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Transient Cervical Nerve Root Compression in the Rat Induces Bilateral Forepaw Allodynia and Spinal Glial Activation: Mechanical Factors in Painful Neck Injuries

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Study Design. An *in vivo* rat model of transient cervical nerve root compression.

Objectives. To investigate the potential for cervical nerve root compression to produce behavioral hypersensitivity and examine its dependence on compression.

Summary of Background Data. Clinically, nerve root injury has been hypothesized as a potential source of neck pain, particularly because cervical nerve roots are at mechanical risk for injury during neck loading. Lumbar radiculopathy models of nerve root ligation show that mechanical allodynia and spinal glial changes depend on nerve root deformation magnitude. However, no investigation has been performed to examine cervical nerve root compression as a cause of pain.

Methods. Two compressive forces (10 and 60 grams force [gf]) were transiently applied to the C7 nerve roots unilaterally using microvascular clips in separate groups (n = 12 each). Sham procedures were also performed in a separate group of rats (n = 12). Bilateral forepaw mechanical allodynia was monitored after surgery for 7 days. On day 7, spinal glial activation was assessed using immunohistochemistry to investigate its dependence on nerve root compressive force, in the context of behavioral hypersensitivity.

Results. Bilateral allodynia was observed following injury, which was significantly ($P < 0.042$) increased over sham and baseline responses. No difference in allodynia was found between the 10 and 60 gf injuries. Astrocytic and microglial activation were observed in the ipsilateral dorsal horn following compression, with only astrocytic activation paralleling allodynia patterns.

Conclusions. Results imply a force threshold exists less than 10 gf for persistent pain symptoms following transient cervical nerve root compression. Findings also suggest that spinal glial activation may be related to behavioral sensitivity and may modulate cervical nerve root mediated pain.

Key words: nerve root, cervical, allodynia, biomechanics, glia, radiculopathy, neck pain. **Spine 2005;30: 1924–1932**

Chronic neck pain has a reported prevalence as high as 30%, with annual costs reaching more than $29 billion.[1] As many as 45% of these cases result from whiplash injuries; yet, painful neck injuries also result from axial loading during recreational accidents and contact sports.[2,3] Although injuries to many spinal tissues, including facet joints, ligaments, and surrounding muscles, can lead to chronic neck pain,[4–8] cervical nerve roots are at particular risk because of their structural frailty and potential for compression resulting from foraminal shape changes during vertebral motions.[9] Coupling the mechanical risk for transient cervical nerve root compression with the known capacity of lumbar nerve roots to elicit low back pain (LBP),[10–12] nerve root compression in the cervical spine is a likely mechanism for producing painful injuries.

Although studies in the lumbar spine have investigated pain symptoms following nerve root ligation,[10–12] responses in the cervical spine cannot simply be assumed as similar scaled versions of the same cascades. In fact, the close proximity to the brain and its supraspinal influences on pain may imply a wholly different or more severe response for these same injuries in the neck. Brachial plexus injury models using compression, ligation, traction, or avulsion have produced neural dysfunction and pain.[13,14] However, these studies do not explicitly investigate mechanical parameters at injury. Ramer *et al*[15] report sensory responses for a cervical nerve root regeneration model without considering the role of mechanics in nerve root compression injury. Currently, no studies show the cervical nerve root's potential for producing pain caused by compressive loading or its dependence on mechanics.

Lumbar radiculopathy models, some with quantifiable injury parameters, show that spinal nerve root injury induces allodynia (*i.e.,* pain caused by a stimulus that does not normally provoke pain) and hyperalgesia (*i.e.,* an increased response to a normally painful stimulus) in the innervated hind paw.[8,12,16–22] Similarly, injury to cervical nerve roots produces sensitivity in the associated dermatomes, which can be assessed and quantified in the forepaw.[23] In clinical cases of radicular pain, the distribution of pain extends from the neck into the arm and hand, allowing forepaw hypersensitivity to serve as an indicator of painful nerve root injury.[24] In LBP models, behavioral hypersensitivity is significantly correlated with nerve root compressive strain magnitudes and spinal inflammatory cytokine messenger ribonucleic acid.[8,12,17,18,20] Many other physiologic re-

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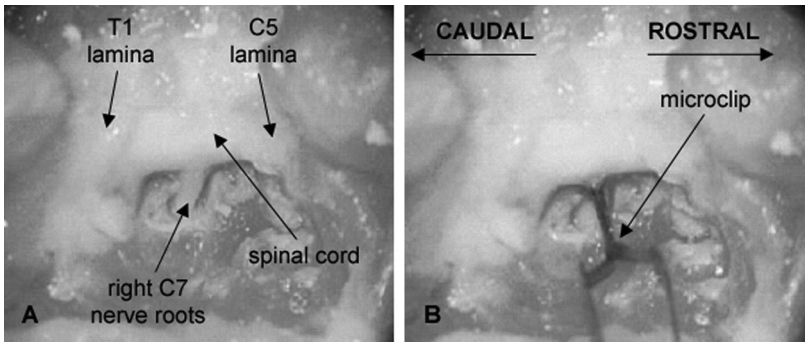

Figure 1. Surgical procedures show the exposure (**A**) and clip compression (**B**) of the right C7 nerve roots. Anatomic landmarks are indicated by arrows (**A**), including the laminae of the C5 and T1 vertebrae superior and inferior to the C6/C7 level, exposing the nerve roots for microclip compression (**B**). The rostral direction is oriented to the right in both images, as labeled in **B**.

sponses, both at the injury site and in the central nervous system (CNS), contribute to behavioral hypersensitivity, including glial cell activation, cytokine up-regulation, Wallerian degeneration, and altered electrophysiology.[10,12,18,20,25–31] These physiologic responses have not been investigated in relation to behavioral changes or injury mechanics for cervical nerve root injury and pain.

Nociceptive responses can lead to central sensitization, causing a decreased threshold and an enhanced responsiveness of the CNS for afferent inputs.[32] Bilateral behavioral hypersensitivity and pain symptoms can result from spinal changes, and have been reported in both animal models of LBP and clinical studies of neck pain.[17,33,34] While suggesting that cervical nerve root compression may elicit bilateral sensitivity, no work has experimentally investigated this or its associated CNS nociceptive responses for painful injuries in the neck.

Therefore, this study investigates whether transient cervical nerve root compression can produce pain in the rat and examines the dependence of behavioral hypersensitivity on compressive force. Behavioral sensitivity is measured and validated by 2 methods of quantifying mechanical allodynia in 2 separate behavioral studies. Spinal glial activation is assessed for insight into potential nociceptive mechanisms contributing to behavioral sensitivity. Efforts are focused on understanding mechanical contributions to painful nerve root compression in the cervical spine.

■ Materials and Methods

All experiments were performed using male Holtzman rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing 275–375 g at the start of the study, housed under US Department of Agriculture and the Association for Assessment and Accreditation of Laboratory Animal Care approved conditions, with a 12–12 hours light-dark cycle, and free access to food and water. All experimental procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee, and performed according to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.[35]

Surgical Procedures and Nerve Root Compression Injury. All procedures were performed with the rats under inhalation anesthesia (4% halothane for induction, 2% for maintenance). Rats were placed in a prone position, and an incision was made in the skin from the base of skull to the bony prominence of the second thoracic vertebra. Muscle and soft tissue were cleared to expose the C6 and C7 laminae, under a surgical microscope (Carl Zeiss, Inc., Thornwood, NY). A C6/C7 hemilaminectomy and partial facetectomy exposed the spinal cord, and C7 dorsal and ventral roots, on the right side. Dura surrounding the nerve root's insertion into the spinal cord was gently ruptured to provide free exposure of the roots (Figure 1A). For nerve root compression, a microvascular clip was applied to the right C7 nerve roots proximal to the dorsal root ganglion (Figure 1B) and was removed after 15 minutes. Two clip magnitudes were used in separate groups: a 10-grams force (gf) clip (WPI, Inc., Sarasota, FL) or a 60-gf clip (Roboz, Inc., Gaithersburg, MD). Light sham procedures involved the same surgery as described previously but without dural rupture or nerve root manipulation. Heavy sham procedures included dural rupture but no further manipulation. Following surgery, wounds were closed using 3-0 polyester suture. Rats were recovered in room air and monitored throughout their recovery.

Surgical procedures were performed in 2 separate studies using techniques for measuring frequency and stimulation threshold of forepaw withdrawals. In the frequency study, animals were divided into 4 surgical groups: 10-gf compression injury (n = 6), 60-gf compression injury (n = 6), light sham (n = 4), and heavy sham (n = 4). In the threshold study, the same injury groups (n = 6 each) were used as well as a light sham group (n = 4).

Mechanical Allodynia. All rats were evaluated for bilateral forepaw mechanical allodynia on days 1, 3, 5, and 7 after surgery. Before injury, animals were acclimated to the testing environment, and baseline measurements recorded. A single tester performed all allodynia testing for this study, blinded to the surgical procedures.

Allodynia methods for quantifying frequency of forepaw withdrawals were adapted from well-established methods used for hind paw evaluation in lumbar pain models[10,11,18,20,36,37] and have been previously implemented by our laboratory to detect forepaw sensitivity.[38] Briefly, after 20 minutes of acclimation, rats were stimulated on the plantar surface of each forepaw using 3 von Frey filaments (0.6, 1.4, and 2.0 g) (Stoelting Co., Wood Dale, IL). Each testing session consisted of 3 rounds of 10 tactile stimulations on each forepaw, separated by 10 minutes. For each filament, the ipsilateral paw was tested before the contralateral paw. The number of responses elicited by the ipsilateral and contralateral forepaws was recorded separately for the 3 filaments after every round. The total number of paw withdrawals was summed for each forepaw of each rat.

To provide validation for the frequency method, allodynia was measured in a second study using Chaplan's up-down

threshold method.[39–42] Each testing session consisted of 3 rounds of 5 stimulations per forepaw, with a series of 9 logarithmically ascending filament strengths (0.4, 0.6, 1.4, 2.0, 4.0, 6.0, 8.0, 15.0, and 26.0 g). The first filament to elicit one withdrawal response was recorded as the threshold, with verification by the next higher filament. Failure to respond to the 26.0 g filament was recorded as a threshold of the next higher filament in the series (60.0 g). An average of the thresholds over 3 rounds was recorded for each forepaw.

Immunohistochemistry. Glial activation was assessed in cervical spinal cord tissue harvested 7 days after surgery. Animals were deeply anesthetized and transcardially perfused with 200 mL phosphate buffered saline (PBS), followed by 300 mL 4% paraformaldehyde in PBS (pH 7.4). Following perfusion, C7 spinal cord tissue was harvested and postfixed in 4% paraformaldehyde for 20 minutes. Samples were then transferred to 30% sucrose/PBS and stored for 3 days at 4°C. Tissue was freeze mounted on cork, with OCT medium (Triangle Biomedical Sciences, Durham, NC) for cryostat sectioning.

Serial C7 spinal cord sections (20 μm) from each rat were prepared for free-floating immunohistochemical staining. 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) was used as a marker of activated microglia (1:500). The avidin-biotin technique was used to localize areas of activation (Vector Labs, Burlingame, CA). For each assay, a negative control with no primary antibody staining and normal naïve controls (n = 3) were included in the analysis for normalization and comparison between groups.

Semiquantitative image analysis methods were used to evaluate the degree of activation in each sample. A representative section from each rat was photographed at 50× magnification using a digital camera and stereomicroscope system (Zeiss, Thornwood, NY). Images were acquired for each of the dorsal and ventral horns on the ipsilateral and contralateral sides relative to injury. The contrast and brightness of each photograph was uniformly adjusted in all images for assessment using Adobe Photoshop (version 7.0, Adobe, San Jose, CA). The degree of activation for each sample was graded by 2 observers blinded to groups, based on cell numbers, compactness, and intensity of staining. An established 4-point scale,[43] previously used by Winkelstein and DeLeo,[44] was used to grade activation intensity. Assessments were made according to that scale with the following levels of gradation: baseline staining (-), mild response (+), moderate response (++), and intense response (+++).

Statistical Analysis. Mechanical allodynia data were averaged for each group. Paw withdrawal frequencies were compared using a 2-way analysis of variance (ANOVA) with repeated measures to determine significant effects of compression force over time followed by a 1-way ANOVA with *post hoc* Bonferroni correction to compare means on days 1, 3, 5, and 7. Nonparametric data from the threshold study were analyzed using a ranked 2-way ANOVA with repeated measures followed by a 1-way ANOVA with *post hoc* Bonferroni correction. All statistical analyses were performed using SYSTAT software (version 10.2, SYSTAT, Richmond, CA), and significance was defined at $P < 0.05$.

■ Results

During surgery and at completion of the study, direct observation of the nerve roots confirmed they were structurally intact for all procedures. After surgery, all rats showed mobility with normal grooming behavior and consistent weight gain. They also showed good head mobility, indicating no adverse effects of the surgical procedures on neck motion.

Mechanical Allodynia

All injured animals showed increased bilateral forepaw allodynia over shams following either 10 or 60-gf compression injury (Figures 2, 3). There was no significant difference in mechanical allodynia between the 2 sham procedures (heavy and light) for any von Frey filament strength; accordingly, both sham groups were considered indistinguishable ($P > 0.45$, data not shown) and combined for behavioral analyses. Ipsilateral sham behaviors were not different from baseline values, except on day 1 for the 1.4-g filament ($P = 0.025$). Contralateral sham behaviors did not differ from baseline, except on day 3 for the 1.4-g filament ($P = 0.007$).

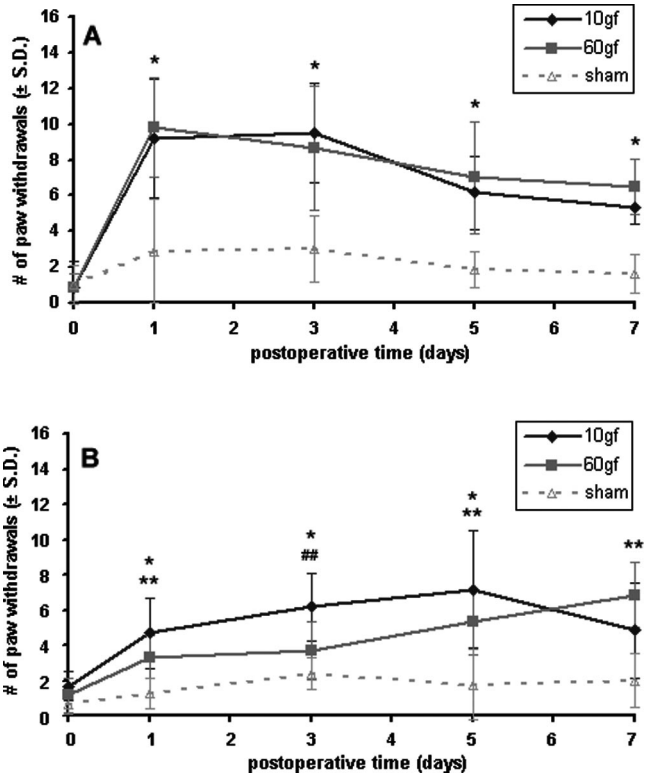

Figure 2. Average forepaw mechanical allodynia shown as the number of paw withdrawals for 30 stimulations with 2.0-g von Frey filament. **A**, Ipsilateral allodynia was significantly increased for both injury magnitudes over sham on all days (*$P < 0.01$). **B**, Contralateral allodynia for 10-gf compression was significantly increased over sham on days 1, 3, and 5 (*$P < 0.002$). Likewise, the 60-gf injury produced significantly more paw withdrawals than sham on days 1, 5, and 7 (**$P < 0.042$). Allodynia was not different between the 2 injury groups, with the exception of day 3 (##$P < 0.03$), as tested on the contralateral paw (**B**). Stimulation with the 1.4 and 0.6-g von Frey filaments produced similar trends in allodynia (data not shown). #, number; SD, standard deviation.

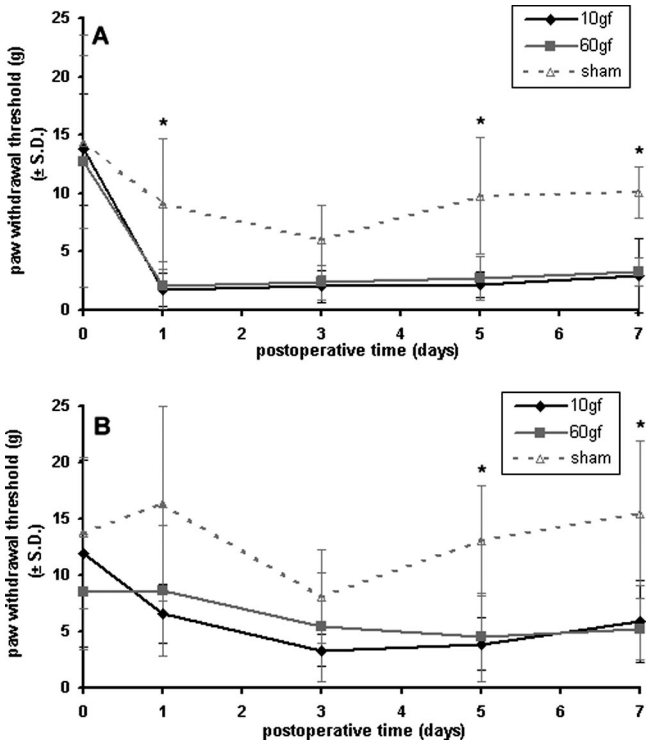

Figure 3. Average forepaw withdrawal threshold for each group shown as average least force von Frey filament to elicit a response.[39] **A**, Threshold for response ipsilateral to injury was significantly decreased compared to sham on days 1, 5, and 7 for both compression forces (*$P < 0.049$). **B**, Threshold for both injury groups was significantly decreased with respect to sham on days 5 and 7 only (*$P < 0.038$). Allodynia was not different between the 2 injury groups on any day for either forepaw. SD, standard deviation.

Ipsilateral forepaw allodynia for both injury groups was significantly increased above baseline and sham levels for the 2.0-g von Frey filament on all days ($P < 0.026$) (Figure 2A). These increases over sham were also detected for both injuries with the 1.4 and 0.6-g von Frey filaments ($P < 0.039$) (data not shown). There was no difference in ipsilateral allodynia between the 2 injury groups at any time ($P > 0.08$) (Figure 2A). These observations were supported by both methods of allodynia testing. Using the threshold method, significant decreases in withdrawal threshold were detected for both the 10 ($P < 0.009$) and 60-gf ($P < 0.049$) injury groups, except on day 3 (Figure 3A). Also, there was no difference between injury groups at any time ($P > 0.659$).

Contralateral forepaw allodynia of the injury groups was less robust than that observed ipsilaterally, yet remained increased above baseline (Figure 2B). This sensitivity was significantly increased over shams on days 1, 3, and 5 for the 10-gf injury ($P < 0.002$) and on days 1, 5, and 7 for the 60-gf injury ($P < 0.042$), for the 2.0-g von Frey filament. Responses to the other von Frey filaments (1.4 and 0.6 g) also evoked later onset of allodynia compared to ipsilateral sensitivity (data not shown). The difference in contralateral allodynia between the 2 injury groups was not statistically significant, with the exception of day 3 for the 2.0-g filament ($P < 0.03$). Withdrawal thresholds for both injury groups differed significantly from sham values on days 5 and 7 ($P < 0.038$), and these groups did not differ from each other on any day ($P > 0.9$) (Figure 3B), confirming the behavioral findings related to clip force in this new model of painful nerve root injury.

Immunohistochemistry

Baseline staining (-) was assigned to samples with glial activation equivalent to or less than that of naïve animals (Figures 4A, E). Representative samples indicating activation with increasing differences in cell count, compactness, and staining intensity are shown in Figure 4.

Astrocytic activation, as measured by GFAP staining, was increased compared to normal in the ipsilateral dorsal horn of the C7 spinal cord on day 7 following injury in all rats undergoing nerve root compression (Figure 5C, D). Staining was intense in both injury groups for most animals (Figure 5C, D; Table 1). Increased reactivity was also apparent in the ipsilateral ventral horns of all injured animals, although to a slightly lesser degree than in the dorsal horn (Table 1). Contralateral astrocytic activation was present in the dorsal horn for both injury groups, although less pronounced than ipsilaterally and sometimes absent. Sham animals showed mild GFAP reactivity in all C7 spinal regions, with slightly increased levels in the dorsal horns of the heavy over light sham groups (Figure 5B, Table 1). Overall, both sham groups showed less astrocytic activation than the injury groups (Table 1).

OX-42 staining on day 7 was less robust than GFAP and was not produced for nerve root compression in all animals (Table 2). OX-42 staining was increased in the ipsilateral dorsal horn for all 60-gf injury animals (Figure 6D), which was not observed in any other group (Table 2). The 10-gf clip injury produced modest but inconsistent reactivity of OX-42 in the ipsilateral dorsal horn (Figure 6C) that was not present in either sham group (Table 2). OX-42 staining of the ipsilateral dorsal horn was minimal in light shams and often at baseline levels for all C7 regions (Table 2). Some activation was observed in the heavy sham group in the ipsilateral dorsal horn, yet levels were mild (Figure 6B, Table 2). Contralateral OX-42 reactivity was minimal in all animals and increased over normal in only a few isolated cases (Table 2).

■ Discussion

To the best of our knowledge, the study presented here is the first to document the existence of forepaw behavioral hypersensitivity as a result of transient cervical nerve root compression. Compression of either 10 or 60 gf is sufficient to induce bilateral mechanical allodynia (Figures 2, 3). However, in this model, allodynia is not differentiated by these applied forces. Contralateral allodynia was produced to a lesser extent than ipsilateral

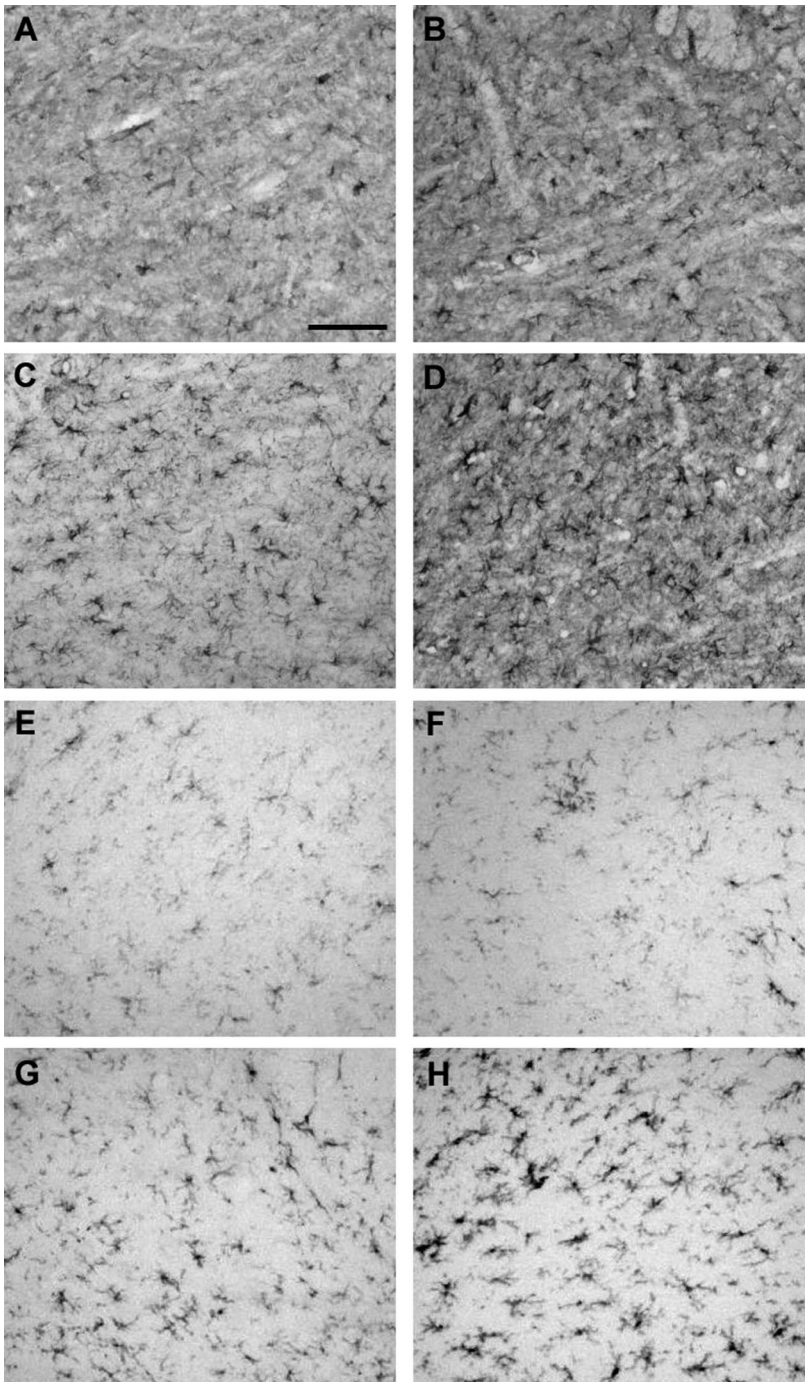

Figure 4. Representative samples of C7 spinal cord used for grading of astrocytic (GFAP) (**A** to **D**) and microglial (OX-42) activation (**E** to **H**). Baseline staining (-) (**A** and **E**) was assigned to samples with immunoreactivity equivalent to or less than that observed for naïve animals. Mild response (+) (**B** and **F**), moderate response (++) (**C** and **G**), and intense response (+++) (**D** and **H**) were assigned to samples with increasing differences in cell count, compactness, and staining intensity. Scale bar = 100 μm.

allodynia and showed a later onset (Figures 2B, 3B). This delay in the development of contralateral allodynia has also been reported for lumbar pain models.[20,33,45] In contrast, Sekiguchi *et al*[22] reported no contralateral allodynia in a rat model of nerve root crush. However, forceps was used to manually crush the L5 nerve root for only 2 seconds, and ipsilateral allodynia was brief and only transient, highlighting the potential modulatory effect that compression duration may have on maintaining behavioral hypersensitivity. Continued research is required to further investigate these and other modulatory factors of injury mechanics on pain. Extending the time for evaluating behavioral sensitivity for cervical nerve root injury would also elucidate factors affecting its chronicity and injury mechanisms.

The existence of bilateral allodynia in this model suggests that spinal mechanisms may drive sensitivity following nerve root injury in the neck. This effect is further supported by bilateral spinal glial activation (Tables 1, 2), particularly astrocytic activation, following injury. Ipsilateral GFAP staining 7 days after injury was increased above normal to the same degree for both compression magnitudes (Figure 5, Table 1), corresponding to similarly increased ipsilateral allodynia responses (Figures 2A, 3A). This result implicates spinal astrocytic activation and its subsequent sequelae as possible mech-

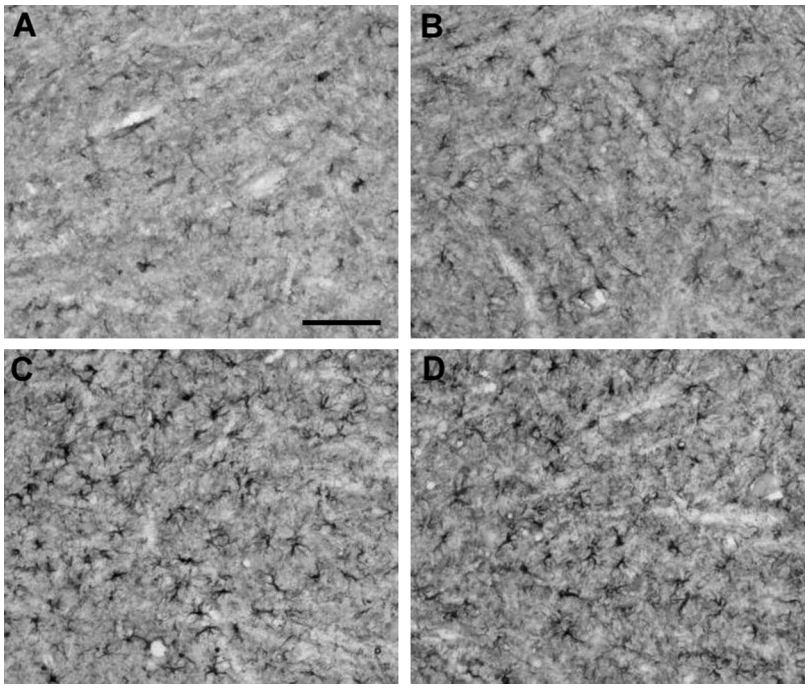

Figure 5. Ipsilateral dorsal horns of representative C7 spinal cord sections stained against GFAP at day 7 after injury. Matching normal naïve samples were assigned baseline levels of staining (**A**). Both sham procedures produced a mild (+) increase in staining for the ipsilateral dorsal horn (**B**) compared to normal. In general, the 10-gf compression produced a modest increase in staining (**C**), while a 60-gf compression consistently produced a moderate (++) to intense (+++) response in the ipsilateral dorsal horn (**D**). Scale bar = 100 μm.

anisms contributing to cervical nerve root-mediated pain. Similarly, GFAP reactivity was observed in the contralateral spinal cord for both forces (Table 1), again following behavioral hypersensitivity patterns (Figures 2B, 3B). Dissociation of GFAP reactivity and compressive force in this study is consistent with our earlier report of astrocytic activation lacking dependence on nerve root injury magnitude in the lumbar spine.[44]

In the present study, OX-42 staining depended on applied force, and contralateral staining was absent or

Table 1. Immunohistochemical Scoring of Staining for GFAP Reactivity on Day 7

		Astrocytic (GFAP) Staining			
		Ipsilateral		Contralateral	
Treatment	ID	DH	VH	DH	VH
Normal	N1	–	–	–	–
	N2	–	–	–	–
	N3	–	–	–	–
Light sham	6	+	+	+	+
	11	+	++	+	+
	12	+	++	+	+
	14	+	+	–	–
Heavy sham	5	++	++	+	+
	13	++	+	+	+
	15	++	+++	+	++
	18	+	–	–	–
10-gf Injury	1	+	+	–	+
	2	+++	++	+	++
	9	+++	++	++	+++
	10	+	+	–	–
	16	+++	+++	+	+
	17	+++	+++	++	++
60-gf Injury	1c	+++	++	++	++
	2c	++	+	+	–
	3	+++	++	+	+
	4	+++	+++	+	++
	7	++	+	–	+
	8	+++	+++	+++	+++

Assessments were made on a 4-point scale with the following levels of gradation: (–) baseline staining, (+) mild response, (++) moderate response, and (+++) intense response.
DH = dorsal horn; ID = animal identification number; VH = ventral horn.

Table 2. Immunohistochemical Scoring of Staining for OX-42 Reactivity on Day 7

		Microglial (OX-42) Staining			
		Ipsilateral		Contralateral	
Treatment	ID	DH	VH	DH	VH
Normal	N1	–	–	–	–
	N2	–	–	–	–
	N3	–	–	–	–
Light sham	6	–	–	–	–
	11	+	–	+	–
	12	+	–	–	+
	14	–	–	–	–
Heavy sham	5	–	–	+	–
	13	++	++	+	+
	15	+	+	–	–
	18	+	–	–	+
10-gf Injury	1	++	++	+	+
	2	+	++	+	–
	9	+	+	–	+
	10	+++	++	+	–
	16	–	–	–	–
	17	–	+	–	–
60-gf Injury	1c	+	++	–	+
	2c	++	++	–	+
	3	+	+	–	–
	4	++	+++	+	+
	7	++	++	+	+
	8	++	+++	++	–

Assessments were made on a 4-point scale with the following levels of gradation: (–) baseline staining, (+) mild response, (++) moderate response, and (+++) intense response.
DH = dorsal horn; ID = animal identification number; VH = ventral horn.

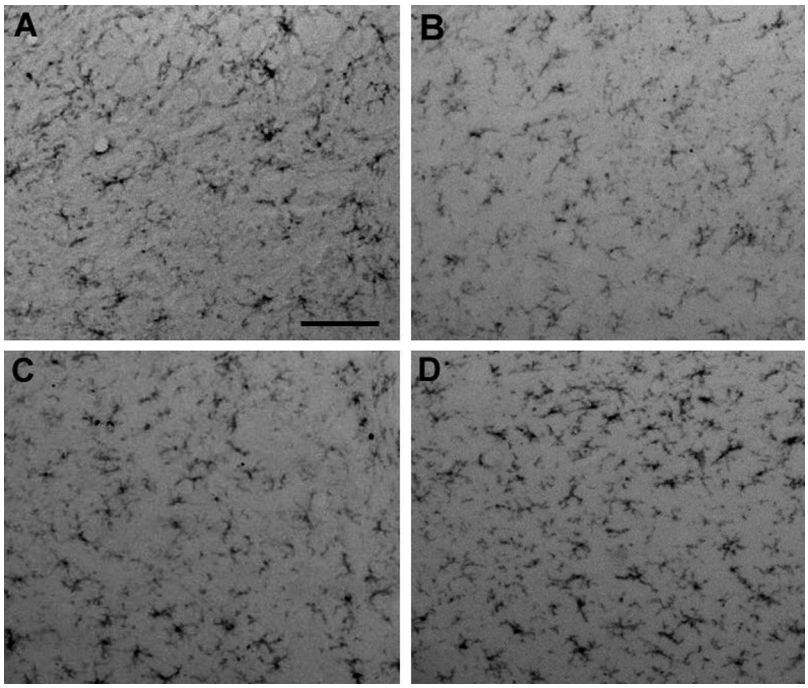

Figure 6. Ipsilateral dorsal horns of representative C7 spinal cord sections stained against OX-42 at day 7 after injury. Sections from naïve normal rats were assigned baseline levels of staining (**A**). Both sham procedures produced only a mild (+) increase in staining intensity (**B**) that was apparent in only some animals. The 10-gf injury induced staining in several animals but did not consistently produce more than a mild (+) response (**C**). The 60-gf injury caused more moderate (++) microglial activation (**D**), with activation of some intensity in all 60-gf injury animals. Scale bar = 100 μm.

mild (Figure 6, Table 2). Although the intent of the 2 sham groups was to investigate whether allodynia results from dural rupture during surgery, the slight differential in microglial activation between the sham groups (Table 2) served to reveal the sensitivity of spinal microglia to nonallodynia producing procedures. Microglial activation, while indicating sensitivity to compressive loading, is here uncoupled with behaviors, implying that microglial responses may not drive behavioral hypersensitivity. Previous studies have also documented this association between microglial activation and perceived CNS injury.[11,43,44] However, despite the 6-fold difference in compression magnitudes, a similarly large differential in microglial activation for the 2 compression groups was not observed (Table 2). Differential spinal glial responses in the context of allodynia and injury force imply that microglia either may not play a dominant role in affecting pain responses following nerve root injury or that a downstream effect of their low level activation may contribute to persistent pain. Further investigation is needed to elucidate the specific nociceptive spinal pathways in these painful injuries, which may be further clarified by assessment at earlier times following injury.

In models of LBP, glial cell activation has induced production and release of proinflammatory cytokines, which are associated with a cascade of other molecular responses, including the release of substance P, up-regulation of major histocompatibility complex class II, and others, driving persistent pain.[10–12,18,20,26,28,29,46,47] Therefore, the presence of glial activation in our model suggests that a cascade of cellular events involving these mechanisms may also occur following cervical nerve root injury.

Although in the current study compressive force was varied, mechanical allodynia and GFAP staining were not different between these injuries (Figures 2, 3, 5). While astrocytic activation may be directly linked to painful outcomes in this study, previous work has identified additional mechanisms of nociception modulated by mechanics, including endoneurial edema, Wallerian degeneration, cytokine production, and electrophysiologic alterations.[12,20,25,27,30,31,48–50] In the Olmarker *et al*[25] model of lumbar root compression, edema increased with magnitude of compression. Chen *et al*[50] reported that at low compression magnitudes, duration of sciatic nerve compression influenced formation of endoneural edema. That model applied a minimum force of 100 gf to a 5-mm nerve segment, mechanically comparable to the 10 gf applied to the 0.5-mm nerve root segment used in our model. Likewise, Kobayashi and Yoshizawa[30] used microvascular clips to compress dog lumbar roots and established a load threshold of 15 gf, which governed vascular permeability of the dorsal root ganglion. A load threshold for behavioral hypersensitivity may likely exist less than the minimal force (10 gf) in our model. Certainly, our data suggest no difference in mechanical allodynia for forces $>$ 10 gf (Figures 2, 3). Further investigations are required to determine this threshold and the effect of mechanics on modulating painful outcomes.

While studies have investigated load-based mechanisms of pain, others have examined mechanical relationships using deformation and/or strain measurements. Cornefjord *et al*[47] reported a significant dependence of nerve conduction velocity on root constriction magnitude after 1 week of compression in a porcine model. Winkelstein *et al*[8,12] showed graded spinal cytokine levels and allodynia according to applied root compressive strain in the rat. Together, these data imply that nerve root deformation may be a better predictive mechanical variable than load regarding modulating behavioral and spinal changes. Preliminary *in*

situ investigations using the 10 and 60-gf microclips described in this study suggest that imposed nerve root strains are in fact similar in these cases (81.7% ± 4.7% and 87.7% ± 3.6%, respectively), despite the 6-fold difference in force,[51] potentially explaining the lack of behavioral differences between the 2 injury groups. However, to date and to our knowledge, no study has investigated behavioral outcomes following transient deformation-controlled injury. Incorporation of compressive deformation measurements and directional components of applied nerve root loading is needed to understand better the injury mechanisms governing pain onset following nerve root compression.

The injury model presented here mimics a unilateral, transient loading of the C7 nerve roots, which produces bilateral behavioral hypersensitivity sustained for 7 days. For these injury conditions, findings suggest that compressive loads ≥10 gf can induce persistent allodynia, which may be mediated by glial activation. This model is not only the first to show behavioral hypersensitivity after transient cervical nerve root loading but also documents the existence of spinal glial changes evident as late as 7 days after nerve root injury. Further manipulation of biomechanical parameters of injury (*i.e.*, duration, rate, stress and strain magnitude) will help determine the mechanical tolerance of this tissue to compression in the context of pain symptoms and specific activation of cellular pathways of nociception.

■ Key Points

- Transient cervical nerve root compression of 10 or 60 gf can produce bilateral behavioral hypersensitivity in the forepaw, lasting at least 7 days after injury.
- Spinal microglial activation indicates a sensitive response to compression magnitude, yet does not match patterns of forepaw mechanical allodynia.
- Spinal astrocytic activation is undifferentiated between the 2 injury groups, following the patterns of behavioral hypersensitivity.
- A load threshold for painful cervical nerve root compression likely exists <10 gf.

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