August 2004

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

Kathryn E. Lee
University of Pennsylvania

John H. Thinnes
University of Pennsylvania

David S. Gokhin
University of Pennsylvania

Beth A. Winkelstein
University of Pennsylvania, winkelst@seas.upenn.edu

Postprint version. Published in *Journal of Neuroscience Methods*, Volume 137, Issue 2, August 30, 2004, pages 151-159.
Publisher URL: http://dx.doi.org/10.1016/j.jneumeth.2004.02.021

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

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 2 separate tensile deformations (low, high; n=5 each) across the C6/C7 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

Comments
Postprint version. Published in *Journal of Neuroscience Methods*, Volume 137, Issue 2, August 30, 2004, pages 151-159.
Publisher URL: http://dx.doi.org/10.1016/j.jneumeth.2004.02.021

A Novel Rodent Neck Pain Model of Facet-Mediated Behavioral Hypersensitivity: Implications for Persistent Pain and Whiplash Injury

Kathryn E. Lee, BS, John H. Thinnes, MS,

David S. Gokhin, Beth A. Winkelstein, PhD*

Department of Bioengineering

University of Pennsylvania

Philadelphia, PA 19104

Number of pages: 26

Number of figures and tables: 5 figures, 1 table

Correspondence*: Beth Winkelstein, PhD
Dept. of Bioengineering
University of Pennsylvania
120 Hayden Hall
3320 Smith Walk
Philadelphia, PA 19104-6392
215-573-4589 (phone)
215-573-2071 (fax)
winkelst@seas.upenn.edu

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 2 separate tensile deformations (*low, high*; n=5 each) across the C6/C7 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.

Key Words: facet joint, biomechanics, pain, strain, neck, whiplash

INTRODUCTION

Chronic pain, of which neck pain comprises nearly 30% of cases, has an estimated annual cost of $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 $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 (Bogduk and Marsland, 1988; Aprill and Bogduk, 1992; Barnsley et al., 1993, 1994; Lord et al., 1996; Grauer et al., 1997; Ono et al., 1997; Yoganandan and Pintar, 1997; Panjabi et al., 1998a, 1998b; Yoganandan et al., 1998; Luan et al., 2000; Winkelstein et al., 2000; Siegmund et al., 2001). 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 (Aprill 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, 1998). 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 (Giles and Harvey, 1987; McLain, 1994; Cavanaugh et al., 1996; Inami et al., 2001; Ohtori 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 (Bogduk and Marsland, 1988; Barnsley et al., 1993; 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; Ono et al., 1997; Kaneoka et al., 1999; Luan et al., 2000; 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; Ono et al., 1997; Yoganandan and Pintar, 1997; Panjabi et al., 1998a, 1998b; Yoganandan et al., 1998; Luan et al., 2000; Winkelstein et al., 2000; Siegmund et al., 2001). 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 subcatastrophic injury for vertebral motions occurring during low-velocity impacts, further implicating the capsule in whiplash-initiated pain (Winkelstein et al., 2000; Siegmund et al., 2001). 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 (Sheather-Reid and Cohen, 1998; Barlas et al., 2000; Ochoa, 2003). 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 (Olmarker et al., 1991; Pedowitz et al., 1992; Matsui et al., 1998; Colburn et al., 1999; Hashizume et al., 2000; Liu et al., 2000;

Winkelstein et al., 2001a; Rutkowski et al., 2002). 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.

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 grams at the start of the study. Animals were housed under USDA & AAALAC-approved conditions with free access to food and water.

Surgical Procedure & 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 paraspinal 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 (Figure 1). Using microforceps, the customized loading device was rigidly attached to the C7 spinous process to hold it fixed during loading. Likewise, microforceps 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 (Figure 2). During facet displacement, the exposure and right facet joint were imaged at 5 frames/sec using a digital video camera, with pixel resolution of 640 x 480 (Pixera Corp., Los Gatos, California).

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 microforceps and interfaced 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 30 seconds, after which the C6 vertebra 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.

Image Analysis. Imposed *in vivo* facet strains were calculated using the C6 and C7 bony markers. Each pair of bony markers was digitized 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 (ℓ_o) and maximal distraction (ℓ_f) configurations (Figure 2). Using the Lagrangian strain calculation, the imposed joint strain was calculated as $\Delta\ell/\ell_o$, where $\Delta\ell = (\Delta\ell_f - \Delta\ell_o)$ is the change in length between the initial and maximal distraction configurations. Spinal rotation angles were calculated as the change in orientation of the joint during distraction and used as a measure of the degree of applied tensile symmetry in the applied injury.

Behavioral Testing. All rats were evaluated for forepaw mechanical allodynia at days 1, 3, 5, and 7, postoperatively. Allodynia was measured as the number of forepaw withdrawals elicited by a defined non-noxious mechanical stimulus and was measured for

both forepaws of each rat. Prior to injury, animals were previously acclimated to the testing environment and tester and baseline measurements were recorded. The same tester, who was blinded to the surgical procedure, performed all behavioral testing for this study. Behavioral testing methods used here for forepaw sensitivity were adapted from well-established methods used for hindpaw evaluation in lumbar pain models (Kim and Chung, 1992; Colburn et al., 1999; Hashizume et al., 2000; Winkelstein et al., 2001b; Decosterd et al., 2002; Rutkowski et al., 2002; Sweitzer et al., 2002). Briefly, 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 forepaw using 1.4 and 2.0 gram von Frey filaments (Stoelting Co., Wood Dale, IL). Prior to surgery, baseline measurements of allodynia obtained using these von Frey filaments were negligible, indicating their being non-noxious. A positive response was counted when the rat emphatically lifted its paw upon stimulation, which was at times accompanied by licking or tightening of the paw.

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 $p<0.05$.

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 I, Figure 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 I). However, neither the applied loading rate (0.09±0.02 mm/s) nor the measured spinal rotation angles (2.93±2.63°) were significantly ($p_{low}=0.283$, $p_{high}=0.340$) different between injury groups (Table I).

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 analysis between groups. For *high* strain injuries, allodynia was immediately increased over baseline on day 1, with only a slight decrease over time (Figure 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 different 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 cumulative hypersensitivity. The data indicate a greater than three-fold increase in total allodynia for a corresponding three-fold increase in applied facet joint strain (Figure 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 (Figure 5A). Total allodynia 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 (Figure 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*.

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 allodynia 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 (Figure 4), implicating this joint in painful neck injuries. The greater than three-fold increase in total allodynia for a three-fold increase in tensile distraction (Figure

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.

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 (Figure 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 (Winkelstein et al., 2000; Siegmund et al., 2001). 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. Panjabi 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 (Watkins et al., 1995; Sweitzer et al., 1999; Hashizume et al., 2000; DeLeo and Yezierski, 2001; Ji and Woolf, 2001; Winkelstein et al., 2001b; Rutkowski et al., 2002). 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; 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.

ACKNOWLEDGEMENTS: This work was funded by grant support from the Whitaker Foundation (RG-02-0311) and a graduate fellowship from the National Science Foundation.

REFERENCES

Aprill C, Bogduk N. The prevalence of cervical zygapophyseal joint pain: a first approximation. Spine 1992;17(7):744-747.

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. J Bone Joint Surg Am 1992;74(10):1464-1471.

Barlas P, Walsh DM, Baxter GD, Allen JM. Delayed onset muscle soreness: effect of an ischaemic block upon mechanical allodynia in humans. Pain 2000;87:221-225.

Barnsley L, Lord S, Bogduk N. Comparative local anaesthetic blocks in the diagnosis of cervical zygapophysial joint pain. Pain 1993;55:99-106.

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

Barnsley L, Lord SM, Wallis BJ, Bogduk N. The prevalence of chronic cervical zygapophysial joint pain after whiplash. Spine 1995;20(1):20-26.

Bogduk N, Marsland A. The cervical zygapophysial joints as a source of neck pain. Spine 1988;13(6):610-617.

Cavanaugh JM, Ozaktay AC, Yamashita HT, King AI. Lumbar facet pain: biomechanics, neuroanatomy and neurophysiology. J Biomech 1996;29(9):1117-1129.

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 1999;0:1-16.

Curatolo M, Petersen-Felix S, Arendt-Nielsen L, Giani C, Zbinden AM, Radanov BP. Central hypersensitivity in chronic pain after whiplash injury. Clin J Pain 2001;17:306-315.

Decosterd I, Allchorne A, Woolf CJ. Progressive tactile hypersensitivity after a peripheral nerve crush: non-noxious mechanical stimulus-induced neuropathic pain. Pain 2002;100(1-2):155-162.

DeLeo JA, Yezierski RP. The role of neuroinflammation and neuroimmune activation in persistent pain. Pain 2001; 91:1-6.

Freeman MD, Croft AC, Rossignol AM, Weaver DS, Reiser M. A review and methodologic critique of the literature refuting whiplash syndrome. Spine 1999;24(1):86-96.

Giles LG, Harvey AR. Immunohistochemical demonstration of nociceptors in the capsule and synovial folds of human zygopophyseal joints. Br J Rheumatol 1987;26(5):362-364.

Grauer JN, Panjabi MM, Cholewicki J, Nibu K, Dvorak J. Whiplash produces an S-shaped curvature of the neck with hyperextension at lower levels. Spine 1997;22(21):2489-2494.

Hashizume H, DeLeo JA, Colburn RW, Weinstein JN. Spinal glial activation and cytokine expression after lumbar root injury in the rat. Spine 2000;25(10):1206-1217.

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 2001;19:593-596.

Ji RR, Woolf CJ. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathologic pain. Neurobiol Dis 2001;8:1-10.

Kaneoka K, Ono K, Inami S, Hayashi K. Motion analysis of cervical vertebrae during whiplash loading. Spine 1999;24(8):763-770.

Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1992;50(3):355-63.

Kivioja J, Rinaldi L, Ozenci V, Kouwenhoven M, Kostulas N, Lindgren U, Link H. Chemokines and their receptors in whiplash injury: elevated RANTES and CCR-5. J Clin Immunol 2001;21(4):272-277.

Liu L, Rudin M, Kozlova EN. Glial cell proliferation in the spinal cord after dorsal rhizotomy or sciatic nerve transection in the adult rat. Exp Brain Res 2000;131(1):64-73.

Lord SM, Barnsley L, Wallis BJ, Bogduk N. Chronic cervical zygapophysial joint pain after whiplash: a placebo-controlled prevalence study. Spine 1996;21(15):1737-1745.

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

Matsui T, Takahashi K, Moriya M, Tanaka S, Kawahara N, Tomita K. Quantitative analysis of edema in the dorsal nerve roots induced by acute mechanical compression. Spine 1998;23(18):1931-1936.

McLain RF. Mechanoreceptor endings in human cervical facet joints. Spine 1994;19(5):495-501.

Ochoa JL. Quantifying sensation: “Look back in allodynia”. Eur J Pain 2003;7:369-374.

Ohtori S, Takahashi K, Chiba T, Yamagata M, Sameda H, Moriya H. Sensory innervation of the cervical facet joints in rats. Spine 2001;26(2):147-150.

Olmarker K, Holm S, Rosenquist AL, Rydevik B. Experimental nerve root compression. A model of acute, graded compression of the porcine cauda equine and an analysis of neural and vascular anatomy. Spine 1991;16(1):61-69.

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. Proceedings of the 41st Stapp Car Crash Conference; Lake Buena Vista, Florida 1997;339-356.

Panjabi MM, Cholewicki J, Nibu K, Grauer JN, Babat LB, Dvorak J. Mechanism of whiplash injury. Clin Biomech 1998a;13:239-249.

Panjabi MM, Cholewicki J, Nibu K, Grauer J, Vahldiek M. Capsular ligament stretches during in vitro whiplash simulations. J Spinal Disord 1998b;11(3):227-32.

Pedowitz R, Garfin S, Massie J, Hargens A, Swenson M, Myers R, Rydevik B. Effects of magnitude and duration of compression on spinal nerve root conduction. Spine 1992;17:194-199.

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 2002;27(15):1604-1613.

Sheather-Reid RB, Cohen ML. Psychophysical evidence for a neuropathic component of chronic neck pain. Pain 1998;75:341-347.

Siegmund GP, Myers BS, Davis MB, Bohnet HF, Winkelstein BA. Mechanical evidence of cervical facet capsule injury during whiplash. Spine 2001;26(19):2095-2101.

Sweitzer S, Colburn R, Rutkowski M, DeLeo J. Acute peripheral inflammation induces moderate glial activation and spinal IL–1β expression that correlates with pain behavior in the rat. Brain Res 1999;829:209-21.

Sweitzer SM, Hickey WF, Rutkowski MD, Pahl JL, DeLeo JA. Focal peripheral nerve injury induces leukocyte trafficking into the central nervous system: potential relationship to neuropathic pain. Pain 2002;100(1-2):163-170.

Takahashi Y, Nakajima Y. Dermatomes in the rat limbs as determined by antidromic stimulation of sensory C-fibers in spinal nerves. Pain 1996;67:197-202.

Watkins LR, Maier SF, Goehler LE. Immune activation: the role of pro-inflammatory cytokines in inflammation, illness responses and pathological pain states. Pain 1995;63:289-302.

Winkelstein BA, Nightingale RW, Richardson WJ, Myers BS. The cervical facet capsule and its role in whiplash injury. Spine 2000;25(10):1238-1246.

Winkelstein BA, Rutkowski MD, Weinstein JN, DeLeo JA. Quantification of neural tissue injury in a rat radiculopathy model: comparison of local deformation, behavioral outcomes, and spinal cytokine mRNA for two surgeons. J Neurosci Meth 2001a;111:49-57.

Winkelstein BA, Rutkowski MD, Sweitzer SM, Pahl JL, DeLeo JA. Nerve injury proximal or distal to the DRG induces similar spinal glial activation and selective cytokine expression but differential behavioral responses to pharmacologic treatment. J Comp Neurol 2001b;439(2):127-139.

Yoganandan N, Pintar FA. Inertial loading of the human cervical spine. J Biomech Eng 1997;119:237-240.

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

Yoganandan N, Pintar FA, Cusick JF. Biomechanical analysis of whiplash injuries using an experimental model. Accid Anal Prev 2002;34:663-671.

FIGURE LEGENDS

Figure 1. Schematic showing surgical setup, with clamps, manual micrometer and LVDT. A surgical microscope is mounted above the loading device for image analysis and strain measurement. The inset picture (left) illustrates the placement of microforceps on the C6 and C7 spinous processes. For the tensile loading protocol used in this study, the C7 spinous process is rigidly held in place while the C6 spinous process is displaced in the rostral direction, producing tension across the joint space.

Figure 2. Representative *in vivo* images (#11, Table I) prior to **(A)** and at maximal **(B)** distraction of the facet joint. Also shown are the bony markers (large black circles) on the C6 and C7 laminae and the corresponding distance between the markers (ℓ_o, ℓ_f) used to calculate joint strains. For #11, a 9.93% facet joint strain was applied.

Figure 3. Representative C6 microforceps displacement data as measured by the LVDT, for #11 (same as Figure 2), during the distraction **(A)**, hold **(B-C)**, and unloading **(D)** sequence. Also shown are the corresponding images taken for strain calculations at each of the reference, loaded, and unloaded conditions.

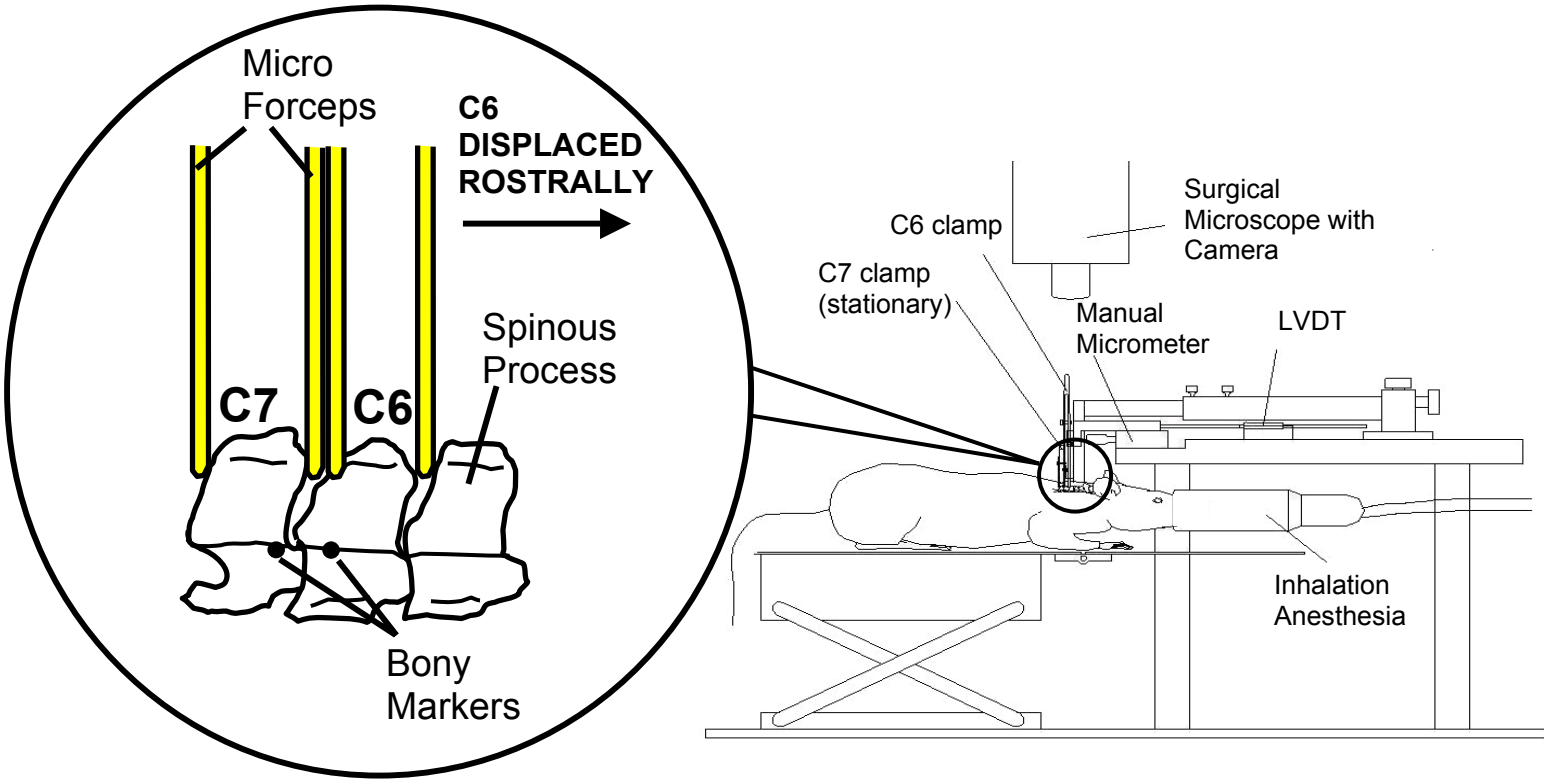
Figure 4. Average mechanical allodynia as measured by the number of forepaw withdrawals for *high, low,* and *sham* injuries. *High* strain injury produced increased allodynia over *low* strain injury and *sham* ($p<0.003$ & $p<0.0005$, respectively) that was maintained over the 7-day testing period, for testing with the 2 g von Frey filament **(A)**.

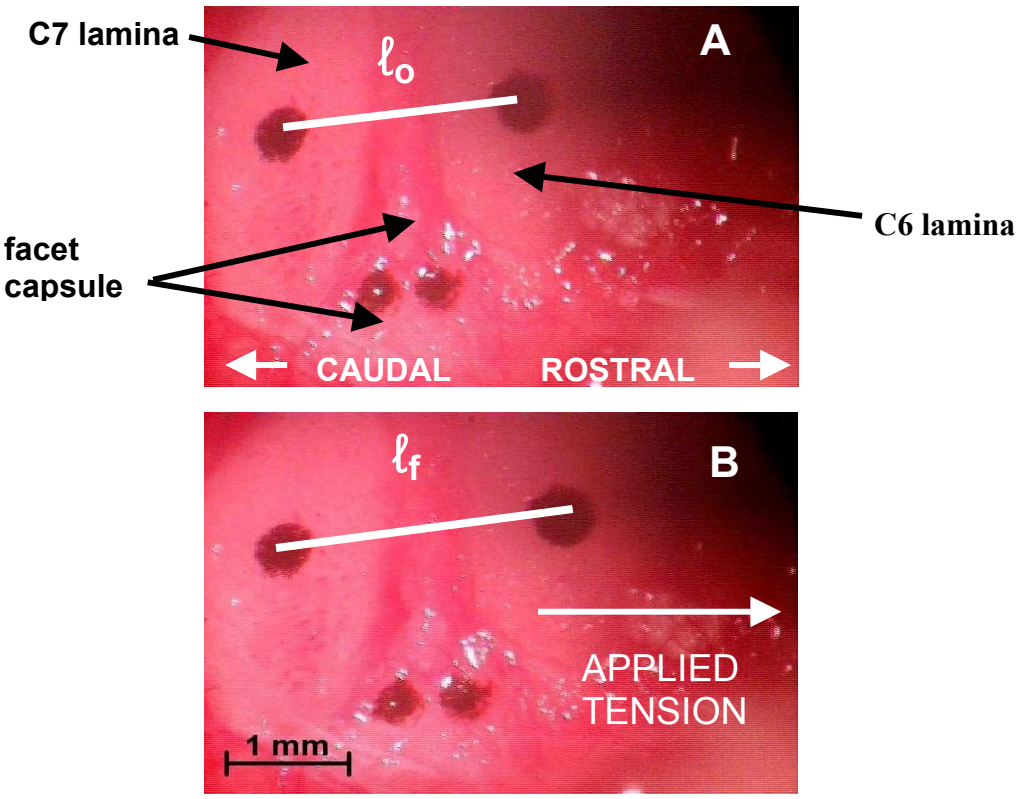
Results were similar for testing with the 1.4 g von Frey filament, with allodynia for *high* strain significantly elevated above *low* and *sham* ($p<0.017$ & $p<0.002$, respectively) **(B)**.

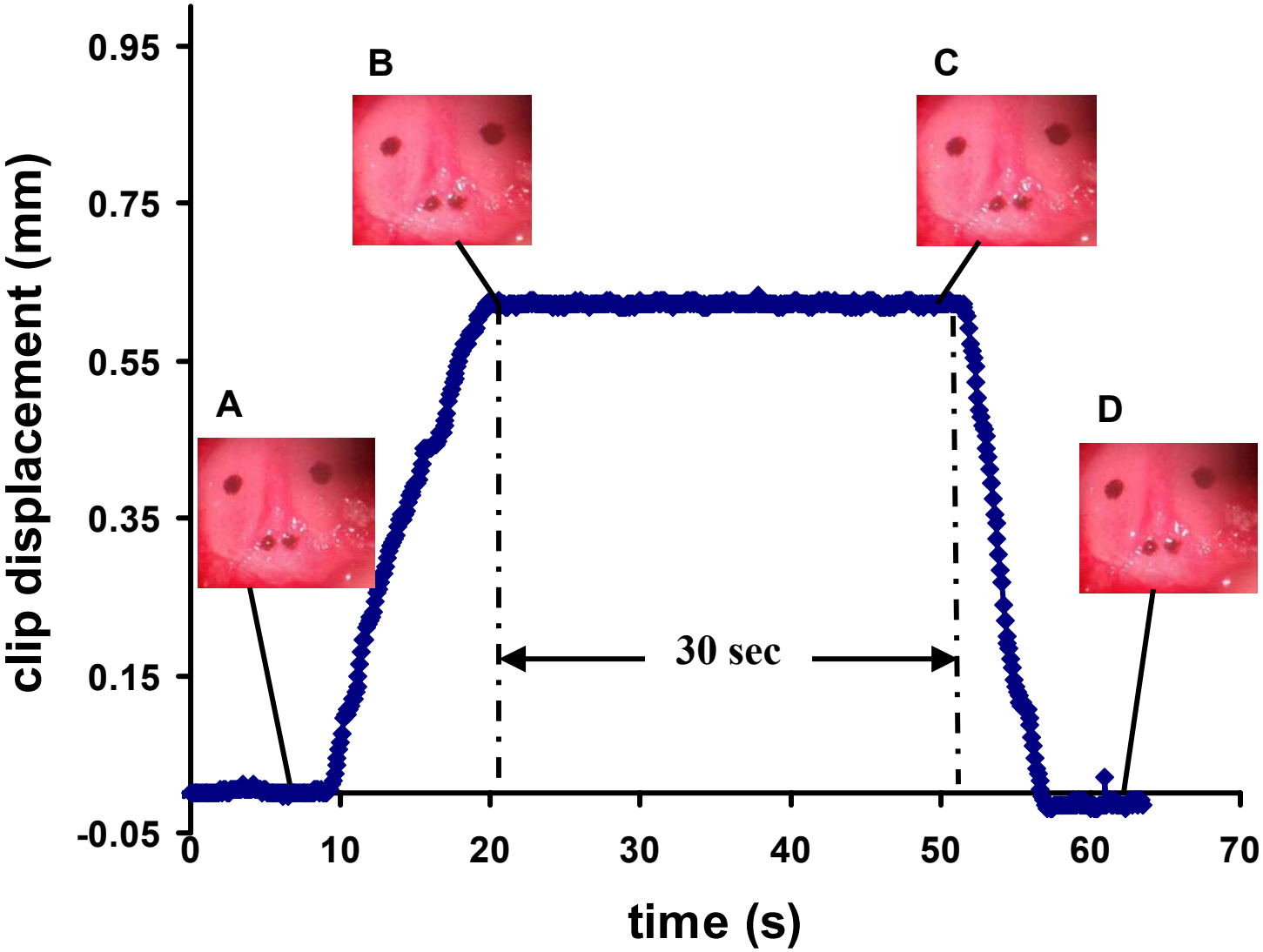
Figure 5. Average total mechanical allodynia for the entire 7-day postoperative testing period for each surgical group using the 2 g **(A)** and 1.4 g **(B)** von Frey filaments. For testing with both filaments, *high* strain injuries resulted in a significant increase in total allodynia over both *low* strain injuries ($p<0.0005$, both filaments) and *sham* injuries ($p<0.0005$, both filaments). Total allodynia in the *low* injury group was significantly elevated above *sham* levels for the 2.0 g filament ($p=0.019$) **(A)**, but not for testing with the 1.4 g filament **(B)**.

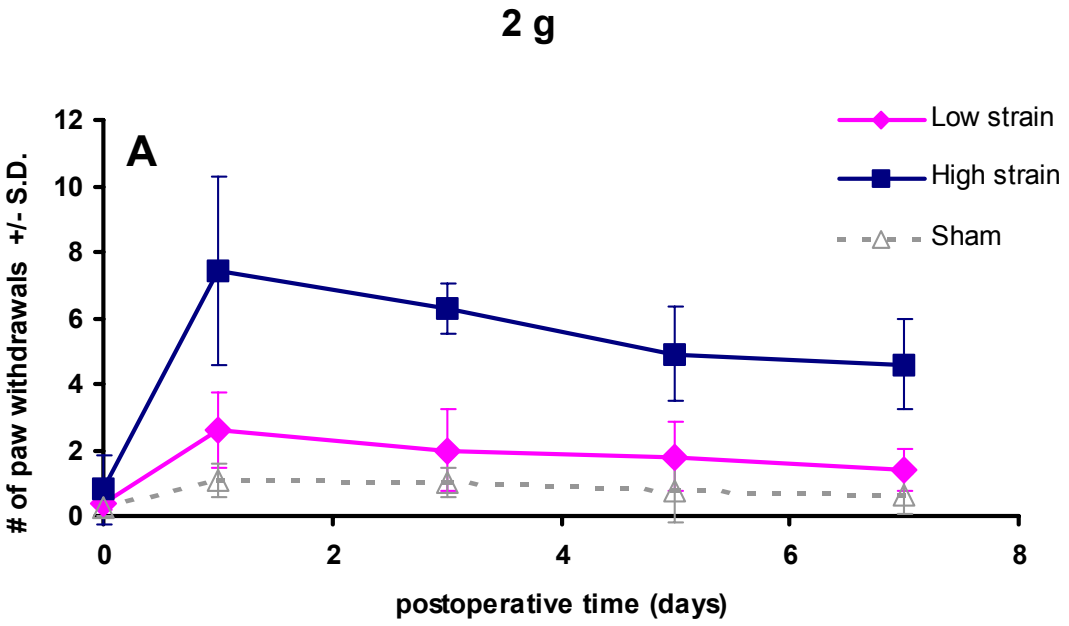
Table I. Summary of imposed facet joint injury mechanics.

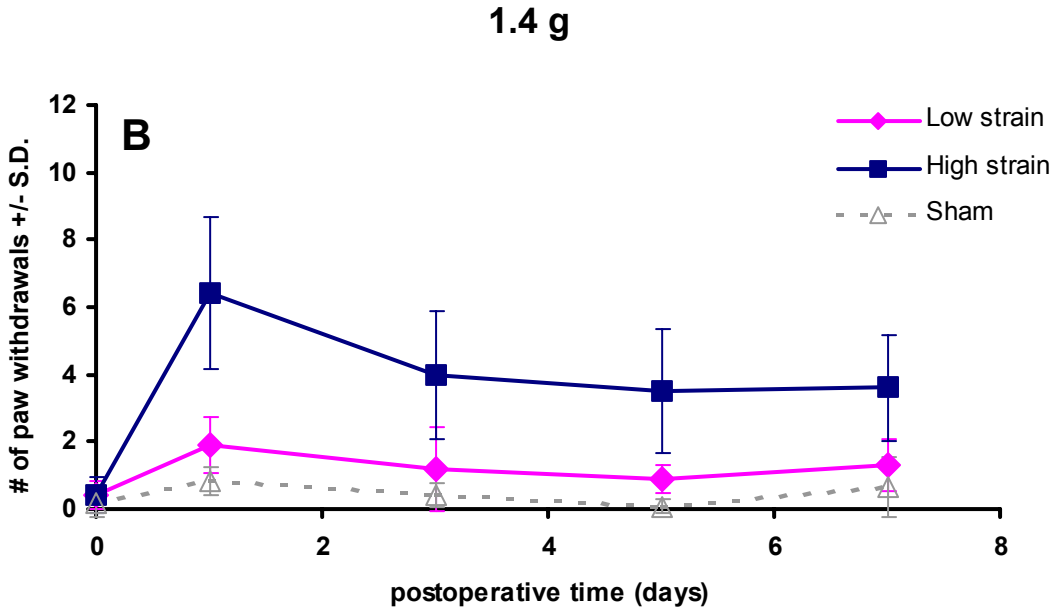
Rat	Rate (mm/s)	Load Time (s)	Clip Disp. (max, mm)	Marker Disp. (max, mm)	Strain (%)
K1	0.08	6.5	0.51	0.31	13.41
K7	0.11	4.4	0.52	0.20	8.79
K11	0.10	4.8	0.51	0.26	13.67
6	0.14	9.1	0.81	0.26	9.50
11	0.06	10.9	0.63	0.20	9.93
***LOW* Avg (SD)**	**0.10 (0.03)**	**7.14 (2.80)**	**0.60 (0.13)**	**0.25 (0.05)**	**11.06 (2.30)**
K4	0.07	12.6	1.22	0.51	37.90
K2A	0.08	18.1	1.66	0.45	34.11
K4A	0.08	14.5	1.44	0.60	34.41
5	0.09	14.2	1.65	0.59	31.77
8	0.09	19.3	1.51	0.61	29.56
***HIGH* Avg (SD)**	**0.08 (0.01)**	**15.74 (2.83)**	**1.50 (0.18)**	**0.55 (0.07)**	**33.55 (3.13)**

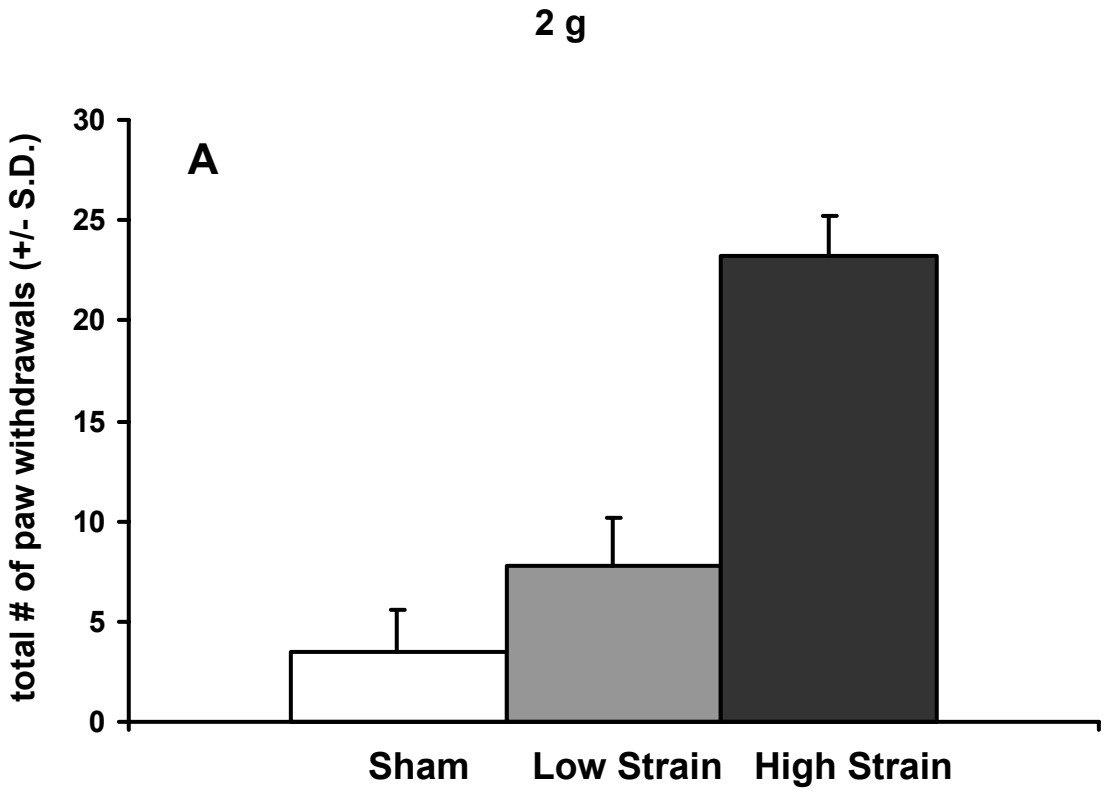

Micro
Forceps
C6
DISPLACED
ROSTRALLY
Spinous
Process
C7
C6
Bony
Markers
C6 clamp
Surgical
Microscope with
Camera
C7 clamp
(stationary)
Manual
Micrometer
LVDT
Inhalation
Anesthesia

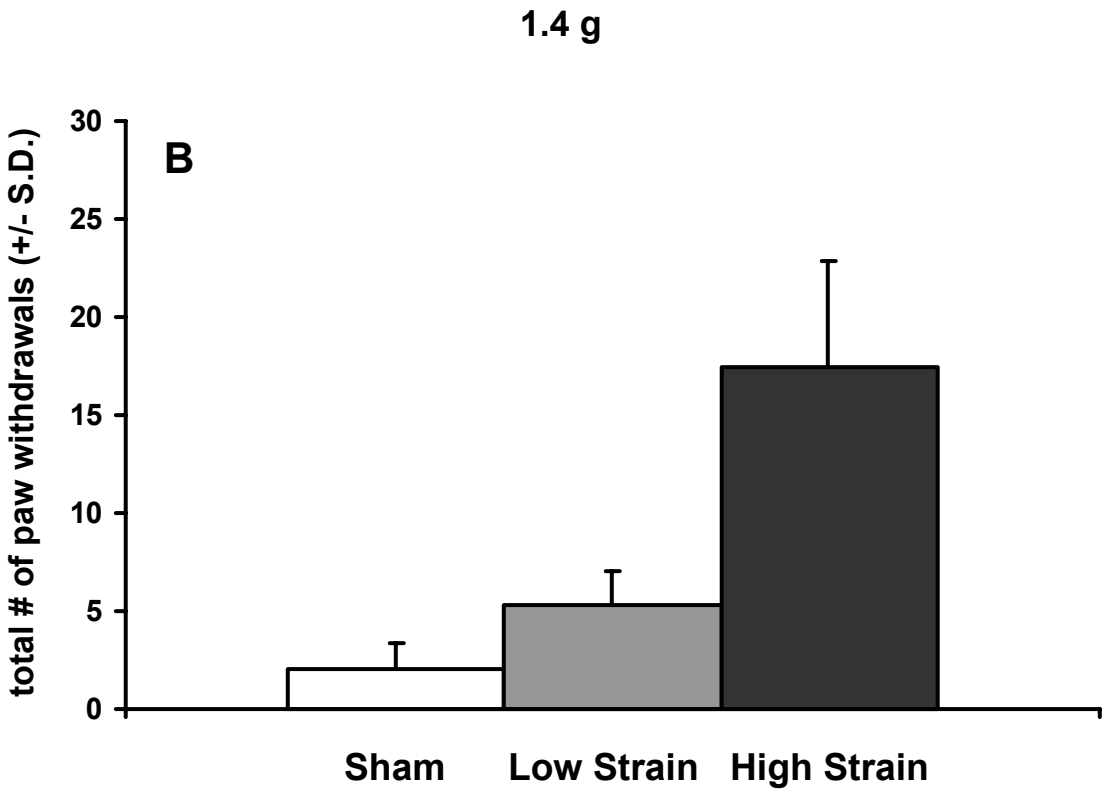

C7 lamina
ℓ_o
A
C6 lamina
facet
capsule
CAUDAL
ROSTRAL
ℓ_f
B
APPLIED
TENSION
1 mm


0.95
0.75
0.55
0.35
0.15
-0.05
clip displacement (mm)
0
10
20
30
40
50
60
70
time (s)
A
B
C
D
30 sec


2 g
A
Low strain
High strain
Sham
# of paw withdrawals +/- S.D.
postoperative time (days)
0
2
4
6
8
10
12


1.4 g
B
Low strain
High strain
Sham
# of paw withdrawals +/- S.D.
postoperative time (days)
0
2
4
6
8
10
12


2 g
A
total # of paw withdrawals (+/- S.D.)
30
25
20
15
10
5
0
Sham
Low Strain
High Strain


1.4 g
B
total # of paw withdrawals (+/- S.D.)
30
25
20
15
10
5
0
Sham
Low Strain
High Strain