., Riley, M.D., Zurcher, N.R., Albrecht, D.S., Vangel, M.G., Rosen, B.R., Napadow, V., Hooker, J.M., 2015. Evidence for brain glial activation in chronic pain patients. *Brain* 138, 604–615.
- Maier, S.F., Wiertelak, E.P., Martin, D., Watkins, L.R., 1993. Interleukin-1 mediates the behavioral hyperalgesia produced by lithium chloride and endotoxin. *Brain Res.* 623, 321–324.
- McMahon, S.B., Cafferty, W.B.J., Marchand, F., 2005. Immune and glial cell factors as pain mediators and modulators. *Exp. Neurol.* 192 (2), 444–462.
- Michell-Robinson, M.A., Touil, H., Healy, L.M., Owen, D.R., Durafourt, B.A., Bar-Or, A., Antel, J.P., Moore, C.S., 2015. Roles of microglia in brain development, tissue maintenance and repair. *Brain* 138, 1138–1159.
- Milligan, E.D., Watkins, L.R., 2009. Pathological and protective roles of glia in chronic pain. *Nat. Rev. Neurosci.* 10, 23–36.
- Moalem, G., Tracey, D.J., 2006. Immune and inflammatory mechanisms in neuropathic pain. *Brain Res. Rev.* 51 (2), 240–264.
- Obata, H., Sakurazawa, S., Kimura, M., Saito, S., 2010. Activation of astrocytes in the spinal cord contributes to the development of bilateral allodynia after peripheral nerve injury in rats. *Brain Res.* 1363, 72–80.
- Prieto, G., Snigdha, S., Baglietto-Vargas, D., Smith, E.D., Berchtold, N.C., Tong, A.J., LaFerla, F.M., Rebeck, J., Cotman, C.W., 2015. Synapse-specific IL-1 receptor subunit reconfiguration augments vulnerability to IL-1 β in the aged hippocampus. *PNAS* 112, E5078–E5087.
- Raghavendra, V., Tanga, F., DeLeo, J.A., 2003. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. *J. Pharmacol. Exp. Ther.* 306 (2), 624–630.
- Ruohonen, S., Jagodi, M., Khademi, M., Taskinen, H.S., Ojala, P., Olsson, T., Roytta, M., 2002. Contralateral non-operated nerve to transected rat sciatic nerve shows increased expression of IL-1 β , TGF- α 1, TNF- α , and IL-10. *J. Neuroimmunol.* 132, 11–17.
- Saab, C.Y., Shamaa, F., El Sabbah, M.E., Safieh-Garabedian, B., Jabbur, S.J., Saadé, N.E., 2009. Transient increase in cytokines and nerve growth factor in the rat dorsal root ganglia after nerve lesion and peripheral inflammation. *J. Neuroimmunol.* 208 (1–2), 94–103.
- Saadé, N.E., Massaad, C.A., Ochoa-Chaar, C.I., Jabbur, S.J., Safieh-Garabedian, B., Atweh, S.F., 2002. Upregulation of proinflammatory cytokines and nerve growth factor by intraplantar injection of capsaicin in rats. *J. Physiol.* 545 (1), 241–253.
- Safieh-Garabedian, B., Poole, S., Allchorne, A., Winter, J., Woolf, C.J., 1995. Contribution of interleukin-1 β to the inflammation-induced increase in nerve growth factor levels and inflammatory hyperalgesia. *Br. J. Pharmacol.* 115, 1265–1275.
- Singhal, G., Jaehne, E.J., Corrigan, F., Toben, C., Baune, B.T., 2014. Inflammasomes in neuroinflammation and changes in brain function: a focused review. *Front. Neurosci.* 8.
- Souter, A.J., Garry, M.G., 2000. Spinal interleukin-1 β reduces inflammatory pain. *Pain* 86 (1–2), 63–68.
- Vezzani, A., Balosso, S., Ravizza, T., 2008. The role of cytokines in the pathophysiology of epilepsy. *Brain Behav. Immun.* 22, 797–803.
- Vitkovic, L., Bockaert, J., Jacque, C., 2000. “Inflammatory” Cytokines: Neuromodulators in normal brain? *J. Neurochem.* 74, 457–471.
- Watkins, L.R., Maier, S.F., 2002. Beyond neurons: evidence that immune and glial cells contribute to pathological pain states. *Physiol. Rev.* 82, 981–1011.
- Wohleb, E.S., McKim, D.B., Sheridan, J.F., Godbout, J.P., 2015. Monocyte trafficking to the brain with stress and inflammation: a novel axis of immune-to-brain communication that influences mood and behavior. *Front. Neurosci.* 8, 447.
- Yabuuchi, K., Nishiyori, A., Minami, M., Satoh, M., 1996. Biphasic effects of intracerebroventricular interleukin-1 β on mechanical nociception in the rat. *Eur. J. Pharmacol.* 300 (1–2), 59–65.
- Zhang, F., Vadakkan, K., Kim, S., Wu, L., Shang, Y., Zhuo, M., 2008. Selective activation of microglia in spinal cord but not higher cortical regions following nerve injury in adult mouse. *Mol. Pain* 4, 15.
- Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 16 (2), 109–110.