TRANSIENT NERVE ROOT COMPRESSION LOAD AND DURATION DIFFERENTIALLY MEDIATE BEHAVIORAL SENSITIVITY AND ASSOCIATED SPINAL ASTROCYTE ACTIVATION AND MGLUR5 EXPRESSION

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Abstract—Injury to the cervical nerve roots is a common source of neck pain. Animal models of nerve root compression have previously established the role of compression magnitude and duration in nerve root-mediated pain and spinal inflammation; yet, the response of the spinal glutamatergic system to transient nerve root compression and its relationship to compression mechanics have not been studied. The glutamate receptor, mGluR5, has a central role in pain, and its expression by neurons and astrocytes in the spinal cord may be pivotal for neuronal-glial signaling. This study quantified spinal GFAP and mGluR5 expression following nerve root compressions of different magnitudes and durations in the rat. Compression to the C7 nerve root was applied for a duration that was either above (10 min) or below (3 min) the critical duration for mediating afferent discharge rates during compression. To also test for the effect of the magnitude of the compression load, either a 10 gf or a 60 gf was applied to the nerve root for each duration. Mechanical allodynia was assessed, and the C7 spinal cord was harvested on day 7 for immunofluorescent analysis. Double labeling was used to localize the expression of mGluR5 on astrocytes (GFAP) and neurons (MAP2). Seven days after injury, 10 min of compression produced significantly greater behavioral sensitivity ($P<0.001$) and spinal GFAP expression ($P=0.002$) than 3 min of compression, regardless of the compression magnitude. Nerve root compression at 60 gf produced a significant increase ($P<0.001$) in spinal mGluR5 for both of the durations studied. There was no difference in the distribution of mGluR5 between astrocytes and neurons following nerve root compression of any type. The glutamatergic and glial systems are differentially modulated by the mechanics of nerve root compression despite the known contribution of glia to pain through glutamatergic signaling. © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: astrocyte, biomechanics, mGluR5, nerve root, pain, radicular.

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Neck pain affects 14–50% of the adult population each year (Côté et al., 2004; Hogg-Johnson et al., 2008). Cervical radiculopathy involves the cervical nerve roots and can arise from a disk herniation, spondylosis, or spinal trauma (Abbed and Coumans, 2007; Krivickas and Wilbourn, 2000; Wainner and Gill, 2000). Painful transient nerve root compression is associated with sustained damage to the axons of the nerve root, as well as with inflammation in the spinal cord (Hubbard and Winkelstein, 2005; Nicholson et al., 2011; Rothman et al., 2009). Although a loss of axonal flow has been demonstrated following nerve root injury, resulting in a decrease in neurotransmitters in the dorsal horn (Hubbard et al., 2008a; Kobayashi et al., 2008), the signaling mechanisms in the spinal cord that are related to nerve root-mediated pain are not well understood.

Primary afferent fibers release glutamate at their synapse with the second order neurons in the dorsal horn of the spinal cord (Basbaum et al., 2009). Studies in the rat demonstrate that glutamate concentration increases in the superficial dorsal horn within 4 days of a chronic peripheral nerve injury, in parallel with the development of behavioral sensitivity (al-Ghoul et al., 1993; Kawamata and Omote, 1996; Somers and Clemente, 2002). The G protein-coupled metabotropic (mGluRs) receptors are thought to play a role in persistent pain (Fisher et al., 2002; Markowitz et al., 2007). Specifically, activation of the two group I receptors, mGluR1 and mGluR5, enhance neuronal excitability (Lesage, 2004). Studies that demonstrate an increase in spinal mGluR5 following peripheral injuries, such as nerve transection, nerve constriction, or facet joint distraction, further suggest a role for this receptor in the maintenance of chronic pain (Dong and Winkelstein, 2010; Gwak and Hulsebosch, 2005; Hudson et al., 2002). To date, however, there is limited information regarding the role of the glutamatergic system in maintaining behavioral sensitivity following nerve root injury.

In the superficial dorsal horn of the spinal cord, mGluR5 is primarily expressed by postsynaptic neurons and astrocytes (D'Antoni et al., 2008; Jia et al., 1999; Lesage, 2004; Pitcher et al., 2007). Spinal astrocytes contribute to pain maintenance following nerve root injury (Colburn et al., 1999; Hashizume et al., 2000; Winkelstein et al., 2002) and act as a primary regulator of extracellular glutamate in the central nervous system (Markowitz et al., 2007). Previous studies demonstrate that following a spinal cord or brain injury, astrocytes in the injured area

increase their expression of mGluR5 (Ferraguti et al., 2001; Gwak and Hulsebosch, 2005). Inhibition of glial cells via minocycline treatment attenuates mechanical allodynia and mGluR5 expression after nerve injury (Osikowicz et al., 2009). Together, these studies provide evidence for glial cell signaling via the glutamatergic system in chronic pain. Although preemptive treatment with minocycline also prevents the development of mechanical allodynia following a nerve root injury (Rothman et al., 2009) and painful nerve root injuries are associated with spinal astrocyte activation (Colburn et al., 1999; Hubbard and Winkelstein, 2005; Rothman et al., 2010), no study has evaluated the role of astrocytic expression of mGluR5 in nerve root-mediated pain.

A model of a transient nerve root compression in the rat has previously established that pain is sensitive to both the magnitude and duration of compression that is applied to the nerve root (Hubbard et al., 2008a,b; Rothman et al., 2010). Using that model, our laboratory recently determined that the critical duration for mediating neuronal signaling during a 10 gf compression to the C7 nerve root is 6.6 ± 3.0 min (Nicholson et al., 2011). Although that duration threshold was determined based on the loading that reduced neuronal discharge rates during the applied compression, axonal damage and behavioral sensitivity were also found to develop following nerve root compressions of longer duration than this threshold and to be absent in compressions applied for shorter durations (Nicholson et al., 2011; Rothman et al., 2010). Further, it is likely that any duration threshold varies with the magnitude of the load applied (Olmarker et al., 1989; Pedowitz et al., 1992; Rydevik et al., 1991); yet, no study has combined the effects of load and duration on the development of behavioral sensitivity and spinal outcomes.

This study varied the load and duration of an applied compression to the nerve root in order to evaluate the effects of compression mechanics on the expression of spinal mGluR5 and astrocyte activation in the context of resulting behavioral sensitivity. In addition, the distribution of spinal mGluR5 across astrocytes and neurons was also quantified. Based on prior studies using transient mechanical insults, compression was applied to the nerve root for a period below (3 min) and above (10 min) the critical compression duration for mediating afferent discharge rates during compression (Nicholson et al., 2011). In addition, both the 10 gf and 60 gf compression loads were applied to determine the effect of magnitude on the development of behavioral and spinal outcomes at each of the two compression durations.

EXPERIMENTAL PROCEDURES

Experiments were performed using male Holtzman rats (300−400 g), housed under USDA- and AAALAC-compliant conditions and given free access to food and water. All procedures were approved by the Institution Animal Care and Use Committee and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmermann, 1983). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Surgical procedures

The C7 dorsal nerve root compression was performed under isoflurane inhalation anesthesia (4% for induction, 2% for maintenance). A dorsal midline incision was made over the cervical spine, and the C6 and C7 vertebrae were exposed by removing the overlying muscle and soft tissue. A C6-C7 hemilaminectomy and facetectomy were then performed on the right side to expose the C7 dorsal nerve root. Compression was applied to the nerve root using clips of either 10 gf (WPI, Inc., Sarasota, FL, USA) or 60 gf (Roboz, Inc., Gaithersburth, MD, USA) compressive loads (Hubbard and Winkelstein, 2005). Each magnitude of compression was applied to the nerve root for either 3 min or 10 min, separately, such that each rat received one of four types of injury (*n*=6/group) as follows: 10 gf compression for 3 min (3 min–10 gf), 10 gf compression for 10 min (10 min–10 gf), 60 gf compression for 3 min (3 min–60 gf), and 60 gf compression for 10 min (10 min–60 gf). Sham procedures (*n*=6) with dorsal nerve root exposure but no compression also were included as controls. Following surgery, all wounds were closed using 3-0 polyester suture and surgical staples. Rats were monitored continuously until they recovered from anesthesia.

Behavioral assessment

Behavioral hypersensitivity was evaluated by measuring bilateral forepaw mechanical allodynia before (baseline) and on days 1, 3, 5, and 7 following surgery (Hubbard and Winkelstein, 2005; Rothman et al., 2005). For each testing session, following 20 min of acclimation, rats were stimulated on the plantar surface of each of the ipsilateral and contralateral forepaws using a 4 g von Frey filament (Stoelting Co., Wood Dale, IL, USA). Each testing session consisted of three rounds of 10 stimulations each, separated by 10 min. The total number of paw withdrawals was recorded for each forepaw of each rat and averaged across each group on each day.

Immunohistochemistry

The C7 spinal cord was harvested on day 7 to evaluate astrocyte activation and mGluR5 expression in the dorsal horn using immunofluorescence. Colabeling techniques were used to also assess the neuronal and astrocytic expression of mGluR5 in the dorsal horn. Rats were deeply anesthetized then transcardially perfused with 200 ml phosphate buffered saline (PBS; Mediatech, Inc., Manassas, VA, USA) followed by 300 ml 4% paraformaldehyde (Sigma, St. Louis, MO, USA). The C7 spinal cord was removed and postfixed in 4% paraformaldehyde overnight. The samples were then cryoprotected in 30% sucrose and embedded in OCT medium (Sakura Finetek, Torrance, CA, USA). Matched spinal cord tissue was also included from normal, naive (*n*=2) rats for comparison.

Axial sections (14 μm) were cryosectioned and thaw-mounted directly onto slides. For staining, sections were blocked using 10% normal goat serum (Vector Laboratories, Burlingame, CA, USA) with 0.3% Triton-X100 (Bio-Rad Laboratories, Hercules, CA, USA) and then incubated overnight at 4 °C in antibodies to rabbit anti-mGluR5 (1:1000; Millipore, Temecula, CA, USA) and either mouse anti-glial fibrillary acidic protein (GFAP) (1:500; Millipore) or mouse anti-microtubule-associated protein (MAP2) (1:200; Covance, Emeryville, CA, USA). Sections were washed with PBS and incubated in the secondary antibodies goat anti-rabbit Alexa Fluor 488 (1:1000; Invitrogen, Carlsbad, CA, USA) and mouse anti-Alexa Fluor 546 (1:1000; Invitrogen). The ipsilateral and contralateral superficial dorsal horns were imaged at 40× magnification from tissue sections from each rat using a Zeiss LSM 510 confocal microscope (Carl Zeiss, Thornwood, NY, USA).

Spinal GFAP and mGluR5 expression were separately analyzed by quantifying the percentage of pixels in each image that were positively immunolabeled for each antibody. The threshold

for determining positive staining was defined based on staining in normal tissue (Abbadie et al., 1996; Romero-Sandoval et al., 2008; Rothman and Winkelstein, 2007). For each antibody, the percent positive pixels were averaged across each of the injury groups and reported as a fold change over sham levels.

In order to evaluate the distribution of mGluR5 expression in astrocytes and neurons in the dorsal horn, the fraction of mGluR5 that colabeled with GFAP or MAP2 was measured by computing the Manders colocalization coefficient (M2) using the JACoP plug-in for ImageJ (NIH, Bethesda, MD, USA) (Bolte and Cordelières, 2006; Dunn et al., 2011; Manders et al., 1993). Specifically, $M2_{GFAP}$ denotes the fraction of the total mGluR5 that is colocalized with GFAP. Similarly, $M2_{MAP2}$ indicates the fraction of the total mGluR5 that is colocalized with MAP2. To determine each M2 coefficient, the number of pixels in each image that colabeled for either mGluR5 and GFAP or mGluR5 and MAP2 was computed as a fraction, from 0 to 1, of the total number of pixels that was positively labeled for mGluR5. A M2 of 0 indicates no colocalization, whereas a coefficient of 1 indicates that 100% of the mGluR5 positive staining colocalizes with GFAP ($M2_{GFAP}$) or MAP2 ($M2_{MAP2}$). For each colocalization coefficient, the fraction of colabeled pixels was averaged across each group.

Statistical analysis

Significant differences in the number of paw withdrawals were determined between groups over time using a two-way, repeated measures analysis of variance (ANOVA). A one-way ANOVA with post hoc Bonferroni correction tested for differences in the number of paw withdrawals between each group on each day. All data are reported as mean±standard deviation, and significance was defined at $P<0.05$ for all statistical analyses. The behavioral and spinal outcomes at day 7 were compared using a two-way ANOVA to test for the effects of compression magnitude (10 gf, 60 gf) and duration (3 min, 10 min). Specifically, separate two-way ANOVAs were performed to test for differences in the number of paw withdrawals, mGluR5 expression, GFAP expression, $M2_{GFAP}$, and $M2_{MAP2}$.

RESULTS

Behavioral sensitivity

Ipsilateral mechanical allodynia was only elicited by compression applied for 10 min (Fig. 1). On day 1, the number of paw withdrawals following a 10 gf compression applied for 10 min was significantly ($P<0.019$) elevated over responses of both sham and compression magnitudes (10 gf, 60 gf) applied for only 3 min (Fig. 1). On days 3, 5, and 7, compression applied for 10 min at either a 10 gf or 60 gf load elicited significantly more ($P<0.014$) paw withdrawals than compression applied for the shorter duration for either
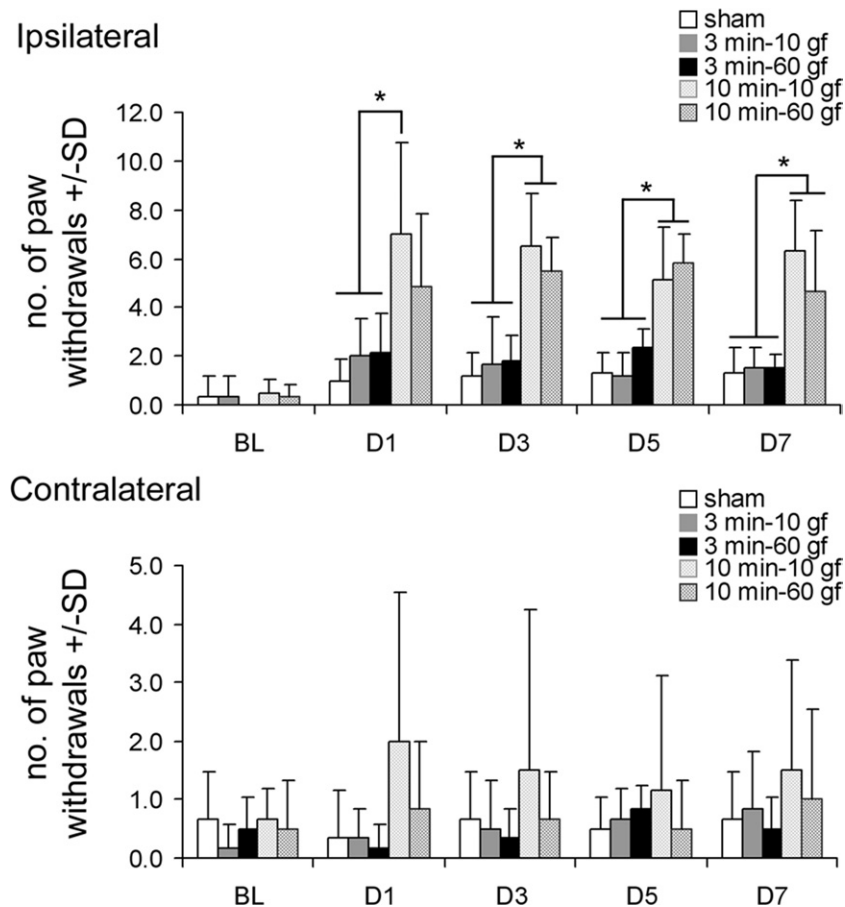

Fig. 1. Average mechanical allodynia assessed in the ipsilateral and contralateral forepaws following sham, 3 min compression of 10 gf (3 min–10 gf; $n=6$), 3 min compression of 60 gf (3 min–60 gf; $n=6$), 10 min compression of 10 gf (10 min–10 gf; $n=6$), or 10 min compression of 60 gf (10 min–60 gf; $n=6$). The number of ipsilateral paw withdrawals significantly (* $P<0.019$) increased following 10 min compressions compared with sham and 3 min compressions, regardless of the compression load applied to the nerve root.

magnitude and also sham responses (Fig. 1). There were no differences in the number of paw withdrawals between either of the 3 min compression groups with each other or with sham at any time point. Likewise, there was no difference in the sensitivity produced by either of the two 10 min compression magnitudes at any time point (Fig. 1). No significant differences were detected in mechanical allodynia measured in the contralateral forepaw (Fig. 1).

At day 7, ipsilateral mechanical allodynia was significantly different ($P<0.001$) for the main effect of duration but not for load ($P=0.243$). There was a 4.6 ± 1.5-fold increase over sham in the number of paw withdrawals elicited by a 10 min, 10 gf compression. Similarly, there was a 4.02 ± 2.1-fold increase in the number of paw withdrawals elicited by a 10 min, 60 gf compression over sham. In contrast, the number of paw withdrawals that were elicited by a 3 min compression relative to sham responses were only 1.1 ± 0.6- and 1.1 ± 0.4-fold more for compressions applied with the 10 gf and 60 gf clips, respectively (Fig. 1).

Astrocyte activation

GFAP staining in the ipsilateral dorsal horn on day 7 significantly ($P=0.002$) increased after a 10 min compression to the nerve root compared with a 3 min compression, regardless of the compression load (Fig. 2). However, no differences in GFAP staining were detected based on the load applied to the nerve root ($P=0.286$). The ipsilateral spinal expression of GFAP following a 10 min compression applied with a 10 gf or 60 gf clip was increased over sham by $38\pm42\%$ and $35\pm44\%$, respectively (Fig. 2C). For the

shorter, 3 min compressions, the ipsilateral GFAP expression only differed from sham by $9\pm33\%$ and $-6\pm37\%$ for each of the compression loads of 10 gf or 60 gf, respectively (Fig. 2C). There was a similar increase in the expression of GFAP in the contralateral dorsal horn following the 10 min compression compared with a 3 min compression, but this was not significant (Fig. 2C). The contralateral expression of GFAP was not different from sham after any nerve root injury. In addition, sham procedures did not alter spinal GFAP expression on either side of the dorsal horn when compared with the GFAP expression in normal tissue (Fig. 2B).

mGluR5 expression

Spinal mGluR5 expression at day 7 in the dorsal horn significantly ($P<0.001$) increased in both of the ipsilateral and contralateral dorsal horns following a 60 gf nerve root compression, regardless of the duration of compression (Fig. 3). No significant differences were detected between 3 min and 10 min of compression for either load ($P=0.623$, ipsilateral; $P=0.370$, contralateral) (Fig. 3). The ipsilateral expression of mGluR5 after a 60 gf compression applied for 3 min or 10 min increased by $147\pm163\%$ and $140\pm151\%$ over sham, respectively (Fig. 3A, C). When compression was applied for these same durations with the 10 gf clip, mGluR5 expression remained at sham levels for both of the 3 min ($-7\pm71\%$) and 10 min ($30\pm99\%$) durations, respectively (Fig. 3A, C). In the contralateral dorsal horn, there was nearly a 150% and 200% increase in mGluR5 over sham following a 60 gf compression applied for each of 3 min and 10 min, respectively (Fig. 3C).
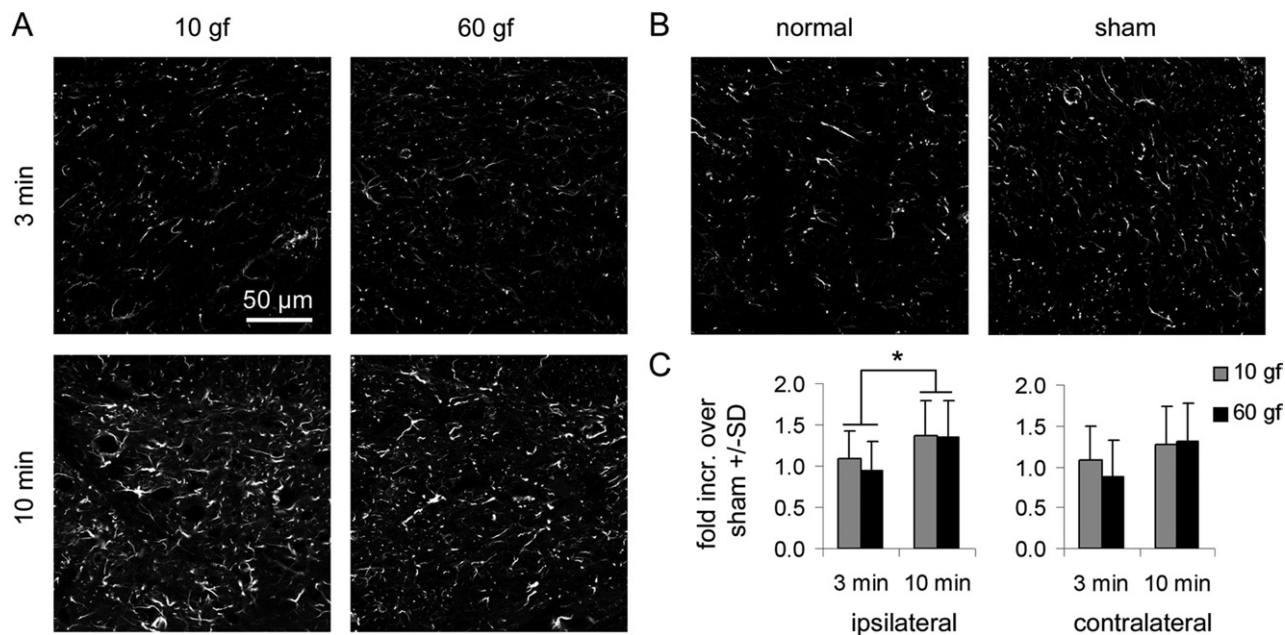

Fig. 2. GFAP expression in the spinal dorsal horn 7 d following nerve root compression is sensitive to the duration of the load applied. (A) Representative images of GFAP immunolabeling in the dorsal horn following 10 gf or 60 gf compression applied for 3 min or 10 min. (B) Representative confocal images of GFAP immunolabeling in the dorsal horn in normal, naive tissue and following sham procedures. (C) Average±standard deviation (SD) of GFAP immunoreactivity expressed as a fold increase over the GFAP expression in sham ($n=6$ rats). GFAP expression in the ipsilateral dorsal horn significantly (* $P=0.002$) increased following 10 min compressions ($n=6$ rats each group) compared with 3 min compressions ($n=6$ rats each group). Scale bar applies to all images.
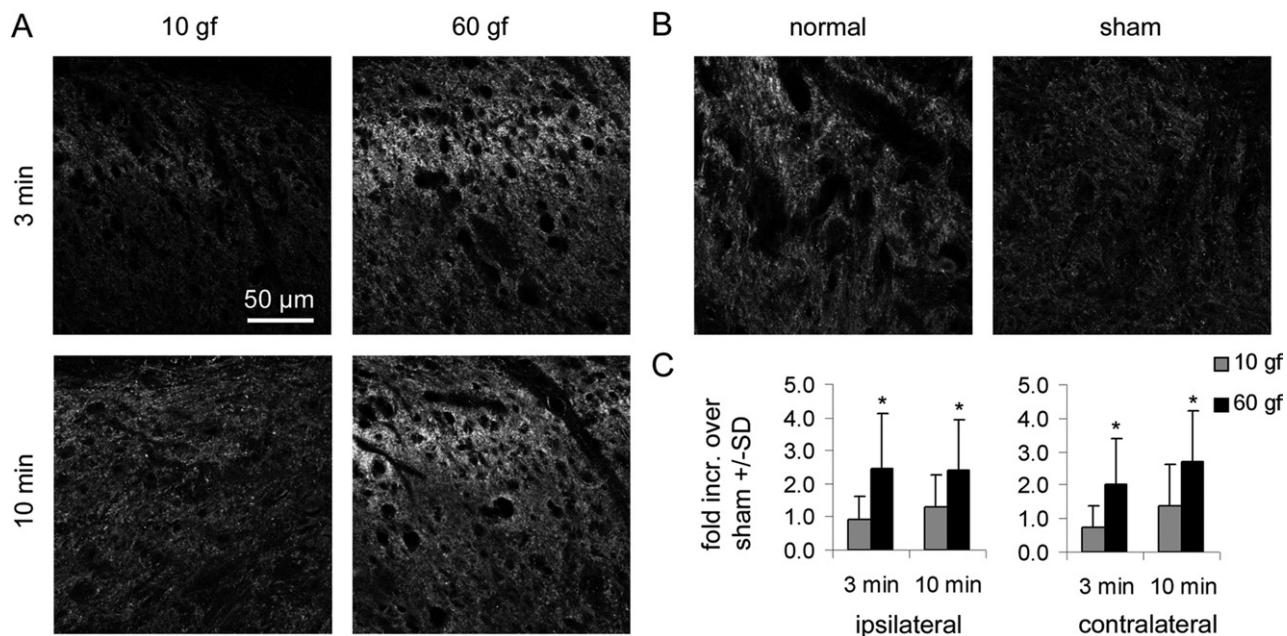
Fig. 3. mGluR5 expression in the spinal dorsal horn 7 d following nerve root compression is sensitive to the magnitude of the load. (A) Representative confocal images of mGluR5 immunolabeling in the dorsal horn following compression applied for 3 min or 10 min with compressive magnitudes of 10 gf or 60 gf. (B) Representative images of mGluR5 immunolabeling in the dorsal horn in normal, naive tissue and following sham. (C) Average positive pixels (mean±SD) immunolabeled for mGluR5 following a nerve root compression relative to sham ($n=6$). Spinal mGluR5 significantly (* $P<0.001$) increased following a 60 gf compression compared ($n=6$ rats each group) with a 10 gf minute compression ($n=6$ rats each group) in both the ipsilateral and contralateral dorsal horns. Scale bar applies to all images.

However, mGluR5 expression remained at sham levels when the compression was applied with a 10 gf load (Fig. 3C). The expression of mGluR5 following sham procedures remained at levels detected in normal, naive tissue (Fig. 3B).

mGluR5 colocalization

The fraction of mGluR5 in the spinal cord that colabeled with either GFAP or MAP2 was not different between any of the groups (Fig. 4). Following sham procedures, the fraction of mGluR5 that colocalized with GFAP was 0.13 ± 0.09, which was similar to that measured in normal tissue (0.16 ± 0.02). Following compression that was applied for 3 min, the amount of mGluR5 that colocalized with GFAP was 0.20 ± 0.14 and 0.17 ± 0.11 in response to a 10 gf and a 60 gf compression, respectively (Fig. 4G). Similarly, the amount of mGluR5 that colocalized with GFAP following compression applied for 10 min with a 10 gf or a 60 gf clip was 0.20 ± 0.15 and 0.16 ± 0.09, respectively (Fig. 4G). When mGluR5 was colabeled with MAP2, there was a similar degree of colocalization measured following sham procedures (0.54 ± 0.27) and in normal tissue (0.50 ± 0.07). Regardless of the type of compression applied to the nerve root, about half of the total mGluR5 expression colocalized with MAP2, and about 20% with GFAP (Fig. 4).

DISCUSSION

This study is the first to evaluate the influence of both the magnitude and the duration of a transient nerve root com-

pression on glial and glutamatergic responses in the spinal cord in the context of pain. All of the nerve root compressions used here elicited a behavioral and/or cellular response, demonstrating that both compression load and duration modulate different aspects of these physiologic responses as late as 7 days after the initial insult (Figs. 1–3). For the loading profiles in this study, both mechanical allodynia and astrocyte activation were sensitive to the duration of compression but not to the load applied (Figs. 1 and 2), which is consistent with previous studies using this same injury model (Hubbard and Winkelstein, 2005; Rothman et al., 2010). Interestingly, spinal astrocyte activation was only evident following a compression scenario that also induced behavioral sensitivity (Figs. 1 and 2), whereas mGluR5 upregulation was not associated with mechanical allodynia (Figs. 1 and 3). This is the first study, to our knowledge, to quantify the expression of mGluR5 in the spinal cord following a transient nerve root compression. Unlike astrocyte activation and behavioral sensitivity, mGluR5 expression was only increased following a 60 gf compression (Fig. 3). Regardless of its overall expression in the spinal cord, the distribution of mGluR5 expression in astrocytes and neurons did not vary between any nerve root compression scenario (Fig. 4).

Mechanical allodynia following a transient nerve root compression was sensitive to the duration, but not the magnitude, of the applied compression (Fig. 1). Previous studies have shown that for nerve root compression magnitudes below 10 gf, behavioral sensitivity is mediated by the magnitude of compression (Hubbard et al., 2008a,b). In those studies, behavioral sensitivity increased with
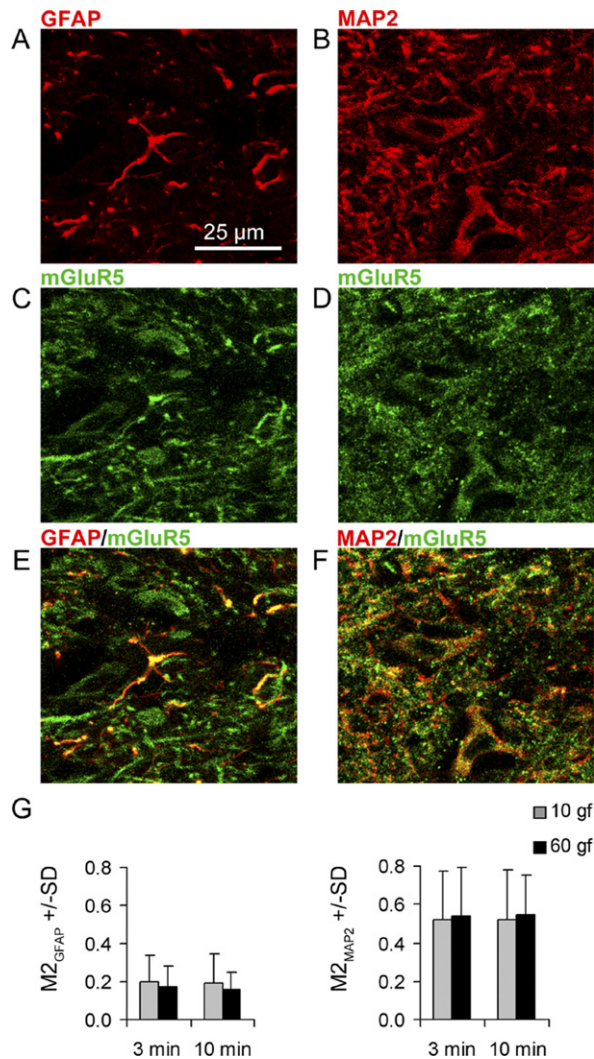
Fig. 4. The fraction of mGluR5 that colocalized with GFAP (M2$_{GFAP}$) and MAP2 (M2$_{MAP2}$) in the dorsal horn on day 7 was not sensitive to either the load or duration of nerve root compression. Representative images labeled for either (A) GFAP or (B) MAP2 and also with (C and D) mGluR5. In the merged images (E and F), yellow indicates areas of colabeling. The fraction of mGluR5 that colocalized with either (E) GFAP or (F) MAP2 was determined by calculating the fraction of colabeled pixels relative to the total number of pixels that labeled for mGluR5. (G) Average M2$_{GFAP}$ and M2$_{MAP2}$ (mean±SD) was not different (n=6 rats each group). Scale bar applies to all images. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

greater nerve root compression loads up to an applied load of 7.8 gf (76.32 mN), beyond which allodynia reached a maximal response, regardless of the application of more severe compressions. Although that magnitude threshold for eliciting maximum sensitivity was based only on a 15 min compression to the root, that work supports the present findings that the behavioral sensitivity resulting from a 10 min nerve root compression is not sensitive to either of the load magnitudes (Fig. 1). Although the 3 min nerve root compression did not induce behavioral sensitivity to the 4 g filament tested here, mechanical hyperalgesia has been reported to develop and persist for at least 14 days after a

short, 2–15 s, crush injury of unspecified load to the nerve root (Sekiguchi et al., 2003, 2009). Consequently, there likely exists a magnitude threshold for eliciting behavioral sensitivity after 3 min of compression to the nerve root that lies above 60 gf. The lack of behavioral sensitivity that was observed following 3 min of compression does not exclude the possibility that there may be a more subtle difference in the behavioral responses induced by these different mechanical insults. Indeed, it is possible that in using additional or more sensitive testing methods, such as Chaplan up-down threshold method (Chaplan et al., 1994; Decosterd and Woolf, 2000), different relationships may have been detected between the different mechanical loading profiles to the nerve root. However, similar trends were found between both testing methods for loads applied for a longer duration than in this study (Hubbard and Winkelstein, 2005). Considering the susceptibility of the nerve root to a broad range of traumas associated both with sporting and automotive events (Krivickas and Wilbourn, 2000; Svensson et al., 1993; Stuber, 2005; Tominaga et al., 2006), the results of the present study begin to identify how the risk of experiencing pain from a mechanical insult of the nerve root may vary across these types of loading scenarios.

Astrocyte activation has been implicated in pain in several animal models of nerve, nerve root, and spinal cord injury (Colburn et al., 1999; Hashizume et al., 2000; Popovich et al., 1997; Watkins and Maier, 2003). In the present study, astrocyte activation was only evident following a painful nerve root compression and was sensitive to the duration (Fig. 2). Although it is not well understood how spinal astrocyte activation is induced by compression of the nerve root, it has been suggested that axonal degeneration is necessary for astrocyte proliferation, which, like reactivity, is a hallmark of the astroglial response to neural injury (Liu et al., 2000; McMahon and Malcangio, 2009). In fact, nerve root compression that elicits an inflammatory response also produces extensive degeneration in the nerve root (Chang and Winkelstein, 2011; Hubbard and Winkelstein, 2005; Hubbard et al., 2008b; Kobayashi et al., 2004, 2005). Yet, the nerve root remains morphologically normal following compressions that also do not produce astrocyte activation (Nicholson et al., 2011; Rothman et al., 2010). Therefore, the presence of astrocyte activation in the spinal cord after a painful 10 min compression to the nerve root may be due to axonal damage in the compressed root. Although the signaling mechanisms for inducing astrocyte activation from such responses is still unknown, the loss of axonal flow and altered expression of spinal neuropeptides, such as calcitonin gene-related peptide and substance P, following nerve root compressions are potential pathways for neuronal activation of glial cells (Chang and Winkelstein, 2011; Hubbard et al., 2008a,b; Kobayashi et al., 2005, 2008; Martin et al., 1992; Ren and Dubner, 2008; Takuma et al., 1996).

The increased expression of mGluR5 in the spinal cord after transient root compression may indicate tissue damage, given that its expression was only increased after the more severe compressions (Fig. 3). In fact, spinal mGluR5

expression following a transient distraction of the facet joint has similarly been shown to correlate with the severity of capsular stretch (Dong and Winkelstein, 2010). Unlike a painful distraction of the facet capsule, increased spinal mGluR5 expression following nerve root compression occurs in the absence of behavioral sensitivity (Figs. 1 and 3; Dong and Winkelstein, 2010). The contribution of mGluR5 to pain may, therefore, be specific to the type of injury. In fact, although the mGluR5 antagonist, MPEP, alleviates mechanical allodynia induced by peripheral inflammation, it has no effect on mechanical allodynia elicited by a nerve ligation (Walker et al., 2001). Yet, thermal sensitivity following a nerve ligation is reversed by the administration of MPEP (Hudson et al., 2002). Therefore, the role of mGluR5 in pain signaling may not only be specific to the type of injury but may also contribute to different pain-processing pathways.

Glutamate sensitization and glial communication with afferent neurons both contribute to pain (Basbaum et al., 2009; Wieseler-Frank et al., 2004; Woolf, 2011). The glutamatergic system signals acute pain not only via the metabotropic glutamate receptors, but also through activation of the ionotropic glutamate receptors, including the NMDA receptor which modulates synaptic strength and contributes to neuronal hyperexcitability and behavioral sensitivity (Lea and Faden, 2001; Leem et al., 2010; Woolf, 2011). Activation of these glutamate receptors is mediated, in part, by astrocytic regulation of extracellular glutamate (Danbolt, 2001; Tao et al. 2005). When astrocytes become activated, as was observed in the present study (Fig. 2), their normal role in regulating extracellular glutamate via the glutamate transporter, GLT1, can be compromised, potentially resulting in an imbalance in synaptic glutamate concentration (Hu et al., 2010; Sung et al., 2003; Xin et al., 2009). Therefore, despite the present findings that the glutamatergic system appears only modulated by injury load and not preferentially in astrocytes in nerve root-mediated pain, altered glutamate signaling via the indirect actions of the astroglial response to nerve root compression may have a role in pain following these types of injuries.

Microglial activation, like mGluR5 expression, has been shown to be more sensitive to tissue injury or severity of injury following a mechanical insult to the root (Colburn et al., 1997; Hubbard and Winkelstein, 2005; Winkelstein et al., 2002). To date, most of the evidence of mGluR5 expression in microglia is limited to cultured cells (Aronica et al., 2001; Byrnes et al., 2009a,b; Loane et al., 2009). Yet, a recent study demonstrated that, even though mGluR5 is not expressed by microglia under normal, physiologic conditions, this receptor is selectively expressed in activated microglia near a chemically induced lesion in the brain of rats (Drouin-Ouellet et al., 2011). Therefore, future studies investigating mGluR5 following nerve root injury should also consider the microglial response. In fact, in the current study, only about 70% of the mGluR5 could be attributed to neurons and astrocytes (Fig. 4). Because microglial activation may be modulated by mGluR5-dependent signaling cascades, microglia may account for the spinal expression of this receptor that was not attributed to astrocytes or neurons in the present study (Byrnes et al., 2009a,b; Maiese et al., 2005). In addition, because microglial proliferation following a nerve root injury is most robust within the first 3 days after injury (Rothman et al., 2009), mGluR5 expression at earlier time points should also be considered. Although mGluR5 expression does not appear to contribute to the behavioral sensitivity that is observed at 7 days after the injury in this model, it may play a role in initiating painful behaviors following a nerve root injury. Together, the results of the present study in the context of the literature begin to identify how the glial response and glutamate signaling interact with each other and contribute to pain following certain nerve root injuries.

CONCLUSIONS

The results of the present study demonstrate that the duration and magnitude of a transient mechanical insult differentially drive behavioral, glial, and glutamatergic outcomes after nerve root loading. The induction of astrocyte activation following only painful nerve root compressions suggests a role for spinal astrocytes in maintaining behavioral sensitivity. Conversely, neither the overall expression of mGluR5 nor the cell-specific expression of mGluR5 demonstrated a clear relationship between the glutamatergic system and the development of pain after nerve root compression. Further studies investigating the temporal response of spinal glutamate receptors and transporters are warranted in order to identify whether glutamate signaling contributes to the initiation and/or maintenance of nerve root-mediated pain. Although the specific contributions of glutamate signaling in this pain model remain undetermined, this is the first study to demonstrate that the glutamatergic system is sensitive to nerve root mechanics.

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