A novel rodent neck pain model of facet-mediated behavioral hypersensitivity: implications for persistent pain and whiplash injury

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Abstract

Clinical, epidemiological, and biomechanical studies suggest involvement of cervical facet joint injuries in neck pain. While bony motions can cause injurious tensile facet joint loading, it remains speculative whether such injuries initiate pain. There is currently a paucity of data explicitly investigating the relationship between facet mechanics and pain physiology. A rodent model of tensile facet joint injury has been developed using a customized loading device to apply two separate tensile deformations (low, high; n = 5 each) across the C6C7 joint, or sham (n = 6) with device attachment only. Microforceps were rigidly coupled to the vertebrae for distraction and joint motions tracked in vivo. Forepaw mechanical allodynia was measured postoperatively for 7 days as an indicator of behavioral sensitivity. Joint strains for high (33.6 ± 3.1%) were significantly elevated (P<0.005) over low (11.1 ± 2.3%). Digitization errors (0.17 ± 0.20%) in locating bony markers were small compared to measured strains. Allodynia was significantly elevated for high over low and sham for all postoperative days. However, allodynia for low injury was not different than sham. A greater than three-fold increase in total allodynia resulted for high compared to low, corresponding to the three-fold difference in injury strain. Findings demonstrate tensile facet joint loading produces behavioral sensitivity that varies in magnitude according to injury severity. These results suggest that a facet joint tensile strain threshold may exist above which pain symptoms result. Continued investigation into the relationship between injury mechanics and nociceptive physiology will strengthen insight into painful facet injury mechanisms.

Keywords: Facet joint; Biomechanics; Pain; Strain; Neck; Whiplash

1. Introduction

Chronic pain, of which neck pain comprises nearly 30% of cases, has an estimated annual cost of US$ 90 billion for treatment and work loss (Freeman et al., 1999). Many chronic spinal pain syndromes remain intractable to treatment, adding to the challenge in managing painful neck injuries. Whiplash injuries and their associated disorders often lead to neck pain and are a widespread problem in today’s society, with an estimated incidence of 4 per 1000 population (Barnsley et al., 1994). As many as 42% of whiplash injuries become chronic, with chronic pain persisting in an estimated 10% of cases (Barnsley et al., 1994). The costs associated with these injuries are staggering, with an estimated US$ 29 billion spent annually on whiplash injuries and their related litigation costs (Freeman et al., 1999). Despite the high incidence of whiplash-associated neck pain, little remains known about the injuries producing these syndromes and the physiologic mechanisms responsible for their persistence.

While several different anatomical structures in the neck have been implicated in whiplash-related pain, clinical, epidemiological and biomechanical studies collectively point to the cervical facet joint as a likely candidate for pain generation due to its mechanical loading during these injuries (Aprill and Bogduk, 1992; Barnsley et al., 1993, 1994; Bogduk and Marsland, 1988; Grauer et al., 1997; Lord et al., 1996; Luan et al., 2000; Ono et al., 1997; Panjabi et al., 1998a,b; Siegmund et al., 2001; Winkelstein et al., 2000; Yoganandan and Pintar, 1997; Yoganandan et al., 1998). In clinical studies of patients reporting painful neck injury, the facet joint has been identified in 25–62% of cases as the site
of pain (April and Bogduk, 1992; Barnsley et al., 1994), with the C5–C7 spinal levels being the most commonly reported site of injury in whiplash (Barnsley et al., 1995; Bogduk and Marsland, 1988). Histologic studies of rabbit, rat, and cadaveric human tissue have identified nociceptive nerve fibers throughout the structures of the facet joint, including the joint’s capsular ligament (Cavanaugh et al., 1996; Giles and Harvey, 1987; Inami et al., 2001; McLain, 1994; Olton et al., 2001). These studies imply that neural input from the facet joint due to loading of the entire joint or any of its tissue elements has the potential for initiating and/or modulating pain sensation. Moreover, anesthetic nerve blocks of painful facet joints offer relief to patients with both general neck pain and whiplash-induced neck pain, suggesting a role for this joint as a pain source (Barnsley et al., 1993; Bogduk and Marsland, 1988; Lord et al., 1996).

Biomechanical studies also provide support for a mechanical role of the facet joint in whiplash injury. “Abnormal” motions in the cervical spine have been hypothesized as mechanisms of whiplash injury (Grauer et al., 1997; Kaneoka et al., 1999; Luan et al., 2000; Ono et al., 1997; Yoganandan et al., 2002). These kinematic patterns include excessive extension of the lower cervical spine, facet joint impingement, synovial fold pinching, and facet capsule stretching (Grauer et al., 1997; Luan et al., 2000; Ono et al., 1997; Panjabi et al., 1998a,b; Siegmund et al., 2001; Yoganandan and Pintar, 1997; Yoganandan et al., 1998; Winkelstein et al., 2000). Also, in isolated cadaveric mechanical studies of the facet capsule in flexion, extension, and combined bending and shear, the capsule has been shown to be at risk for subacatastrophic injury for vertebral motions occurring during low-velocity impacts, further implicating the capsule in whiplash-initiated pain (Siegmund et al., 2001; Winkelstein et al., 2000). However, despite the abundance of evidence suggesting involvement of the facet joint and its capsule in whiplash injury and neck pain, no studies have specifically investigated the role of facet-mediated injury in the generation and/or maintenance of neck pain and its associated symptoms.

Rodent pain models provide useful tools for examining painful injuries, with particular utility in linking nociceptive and physiologic responses to behavioral outcomes. For example, in low back pain models, behavioral hypersensitivity is commonly measured by mechanical allodynia (an increased sensitivity to a non-noxious stimulus), observed in the dermatome of the injured neural tissue (Colburn et al., 1999; Hashizume et al., 2000). Allodynia is measured by the frequency of paw withdrawals elicited by stimulation with otherwise non-noxious von Frey filaments (Hashizume et al., 2000) and is a useful behavioral outcome as it is also representative of clinical symptoms observed in chronic pain patients and provides a gauge of nociceptive responses (Barlas et al., 2000; Ochou, 2003; Sheather-Reid and Cohen, 1996). In the rat, the same spinal nerves that innervate the lower cervical spine also innervate the shoulder and forepaw (Takahashi and Nakajima, 1996), allowing for the measurement of forepaw allodynia as an indicator of increased behavioral sensitivity after facet joint injury. Many studies have used in vivo pain models to examine the relationship between mechanical injury to nerves and nerve roots in the lumbar spine and the ensuing physiologic and behavioral responses (Colburn et al., 1999; Hashizume et al., 2000; Liu et al., 2000; Matsu et al., 1998; Olmarker et al., 1991; Pedowitz et al., 1992; Rutkowski et al., 2002; Winkelstein et al., 2001a). In addition, altered electrophysiology has been shown to result from direct mechanical stimulation of lumbar facet capsules (Avramov et al., 1992; Cavanaugh et al., 1996), yet this work did not directly investigate the joint’s potential for generating pain. While there is an extensive body of work investigating chronic low back pain and painful neural injuries, efforts to define chronic neck pain mechanisms for cervical facet injuries and the role of biomechanical loading of this joint in neck pain are lacking.

Therefore, the goal of this study was to develop a repeatable in vivo rat model of controlled mechanically-induced painful facet joint tension injury. This study presents a novel rodent facet distraction model, with pain responses for two separate severities of C6/C7 tensile facet capsule injury. The effect of joint strain magnitude was examined in the context of resulting behavioral hypersensitivity, as measured by forepaw mechanical allodynia. These preliminary efforts provide an early basis for simultaneous investigation of the biomechanics of whiplash injuries with physiologic mechanisms of nociception and pain.

2. Materials and methods

All experimental procedures have been approved by the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC). Experiments were performed using male Holtzman rats, weighing 275–350 g, at the start of the study. Animals were housed under USDA and AAALAC-approved conditions with free access to food and water.

2.1. Surgical procedure and tensile facet injury

All procedures were performed under inhalation anesthesia (4% halothane for induction, 2.5% for maintenance). Rats were placed in a prone position and the paraspinous musculature separated from the spinous processes from C4–T2. The laminae, facet joints and spinous processes at C6–C7 were carefully exposed bilaterally under a surgical microscope (Carl Zeiss Inc., Thornwood, NY). The interspinous ligament and ligamentum flavum were minimally resected at C6/C7 to facilitate device attachment to each of the C6 and C7 spinous processes (Fig. 1). Using microforces, the customized loading device was rigidly attached to the C7 spinous process to hold it fixed during loading. Likewise, microforces were also attached to the C6
spinous process, allowing for its rostral translation. Acrylic black paint marks (diameter = 0.36 ± 0.20 mm) were applied to the right C6 and C7 laminae and the C6/C7 facet capsular ligament, to allow motion tracking (Fig. 2). During facet displacement, the exposure and right facet joint were imaged at five frames/s using a digital video camera, with pixel resolution of 640 × 480 (Pixera Corp., Los Gatos, CA).

Facet capsule tension was imposed according to one of the following procedures: (1) low tensile strain (target 10%) (n = 5), (2) high tensile strain (target 30%) (n = 5), or (3) sham (device attachment only) (n = 6). Briefly, a manual micrometer (Newport Corp., Irvine, CA) was used to apply quasistatic tensile displacement and return of the C6/C7 facet joint, imposing the target tensile strain across the facet capsule. The micrometer was rigidly coupled to the C6 mi-
crofroteps and interacted with a linear variable differential transducer (LVDT) (MicroStrain Inc., Williston, VT) that recorded clip displacements at 10 Hz. During tension, the facet joint was distracted to the desired target strain magnitude (low, high) and held for 30s, after which the C6 verte-
bra was translated back to its initial position, unloading the facet joint. All data acquisition was synchronized in time. Sham surgeries consisted of device attachment only, for the same duration as in the tension groups. Wounds were closed with silk suture and surgical staples. Rats were allowed to recover in room air and were monitored during recovery.

2.2. Image analysis

Imposed in vivo facet strains were calculated using the C6 and C7 bony markers. Each pair of bony markers was dig-
itized using Scion Image (Scion Corp., Frederick, MD) in their initial configuration as well as at the maximal loading condition. In the initial image, three points were selected on each bony marker and three lines created to connect each set of points. Lengths were defined as the distance between the bony markers for each of their initial (ℓ0) and maximal dis-
traction (ℓ) configurations (Fig. 2). Using the Lagrangian strain calculation, the imposed joint strain was calculated as Δℓ/ℓ0, where Δℓ = (Δℓf – Δℓ0) is the change in length between the initial and maximal distortion configurations. Spinal rotation angles were calculated as the change in orien-
tation of the joint during distraction and used as a measure of the degree of applied tensile symmetry in the applied injury.

2.3. Behavioral testing

All rats were evaluated for forepaw mechanical allody-
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2.4. Statistical analysis

Mechanical parameters of the injury application were compared for the two injury groups using a Student’s t-test. Allodynia responses in the right and left forepaws were compared using a paired t-test. To compare the effects of surgery type on mechanical allodynia across all groups, a repeated analysis of variance (ANOVA) with post hoc Bonferroni correction was used. All statistical analyses were performed using SYSTAT (SYSTAT Software Inc., Richmond, CA) and significance was defined as P < 0.05.

3. Results

During loading, no observable damage of the facet joint capsule was observed. Also, at the completion of the study, examination of the facet capsule under a surgical microscope indicated no gross mechanical injury to the capsule in any of the animals. After surgery, all rats demonstrated normal functioning with grooming and consistent weight gain. They showed good head mobility, indicating that there were no adverse effects of the procedures on neck mobility.

For animals in both facet strain groups (low, high), clip displacement was obtained from LVDT data and the bony marker displacement from digitized images (Table 1, Fig. 3). Digitization error in locating bony markers was small (0.17 ± 0.20%) compared to imposed strains. Mean applied strain in the low and high groups was 11.1 ± 2.3 and 33.6 ± 3.1%, respectively, and was significantly different between the two groups (P < 0.0005) (Table 1). However, neither the applied loading rate (0.09 ± 0.02 mm/s) nor the measured spinal rota-
tion angles (2.93 ± 2.63°) were significantly (Phigh = 0.283, Pratio = 0.340) different between injury groups (Table 1).

Mechanical allodynia was not significantly different in the left and right forepaws for either tension injury or shams (both filament strengths, data not shown). As such, left and right allodynia responses for each rat were averaged for anal-
ysis between groups. For high strain injuries, allodynia was immediately increased over baseline on day 1, with only a slight decrease over time (Fig. 4). Allodynia for high strain was significantly elevated over both low strain (P < 0.017, 1.4 g; P < 0.003, 2 g) and sham (P < 0.002, 1.4 g; P < 0.0005, 2 g) for the entire postoperative period. However, allodynia in the low strain group was not significantly dif-
ferent from sham for any time point using either von Frey filament. Sham responses were low and not different from baseline values.

Total mechanical allodynia over the entire postoperative period was calculated for each animal as a measure of cu-
mulative hypersensitivity. The data indicate a greater than threefold increase in total allodynia for a corresponding three-fold increase in applied facet joint strain (Fig. 5). Total allodynia for high facet strain was significantly greater than for low strain injury (P < 0.0005) and sham (P < 0.0005) for testing with a 2.0 g von Frey filament (Fig. 5A). Total
Table 1
Summary of imposed facet joint injury mechanics

<table>
<thead>
<tr>
<th>Rat</th>
<th>Rate (mm/s)</th>
<th>Load time (s)</th>
<th>Clip displacement (max, mm)</th>
<th>Marker displacement (max, mm)</th>
<th>Strain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>0.08</td>
<td>6.5</td>
<td>0.51</td>
<td>0.31</td>
<td>13.41</td>
</tr>
<tr>
<td>K7</td>
<td>0.11</td>
<td>4.4</td>
<td>0.52</td>
<td>0.20</td>
<td>8.79</td>
</tr>
<tr>
<td>K11</td>
<td>0.10</td>
<td>4.8</td>
<td>0.51</td>
<td>0.26</td>
<td>13.67</td>
</tr>
<tr>
<td>6</td>
<td>0.14</td>
<td>9.1</td>
<td>0.81</td>
<td>0.26</td>
<td>9.50</td>
</tr>
<tr>
<td>11</td>
<td>0.06</td>
<td>10.9</td>
<td>0.63</td>
<td>0.20</td>
<td>9.93</td>
</tr>
<tr>
<td>Low average (S.D.)</td>
<td>0.10 (0.03)</td>
<td>7.14 (2.80)</td>
<td>0.60 (0.13)</td>
<td>0.25 (0.05)</td>
<td>11.06 (2.30)</td>
</tr>
<tr>
<td>K4</td>
<td>0.07</td>
<td>12.6</td>
<td>1.22</td>
<td>0.51</td>
<td>37.90</td>
</tr>
<tr>
<td>K2A</td>
<td>0.08</td>
<td>18.1</td>
<td>1.66</td>
<td>0.45</td>
<td>34.11</td>
</tr>
<tr>
<td>K4A</td>
<td>0.08</td>
<td>14.5</td>
<td>1.44</td>
<td>0.60</td>
<td>34.41</td>
</tr>
<tr>
<td>5</td>
<td>0.09</td>
<td>14.2</td>
<td>1.65</td>
<td>0.59</td>
<td>31.77</td>
</tr>
<tr>
<td>8</td>
<td>0.09</td>
<td>19.5</td>
<td>1.51</td>
<td>0.61</td>
<td>29.56</td>
</tr>
<tr>
<td>High average (S.D.)</td>
<td>0.08 (0.01)</td>
<td>15.74 (2.83)</td>
<td>1.50 (0.18)</td>
<td>0.55 (0.07)</td>
<td>33.55 (3.13)</td>
</tr>
</tbody>
</table>

alldynia in the low injury group was also significantly increased over sham (P = 0.019). The same trends were observed for each of the three groups for testing with the 1.4 g filament (Fig. 5B), with high significantly elevated over low (P < 0.0005) and sham (P < 0.0005). However, using the 1.4 g filament there was no significant difference between sham and low.

4. Discussion

To the best of our knowledge, this study is the first to demonstrate a relationship between controlled mechanical facet joint injury and behavioral outcomes suggestive of pain symptoms. The results of this study demonstrate that mechanical alldynia is produced in the forepaw following tensile loading of the C6/C7 facet joint and that the behavioral sensitivity response varies in magnitude depending on the degree of capsule distraction (Fig. 4), implicating this joint in painful neck injuries. The greater than three-fold increase in total alldynia for a three-fold increase in tensile distraction (Fig. 5) further suggests a direct relationship may exist between mechanical loading of this joint and the nature of the resulting behavioral sensitivity. Moreover, the lack of significant difference in hypersensitivity produced between the sham and low tension groups further suggests that a mechanical threshold may exist for tensile loading to the C6/C7 facet joint above which persistent pain symptoms result.
Fig. 4. Average mechanical allodynia as measured by the number of forepaw withdrawals for high, low, and sham injuries. High strain injury produced increased allodynia over low strain injury and sham (P < 0.003 and <0.0005, respectively) that was maintained over the 7-day testing period, for testing with the 2 g von Frey filament (A). Results were similar for testing with the 1.4 g von Frey filament, with allodynia for high strain significantly elevated above low and sham (P < 0.017 and <0.002, respectively) (B).

The facet injury device presented here provides utility for applying controlled and repeatable facet joint distraction, with control of mechanical injury parameters, such as magnitude of distraction (and strain), rate of distraction, and hold duration. In fact, the precision errors for low and high applied strain (2.9 and 3.7%, respectively) were low, confirming an ability to apply a target strain across a given joint in vivo. The imposed joint injury was primarily tensile in nature, with little off-axis rotation. Off-axis rotation angles were small (2.93 ± 2.64°), confirming that joint distraction was symmetric along the spinal axis. The lack of difference in allodynia responses between the right and left forepaws further supports that a symmetric injury was imposed. In addition, it should be noted that the sham procedure involved the same ligament resection and device attachment as that of the low and high strain groups but with no imposed strain. Allodynia responses following sham procedures were not different from baseline values (Fig. 4), suggesting that the tensile distraction of this joint is necessary to produce pain responses.

Of note, the joint injuries imposed in this study were primarily tensile across the joint, without directly incorporating a sagittal bending component. However, the maximum capsular strain created by whiplash-like bending motions has been previously reported as 12 and 11.6% for flexion and extension, respectively (Winkelstein et al., 2000), which are comparable to the low capsule strain condition of this study.

This study suggests that a threshold for pain behaviors due to tensile facet capsule injury may exist between 11 and 33% strain across the C6/C7 joint. In studies of isolated middle and lower cadaveric cervical motion segments, the maximum principal strains in the capsule were 11.6 and 16.8%, for pure extension and combined shear and extension, respectively (Siegmund et al., 2001; Winkelstein et al., 2000). These values are comparable to the 11% strain for low injury in the current study and suggest that individuals undergoing these capsule strains may not experience pain symptoms after injury. Punjabi et al. (1998b) reported peak facet capsule strains of 29.5 and 35.4% at the C6/C7 joint level for 6.5 and 10.5 G accelerations, respectively, using mini-sled tests of human cadaveric head-neck specimens. In addition, Winkelstein et al. (2000) reported mean subcatastrophic failure capsule strains as low as 35% in tension for
isolated cadaveric cervical motion segments. For these subcatastrophic failures, capsular ligaments remained grossly intact, but the mechanics suggested microscopic failures may have occurred. Taken together with the findings of our current study from the high facet strain group, these data suggest that under such loading conditions in whiplash injury pain symptoms may be produced. Likewise, the cadaveric data provide mechanical context suggesting that subcatastrophic injuries to the capsular ligament may be produced in this animal model.

While many earlier studies have implicated the facet joint in neck pain, this study provides direct evidence for its involvement in producing neck pain by demonstrating behavioral hypersensitivities after joint injury. In previous studies using animal models of low back pain, mechanical allodynia has been correlated with and is hypothesized to be due to a host of physiologic changes in the central nervous system. Among these nociceptive responses are neuronal plasticity, glial cell activation and cytokine upregulation (DeLeo and Yezierski, 2001; Hashizume et al., 2000; Ji and Woolf, 2001; Rutkowski et al., 2002; Sweitzer et al., 1999; Watkins et al., 1995; Winkelstein et al., 2001b). In addition, animal models specifically investigating changes in neural electrical activity following lumbar facet capsule stretching have demonstrated alterations in neurophysiology for applied loading (Avramov et al., 1992; Cavanaugh et al., 1996). Together, these molecular and cellular changes contribute to central sensitization and persistent pain. Indeed, in clinical research, central sensitization has been hypothesized as a mechanism of chronic pain after whiplash injury (Barlas et al., 2000;
References

Curatolo et al., 2001; Kivioja et al., 2001). The results presented here demonstrate increased allodynia after facet injury and serve to further support facet joint involvement in neck pain. While this study has not reported a relationship between allodynia and spinal nociceptive changes, these efforts are the focus of ongoing work in our laboratory. This model of facet joint-mediated behavioral hypersensitivity serves as a useful tool to further investigate the relationship between facet joint loading and pain. While this study has explicitly examined and quantified facet joint and capsular ligament mechanics in the context of pain symptoms, it is recognized that the scenarios imposed may also load additional anatomical structures in the spine (e.g. intervertebral disc, nerve root) due to bending in the sagittal plane. Because injury to such structures may also contribute to neck pain, efforts in our laboratory are also focused on characterizing associated tissue loading in this model and investigating other spinal structures’ ability to generate behavioral hypersensitivities in this animal model. Future studies examining particular aspects of injury biomechanics, such as loading rate and duration, will allow a more complete characterization of the relationship between pain and injury. In particular, further studies into the physiologic responses of this painful joint injury will undoubtedly be useful for understanding the mechanisms of chronic neck pain and development of potentially effective therapeutic interventions.

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