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Joint Distraction Magnitude Is Associated With Different Behavioral Outcomes and Substance P Levels for Cervical Facet Joint Loading in the Rat

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Abstract: The facet joint is a common source of pain in both the neck and low back, and can be injured by abnormal loading of the spinal joints. Whereas a host of nociceptive changes including neuronal activation, neuropeptide expression, and inflammatory mediator responses has been reported for rat models of joint pain, no such responses have been explicitly investigated or quantified for painful mechanical injury to the facet joint. Two magnitudes of joint loading were separately imposed in a rat model of cervical facet joint distraction: Painful and nonpainful distractions. Behavioral outcomes were defined by assessing mechanical hyperalgesia in the shoulders and forepaws. Substance P (SP) mRNA and protein levels were quantified in the dorsal root ganglion (DRG) and spinal cord at days 1 and 7 following distraction. Painful distraction produced mechanical hyperalgesia that was significantly greater ($P < .010$) than that for a nonpainful distraction. Painful distraction significantly increased spinal SP mRNA ($P = .048$) and SP protein expression in the DRG ($P = .013$) at day 7 compared to nonpainful distraction. However, spinal SP protein for painful distraction was significantly less ($P = .024$) than that for nonpainful distraction at day 1. Joint distractions producing different behavioral outcomes modulate SP mRNA and protein in the DRG and spinal cord, suggesting that SP responses may be involved with different temporal responses in painful joint loading.

***Perspective:** SP mRNA and protein in the DRG and spinal cord are quantified at 2 time points after cervical facet joint distractions that separately do or do not produce mechanical hyperalgesia. Studies describe a role for SP to contribute to pain produced by mechanical joint loading.*

***Key words:** Facet, pain, substance P, hyperalgesia, neck, joint.*

The facet joint has been reported to contribute to persistent neck and low back pain.[3,5,57] Studies identify this joint as the site of chronic pain in up to 62% of the neck pain cases and 45% of the low back pain cases.[3,5,57] Biomechanical studies suggest that facet-mediated pain may result from altered vertebral motions that can increase mechanical loading to the facet and its capsule.[16,35,54,66,67,68,86,87] Increases in behavioral hypersensitivity, neuronal activation, and inflammatory mediator expression in the joint and spinal cord have all been reported for rat models of joint inflammation and arthritis.[24,31,81,88] However, although those studies support a role for inflammation and/or joint afferents to produce pain as a result of mechanical loading to joints, little is known about the relationships between joint-loading conditions, pain, and related physiologic sequelae.

Cervical and lumbar facet joints have been implicated in nociceptive signaling for mechanical joint loading, via capsule afferents sensing loading and initiating physiologic responses.[4,11,12,30,52,53,84] Both cervical and lumbar joints and their capsules are innervated by Aδ and C fibers,[11,12,30,53] some of which are reactive for the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP).[6,21,23,33] SP is a neurotransmitter and nociceptive neuromodulator,[59] suggesting a means by which facet joint loading or inputs can induce pain. In particular, application of SP to nociceptor receptive fields

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in the rat lumbar facet joint increases the afferent discharge rate in nociceptors,[84] demonstrating that joint afferents can become sensitized. Cervical and lumbar facet capsule afferents are also activated by tensile joint loading and can remain sensitized following unloading,[4,11,12,52,53] suggesting that joint loading may elicit pain via capsule afferents. Despite demonstration of neuropeptide immunoreactivity in facet joint afferents and indirect implication of SP in facet-mediated pain, the relationship between SP in the nervous system and quantitative pain outcomes remains uncharacterized for this joint.

Several studies demonstrate that SP is associated with, and directly modulates, spinal and supraspinal signaling in painful peripheral injury.[1,55,79,85] Gene upregulation and enhanced protein expression of SP and its receptor are produced in the dorsal root ganglion (DRG) and spinal cord in pain models of partial nerve injury and inflammation.[18,55,79,80] Painful arthritis and joint inflammation models demonstrate altered SP expression in both spinal cord and synovial tissues,[37,58] suggesting that pain after a mechanical joint injury may also involve a combination of local and spinal neuropeptide responses. Although there is support for altered neuropeptide expression in modulating neuropathic and inflammatory pain, SP mRNA and protein levels in the dorsal root ganglion and spinal cord after joint distraction are undefined.

Our lab has previously developed a rat model of painful cervical facet joint-mediated injury, in which the resultant behavioral hypersensitivity (ie, mechanical allodynia) is dependent on the magnitude of applied vertebral distraction.[46,47] Mechanical allodynia represents enhanced nociceptive processing and is commonly used as an indicator of pain outcomes in a variety of other models of radiculopathy, neuropathy, and inflammatory pain.[15,70,71,72,73,89,90] In the previous studies using this facet joint distraction model, vertebral distractions of 0.7 mm across the C6/C7 facet joint produce mechanical allodynia that lasts for up to 2 weeks, although distractions of 0.2 mm do not produce any allodynia that differs from either baseline (uninjured) or sham responses at any time point.[46,47,48] Accordingly, those results suggest that distractions of 0.7 mm across the facet joint can be assumed to produce painful outcomes, whereas distractions of 0.2 mm do not produce painful outcomes. Although those data suggest that the presence and extent of pain symptoms depend on the severity of joint distraction, the mechanisms by which certain facet distractions produce symptoms remain undefined. This study tested the hypothesis that behavioral hypersensitivity after painful facet joint distraction is associated with increases in SP mRNA and protein in the DRG, and delayed spinal modifications of SP. As such, shoulder and forepaw hyperalgesia were measured to assess behavioral outcomes following different joint distraction severities. SP mRNA and protein levels were quantified in the DRG and spinal cord at 2 time points (days 1 and 7) after joint distractions that separately did and did not result in behavioral hypersensitivity. These studies offer insight into the potential involvement of SP in the DRG and spinal cord in painful outcomes after facet distraction and may provide context for additional investigations into mechanisms underlying these painful symptoms.

Methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.[91] Male Holtzman rats (Harlan Sprague-Dawley, Indianapolis, IN) (424 $\pm$ 32 g) were housed under USDA- and AAALAC-compliant conditions.

General Surgical Procedures

All surgical procedures were performed under inhalation isoflurane anesthesia (4% for induction, 2.5% for maintenance). A controlled bilateral cervical facet joint distraction was applied using a rat model, as previously described.[46,47] Briefly, the C6 and C7 laminae and facet joints were exposed and cleared, and a loading device was attached to the C6 and C7 spinous processes. The device translated the C6 spinous process rostrally while holding the C7 spinous process fixed, distracting the joint. Two subfailure distraction paradigms were separately imposed across the facet joint in these studies: (1) a painful distraction (0.7 mm), and (2) a nonpainful distraction (0.2 mm) (n = 18, each group).[46,47,48] Sham surgeries (n = 18) were also performed to control for the physiologic effects of surgery; for sham, the device was attached but no distraction was imposed. Following surgery, wounds were closed and rats were recovered in room air.

Vertebral distraction and applied force were measured during loading in order to verify that the imposed distractions for the 2 groups were mechanically different. Polystyrene particles were placed at the center of the C6 and C7 laminae and images of the laminae were acquired during distraction. Image tracking software (Image Pro Plus; Media Cybernetics, Inc., Silver Spring, MD) was used to locate marker centroids for motion tracking. Applied joint distraction was defined as the maximum separation of the C6 laminae relative to the C7 laminae. The applied force during joint distraction was defined as the peak force supported by the ligament during the distraction procedure.

Behavioral Assessment

Mechanical hyperalgesia was quantified as a measure of behavioral hypersensitivity in the bilateral shoulders and forepaws of each rat to verify that behavioral results in this study correspond to previously reported outcomes using these distraction conditions.[46,47] Specifically, hyperalgesia was assessed using a modified version of Chaplan's up/down method to quantify the threshold for tactile sensitivity to a stimulus that is normally noxious.[13,28] For this method, a lower response threshold represents greater behavioral hypersensitivity;[13] specific

methods and anatomic testing locations have been described previously for this model[48]. Briefly, response thresholds were measured separately for each of the left and right shoulders and forepaws using a series of logarithmically ascending von Frey filament strengths (0.4, 0.6, 1.4, 2.0, 4.0, 6.0, 8.0, 15.0, and 26.0 g) (Stoelting, Wood Dale, IL).[28,48] Response thresholds were measured on 3 consecutive days prior to surgery to obtain baseline values, and on postoperative days 1, 3, 5, and 7. Each testing session consisted of 3 rounds of 5 stimulations to each region using a series of ascending von Frey filaments (0.4–26.0 g). For each round, the lowest filament to elicit a positive response was recorded as the response threshold if the next larger filament also evoked a positive response. A positive response was defined as vigorous shaking of the shoulder or emphatic lifting of the forepaw, which was generally accompanied by licking of the affected area, and in some cases by vocalization as well. If the rat failed to respond to any filament, the maximum filament strength (26.0 g) was recorded as the threshold for that round. The thresholds over the 3 rounds were separately averaged for each shoulder and forepaw to obtain a single value at that location for each rat.

Tissue Processing and SP Characterization

Spinal cord segments and DRGs were harvested at the C6 and C7 spinal levels of each animal for processing to quantify SP mRNA and protein levels in each group (painful, nonpainful, sham) at 2 time points (day 1, day 7). After behavioral assessment on day 1 (n = 8/group) or day 7 (n = 10/group), rats were anesthetized with sodium pentobarbital (65 mg/kg) and transcardially perfused with phosphate-buffered saline. Whole spinal cord segments and the DRGs from the right side were harvested from the C6 and C7 spinal levels, and immediately frozen on dry ice. Tissue was stored at –80°C until further processing.

The C6 DRG and spinal cord samples were analyzed by real-time PCR to quantify SP mRNA levels. Total RNA was extracted from tissue samples using the TRIzol isolation system (Life Technologies, Rockville, MD) according to manufacturer's instructions. Total RNA (2 μg) from each sample was used to generate single-strand cDNA using the MultiScribe Reverse Transcriptase system (Applied Biosystems, Foster City, CA). A custom gene expression assay (Applied Biosystems Assay #4331183) was used to probe SP mRNA. Primers and probes for the internal housekeeping gene, 18s, have been previously reported (Forward primer: 5'- CGGCTACCACATCCAAGGAA-3'; Reverse primer: 5'-GCTGGAATTACCGCGGCT-3'; Probe: 5'-CACCAGACTTGCCCTC-3').[51] Taqman real-time PCR was performed using an ABI-7300 system (Applied Biosystems); 1.5 μL of sample cDNA was used for each 25-μL reaction and all reactions were performed in duplicate. Cycle conditions were: 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, and 60°C for 60 seconds. For all samples, the target gene expression was normalized to the internal housekeeping gene (18s) expression in that sample using the ΔΔCt method;[50] the mRNA level for each sample in the painful and nonpainful groups is reported as the fold-difference in expression relative to the average expression of sham samples. Fold-differences in SP mRNA were averaged for each distraction group (painful, nonpainful) in each tissue type (DRG, spinal cord) at each time point (day 1, day 7).

Quantitative determination of SP protein was performed on the C7 DRG and spinal cord by quantitative ELISA assay. Tissue was homogenized in buffer (10 mM Tris, pH 7.4) and centrifuged at 16,000 *g* for 15 minutes at 4°C. Tissue protein concentration was determined using an ELISA for rat SP (MD Biosciences, St. Paul, MN), according to the manufacturer's instructions. Each sample was analyzed in duplicate. SP protein quantification was determined by comparing samples to the standard curve from the assay kit and data are reported as a fold-difference in expression for each sample relative to the average protein for sham samples. Fold-differences in SP protein were averaged for each distraction group in each tissue type at each time point.

Statistical Analysis

All statistical analyses were performed using JMPIN 4.0.4 (SAS Inc, Cary, NC) and significance was defined as $P < .05$. Mechanical data were compared between distraction groups using t-tests. Hyperalgesia thresholds were compared between the left and right forepaws and the left and right shoulders using paired t-tests to test for asymmetry in each anatomic region. A multivariate repeated measures analysis was used to compare sensitivity thresholds between groups at each postoperative day. To test whether painful joint distraction affects SP gene expression, SP mRNA levels were separately compared in each tissue type (DRG, spinal cord) using a 2-way ANOVA to account for differences between group and time point. Similarly, the same comparisons were performed for SP protein responses.

Results

Facet Joint Distraction Mechanics

The different distraction inputs across the facet joint produced different mechanical and behavioral outcomes. The mean vertebral distraction for the painful group was 0.67 ± 0.06 mm, and was significantly greater ($P < .005$) than mean distractions measured for the nonpainful group (0.20 ± 0.04 mm). Likewise, the average force supported by the ligament for painful distraction (4.00 ± 1.81 N) was also significantly greater ($P < .005$) than, and twice as large as, the corresponding force generated for nonpainful distraction (1.92 ± 1.18 N).

Mechanical Hyperalgesia

Behavioral hypersensitivity patterns after joint distraction were different for the 2 distraction groups. Hyperalgesia response thresholds were not significantly different between the right and left shoulders, or the right and left forepaws for any group; as such, data were separately averaged for the shoulder and forepaw

regions for each rat. Response thresholds in both anatomic regions were high for sham and not different from the baseline response at any time point (Fig 1). In contrast, painful distraction across the joint produced an immediate reduction in response threshold in both the shoulder and forepaw that persisted over the 7-day evaluation period (Fig 1). The response threshold for painful distraction was significantly lower than sham at days 3, 5, and 7 ($P < .026$) in the shoulder (Fig 1A), and at all time points ($P < .005$) in the forepaw (Fig 1B). In contrast, response thresholds after nonpainful joint distraction were high in both regions and not different from sham at any day (Fig 1). The behavioral hypersensitivity produced in the painful group was significantly greater (as reflected by a decrease in response threshold) than the corresponding nonpainful response at days 5 and 7 in the shoulder ($P < .006$) (Fig 1A) and for all days ($P < .010$) in the forepaw (Fig 1B).

Quantification of SP mRNA in the DRG and Spinal Cord

SP mRNA was detected in the DRG and spinal cord of all rats. SP mRNA levels were not different in sham compared to normal levels in either tissue at any time point.

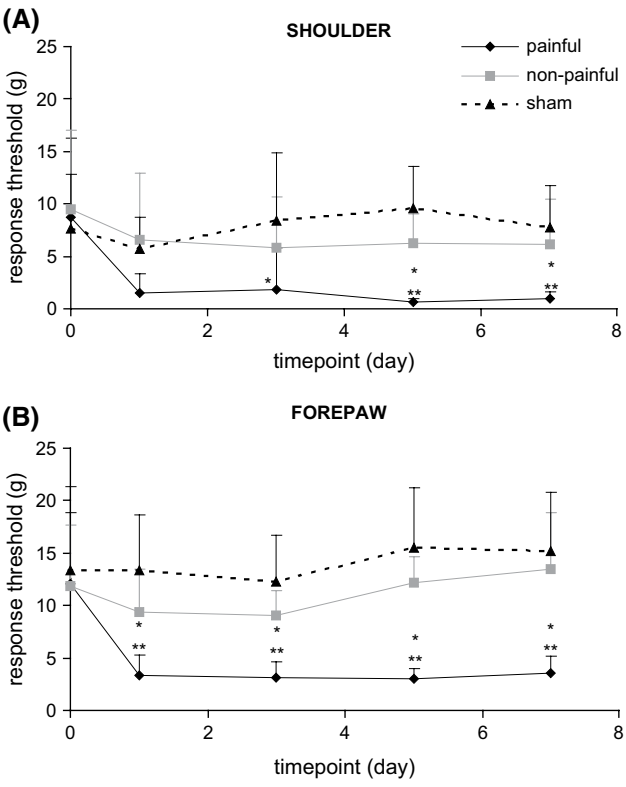

Figure 1. Mechanical hyperalgesia as measured by the average response threshold to von Frey filament stimulation in the shoulder **(A)** and forepaw **(B)**. Increased sensitivity corresponds to a reduced response threshold. **(A)** Painful distraction significantly reduced (*$P < .026$) shoulder thresholds below sham for days 3, 5, and 7, and below nonpainful distraction (**$P < .006$) on days 5 and 7. **(B)** Likewise, painful distraction significantly decreased (*$P < .005$) forepaw thresholds below sham and nonpainful distraction (**$P < .010$) for all days.

In the DRG, SP mRNA levels at day 1 were not different between painful and nonpainful distraction (Fig 2A). However, despite the fact that SP mRNA in the DRG for painful distraction was unchanged over time, it was significantly increased ($P = .027$) from day 1 to day 7 following a nonpainful distraction (Fig 2A). There was no difference in spinal SP mRNA between painful and nonpainful distraction at day 1. However, by day 7, spinal mRNA levels were significantly greater ($P = .048$) for a painful distraction than for a nonpainful one (Fig 2B).

Quantification of SP Protein in the DRG and Spinal Cord

SP protein was also detected in DRG and spinal cord for these studies. SP protein in the DRG was greater for a painful distraction than for a nonpainful one at both time points, although this increase was only significant ($P = .013$) at day 7 (Fig 3A). At day 7, SP protein in the DRG after painful distraction was nearly twice that of levels from either nonpainful distraction or sham. Spinal SP was generally not different from sham for both groups at both time points. Yet, at day 1, SP protein was significantly different ($P = .015$) between painful and nonpainful distractions (Fig 3B). By day 7, however, protein levels produced by a nonpainful distraction were significantly lower ($P = .024$) than on day 1, and no longer different from painful distraction.

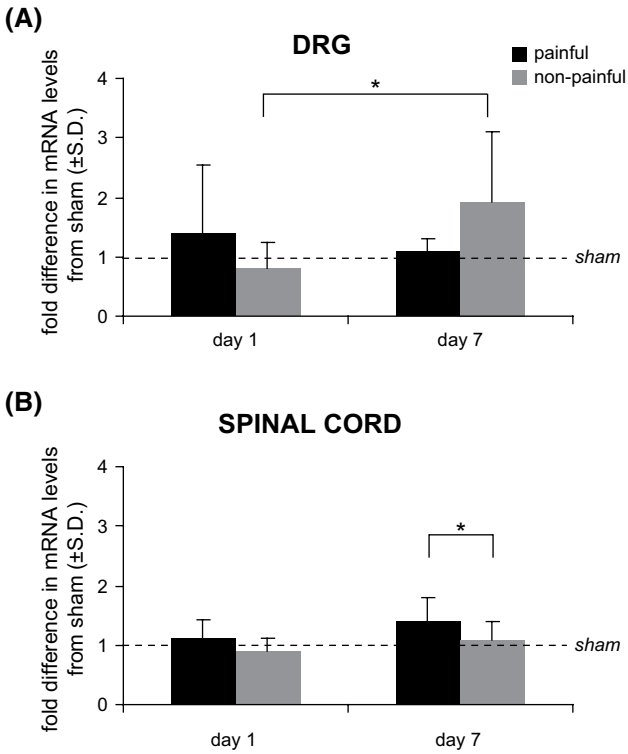

Figure 2. Quantification of SP mRNA in the DRG **(A)** and spinal cord **(B)** at days 1 and 7 after facet distraction. **(A)** Nonpainful distraction significantly increased (*$P = .027$) mRNA from day 1 to day 7 in the DRG. **(B)** In the spinal cord, SP mRNA for painful distraction was significantly greater (*$P = .048$) than nonpainful at day 7. The dashed line indicates sham SP mRNA levels.

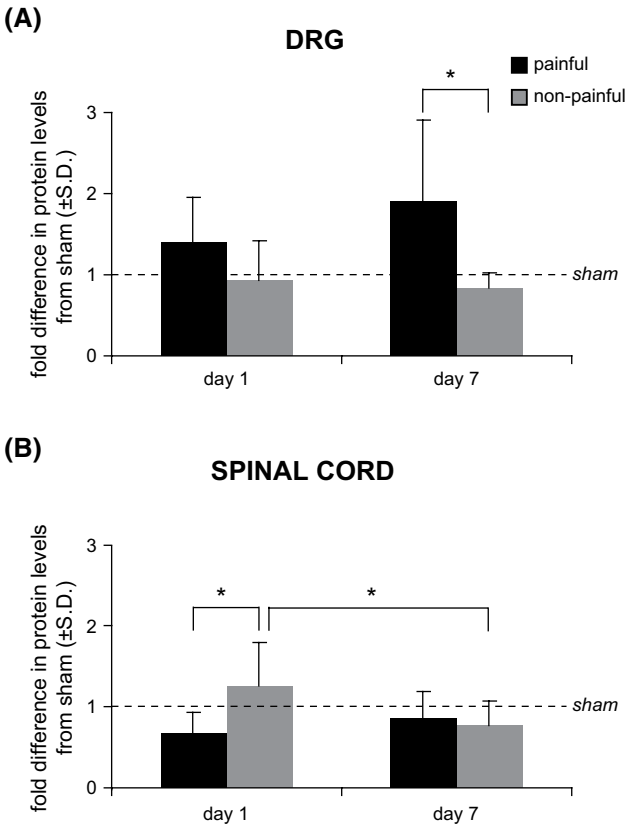

Figure 3. SP protein in the DRG **(A)** and spinal cord **(B)** at days 1 and 7 after distraction. **(A)** SP protein for painful distraction was significantly increased (*P = .013) in the DRG above nonpainful distraction at day 7. **(B)** Spinal SP protein for painful distraction was significantly lower (*P = .015) than nonpainful at day 1. SP protein for nonpainful distraction was significantly decreased (*P = .024) from day 1 to day 7 in the spinal cord. The dashed line indicates sham SP protein levels.

Discussion

This study is the first to demonstrate that cases of painful and nonpainful mechanical loading to the cervical facet joint are associated with different patterns of SP mRNA and protein in the DRG and spinal cord (Figs 2 and 3). In the DRG, SP mRNA increases over time for nonpainful joint distraction, yet remains unchanged for painful distractions (Fig 2A). This differential response in the gene for SP suggests that painful distraction of this joint may induce axonal dysfunction that subsequently disrupts the normal mRNA production in the DRG.[34] In contrast, SP mRNA in the spinal cord is generally unchanged for both types of distractions over time (Fig 2B). Taken together, these data support the assertion that the magnitude of joint distraction affects SP mRNA production more strongly in the DRG than in the spinal cord for this model. SP protein, however, appears to depend on the severity of joint distraction in both the DRG and spinal cord (Fig 3). Painful distraction increases SP protein in the DRG at day 7 and decreases spinal SP protein at day 1 (Figs 3A and 3B), suggesting that SP protein may be utilized differently in the DRG and spinal cord in response to cervical facet joint loading. For this study, pain outcomes following facet joint distraction were assessed by quantifying mechanical hyperalgesia, which is representative of clinically observed symptoms in neck pain patients[26,32,78] and has been used to assess sensitivity in existing neck and low back pain models.[7,14,25,28,70] This study used only a single distraction magnitude to represent each of the painful (0.7 mm) or nonpainful (0.2 mm) joint-loading scenarios. Given that a threshold has not been identified for joint-distraction magnitudes that consistently produce pain, there are likely additional magnitudes of distraction that may correspond to either a painful or nonpainful outcome that were not investigated in the current study. Regardless, the studies presented here provide evidence that SP mRNA in the DRG and SP protein in the DRG and spinal cord are likely involved in facet-mediated pain, but that those neuropeptide responses may not be the sole regulator and/or modulator of nociception.

Interestingly, SP mRNA increases temporally in the DRG for a nonpainful joint distraction, but remains unchanged over time for a painful distraction (Fig 2A). Other studies have reported that neuropeptide (CGRP) and preprotachykinin A (an SP precursor) mRNA are increased in the DRG in rat models of joint inflammation,[8,22] which could suggest that SP mRNA may likely also be increased in the DRG for cases of joint inflammation. However, the initial mode of injury in the current model was mechanical joint loading, producing SP mRNA in the DRG that was observed only for nonpainful distractions and not for painful cases (Fig 2A). A possible explanation for this apparent disconnect in SP and pain responses observed in this model is that the *mechanical severity* of a painful distraction may actually disrupt the ability of capsule afferents to produce mRNA via their physical injury. Both group III and IV nociceptors in the cervical facet capsule are activated at capsule strains greater than 10.7 $\pm$ 3.3% and 10.0 $\pm$ 4.6%, respectively, in a goat model of facet joint distraction.[52,53] Previous work with the current model has also demonstrated that both of the distraction conditions used in this study produce strains in the capsule that exceed those strain thresholds for nociceptor activation,[47] suggesting that nociceptors may be activated for both conditions in the current study. As such, nociceptor activation alone may not be the sole specific contributor to the nociceptive cascade that is directly responsible for producing the mechanical hyperalgesia observed for painful loading in our model. The painful loading condition used here produces capsule strains of 48 $\pm$ 16%,[69] which are within the range previously hypothesized to alter axonal morphology in the capsule (62%—82%) in the goat model of facet joint loading.[34] In that goat model, those capsule strains produced axonal swellings and terminal retraction, both of which are indicative of loading-induced axotomy.[34] Although the current study did not examine nerve fibers in the capsule for evidence of damage or the corresponding neuronal responses in the DRG, the painful distraction condition used here produces capsule strains within that range hypothesized to produce morphologic damage[47] and sufficient to activate 1 component of the neuronal stress response in the DRG.[17] Taking those studies together with reports

in the literature, it is possible that this painful cervical facet joint-loading condition may induce axonal damage in the afferents of the capsule directly or in the adjacent neurons in the DRG that may prevent their normal functioning and may potentially affect the ability of those neurons to produce SP. Direct mechanical compression of either the nerve root or DRG induces edema and vascular permeability,[40,41,42] preventing the normal functioning of afferent fibers. Moreover, recent work with a model similar to the 1 used here demonstrates that painful cervical facet distraction increases expression of a marker of the activated stress response in neurons of the DRG,[17] further suggesting that the afferents innervating the facet joint may actually be indirectly damaged by the capsule's undergoing mechanical loading, and such loading may subsequently modulate neuronal responses in the DRG. Taken together with the literature, our SP mRNA data suggest there may be a neuropathic component to outcomes after painful facet distraction, and in particular, that painful magnitudes of joint loading may incite dysfunction of mRNA production in affected joint afferents.

Despite the current results suggesting that painful joint distraction may disrupt SP mRNA in the DRG, they do not clarify the specific cell types that may be responsible for this disruption. The techniques used here prevent the identification of specific responses of different neuronal populations, or even nonneuronal cell types. Previous studies report phenotypic shifts in DRG neurons after both lumbar and cervical facet joint incision and inflammation, and sciatic nerve injury.[49,64,65] In those studies, a subpopulation of large-diameter (likely Aβ) fibers in the DRG showed a phenotypic switch toward either increased CGRP- or brain-derived neurotrophic factor-immunoreactivity after introduction of the mechanical or inflammatory stimulus. Although those reports suggest that after certain peripheral stimuli, large-diameter afferents may have the capability to act as additional nociceptive transmitters, the phenotypic responses of individual neuronal populations in the current model of facet-mediated pain remains undetermined. Such studies would prove useful in identifying relevant cellular and molecular targets for intervention. In addition, the current study examined the SP response in the DRG and spinal cord only, but did not measure responses in the facet joint. Indeed, decreased SP has been documented in the afferents and synovia of joints from rheumatoid arthritis patients and in a rat model of monoarthritis,[38,58] further supporting our finding that painful facet loading does not significantly increase SP mRNA production in the DRG (Fig 2A). Quantification of SP responses in the joint after its distraction would provide a more comprehensive description of the relationship between SP responses throughout the peripheral and central nervous systems for this painful syndrome.

SP protein is increased in the DRG at day 7 after painful distraction (Fig 3A) as reported in other models of painful neuropathy and joint inflammation.[8,55,77] However, in the current model, mRNA for SP is not similarly increased in the DRG at that same time point (Fig 2A), suggesting that the elevated SP protein is not attributable to an upregulation of SP production in the DRG. In peptidergic neurons, SP mRNA is transported from the nucleus to the cytoplasm, where it produces prepropeptides that are transported along the axon, differentially processed into SP protein, and released from the nerve terminal.[76] The observed decrease in mRNA in this study may be due to distraction-induced axonal mechanical injury for the painful condition that disrupts the production or trafficking of mRNA along those injured axons. Decreases in mRNA in the DRG have been reported for genes involved in pain modulation, including CGRP, brain-derived neurotrophic factor, and the μ-opioid receptor, in cases of mechanical nerve injury.[19,45,61] Those authors explicitly hypothesized axonal damage as a potential mechanism of decreased mRNA, which supports the finding in our study. Accordingly, the associated increase in SP protein suggests that the protein may come from a nonresident cell source. SP can be released from a number of cells that are involved in inflammation, including astrocytes, microglia, monocytes, and macrophages;[59] the current data suggest that those cells may release SP in the DRG to regulate and/or promote inflammation for painful joint conditions. Tumor necrosis factor-alpha (TNFα)-receptor immunoreactivity in the DRG has been reported to increase after incision of the facet capsule;[74] mRNA for the proinflammatory cytokine TNFα is also increased in the DRG after painful distraction in the current facet model.[48] In conjunction with evidence of inflammation in the DRG for painful conditions and injury to the facet joint, our data may indicate that although protein for each type of distraction is individually unchanged over time in the DRG, the significant difference between groups at day 7 (Fig 3A) may point to a role for SP in modulating inflammatory responses contributing to pain.

There is also a difference in spinal protein levels at day 1 for the 2 distractions cases; nonpainful distraction levels are significantly greater than painful levels. In fact, painful levels are actually reduced below sham levels (Fig 3B). That decrease in spinal SP protein for painful distraction below sham levels is consistent with previous reports of decreased SP protein expression for other models of painful neural injuries in the cervical and lumbar spines,[29,42,56,62] and may be a consequence of decreased mRNA production, increased protein utilization in the spinal cord, and/or injury-induced axonal dysfunction that can affect protein transport.[2,9,39,43,44] SP is also reported to be involved in regulating the glial response to injury in the CNS.[59] We have previously observed increased spinal astrocytic activation for the painful distraction condition in the current model;[47,83] as such, it is possible that painful distraction may require increased utilization of spinal SP to regulate the enhanced spinal glial reactivity, which may in part explain the depletion of spinal SP protein observed at day 1 (Fig 3B). Although this study did probe SP, it did not examine the response of the SP receptor, neurokinin-1 (NK-1), after facet joint distraction. Numerous studies implicate spinal NK-1 mRNA and protein expression as directly contributing to pain in models of inflammation, arthritis, and

neuropathy,[10,18,20,36,80] suggesting that modulating the NK-1 response may alleviate behavioral hypersensitivity following injury. Yet, there are conflicting reports of the efficacy of either SP or NK-1 treatments for alleviating pain symptoms; whereas administration of their antagonists has either reduced or prevented the development of behavioral symptoms in animal models of joint pain and neuropathy,[27,60,63,75,79,82] clinical trials of NK-1 antagonists have yet to demonstrate reliable evidence of pain alleviation.[37,59] In conjunction with our data that the SP responses (mRNA and protein) do not directly relate to painful conditions (Figs 2 and 3), the results of antagonist studies suggest that targeting SP and/or NK-1 may not actually be the most appropriate therapeutic intervention for joint-mediated pain.

These data support a role for SP in cervical facet-mediated pain; however, it is likely that SP is not the exclusive contributor to modulating pain outcomes in mechanical joint injury. There may be accompanying responses in either the DRG or spinal cord from inflammatory mediators, components of the stress response, and/or other neuropeptides and their receptors that are more directly associated with facet pain. Moreover, studies are needed to characterize the complicated relationships between the numerous potential cellular and molecular pain mediators in the context of facet injury. Regardless, by identifying differential SP responses for painful and nonpainful joint distractions, these findings supply foundational evidence of modifications of a common nociceptive mediator, both locally and spinally, for joint pain.

References

1. Allen BJ, Rogers SD, Ghilardi JR, Menning PM, Kuskowski MA, Basbaum AI, Simone DA, Mantyh PW: Noxious cutaneous thermal stimuli induce a graded release of endogenous substance P in the spinal cord: Imaging peptide action in vivo. J Neurosci 17:5921-5927, 1997

2. Allen BJ, Li J, Menning PM, Rogers SD, Ghilardi J, Mantyh PW, Simone DA: Primary afferent fibers that contribute to increased substance P receptor internalization in the spinal cord after injury. J Neurophysiol 81:1379-1390, 1999

3. Aprill C, Bogduk N: The prevalence of cervical zygapophyseal joint pain. A first approximation. Spine 17:744-747, 1992

4. Avramov AI, Cavanaugh JM, Ozaktay CA, Getchell TV, King AI: The effects of controlled mechanical loading on group-II, III, and IV afferent units from the lumbar facet joint and surrounding tissue. An in vitro study. J Bone Joint Surg Am 74:1464-1471, 1992

5. Barnsley L, Lord S, Bogduk N: Whiplash injury. Pain 58: 283-307, 1994

6. Beaman DN, Graziano GP, Glover RA, Wojtys EM, Chang V: Substance P innervation of lumbar spine facet joints. Spine 18:1044-1049, 1993

7. Bennett GJ, Xie YK: A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33:87-107, 1988

8. Bulling DG, Kelly D, Bond S, McQueen DS, Seckl JR: Adjuvant-induced joint inflammation causes very rapid transcription of beta-preprotachykinin and alpha-CGRP genes in innervating sensory ganglia. J Neurochem 77:372-382, 2001

9. Cahill CM, Coderre TJ: Attenuation of hyperalgesia in a rat model of neuropathic pain after intrathecal pre- or post-treatment with a neurokinin-1 antagonist. Pain 95: 277-285, 2002

10. Castro AR, Pinto M, Lima D, Tavares I: Imbalance between the expression of NK1 and GABAB receptors in nociceptive spinal neurons during secondary hyperalgesia: A c-Fos study in the monoarthritic rat. Neuroscience 132: 905-916, 2005

11. Cavanaugh JM, el-Bohy A, Hardy WN, Getchell TV, Getchell ML, King AI: Sensory innervation of soft tissues of the lumbar spine in the rat. J Orthop Res 7:378-388, 1989

12. Cavanaugh JM, Ozaktay AC, Yamashita HT, King AI: Lumbar facet pain: Biomechanics, neuroanatomy and neurophysiology. J Biomech 29:1117-1129, 1996

13. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL: Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 53:55-63, 1994

14. Colburn RW, Rickman AJ, DeLeo JA: The effect of site and type of nerve injury on spinal glial activation and neuropathic pain behavior. Exp Neurol 157:289-304, 1999

15. Decosterd I, Woolf CJ: Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain 87: 149-158, 2000

16. Deng B, Begeman PC, Yang KH, Tashman S, King AI: Kinematics of human cadaver cervical spine during low speed rear-end impacts. Stapp Car Crash J 44:171-188, 2000

17. Dong L, Odeleye AO, Jordan-Sciutto KJ, Winkelstein BA: Painful facet joint injury induces neuronal stress activation in the DRG: Implications for cellular mechanisms of pain. Neurosci Lett 443:90-94, 2008

18. Doyle CA, Hunt SP: Substance P receptor (neurokinin-1)-expressing neurons in lamina I of the spinal cord encode for the intensity of noxious stimulation: A c-Fos study in rat. Neuroscience 89:17-28, 1999

19. Dumoulin FL, Raivich G, Streit WJ, Kreutzberg GW: Differential regulation of calcitonin gene-related peptide (CGRP) in regenerating rat facial nucleus and dorsal root ganglion. Eur J Neurosci 3:338-342, 1991

20. Duric V, McCarson KE: Hippocampal neurokinin-1 receptor and brain-derived neurotrophic factor gene expression is decreased in rat models of pain and stress. Neuroscience 133:999-1006, 2005

21. el-Bohy A, Cavanaugh JM, Getchell ML, Bulas T, Getchell TV, King AI: Localization of substance P and neurofilament immunoreactive fibers in the lumbar facet joint capsule and supraspinous ligament of the rabbit. Brain Res 460:379-382, 1988

22. Garrett NE, Kidd BL, Cruwys SC, Tomlinson DR: Changes in preprotachykinin mRNA expression and substance P levels in dorsal root ganglia of monoarthritic rats: Comparison

with changes in synovial substance P levels. Brain Res 675: 203-207, 1995

23. Giles LG, Harvey AR: Immunohistochemical demonstration of nociceptors in the capsule and synovial folds of human zygapophyseal joints. Br J Rheumatol 26:362-364, 1987

24. Hartwig AC, Mathias SI, Law AS, Gebhart GF: Characterization and opioid modulation of inflammatory temporomandibular joint pain in the rat. J Oral Maxillofac Surg 61: 1302-1309, 2003

25. Hashizume H, DeLeo JA, Colburn RW, Weinstein JN: Spinal glial activation and cytokine expression after lumbar root injury in the rat. Spine 25:1206-1217, 2000

26. Herren-Gerber R, Weiss S, Arendt-Nielsen L, Petersen-Felix S, Di Stefano G, Radanov BP, Curatolo M: Modulation of central hypersensitivity by nociceptive input in chronic pain after whiplash injury. Pain 5:366-376, 2004

27. Hong SK, Han JS, Min SS, Hwang JM, Kim YI, Na HS, Yoon YW, Han HC: Local neurokinin-1 receptor in the knee joint contributes to the induction, but not maintenance, of arthritic pain in the rat. Neurosci Lett 322:21-24, 2002

28. Hubbard RD, Winkelstein BA: Transient cervical nerve root compression in the rat induces bilateral forepaw allodynia and spinal glial activation: Mechanical factors in painful neck injuries. Spine 30:1924-1932, 2005

29. Hubbard RD, Chen Z, Winkelstein BA: Transient cervical nerve root compression modulates pain: Load thresholds for allodynia and sustained changes in spinal neuropeptide expression. J Biomech 41:677-685, 2008

30. Inami S, Shiga T, Tsujino A, Yabuki T, Okado N, Ochiai N: Immunohistochemical demonstration of nerve fibers in the synovial fold of the human cervical facet joint. J Orthop Res 19:593-596, 2001

31. Inglis JJ, Nissim A, Lees DM, Hunt SP, Chernajovsky Y, Kidd BL: The differential contribution of tumour necrosis factor to thermal and mechanical hyperalgesia during chronic inflammation. Arthritis Res Ther 7:R807-R816, 2005

32. Jull G, Sterling M, Kenardy J, Beller E: Does the presence of sensory hypersensitivity influence outcomes of physical rehabilitation for chronic whiplash? –A preliminary RCT. Pain 129:28-34, 2007

33. Kallakuri S, Singh A, Chen C, Cavanaugh JM: Demonstration of substance P, calcitonin gene-related peptide, and protein gene product 9.5 containing nerve fibers in human cervical facet joint capsules. Spine 29:1182-1186, 2004

34. Kallakuri S, Singh A, Lu Y, Chen C, Patwardhan A, Cavanaugh JM: Tensile stretching of cervical facet joint capsule and related axonal changes. Eur Spine J 17:556-563, 2008

35. Kaneoka K, Ono K, Inami S, Hayashi K: Motion analysis of cervical vertebrae during whiplash loading. Spine 24: 763-769, 1998

36. Kar S, Rees RG, Quirion R: Altered calcitonin gene-related peptide, substance P and enkephalin immunoreactivities and receptor binding sites in the dorsal spinal cord of the polyarthritic rat. Eur J Neurosci 6:345-354, 1994

37. Keeble JE, Brain SD: A role for substance P in arthritis? Neurosci Lett 361:176-179, 2004

38. Kidd BL, Morris VH, Urban L: Pathophysiology of joint pain. Ann Rheum Dis 55:276-283, 1996

39. Kirita T, Takebayashi T, Mizuno S, Takeuchi H, Kobayashi T, Fukao M, Yamashita T, Tohse N: Electrophysiologic changes in dorsal root ganglion neurons and behavioral changes in a lumbar radiculopathy model. Spine 32: E65-E72, 2007

40. Kobayashi S, Yoshizawa H, Hachiya Y, Ukai T, Morita T: Vasogenic edema induced by compression injury to the spinal nerve root. Distribution of intravenously injected protein tracers and gadolinium-enhanced magnetic resonance imaging. Spine 18:1410-1424, 1993

41. Kobayashi S, Yoshizawa H: Effect of mechanical compression on the vascular permeability of the dorsal root ganglion. J Orthop Res 20:730-739, 2002

42. Kobayashi S, Yoshizawa H, Yamada S: Pathology of lumbar nerve root compression. Part 1: Intraradicular inflammatory changes induced by mechanical compression. J Orthop Res 22:170-179, 2004

43. Kobayashi S, Kokubo Y, Uchida K, Yayama T, Takeno K, Negoro K, Nakajima H, Baba H, Yoshizawa H: Effect of lumbar nerve root compression on primary sensory neurons and their central branches: changes in the nociceptive neuropeptides substance P and somatostatin. Spine 30:276-282, 2005

44. Kobayashi S, Sasaki S, Shimada S, Kaneyasu M, Mizukami Y, Kitade I, Ogawa M, Kawahara H, Baba H, Yoshizawa H: Changes of calcitonin gene-related peptide in primary sensory neurons and their central branch after nerve root compression of the dog. Arch Phys Med Rehabil 86:527-533, 2005

45. Kohno T, Ji RR, Ito N, Allchorne AJ, Befort K, Karchewski LA, Woolf CJ: Peripheral axonal injury results in reduced μ opioid receptor pre- and post-synaptic action in the spinal cord. Pain 117:77-87, 2005

46. Lee KE, Thinnes JH, Gokhin DS, Winkelstein BA: A novel rodent neck pain model of facet-mediated behavioral hypersensitivity: Implications for persistent pain and whiplash injury. J Neurosci Methods 137:151-159, 2004

47. Lee KE, Davis MB, Mejilla RM, Winkelstein BA: In vivo cervical facet capsule distraction: Mechanical implications for whiplash and neck pain. Stapp Car Crash J 48:373-395, 2004

48. Lee KE, Davis MB, Winkelstein BA: Capsular ligament involvement in the development of mechanical hyperalgesia after facet joint loading: behavioral & inflammatory outcomes in a rodent model of pain. J Neurotrauma 25: 1383-1393, 2008

49. Li WP, Xian C, Rush RA, Zhou XF: Upregulation of brain-derived neurotrophic factor and neuropeptide Y in the dorsal ascending sensory pathway following sciatic nerve injury in rat. Neurosci Lett 260:49-52, 1999

50. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-408, 2001

51. Lossos IS, Czerwinski DK, Wechser MA, Levy R: Optimization of quantitative real-time RT-PCR parameters for the study of lymphoid malignancies. Leukemia 17:789-795, 2003

52. Lu Y, Chen C, Kallakuri S, Patwardhan A, Cavanaugh JM: Neurophysiological and biomechanical characterization of goat cervical facet joint capsules. J Orthop Res 23:779-787, 2005

53. Lu Y, Chen C, Kallakuri S, Patwardhan A, Cavanaugh JM: Neural response of cervical facet joint capsule to stretch:

A study of whiplash pain mechanism. Stapp Car Crash J 49: 49-65, 2005

54. Luan F, Yang KH, Deng B, Begeman PC, Tashman S, King AI: Qualitative analysis of neck kinematics during low-speed rear-end impact. Clin Biomech 15:649-657, 2000

55. Ma W, Bisby MA: Increase of preprotachykinin mRNA and substance P immunoreactivity in spared dorsal root ganglion neurons following partial sciatic nerve injury. Eur J Neurosci 10:2388-2399, 1998

56. Malmberg AB, Basbaum AI: Partial sciatic nerve injury in the mouse as a model of neuropathic pain: Behavioral and neuroanatomical correlates. Pain 76:215-222, 1998

57. Manchikanti L, Pampati V, Fellows B, Bakhit CE: Prevalence of lumbar facet joint pain in chronic low back pain. Pain Physician 2:59-64, 1999

58. Mapp PI, Kidd BL, Gibson SJ, Terry JM, Revell PA, Ibrahim NB, Blake DR, Polak JM: Substance P-, calcitonin gene-related peptide- and C-flanking peptide of neuropeptide Y-immunoreactive fibres are present in normal synovium but depleted in patients with rheumatoid arthritis. Neuroscience 37:143-153, 1990

59. Marriott I: The role of tachykinins in central nervous system inflammatory responses. Front Biosci 9:2153-2165, 2004

60. Massaad CA, Safieh-Garabedian B, Poole S, Atweh SF, Jabbur SJ, Saade NE: Involvement of substance P, CGRP and histamine in the hyperalgesia and cytokine upregulation induced by intraplantar injection of capsaicin in rats. J Neuroimmunol 153:171-182, 2004

61. Michael GJ, Averill S, Shortland PJ, Yan Q, Priestley JV: Axotomy results in major changes in BDNF expression by dorsal root ganglion cells: BDNF expression in large trkB and trkC cells, in pericellular baskets, and in projections to deep dorsal horn and dorsal column nuclei. Eur J Neurosci 11:3539-3551, 1999

62. Munglani R, Harrison SM, Smith GD, Bountra C, Birch PJ, Elliot PJ, Hunt SP: Neuropeptide changes persist in spinal cord despite resolving hyperalgesia in a rat model of mononeuropathy. Brain Res 743:102-108, 1996

63. Noguchi K, Kowalski K, Traub R, Solodkin A, Iadarola MJ, Ruda MA: Dynorphin expression and Fos-like immunoreactivity following inflammation induced hyperalgesia are colocalized in spinal cord neurons. Brain Res Mol Brain Res 10:227-233, 1991

64. Ohtori S, Takahasi K, Moryia H: Inflammatory pain mediated by a phenotypic switch in brain-derived neurotrophic factor-immunoreactive dorsal root ganglion neurons innervating the lumbar facet joint in rats. Neurosci Lett 323: 129-132, 2002

65. Ohtori S, Takahashi K, Moriya H: Calcitonin gene-related peptide immunoreactive DRG neurons innervating the cervical facet joints show phenotypic switch in cervical facet injury in rats. Eur Spine J 12:211-215, 2003

66. Ono K, Kaneoka K, Wittek A, Kajzer J: Cervical injury mechanism based on the analysis of human cervical vertebral motion and head-neck-torso kinematics during low speed rear impacts. Stapp Car Crash J 41:339-356, 1997

67. Panjabi MM, Cholewicki J, Nibu K, Grauer JN, Vahldiek M: Capsular ligament stretches during in vitro whiplash simulations. J Spinal Disord 11:227-232, 1998

68. Pearson AM, Ivancic PC, Ito S, Panjabi MM: Facet joint kinematics and injury mechanisms during simulated whiplash. Spine 29:390-397, 2004

69. Quinn KP, Lee KE, Ahaghotu CC, Winkelstein BA: Structural changes in the cervical facet capsular ligament: Potential contributions to pain following subfailure loading. Stapp Car Crash J 51:169-187, 2007

70. Ramer MS, Priestley JV, McMahon SB: Functional regeneration of sensory axons into the adult spinal cord. Nature 403:312-316, 2000

71. Ringkamp M, Eschenfelder S, Grethel EJ, Habler HJ, Meyer RA, Janig W, Raja SN: Lumbar sympathectomy failed to reverse mechanical allodynia- and hyperalgesia-like behavior in rats with L5 spinal nerve injury. Pain 79:143-153, 1999

72. Rothman SM, Kreider RA, Winkelstein BA: Spinal neuropeptide responses in persistent and transient pain following cervical nerve root injury. Spine 30:2491-2496, 2005

73. Rutkowski MD, Winkelstein BA, Hickey WF, Pahl JL, DeLeo JA: Lumbar nerve root injury induces central nervous system neuroimmune activation and neuroinflammation in the rat: Relationship to painful radiculopathy. Spine 27: 1604-1613, 2002

74. Sakuma Y, Ohtori S, Miyagi M, Ishikawa T, Inoue G, Doya H, Koshi T, Ito T, Yamashita M, Yamauchi K, Suzuki M, Moriya H, Takahashi K: Up-regulation of p55 TNF alpha-receptor in dorsal root ganglia neurons following lumbar facet joint injury in rats. Eur Spine J 16:1273-1278, 2007

75. Sluka KA, Westlund KN: Behavioral and immunohistochemical changes in an experimental arthritis model in rats. Pain 55:367-377, 1993

76. Snijdelaar DG, Dirksen R, Slappendel R, Crul BJP: Substance P. Eur J Pain 4:121-135, 2000

77. Staton PC, Wilson AW, Bountra C, Chessell IP, Day NC: Changes in dorsal root ganglion CGRP expression in a chronic inflammatory model of the rat knee joint: Differential modulation by rofecoxib and paracetamol. Eur J Pain 11:283-289, 2007

78. Sterling M, Jull G, Kenardy J: Physical and psychological factors maintain long-term predictive capacity post-whiplash injury. Pain 122:102-108, 2006

79. Takeda M, Tanimoto T, Nasu M, Ikeda M, Kadoi J, Matsumoto S: Activation of NK1 receptor of trigeminal root ganglion via substance P paracrine mechanism contributes to the mechanical allodynia in the temporomandibular joint inflammation in rats. Pain 116:375-385, 2005

80. Taylor BK, McCarson KE: Neurokinin-1 receptor gene expression in the mouse dorsal horn increases with neuropathic pain. J Pain 5:71-76, 2004

81. Vasquez E, Bar KJ, Ebersberger A, Klein B, Vanegas H, Schaible HG: Spinal prostaglandins are involved in the development but not the maintenance of inflammation-induced spinal hyperexcitability. J Neurosci 21:9001-9008, 2001

82. Vera-Portocarrero LP, Westlund KN: Attenuation of nociception in a model of acute pancreatitis by an NK-1 antagonist. Pharmacol Biochem Behav 77:631-640, 2004

83. Winkelstein BA, Santos DG: An intact facet capsular ligament modulates behavioral sensitivity and spinal glial activation produced by cervical facet joint tension. Spine 33:856-862, 2008

84. Yamashita T, Cavanaugh JM, el-Bohy AA, Getchell TV, King AI: Mechanosensitive afferent units in the lumbar facet joint. J Bone Joint Surg Am 72:865-870, 1990

85. Yezierski RP, Yu CG, Mantyh PW, Vierck CJ, Lappi DA: Spinal neurons involved in the generation of at-level pain following spinal injury in the rat. Neurosci Lett 361:232-236, 2004

86. Yoganandan N, Cusick JF, Pintar FA, Rao RD: Whiplash injury determination with conventional spine imaging and cryomicrotomy. Spine 26:2443-2448, 2001

87. Yoganandan N, Pintar FA, Klienberger M: Cervical spine vertebral and facet joint kinematics under whiplash. J Biomech Eng 120:305-307, 1998

88. Zhang GH, Min SS, Lee KS, Back SK, Yoon SJ, Yoon YW, Kim YI, Na HS, Hong SK, Han HC: Intraarticular pretreatment with ketamine and memantine could prevent arthritic pain: Relevance to the decrease of spinal c-fos expression in rats. Anesth Analg 99:152-158, 2004

89. Zhang Z, Zhang ZY, Fauser U, Schluesener HJ: Mechanical allodynia and spinal up-regulation of P2X4 receptor in experimental autoimmune neuritis rats. Neuroscience 152: 495-501, 2008

90. Zhao C, Chen L, Tao YX, Tall JM, Borzan J, Ringkamp M, Meyer RA, Raja SN: Lumbar sympathectomy attenuates cold allodynia but not mechanical allodynia and hyperalgesia in rats with spared nerve injury. J Pain 8:931-937, 2007

91. Zimmermann M: Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16:109-110, 1983