Capsular Ligament Involvement in the Development of Mechanical Hyperalgesia after Facet Joint Loading: Behavioral and Inflammatory Outcomes in a Rodent Model of Pain

Kathryn E. Lee, 1 Martin B. Davis, 2 and Beth A. Winkelstein 1,2

Abstract
Whiplash injury can produce pain in the neck, arm, and hand, and has been associated with inflammation. However, the relationship between inflammatory responses and pain symptoms remains unknown, hindering the development of appropriate therapeutics for whiplash symptoms. Two joint loading paradigms were used separately in an established rat model of painful cervical facet joint distraction to apply: (1) gross failure, and (2) subfailure distraction of the facet capsular ligament. Behavioral outcomes were compared to determine whether more severe mechanical loading produces greater pain by measuring mechanical hyperalgesia in the shoulder and forepaws. Inflammatory mediators (glia and cytokines) were quantified in the spinal cord and dorsal root ganglion (DRG) after injury. Subfailure loading produced sustained hyperalgesia in the shoulder and forepaw that was significantly greater ($p < 0.042$) than sham, while an induced capsule failure produced only transient, yet significant ($p < 0.021$), mechanical hyperalgesia. The absence of hyperalgesia after ligament failure suggests this type of injury may interrupt nociceptive input from the capsule, which is likely necessary to produce sustained pain symptoms. Glial mRNA was significantly increased ($p < 0.043$) in the spinal cord after ligament failure, but remained unchanged in the DRG. Cytokine mRNA levels in the spinal cord and DRG were also significantly elevated after facet ligament failure, but not after painful subfailure loading. Findings suggest that different joint loading scenarios produced varied inflammatory responses in the CNS. These data support existing clinical reports suggesting that therapeutic interventions directed at the facet capsule may be effective in treating this painful injury.

Key words: cytokines; facet; glia; neck; pain

Introduction
Clinical studies identify the cervical facet joint as the source of pain in up to 62% of chronic neck pain patients (Aprill et al., 1992; Barnsley et al., 1994). Anesthetic nerve blocks of painful facet joints provide relief for both whiplash-induced and idiopathic neck pain (Barnsley et al., 1993, 1994, 1995; Bogduk et al., 1988; Lord et al., 1996a). Injurious loading to the cervical facet joint and its capsular ligament are common mechanisms producing neck pain, and can occur from whiplash and/or other non-contact neck injuries (Barnsley et al., 1993, 1994, 1995; Lord et al., 1996a). Biomechanical studies strongly suggest that the capsular ligament is at risk for painful mechanical injury because it undergoes excessive stretching during many neck loading scenarios (Deng et al., 2000; Kaneoka et al., 1999; Luan et al., 2000; Ono et al., 1997; Panjabi et al., 1998; Pearson et al., 2004; Stemper et al., 2005; Sundararajan et al., 2004; Yoganandan et al., 1998, 2001, 2002). Despite clinical and biomechanical evidence suggesting the facet joint and its ligament as producing neck pain, the mechanisms contributing to facet joint injury and the associated physiologic sequelae for this painful syndrome remain largely inferential.

Neck pain is the most commonly reported symptom after whiplash; pain is typically most severe at the back of the neck but can radiate to the head, shoulder, or arm, and into the hand or trunk (Barnsley et al., 1994; Kasch et al., 2001; Koelbaek Johansen et al., 1999; Mayou et al., 1996; Radianov

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et al., 1993, 1995; Sturzenegger et al., 1994, 1995). Whiplash patients also demonstrate sensitivity to pressure in the neck, shoulder, and into the arm and leg (Greening et al., 2005; Kasch et al., 2001; Scott et al., 2005; Sterling et al., 2003, 2004, 2006; Sterner et al., 2001; Banic et al. (2004) and Curatolo et al. (2001) reported reduced electrical stimulation thresholds in the neck, arm, and leg in whiplash patients. These studies suggest that the central nervous system (CNS) may be responsible for the type and distribution of symptoms. However, testing this hypothesis requires an in vivo model to simultaneously investigate behavioral hypersensitivity (pain symptoms) and cellular and molecular responses in the CNS. There are no relevant assessments to measure behavioral sensitivity in the neck and shoulder region in rodents in the context of spinal injury.

Our lab previously developed an in vivo model of persistent facet joint mediated pain in the rat. Significant behavioral hypersensitivity in the forepaws is produced after a facet distraction below capsule failure, persisting for nearly 4 weeks (Rothman et al., 2008). Mechanical allodynia depends on the magnitude of stretch that is applied to the capsular ligament (Lee et al., 2004b, c), suggesting that the capsule may transduce mechanical stimuli and contribute to pain. In contrast, no forepaw mechanical allodynia is produced if the same distraction is applied to the facet joint after transecting the capsular ligament (Winkelstein et al., 2008), further supporting the hypothesis that an intact capsule is necessary for development of allodynia. However, capsular ligament ruptures have been produced in cadaveric whiplash simulations (Yoganandan et al., 2001); that type of capsule injury has the potential to lead to clinical instability and/or increased flexibility in the spine after whiplash (Herkowitz et al., 1984; Panjabi et al., 2006; Pearson et al., 2005). Moreover, Lu et al. (2005) reported prolonged neural afterdischarges in dorsal nerve roots after facet joint distractions sufficient to produce capsule tears in a goat model. Those authors hypothesized that such robust afterdischarges may initiate prolonged changes in spinal excitability that could ultimately develop into persistent pain. However, it remains to be seen if facet capsule failure is indeed a more severe injury than some subfailure joint loading cases in terms of behavioral outcomes or associated inflammatory responses.

Proinflammatory cytokines contribute to persistent pain. Cytokines are synthesized by astrocytes, microglia, and macrophages, and can induce activation and/or expression of a host of pain mediators throughout the nervous system (Czeschik et al., 2008; DeLeo et al., 1996, 1997; Guo et al., 2007; Scholz et al., 2007; Sommer et al., 2004; Sweitzer et al., 1999). Cytokines can modulate neuronal activity, induce the release of other additional cytokines, and elicit prostaglandin production in non-neuronal cells (Bianchi et al., 1998; Scholz et al., 2007; Sommer et al., 2004), sensitizing primary afferents following injury and facilitating pain. Whiplash patients exhibit elevated levels of blood mononuclear cells expressing the cytokines IL-6, IL-10, and RANTES (Kivioja et al., 2001a,b), as well as increases in IL-6 protein in the trapezius muscle (Gerdel et al., 2008), implicating cytokines as potentially important contributors to whiplash-related pain. Miyagi et al. (2006) demonstrated that lumbar facet joint incision in the rat upregulates glial TNF-α in the associated dorsal root ganglion (DRG), supporting the involvement of inflammatory cells and cytokines in the DRG after painful facet joint injury. Despite the known role of cytokines in persistent pain and the limited characterization of cytokine responses in facet injury, no study has explicitly quantified inflammatory responses of glial cells and cytokines in the context of the pathogenesis of painful facet-mediated injury.

This study investigated the hypothesis that a more severe mechanical injury, such as capsular ligament failure, produces more severe pain outcomes and associated inflammation. Two groups were used to test this hypothesis: in one group, the facet joint was loaded until gross ligament failure was produced to mimic the extreme injury scenarios in which ligament integrity is not maintained. In a second group, the joint was loaded to a subfailure level, maintaining ligament integrity, but producing pain (Lee et al. 2004b, c). Sensitivity in the neck and shoulders was evaluated as a novel behavioral assessment to quantify pain symptoms for a clinically-relevant symptom presentation. Mechanical hyperalgesia was measured in anatomic regions mimicking those monitored in clinical studies—in the region of the back of the neck and shoulders—as well as in the forepaw. In addition, it was also hypothesized that inflammatory responses in the spinal cord and DRG are associated with the persistence of pain symptoms. As such, mRNA levels for inflammatory cytokines (tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6)) and glial and macrophage markers (glial fibrillary acidic protein (GFAP), integrin alpha M (ITGAM)) were quantified in the DRG and spinal cord at day 7 after joint loading in both groups.

Methods

All experimental procedures were approved by our Institutional Animal Care and Use Committee and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmermann, 1983). Male Holtzman rats (421 ± 16 g) were housed under USDA- and AAALAC-compliant conditions, with free access to food and water.

General surgical procedures

All surgical procedures were performed under inhalation isoflurane anesthesia (4% for induction, 2.5% for maintenance). Methods to impose controlled bilateral cervical facet joint distraction in the rat have been previously described (Lee et al., 2004b,c). The C6/C7 facet joints underwent bilateral tensile joint distraction imposed using a customized loading device. Following surgery, wounds were closed.

To investigate the hypothesis that capsule failure produces more severe behavioral and inflammatory responses than subfailure loading of the capsule, two loading paradigms were used. A subfailure distraction of 0.7 mm was imposed (n = 6, subfailure); joint distractions between 0.6—0.9 mm have been shown to reliably elicit persistent mechanical alldynia in the forepaws of the rat (Lee et al., 2004b,c; Rothman et al., 2008). In the second loading paradigm, joint distraction was imposed in a separate group of rats until the facet capsule was completely ruptured (n = 6, failure), modeling a potential injury scenario in which capsule integrity is disrupted during neck loading. Capsule failure was identified by mechanical evidence of decreased tensile force with continued distraction, and was confirmed by visual inspec-
tion of a capsule tear. Sham surgeries were also performed (n=6, sham) to control for the physiologic effects of surgery, exposing the joints and attaching to the loading device, but with no joint distraction.

**Mechanical hyperalgesia assessment**

Mechanical hyperalgesia was assessed in each rat in the areas of the right and left shoulders (Fig. 1) and in both forepaws (Lee et al., 2004b), mimicking testing methods used in clinical studies assessing pressure pain thresholds in the back of the neck, shoulders, and the arm (Koelbaek Johansen et al., 1999; Scott et al., 2005; Sterling et al., 2003, 2004, 2006). These anatomical regions are innervated by the same spinal nerves that innervate the C6/C7 facet joint and capsule (Takahashi et al., 1996). Mechanical hyperalgesia thresholds were measured using a modified version of Chaplan’s up-down method (Chaplan et al., 1994; Decosterd et al., 2000; Hubbard et al., 2005); thresholds were measured separately for each of the left and right shoulders and forepaws using a series of von Frey filaments (Stoelting; Wood Dale, IL). Hyperalgesia measurements were taken repeatedly prior to surgery to obtain baseline thresholds for each region as a measure of normal sensitivity, and also on postoperative days 1, 3, 5, and 7. Each testing session comprised of three rounds of five stimulations at each location (right shoulder, left shoulder, right forepaw, left forepaw) using a series of ascending filament strengths (0.4—26.0 g) (Hubbard et al., 2005). For each round, the shoulders were tested first, then the forepaws, with the order of side tested randomized for each location. For each location, the first filament to elicit a positive response was recorded as the threshold if the next larger filament also evoked a positive response. For shoulder stimulations, a positive response was counted when the rat vigorously shook or moved its shoulder upon stimulation. For the forepaw, a positive response was counted as an emphatic lifting of the paw (Chaplan et al., 1994; Decosterd et al., 2000; Hubbard et al., 2005). Failure to respond to any of the filaments was recorded as a threshold value of the maximum filament strength (26.0 g). The average threshold over the three rounds was recorded for each location for each rat.

**Mechanical data and image analysis**

Mechanical data describing facet joint kinematics were measured to verify that similar loading was imposed for all rats in the subfailure group and to evaluate whether those distractions were mechanically less severe than those imposed for the failure group. Force and joint displacement data acquisition methods have been previously described (Lee et al., 2004b, 2006). Image data were acquired during joint distraction and synchronized with the mechanical data; joint distraction was defined as the maximum relative displacement of the C6 and C7 laminae (Lee et al., 2004b, 2006). Force data were combined with the joint displacement data to identify capsule failure for the failure group (Lee et al., 2006) (Fig. 2).

**Tissue processing and real-time polymerase chain reaction**

Real-time polymerase chain reaction (PCR) was performed on spinal cord and DRG tissue to define glial and cytokine mRNA levels following facet joint injury. After behavioral assessment on day 7, rats were deeply anesthetized with sodium pentobarbital (65 mg/kg) and transcardially perfused with phosphate buffered saline. The right half of the C6 spinal cord segment was harvested, and immediately frozen on dry ice. Likewise, the right C7 DRG was identified, harvested and frozen.

Total RNA was extracted using the TRIzol® isolation system (Life Technologies, Rockville, MD) according to manufacturer instructions. Total RNA concentration and quality were measured using a NanoDrop spectrometer (NanoDrop Technologies, Wilmington, DE). Total RNA (2 μg) was used for reverse transcription with the MultiScribe™ Reverse Transcriptase system (Applied Biosystems, Foster City, CA). Single-strand synthesized cDNA was used for real-time PCR.
Primers were custom-designed and synthesized (Applied Biosystems); primer sequences for glial and macrophage markers (GFAP, ITGAM) and pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) probed in these studies are listed in Table 1. SYBR-Green® 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. Each sample was run in duplicate. Samples were denatured at 95°C for 10 min, followed by 40 cycles of amplification at 95°C for 15 sec, and annealed at 60°C for 60 sec. For each sample, the target gene expression was separately normalized to the corresponding housekeeping gene (CyA) expression in that sample using the ΔΔCt method (Livak et al., 2001) and reported as the fold-difference in expression relative to normal (uninjured) control tissue (n = 2 rats). Fold-differences were measured for each target gene for each tissue sample (DRG, spinal cord) and averaged across each injury group (subfailure, failure, sham).

Statistical analysis

All statistical analyses were performed using SYSTAT (Richmond, CA), and significance was defined as p < 0.05. Joint distraction between the subfailure and failure groups was compared using a t-test. Mechanical hyperalgesia was compared between anatomical sides using paired t-tests to test for asymmetry in each region for all rats. A two-way ANOVA with post-hoc Bonferroni correction compared response thresholds at each anatomic region to account for the effect of injury group (subfailure, failure, sham) over time. mRNA levels for each gene were compared between failure and subfailure groups using a Student’s t-test.

**Results**

After surgery, all rats demonstrated normal functioning with grooming, consistent weight gain, and good head mobility, indicating that surgical procedures produced no adverse effects on neck mobility. The mean vertebral distraction for the subfailure group was 0.72 ± 0.02 mm. The failure group underwent 1.26 ± 0.63 mm of distraction before the capsular ligament ruptured; joint displacement at failure was significantly greater (p = 0.033) than subfailure displacements. For all capsule failures, force increased steadily with applied joint displacement until it abruptly dropped off.

![FIG. 2. A representative loading trace showing C6 displacement during joint distraction, as measured by an LVDT, and resultant force across the C6/C7 facet joint. Facet capsular ligament failure is indicated on the trace by an arrow.](image-url)

**Table 1. Specific Primer Sequences for Reverse Transcription–Polymerase Chain Reaction (RT-PCR) Assays**

<table>
<thead>
<tr>
<th>Gene</th>
<th>NCBI accession number</th>
<th>Forward sequence</th>
<th>Reverse sequence</th>
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<tbody>
<tr>
<td>GFAP</td>
<td>NM 017009</td>
<td>5′-CATCGAGATCGGCCACCTACA-3′</td>
<td>3′-GGAATGGTGATCGCTTTC-5′</td>
</tr>
<tr>
<td>ITGAM</td>
<td>NM 012711</td>
<td>5′-GCTGAATGGAAGGACAAAAACTG-3′</td>
<td>3′-GGCCCCATTGATTTTCTGAA-5′</td>
</tr>
<tr>
<td>TNF-α</td>
<td>NM 012675</td>
<td>5′-ATCATCTTTCACACCTCGATGAC-3′</td>
<td>3′-CTGCTCTCTCTCTCTCTCT-5′</td>
</tr>
<tr>
<td>IL-1β</td>
<td>NM 031512</td>
<td>5′-CACCTCTCAAGCAGAGCACAG-3′</td>
<td>3′-GGTTCTGATGGTGAAGTCAAC-5′</td>
</tr>
<tr>
<td>IL-6</td>
<td>NM 012589</td>
<td>5′-AGTTCGGAGGCTTAAATTACATATGTC-3′</td>
<td>3′-GCCATTGCAAACTCGAGTCAA-5′</td>
</tr>
<tr>
<td>CyA</td>
<td>NM 017101</td>
<td>5′-TACGTGACTGCAAGGACTG-3′</td>
<td>3′-CTTCTTGTGCTGATCCATTCC-5′</td>
</tr>
</tbody>
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GFAP, glial fibrillary acidic protein; ITGAM, integrin alpha M; TNF, tumor necrosis factor; IL, interleukin; CyA, cytokine A.
when the capsular ligament failed (Fig. 2). The average force at capsule failure (7.74 ± 2.14 N) was significantly greater \((p = 0.003)\) than the corresponding force supported by the ligament for subfailure distraction (3.48 ± 0.47 N).

Stimulation thresholds for mechanical hyperalgesia were not significantly different between the right and left shoulders, or the right and left forepaws in any injury group; those data were averaged for each anatomic region (shoulder, forepaw) for each rat. Response thresholds for stimulation in both anatomic regions remained high for sham and not different from baseline (unoperated) responses at any time point (Fig. 3). In contrast, the subfailure group exhibited an immediate reduction in response thresholds for the shoulder and forepaw that persisted over the evaluation period (Fig. 3). The response threshold for subfailure was significantly less than for sham at all time points for the shoulder \((p < 0.042)\) and forepaw \((p < 0.004)\) regions (Fig. 3).

Failure of the facet capsule during distraction did not produce a persistent reduction in response threshold in either the shoulder or forepaw (Fig. 3). Transient reductions in response thresholds were produced in both regions that were significantly less \((p < 0.025)\) than sham only on days 1 and 3 (Fig. 3). Mechanical hyperalgesia for failure returned to sham levels, and was significantly different from subfailure on days 5 and 7 in the shoulder \((p < 0.020)\), and on days 3, 5, and 7 in the forepaw \((p < 0.021; Fig. 3)\).

**FIG. 3.** 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) Subfailure loading significantly reduced \((p < 0.004)\) shoulder thresholds below sham for all days, and below failure \((p < 0.020)\) on days 5 and 7. Capsule failure significantly decreased \((p < 0.002)\) thresholds below sham on days 1 and 3. (B) Likewise, subfailure loading significantly decreased \((p < 0.004)\) forepaw thresholds below sham for all days and below failure \((p < 0.021)\) on days 3, 5, and 7. Failure thresholds were significantly \((p < 0.025)\) lower than sham on days 1 and 3.
The glial markers and cytokines probed here were detected in the spinal cord of all groups. In all cases, mRNA levels for sham were not significantly different from normal levels. Both GFAP and ITGAM mRNA levels increased for each distraction group (subfailure, failure) compared to sham, but this increase was significant (\( p = 0.010 \) for GFAP, \( p = 0.043 \) for ITGAM) only for failure (Fig. 4). While spinal genes for glial activation were greater for failure than for painful subfailure, this increase was significant only for GFAP (\( p = 0.025 \); Fig. 4). Spinal TNF-\( \alpha \) mRNA levels followed the same trend; both failure and subfailure were slightly elevated above sham, with failure producing a significant increase (\( p = 0.043 \)) over sham (Fig. 5A). In contrast, IL-1\( \beta \) mRNA in the spinal cord did not show the same graded response for capsule injury. IL-1\( \beta \) mRNA after failure was significantly greater (\( p = 0.045 \)) than for subfailure joint loading and was nearly twice that for sham (Fig. 5A). There was no difference between any group for spinal IL-6 mRNA, which displayed considerable variability between individual responses in all groups.

Genes probed in this study were also detected in the DRG of all groups. However, there was no significant difference in expression of either glial marker between any of the groups (data not shown). Moreover, cytokine mRNA levels in sham were not significantly different from normal DRG levels. TNF-\( \alpha \) mRNA levels in the DRG followed similar trends as observed in the spinal cord; TNF-\( \alpha \) mRNA levels in the DRG for both injuries were greater than sham (Fig. 5B). However, there was no significant difference between groups, despite failure being the highest. Both IL-1\( \beta \) and IL-6 mRNA levels were lower following a subfailure distraction than either failure or sham (Fig. 5B). This difference between failure and subfailure was significant (\( p = 0.049 \)) for IL-6. Overall, across all tissues and target genes, capsule failure produced during loading resulted in the greatest variability in responses (Figs. 4 and 5).

Discussion

The behavioral findings of this in vivo model demonstrate that subfailure distraction of the facet joint and its capsular ligament can produce mechanical hyperalgesia that mimics symptom presentation observed in whiplash and neck pain (Fig. 3). Data support that the persistence of mechanical hyperalgesia depends on whether the capsular ligament is kept intact during and after facet joint loading (Fig. 3). If the capsule is ruptured during loading, hyperalgesia in both the shoulder and forepaw is only transient and short-lasting (Fig. 3), suggesting that continuous sensory input from an intact capsule may be necessary for sustained pain symptoms. Both types of facet joint injuries (failure, subfailure), that produced persistent and transient hyperalgesia, are sufficient to slightly increase gene transcription profiles of glial markers (GFAP, ITGAM) in the spinal cord (Fig. 4). In addition, transcription of three pro-inflammatory cytokines, TNF-\( \alpha \), IL-1\( \beta \), and IL-6, is altered in both the spinal cord and DRG (Fig. 5). In general, different trends in expression were observed for each type of injury and across all inflammatory markers, suggesting a complicated cellular cascade for each injury and each symptomatic outcome. The combination of behavioral and gene expression data presented here suggests that different signals from the capsule are required to maintain pain after facet joint distraction injury, and that inflammatory responses may in part influence the transient mechanical hyperalgesia observed following facet capsule failure for this injury model.

Mechanical hyperalgesia is produced in both the shoulder and the forepaw following facet joint distraction in this model (Fig. 3). Clinical studies show that whiplash patients also present with pain in these regions (Barnsley et al., 1994; Kasch et al., 2001; Koelbaek Johansen et al., 1999; Mayou et al., 1996; Radanov et al., 1993, 1995; Sturzenegger et al., 1994, 1995). Anatomically widespread sensitivity is characteristic of whiplash patients, in particular, and not normally seen for those patients whose neck pain is idiopathic (Scott et al., 2005). Given this distinction, our demonstration of shoulder pain following facet-mediated injury suggests that this model has utility in examining whiplash-specific neck pain mechanisms. Although neck pain is a primary symptom of whiplash, we chose to measure hyperalgesia in the shoulder region instead, in order to avoid the potential confound of detecting primary hyperalgesia due to a surgical incision (Duarte et al., 2005), because the shoulder is within the same region instead, in order to avoid the potential confound of detecting primary hyperalgesia due to a surgical incision (Duarte et al., 2005), because the shoulder is within the same

FIG. 4. Quantification of GFAP and ITGAM mRNA in the spinal cord at day 7 after injury. Failure significantly increased levels of both GFAP (\( \# p = 0.010 \)) and ITGAM (\( \# p = 0.043 \)) mRNA over sham. Subfailure GFAP mRNA was significantly lower than (\( \dagger p = 0.025 \)) failure. A value of 1 indicates normal (uninjured) mRNA levels for both markers.
dermatome as the C6/C7 facet joint (Takahashi et al., 1996), and whiplash patients also routinely experience pain and sensitivity in the shoulder (Barnsley et al., 1994; Kasch et al., 2001; Koelbaek Johansen et al., 1999; Mayou et al., 1996; Radanov et al., 1993, 1995; Sturzenegger et al., 1994, 1995).

The absence of shoulder responses in sham (Figs. 1 and 3) supports that this region is sufficiently far from the surgical incision to avoid either primary or secondary hyperalgesia due to surgery. The prolonged sensitivity in the shoulder and forepaw regions demonstrates that mechanical hyperalgesia is uniform throughout a dermatomal region and also validates the use of forepaw hyperalgesia as a measure of sensitivity following certain cervical spine injuries.

Loading the capsule until it undergoes mechanical failure produces only transient hyperalgesia (Fig. 3), while persistent mechanical hyperalgesia is observed only for loading that remains below tissue failure. This is the first demonstration that joint loading severe enough to actually rupture the capsule does not produce sustained behavioral hypersensitivity (i.e., pain symptoms). These data suggest that joint loading that produces capsule rupture, while a more severe mechanical injury, may not independently produce more extensive or worse pain symptoms; in contrast, less severe loading scenarios to the capsule without its rupture may actually produce greater and longer-lasting symptoms. Taken together, these behavioral data may help explain the variability in pain symptom extent and duration reported for individuals undergoing similar whiplash exposures. These findings support existing clinical data suggesting that eliminating sensory inputs in the facet may alleviate sensitivity.

Percutaneous radio-frequency neurotomy that eliminatesafferent signaling from symptomatic joints in whiplash patients with neck pain and can alleviate pain (Lord et al., 1995, 1996b). Up to 70% of patients receiving dorsal rami medial branch neurotomies for painful joints reported complete relief for up to two years (Lord et al., 1995, 1996b), and modulating sensory inputs to the joint even at time points sufficiently after an injury can be effective in providing temporary, yet complete, pain relief (Barnsley et al., 1993, 1995; Lord et al., 1995, 1996b). The behavioral assessments in the current study were performed for only 7 days, so long-term outcomes after capsule failure remain to be determined.

**FIG. 5.** Quantification of pro-inflammatory cytokine mRNA levels in the spinal cord (A) and DRG (B) at 7 days following injury. (A) Failure significantly increased TNF-α levels over sham (#p = 0.043) and IL1–β levels over subfailure (*)p = 0.045) in the spinal cord. (B) There was no significant difference between any group for either TNF-α or IL1–β mRNA in the DRG. There was a significant increase (*)p = 0.049) in IL-6 mRNA after failure compared to subfailure. The dashed line indicates mRNA levels in normal tissue for each cytokine.
However, our behavioral data support the hypothesis that sensory afferents in the capsule likely continuously relay nociceptive information to maintain a painful syndrome, and elimination of these afferents may potentially be an effective therapeutic approach for treating this syndrome.

Spinal glial activation has been demonstrated in rodent models of painful neuropathy and radiculopathy (Colburn et al., 1999; Hashizume et al., 2000; Hubbard et al., 2005; Liu et al., 2000; Sweitzer et al., 1999; Winkelstein et al., 2002), and leads to synthesis and secretion of proinflammatory mediators, including cytokines, nitric oxide, and prostaglandins. In the current study, the increase in spinal GFAP mRNA after a facet injury that does not produce persistent pain (i.e., failure) (Fig. 4) suggests that increases in spinal astrocytes alone may not be sufficient to maintain mechanical hyperalgesia. Our data extend previous findings with this model, in which spinal GFAP activation increased after painful subfailure loading (Lee et al., 2004b; Winkelstein et al., 2008); this activation of astrocytes was suggested to play a role in maintaining pain following facet injury. However, prolonged behavioral hypersensitivity was not observed after gross capsule rupture in the current study or in previous studies in which the capsule was transected after it underwent a painful distraction (Winkelstein et al., 2008), implying that ligament integrity must be maintained after injury in order to produce persistent pain. In contrast, the spinal ITGAM mRNA increases after failure of the capsule (Fig. 4) demonstrate greater production of microglia in the spinal cord following that mechanically severe injury; but, the absence of hyperalgesia in that case suggests that microglia production may not directly relate to pain in this model. Spinal microglial activation has been previously shown not to be different between painful and non-painful facet joint distractions at either day 7 or 14 post-injury (Lee et al., 2004b; Winkelstein et al., 2008), further supporting this notion. It is possible that for facet injury, neither production nor activation of spinal microglia is sufficient to maintain pain symptoms. Because glial mRNA levels are increased for loading-to-failure, despite the absence of persistent mechanical hyperalgesia, this may indicate that while the injury is perceived as being severe enough to initiate spinal responses, such responses may not specifically drive the pain outcomes in this model.

Proinflammatory cytokines are synthesized and released in painful tissue inflammation, peripheral nerve inflammation, peripheral and spinal nerve trauma, spinal cord inflammation, and spinal cord trauma (Bianchi et al., 1998; De Jongh et al., 2003; DeLeo et al., 2002; Flatters et al., 2003; Guo et al., 2007; Hashizume et al., 2000; Lee et al., 2004a; Marchand et al., 2005; Rothman et al., 2005; Rutkowski et al., 2002; Schafers et al., 2003a; Sommer et al., 2004; Sweitzer et al., 1991, 2001; Watkins et al., 1995; Wieseler-Frank et al., 2004). Cytokine release can modulate the activity of neurons, macrophages, prostaglandins, and cytokines, all of which help to sensitize primary afferents and facilitate pain (Bianchi et al., 1998; Scholz et al., 2007; Sommer et al., 2004). In general, the current data show an increase in cytokine mRNA in both the spinal cord and DRG after capsule failure, despite the absence of prolonged mechanical hyperalgesia (Figs. 3 and 5). In particular, the relative increase in spinal IL-1β and IL-6 in the DRG after capsule failure over subfailure suggests that these two cytokines may be sensitive to the magnitude and extent of tissue injury, with greater injury initiating greater cytokine transcription. While cytokine expression has been correlated with allodynia and hyperalgesia in neuropathic and radiculopathic pain models (George et al., 1999; Schafers et al., 2003a,b; Sweitzer et al., 2001), the present data suggest that for painful joint injury, such spinal cytokine regulation may not drive the maintenance of mechanical hyperalgesia. However, given the demonstrated cytokine involvement in other pain models, and knowing that cytokine antagonists can prevent pain symptom development (Lindenlaub et al., 2000; Schafers et al., 2003b; Sommer et al., 2001a,b), cytokines may be involved in mediating some component of the pain cascade following facet injury. Studies of other tissues, time points, cytokines, and cell-specific responses would provide additional insight. While subfailure distraction of the capsule is sufficient to produce prolonged mechanical hyperalgesia, it did not elicit robust cytokine responses in either tissue probed. However, this study quantified mRNA only, and not protein expression, and only for one time point; it is possible that the induction of cytokine gene transcription and/or protein release for this painful subfailure injury may have been missed. TNF-α mRNA peaks in the DRG at 1–3 days after chronic nerve constriction (Lee et al., 2004a) while IL-6 peaks at 1 day (Murphy et al., 1995). In addition, certain of these inflammatory responses may be localized to the facet joint and may be more robust at that site as well. Certainly, clinical studies report increased cytokine levels in the cartilage and synovial fluid of joints in facet disorders (Igarashi et al., 2004), implicating local cytokines in the pathogenesis of painful joint injury. In conjunction with those clinical data, the combination of DRG and spinal cord cytokine data presented here suggests inflammatory mechanisms may play a role in mediating some components of pain that present following certain facet-mediated injuries.

Profiles of several cytokines have been characterized in limited studies of whiplash patients. Kivioja et al. (2001a,b) reported increased mononuclear cells in blood expressing TNF-α, IL-6, IL-10, and the chemokine RANTES in whiplash patients. Also, interlestitial concentrations of IL-6 in the trapezius muscle are elevated in whiplash patients (Gerdle et al., 2008). Despite the suggestion of local and widespread inflammation in this injury, there is currently no effective pharmacologic method to treat whiplash-related pain. There is conflicting evidence on the prolonged efficacy of steroid and botulinum toxin injections (Braker et al., 2008; Freund et al., 2002; Juan, 2004; Padberg et al., 2007) into tender muscles after whiplash. Injection of anesthetic agents into symptomatic joints generally relieves pain, but only for a few months (Bogduk et al., 1988; Barnsley et al., 1993, 1994, 1995). Integrating clinical reports of cytokine modulation following whiplash injury with our data, the cytokine family is a likely contributor to whiplash-related neck pain, and local delivery of cytokine antagonists may be a useful method for relief in this painful syndrome. Further studies are needed to determine the extent to which the peripheral and central immune responses specifically contribute to this pathologic state, and to define relevant mechanistic pathways in the context of developing therapeutic interventions.

While many earlier reports have implicated the facet capsule in transmitting nociceptive signals to the CNS, this study supports the capsule’s direct involvement in pain following joint injury by demonstrating that joint distractions creating
capsule failure produce only transient hyperalgesia. This behavioral outcome implies that continued capsule integrity may be necessary to produce persistent pain. Loading the capsular ligament to its mechanical failure increases cytokine mRNA levels in the spinal cord and DRG, despite an absence of behavioral hypersensitivity. Additionally, IL-6 mRNA may potentially be involved in pain production and/or maintenance following painful subfailure joint loading (Fig. 5), as this cytokine exhibits the greatest increase (though not significant) in both the spinal cord and DRG. Further studies are necessary to fully characterize cytokine protein expression following facet injury and to determine the relationship between transcription and translation of relevant inflammatory mediators. Nonetheless, overall these results suggest a specific role for the facet capsular ligament in transducing mechanical facet joint distraction into inflammatory cascades and associated pain symptoms, and provide support for future investigations of therapeutic interventions that may be targeted towards the immune response.

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Author Disclosure Statement

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