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An Intact Facet Capsular Ligament Modulates Behavioral Sensitivity and Spinal Glial Activation Produced by Cervical Facet Joint Tension

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Study Design. *In vivo* experiments using a rat model of painful facet joint distraction.

Objective. To determine whether tension of the facet capsular ligament is requisite for producing pain for joint loading and to define effects on spinal glial activation.

Summary of Background Data. Cervical facet joint loading may initiate pain for certain conditions. While facet capsule tension has been proposed as requisite for pain, this hypothesis has not been tested.

Methods. Using an established rat model of painful C6–C7 distraction, tension was applied after transection of the left facet capsule; the right capsule remained intact. Each rat (n = 8) received the same distraction simultaneously applied across both the intact and cut capsules. Sham procedures were performed on separate rats (n = 4) with no joint distraction. Bilateral forepaw mechanical allodynia was measured as a pain outcome. Cervical spinal cord tissue (C7) was harvested on day 7 to detect glial reactivity using immunohistochemistry.

Results. Distraction mechanics were consistent with conditions eliciting persistent behavioral hypersensitivity. Allodynia was produced for an intact capsule and was significantly elevated over both the cut capsule ($P < 0.004$) and sham ($P < 0.002$). Transecting the capsule before distraction did not produce elevated allodynia, except on day 7. Spinal astrocytic reactivity paralleled allodynia; glial fibrillary acidic protein expression for an intact capsule was significantly greater than the cut and sham responses ($P < 0.04$), with no difference observed between the cut and sham spinal astrocytic reactivity. Spinal microglial activation did not differ between groups.

Conclusion. Results suggest ligament tension may be required to produce pain from facet joint loading. Further studies of other cellular responses are needed to define the mechanisms of painful facet joint injury.

Keywords: facet joint, pain, whiplash, glia, capsular ligament. **Spine 2008;33:856–862**

The cervical facet joint has been reported as the most common source of neck pain.[1–3] Although there are varied reports of the exact incidence of facet joint-mediated neck pain, this joint has been identified as the site of pain in 25% to 62% of neck pain cases.[3–5] In particular, injury to the facet joint and its capsule has been reported to have a role in whiplash and other painful neck injuries.[6–12] This is due in part to the ligament's risk for mechanical injury during spinal loading and vertebral motions. The dorsal and lateral aspects of the facet capsule undergo injurious motions and loading during whiplash[9,10,13,14]; but, cadaveric investigations have not determined a relationship between mechanical loading and the potential for pain. Those studies do provide strong mechanical evidence suggesting stretch of the facet capsular ligament as a mechanism by which pain is produced during certain motions for the cervical spine and the facet joints. Anesthetic nerve blocks of painful facet joints provide relief for whiplash-induced neck pain, further supporting a role for this joint as a source of pain.[3] However, it remains unknown whether loading to the facet capsule is a necessary condition to produce facet joint-mediated pain symptoms.

Both histologic and electrophysiological studies provide evidence of nerve fibers in the facet capsular ligament and suggest its potential to generate pain. Nerve fibers have been identified throughout the facet joint in the rat, rabbit and human, with pain fibers located in the capsular ligament.[15–23] Studies have also demonstrated activation of afferents for applied unspecified compression, tension and manipulation of the lumbar facet joint,[15–17,24,25] implying that neural inputs are generated by mechanical stimulation of the fibers in the facet capsule due to joint motions and ligament loading. Yet, while ligament stretch has been suggested as a requisite to initiate pain in facet-mediated pain syndromes, this hypothesis has not been investigated explicitly in the context of controlled joint mechanics or pain symptoms.

Despite growing evidence suggesting involvement of the cervical facet capsule in painful mechanical neck injuries, no study has specifically investigated whether loading to the capsule itself is required to generate behavioral hypersensitivity due to joint distraction. Therefore, the goal of this study was to test whether painful facet joint distraction is transduced via stretching of the intact facet capsule. Using a cervical facet joint distraction known to produce persistent mechanical allodynia in an established pain model in the rat,[26,27] matched studies of both intact and transected facet capsules were performed. The resulting behavioral and spinal glial out-

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comes were compared to determine if capsule distraction is necessary for facet joint-mediated pain.

■ Methods

Experimental procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee and adhered to the guidelines of the Committee for Research and Ethical Issues of the IASP.[28] Male Holtzman rats (404 ± 17 g) were housed under USDA and AAALAC-compliant conditions with free access to food and water.

Surgical Procedures, Tensile Facet Loading and Analysis

All surgical procedures were performed under inhalation halothane anesthesia (4% induction, 2.5% maintenance). Surgical procedures were the same as previously described to impose a bilateral painful cervical facet joint distraction injury.[26,27] Briefly, bilateral cervical facet joints were exposed and controlled quasistatic distraction was applied across the C6/C7 joint via a customized loading device. The C7 spinous process was held fixed and C6 was distracted in the rostral direction away from C7. For each joint loading case, the C6 facet was distracted by 0.6 mm, held for 30 seconds, and returned to its initial position, unloading the facet joint. This displacement consistently produces persistent behavioral sensitivity, while not causing a failure of the ligament tissue.[26,27] For this study, tension was applied after transection of the dorsal aspect of the left facet capsule; the right joint remained intact. In this way, the same distraction was applied across both the right (intact) and left (cut) capsules for each rat (n = 8). Sham surgeries (n = 4) were performed separately with no joint distraction.

Biomechanical data were measured during joint loading. A micrometer rigidly coupled to C6 measured vertebral displacements. Acrylic black paint markings (diameter = 0.17 ± 0.01 mm) were applied to the C6 and C7 laminae for bony motion tracking and the vertebrae were imaged using a digital video camera (1280 × 1024 pixel; QImaging, B.C. Canada) during loading. From these images, displacement vectors and joint distractions were calculated. Maximal applied tensile force was measured; stiffness of the C6–C7 motion segment was quantified as the slope of the curve relating applied force to vertebral displacement, from 20 to 100% of maximal distraction.[29] Spinal rotation angles were calculated to quantify the symmetry of loading along the spinal long-axis. The alignment angle was quantified as the change in orientation of the line connecting the bony markings on C6 and C7. For a symmetrically applied distraction, this angle is close to zero (aligned with the spine's long-axis); in previous studies of bilaterally intact joints this angle was 2.3 ± 2.5°.[26]

Behavioral Testing

Mechanical allodynia was assessed to quantify behavioral hypersensitivity in both forepaws of each rat.[26,27,30,31] Two von Frey filaments (2 and 4 g; Stoelting Co., Wood Dale, IL) were used to measure mechanical allodynia, as the number of forepaw withdrawals elicited by a non-noxious mechanical stimulus. Allodynia was measured before injury and on each of postoperative days 1, 3, 5, and 7. Before injury, rats were acclimated to the testing environment and tested for 3 days and baseline (day 0) measurements were recorded. In each testing session, rats were subjected to 3 rounds, separated by 10 minutes each, of 10 tactile stimulations to the plantar surface of each of the right (intact) and left (cut) forepaws. A positive response was counted when the rat emphatically lifted its paw on stimulation, which was accompanied by licking or tightening of the paw. The average response for each of the cut and intact paws was determined for each day.

Immunohistochemistry

Glial activation was assessed in cervical spinal cord tissue (C7) harvested 7 days after surgery. Animals were deeply anesthetized and transcardially perfused with 200 mL of phosphate buffered saline (PBS) followed by 300 mL of 4% paraformaldehyde in PBS (pH 7.4). Tissue was harvested, postfixed in 4% paraformaldehyde for 20 minutes, transferred to 30% sucrose/PBS and stored for 3 days at 4°C. Serial C7 spinal cord sections (20 μm) from each rat underwent standard free-floating immunohistochemistry procedures.[30,32–34] A polyclonal antibody to glial fibrillary acidic protein (GFAP) (Dako, Carpinteria, CA) was used as a marker of activated astrocytes (1:20,000). A monoclonal antibody (OX-42) to CR3/CD11b (BD Pharmingen, San Diego, CA) served as a marker of activated microglia (1:500). Areas of activation were localized using the avidin-biotin technique (Vector Labs, Burlingame, CA). Normal naïve spinal cord (n = 3 rats) tissue and samples not incubated in primary antisera were included for normalization and control.

The dorsal horns (cut and intact) of 3–4 axial sections from each rat were imaged at 50X for analysis of immunostaining. Analysis was performed on the superficial laminae (LI-LIII) of each dorsal horn, corresponding to a pixel area of 300 × 700. For each image, contrast and brightness were uniformly adjusted using Adobe Photoshop v7.0 (Adobe, San Jose, CA); customized MATLAB code separately quantified the amount of GFAP and OX-42 reactivity as a percentage of the uniform dorsal horn area. For the sham group, sections were randomly selected from the right or left side of the dorsal horn. Averages of reactivity as a percentage of the superficial dorsal horn were determined for each of the intact, cut, and sham groups.

Statistical Analyses

To compare mechanical allodynia across all groups, a repeated analysis of variance with *post hoc* Bonferroni correction was used. Average GFAP and OX-42 reactivity were also compared between groups using an analysis of variance, with *post hoc* Bonferroni test. Statistical analyses were performed using SYSTAT (SYSTAT Software Inc., Richmond, CA); significance was defined at $P < 0.05$.

■ Results

For all cases of a cut capsule, it was verified that the capsule had been fully transected before vertebral distraction. At the completion of the applied distraction, the right capsule was verified as being intact by inspection under a microscope. The mechanical load-displacement data confirmed that no ligament failure or rupture occurred during any of the distractions. Joint mechanics were consistent with previously reported conditions known to produce persistent allodynia in this rat model.[26,27] Mean applied joint distraction was 0.66 ± 0.11 mm (Table 1). Mean distraction rate was 0.07 ± 0.02 mm/s; mean applied force was 2.48 ± 1.25 N and mean tensile stiffness was 1.10 ± 0.18 N/mm (Table 1). Despite a unilateral cut left capsule, distractions were primarily oriented along the long-axis of the spine, with the mean displacement vector 1.45 ± 0.62 degrees off the rostral-caudal axis

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Table 1. Summary of Mechanical Response Parameters of Distraction Tests

Specimen	Weight (g)	Vertebral Distraction (mm)	Maximum Tensile Load (N)	Tensile Stiffness (N/mm)	Alignment Angle* (°)
53†	416	0.64	—	—	1.75
54	380	0.84	2.48	1.11	1.40
55	408	0.56	1.68	1.11	−0.92
58	406	0.77	1.04	0.90	−2.24
59‡	398	N/A	1.11	0.87	N/A
63	404	0.68	3.41	1.13	−0.91
64	380	0.51	3.43	1.20	2.19
65	380	0.62	4.22	1.38	−0.77
Average (SD)	397 (15)	0.66 (0.11)	2.48 (1.25)	1.10 (0.18)	1.45 (0.62)

*(+) and (−) rotation angles are provided for individual specimens. The average angle was determined using the absolute value of each rotation angle magnitude.
†Load cell data were not available. Therefore, stiffness could not be calculated.
‡Video data were not available. Measurement of vertebral marker displacement was not possible. Forceps distractions for this test were consistent with other tests so applied tension across the joint was considered comparable.

(Table 1). This small angle deviation from the spine's long-axis indicates that the applied distraction was symmetric across both bilateral joint articulations. The individual alignment angles in this study (Table 1) were well within the range of previous studies of intact joints (−2.4–7 degrees) and had a lower magnitude and variation.[18]

Joint distraction produced increased mechanical allodynia in the forepaw on the side with the intact capsule (Figure 1). However, allodynia in the forepaw of the cut

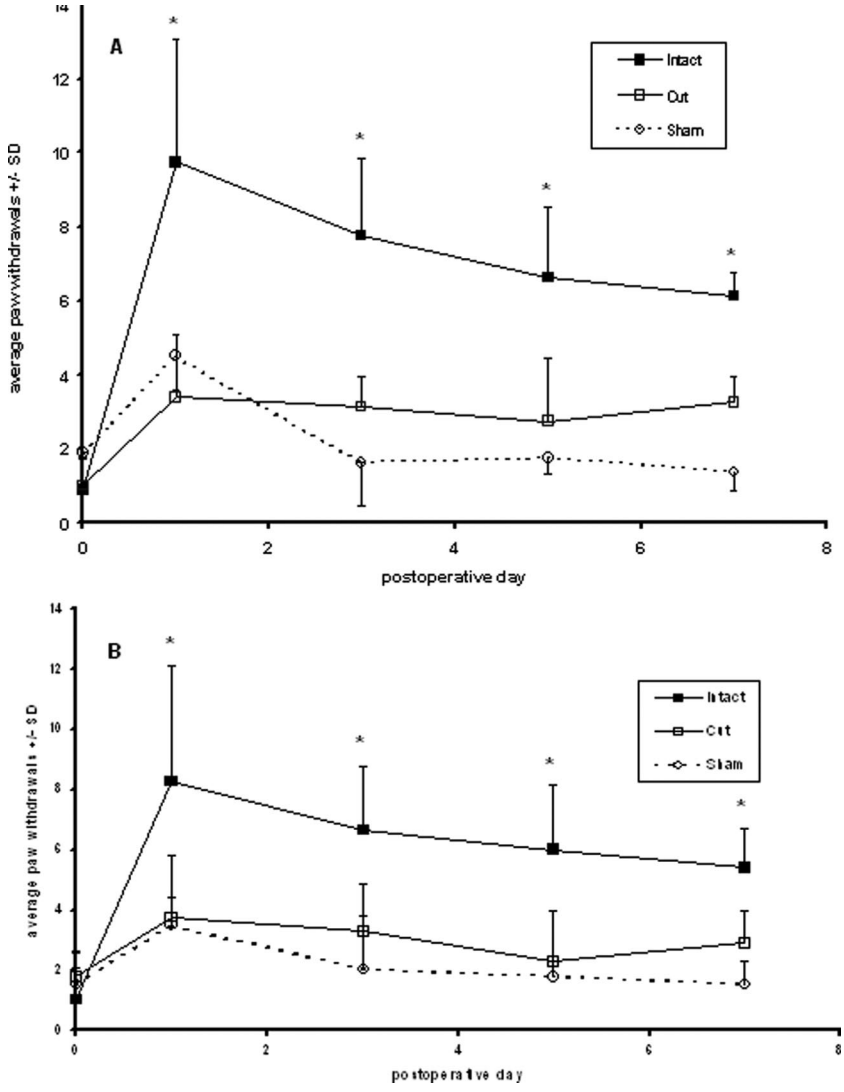

Figure 1. **A**, Average forepaw allodynia for intact, cut and sham groups using a 4 g von Frey filament. Allodynia for intact was significantly elevated (*$P <$ 0.0001) over both cut and sham on all days. Allodynia for cut was not different from sham, except on day 7. **B**, Allodynia responses were similar using a 2 g von Frey filament, with intact being significantly elevated (*$P <$ 0.004) over both cut and sham on all days, and no differences in allodynia between cut and sham on any day.

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side remained low following distraction and comparable to sham (Figure 1). The baseline responses (day 0) for all rats in both forepaws were not significantly different from each other (Figure 1). Allodynia on the left and right for sham was not different; so, sham responses were averaged for both paws to compare with the distraction groups. Allodynia on the intact side was significantly greater than both cut ($P < 0.0001$ for 4 g; $P < 0.004$ for 2 g) and sham ($P < 0.0001$ for 4 g; $P < 0.002$ for 2 g) on all days (Figure 1). Allodynia was not present if the capsule was transected before joint distraction (cut); allodynia was not elevated compared to sham, except on day 7 for the 4 g von Frey filament ($P = 0.001$; Figure 1). Allodynia for sham was low and not different from baseline, except on day 1 ($P < 0.01$).

Facet joint distraction produced differential spinal glial responses on day 7 (Figures 2–5). GFAP reactivity followed allodynia, with activation in the dorsal horn greatest for the intact capsule (Figures 2 and 3). Astrocytic reactivity (8.4% ± 3.4%) resulting from tension across an intact capsule was 1.4 times that produced for tension across a cut capsule (6.0% ± 2.5%); this increase was significant ($P = 0.04$) (Figures 2 and 3). While GFAP reactivity for intact was significantly greater ($P = 0.03$) than sham, spinal astrocytic activation for the cut side was only modestly elevated over sham. No differences in OX-42 reactivity were observed between any of the groups (Figures 4 and 5).

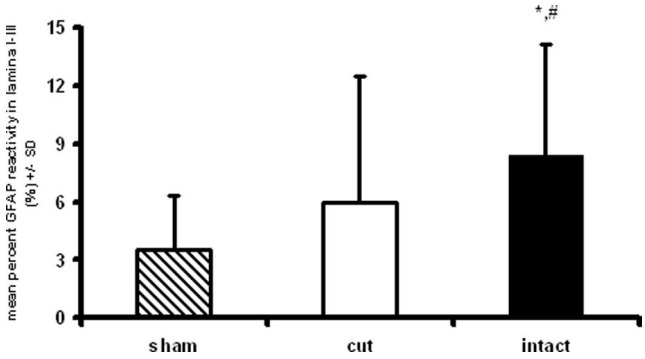

Figure 3. Average percent of the dorsal horn detected as reactive to GFAP at day 7 for sham, cut, and intact groups. The percent of LI-LIII expressing GFAP in the dorsal horn of the intact capsule side was significantly elevated ($P < 0.04$) over sham (*) and cut (#). However, cut was not different from sham.

Discussion

This study is the first to demonstrate that distraction across the cervical facet joint does not produce sustained

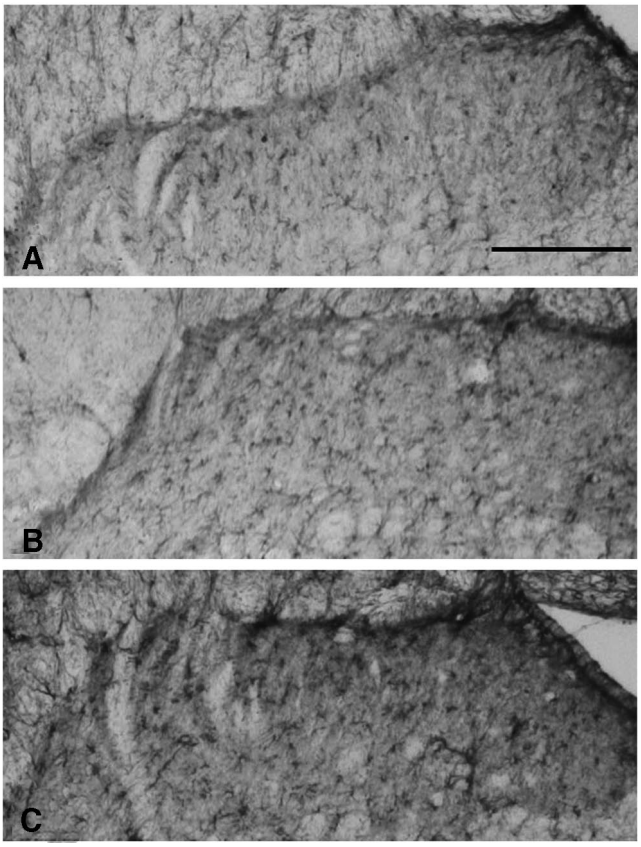

Figure 2. Images of the superficial dorsal horn (LI-LIII) of representative C7 spinal cord sections stained against GFAP at day 7 after injury. Sections from normal naïve rats were used to assign control levels of baseline staining. **A**, Sham produced only mild staining. GFAP reactivity in the dorsal horn of the cut side (**B**) was lower than that of the intact side (**C**). **A**, Scale bar = 200 μm, applies to all.

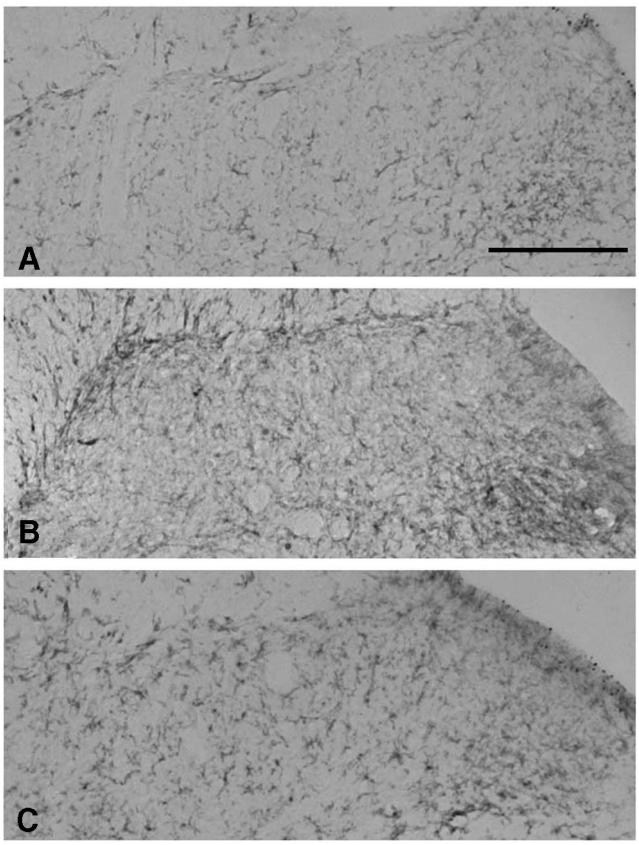

Figure 4. Images of the superficial dorsal horn regions (LI-LIII) of representative C7 spinal cord sections stained against OX-42 at day 7 after injury. There was no difference in OX-42 staining between sham (**A**), cut (**B**) or intact (**C**) side. **A**, Scale bar = 200 μm, applies to all.

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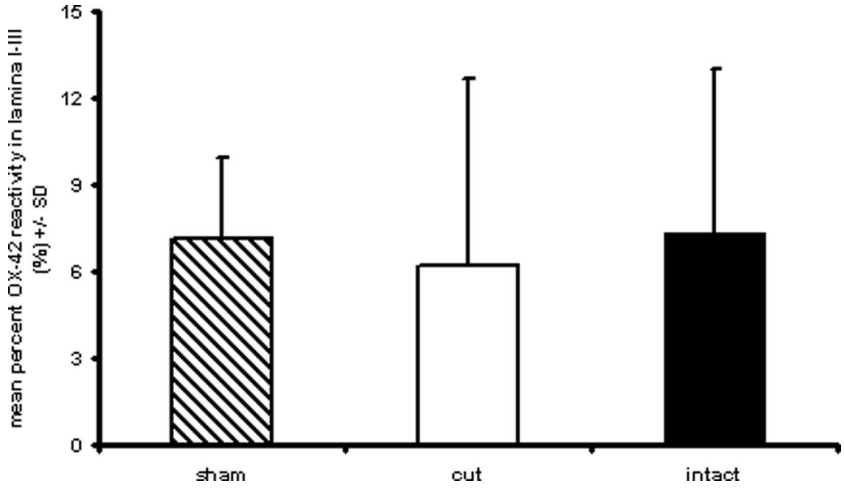

Figure 5. Average percent of the dorsal horn detected as reactive to OX-42 at day 7, for each of sham, cut, and intact groups. OX-42 staining was unchanged for the different facet joint tension groups.

behavioral sensitivity if the capsule is not intact (Figure 1). Sustained and elevated mechanical allodynia is produced in the forepaw following tensile loading of the C6/C7 facet joint if the capsule is intact and able to undergo tension produced by the joint's motions. For the case in which the capsule is transected before distraction, allodynia is not different from sham or unoperated baseline control cases (Figure 1). The mechanical loading conditions and joint responses measured here (0.66 mm, 0.07 mm/s; Table 1) are comparable to those previously reported to produce bilateral mechanical allodynia for intact capsules (0.57 mm, 0.08 mm/s).[26,27] These biomechanical findings support the assumption that both the intact and cut joints undergo comparable joint loading conditions.

Allodynia produced for the intact group is comparable to behavioral responses previously reported for this distraction model.[26] Facet distraction at the magnitude used in this study (0.66 mm; 2.48 N; Table 1) is significantly less than those conditions which produce rupture or failure of the facet capsule,[29] suggesting that the allodynia produced by tension across the intact capsule is mediated by the activation of capsule nerve fibers in the facet capsular ligament. No allodynia is produced in the absence of such a loading route across the ligament (*i.e.*, for cut) (Figure 1). These behavioral findings imply a role for the facet capsule in detecting and transducing a painful loading condition in the joint.

Firing of facet capsule A- and C-fiber afferents can be evoked as a result of tension and direct pinching of the capsule in both the lumbar and cervical spines.[15,17,35–37] Recently, work with a caprine model of facet joint distraction has identified that sensory receptors in the dorsal aspect of the cervical facet can signal graded loading paralleling applied ligament distractions; for sufficiently severe tension conditions, afferents can produce afterdischarges even after unloading of the joint.[35–37] While that collection of studies documents the electrophysiological consequences of facet joint distraction and supports the hypothesis that stretching the facet capsule and its nerve fibers could initiate pain for joint loading, those studies do not provide a direct link to behavioral sensitivity (pain symptoms). Our study demonstrates that for identical distraction conditions, the connectivity of the intact capsular ligament appears to be a requirement for initiation of sustained mechanical allodynia. Without an intact capsule, the local tension imposed by distracting the bones of the facet joint is not transferred to the nerve fibers in the ligament to detect the painful loading condition.

The spinal glial responses in the conditions of loading applied here further support the capsule's involvement in mediating pain for facet joint loading. Different patterns of spinal astrocytic and microglial reactivity are produced for the intact and cut capsule conditions relative to sham. No changes in dorsal horn microglial reactivity are produced for any of the procedures (Figures 4 and 5), despite the different injury scenarios and allodynia responses (Figure 1). This response of spinal microglial is consistent with other studies of neuropathic pain which indicate a lack of direct correlation between this spinal cell type and either injury mechanics or resulting allodynia.[30,34] These findings further support that spinal microglial activation in pain may not be a direct result of painful injury nor be responsible for directly modulating nociception. Further studies are needed to more fully understand the role of these immune cells in whiplash and other painful spine injuries. In contrast, astrocytic activation does demonstrate differential responses for different loading cases in relationship to pain. Distraction across the cut capsule does not produce allodynia or GFAP reactivity different from sham (Figures 1 and 3). In contrast, GFAP expression is significantly elevated for the intact capsule and is in agreement with increased spinal GFAP for painful loading observed as late as day 14 in this model.[26] The trend of spinal GFAP, but not OX-42, expression paralleling allodynia patterns is consistent with reports that GFAP is elevated only in the pain-producing distraction conditions for the facet joint.[26] Taken together, the behavioral and spinal astrocytic data suggest that activation of spinal astrocytes may be required for establishing pain from facet-

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mediated mechanisms and that their continued activation may drive the maintenance of allodynia. This study investigated only a single time point following loading to a facet joint that was not intact; further studies are needed to define the direct role of these cells in this pain syndrome.

Activation of neurons and glia in the spinal cord, together with increased expression of neuropeptides, cytokines, and cellular adhesion molecules, has been demonstrated in many types of painful peripheral injury models of pain. In particular, inflammatory and neuromodulatory responses are observed in the contralateral spinal cord in a host of other unilateral injury models of pain.[30,32–34,38–41] In that context, it should be noted that the glial activation detected in the spinal cord on the cut side may not reflect independent expression of cellular activity and may be greater in this study due to contralateral spinal effects of the intact capsule. While this is a limitation of the present study, the fact that both the allodynia and glial responses in that side are not different from sham imply that the potential contralateral effects are minimal. In fact, the differences in allodynia and GFAP reactivity are significant for the cut and intact forepaws (Figures 1 and 3). Contralateral astrocytic reactivity in this model may not be responsible for driving behavioral sensitivity since allodynia on the contralateral paw (cut, in this case) was minimal. However, astrocytic activation is only 1 of a host of spinal responses in the nociceptive cascade that contributes to pain.[30–34,38,39,41,42] Certainly, additional cell-types and their responses can also contribute to pain and should not be ruled out as contributing to sensitivity produced in this model. Future studies investigating these and other cellular responses in this model will provide further information about both the mechanisms of injury and spinal reactivity leading to behavioral sensitivity from facet-mediated loading.

Although previous epidemiologic, biomechanical and clinical studies have implicated the facet joint in neck pain, this work provides evidence for its involvement in producing pain by demonstrating a lack of behavioral hypersensitivity for joint loading after capsule transection. Animal models have investigated changes in neural activity following facet capsule stretch, demonstrated alterations in neurophysiology for ligament loading, and produced sustained mechanical allodynia for capsule distraction. However, no study has investigated whether the capsule, and loading applied to it, are required for generating painful mechanical loading signals which are manifest as pain symptoms. These results demonstrate increased allodynia after facet joint tension, suggest astrocytes as a possible spinal glial mediator of such painful injury, and provide further support for facet capsule involvement in pain from mechanical neck injury. Additional research investigating tissue and cellular responses, both in the capsule and the spinal cord and central nervous system, will define a more complete understanding of the relationship between facet joint loading, injury, and pain.

■ Key Points

- Transection of the facet capsular ligament does not allow production of allodynia for painful facet joint distaction.
- Sustained spinal astrocytic activation is produced for painful facet joint distraction but is not produced for a transected capsule.
- Spinal microglial activation is not altered at day 7 for facet joint distraction.
- An intact facet capsular ligament is requisite for joint tension to produce mechanical allodynia and spinal glial modifications.

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