

## A Rat Model of Radicular Pain Induced by Chronic Compression of Lumbar Dorsal Root Ganglion with SURGIFLO™

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**Background:** Radicular pain is a common and debilitating clinical pain condition. To date, the mechanisms of radicular pain remain unclear, partly because of the lack of suitable preclinical models. The authors report a modified rat model of radicular pain that could mimic a subset of clinical radicular pain conditions induced by the soft tissue compression on dorsal root ganglion.

**Methods:** A rat model of radicular pain was produced by infiltrating the L5 intervertebral foramen with 60  $\mu$ l of a hemostatic matrix (SURGIFLO™; Johnson & Johnson, Somerville, NJ) resulting in chronic compression of lumbar dorsal root ganglion. Thermal hyperalgesia and mechanical allodynia were measured with or without epidural treatment with triamcinolone. Western blot was used to assess the expression of the NR1 subunit of the *N*-methyl-D-aspartate receptor and inhibitory factor  $\kappa\beta\alpha$ , an inflammatory marker, within the affected L5 dorsal root ganglion and spinal cord dorsal horn.

**Results:** Chronic compression of lumbar dorsal root ganglion resulted in: (1) persistent mechanical allodynia and thermal hyperalgesia up to 4 or 5 postoperative weeks and (2) up-regulation of the *N*-methyl-D-aspartate receptor and inhibitory factor  $\kappa\beta\alpha$  within the ipsilateral L5 dorsal root ganglion and spinal cord dorsal horn. Epidural administration of triamcinolone (6.25–100  $\mu$ g) on postoperative day 3 dose-dependently attenuated both thermal hyperalgesia and mechanical allodynia in rats with chronic compression of lumbar dorsal root ganglion.

**Conclusion:** The data suggest that this modified rat model of chronic compression of lumbar dorsal root ganglion may be a useful tool to explore the mechanisms as well as new therapeutic options of radicular pain.

RADICULAR low back pain often results from focal compression of a nerve root, dorsal root ganglion (DRG), and/or the sciatic nerve itself. This clinical condition is associated with severe pain that radiates to the affected lower extremity. A herniated disc and spinal or foraminal stenosis are some of the common etiologies of radicular pain.<sup>1,2</sup> Although epidural steroid injection, medical treatment, and surgical intervention have been successfully used in many cases, radicular pain remains a common chronic pain condition that is sometimes refractory to current treatment modalities. To date, the mechanisms of radicular pain remain unclear, partly because of the lack of suitable preclinical models.

It has been reported that thermal and mechanical hyperalgesia can be produced in rats after chronic compression injury of DRG resulting from implantation of a metal rod into the L4 and L5 intervertebral foramina.<sup>3,4</sup> This animal model produces behavioral and electrophysiological changes indicative of radicular pain and mimics certain clinical cases of DRG compression, particularly with a metal instrument (e.g., lumbar spine fusion with metal hardware). Because the texture of a metal rod differs from that of soft tissue such as a herniated disc, appropriate animal models of radicular pain closely mimicking DRG and/or nerve root compression by a disc or foraminal stenosis would be desirable.

In the present study, we sought to develop a rat model of radicular pain through chronic compression of lumbar DRG (CCD) by using a hemostatic matrix (SURGIFLO™; Johnson & Johnson, Somerville, NJ). SURGIFLO™ is a sterile hemostatic material made from a bioreabsorbable gelatin matrix and has been extensively and safely used in surgical operations.<sup>5-7</sup> The liquid form of this material allows free injection to a target site. Once in contact with tissue, the material hardens within a minute or so, producing progressive and persistent compression of the target site (e.g., the neuroforaminal and DRG area). Chronic compression of the unilateral L5 DRG area using SURGIFLO™ produced persistent thermal hyperalgesia and mechanical allodynia, which was associated with an up-regulated expression of both the *N*-methyl-D-aspartate (NMDA) receptor and inhibitory factor  $\kappa\beta\alpha$  ( $1\kappa\beta\alpha$ ), a proposed inflammatory marker,<sup>8,9</sup> within the ipsilateral L5 DRG and spinal cord dorsal horn. The CCD-induced hyperalgesia and allodynia were also reduced by epi-

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dural administration of triamcinolone, a commonly used glucocorticoid steroid.

## Materials and Methods

### Animal Surgery

Adult male Sprague-Dawley rats weighing 275–325 g (Charles River Laboratories, Wilmington, MA) were used. The animal room was artificially lighted from 7:00 AM to 7:00 PM. Rats were housed in individual cages with free access to water and food pellets. Room temperature was maintained at 24°C. The experimental procedure was carried out in accordance with the protocol approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee (Boston, MA).

The surgical procedure was performed aseptically under pentobarbital anesthesia (50 mg/kg intraperitoneally). In a previous study, metal rods were inserted into intervertebral foramina to induce nerve root/DRG compression.<sup>3</sup> In this study, we used a hemostatic matrix (SURGIFLO™) to induce a focal compression on one side of the L5 DRG/exiting nerve root. To inject SURGIFLO™, paraspinal muscles were separated from the mammillary process and the transverse process to expose the left L5 intervertebral foramen (fig. 1A). A stainless 22-G steel needle, with a blunt angle to avoid tissue penetration, was gently and slowly inserted approximately 4 mm into the L5 intervertebral foramen. The needle was inserted at a 30–40° angle toward the dorsal middle line and 10–15° below the vertebral horizontal line. Care was taken to minimize contact with the existing nerve root/DRG by monitoring twitches of ipsilateral hind paw muscles. When small twitches were noted, the needle was either slightly withdrawn or redirected. SURGIFLO™ (60  $\mu$ l) was slowly injected (within 1–2 min) into the left L5 intervertebral foramen, which was often accompanied by one or two mild twitches of ipsilateral

hind paw muscles. After the injection, the muscle and skin layers were closed with 6.0 nylon sutures. Sham operation was performed following the same surgical procedure but without the SURGIFLO™ injection. Baytril (enrofloxacin, 2.5 mg/kg intramuscularly; Bayer Health Care, Pittsburgh, PA) was administered immediately after surgery to prevent infection.

In our pilot experiment, we tried different volumes of SURGIFLO™ for the injection. If the volume was too high (>60  $\mu$ l), some liquid could spread to the nearby vertebral canal before it hardened, and rats could express opisthotonos or paralysis in both hind paws. To the contrary, a smaller volume (e.g., 30  $\mu$ l) of SURGIFLO™ produced only mild and inconsistent behavioral changes. The reported volume (60  $\mu$ l) of SURGIFLO™, when slowly injected within 1–2 min, resulted in quick hardening of the material and reliable behavioral changes for weeks without limb paralysis. The location of the hardened SURGIFLO™ plaque was on the L5 DRG/exiting nerve root (fig. 1B), as confirmed by laminectomy at the time of autopsy after the rats were sacrificed for tissue harvesting. The size of the hardened SURGIFLO™ was approximately 4–5 mm in diameter 2–3 min after the injection, as confirmed before the skin incision was closed. This size of SURGIFLO™ plaque remained intact and its size and location were not changed up to 3–4 wk after the injection, indicating a very slow absorption of the material at this location in rats. To confirm whether the behavioral change after the injection was related to the compression induced by the hardened SURGIFLO™ at the DRG site, SURGIFLO™ was injected into the surrounding tissue outside the intended DRG area. These rats did not show allodynia and hyperalgesia, as demonstrated in our pilot experiment.

### Implantation of an Epidural Catheter

For those rats receiving an epidural injection, an epidural catheter was inserted under the same surgical condition. A PE-10 tube (outer diameter 0.61 mm) (Clay Adams, Parsippany, NJ) was inserted according to the method described previously.<sup>10</sup> Briefly, a 1- to 2-cm mid-line skin incision was made at the most prominent thoracic spinal process (T13). Using a pair of microscissors, a small hole was made in the middle of ligament flavum, and a PE-10 tube was gently advanced approximately 3 cm caudally into the epidural space with the catheter tip being placed at the level between the L4 and L5 nerve roots. The proximal end of the epidural catheter was tunneled subcutaneously and secured to the posterior cervical area to facilitate epidural injection. Incisions were closed with a 6.0 nylon suture or wound clip. To confirm correct epidural catheter placement, negative aspiration of spinal fluid was confirmed after each catheter implantation, and 2% lidocaine (0.15 ml) was injected through the catheter after the complete recovery from pentobarbital anesthesia. Correct epidural catheter placement was confirmed when paralysis was present in

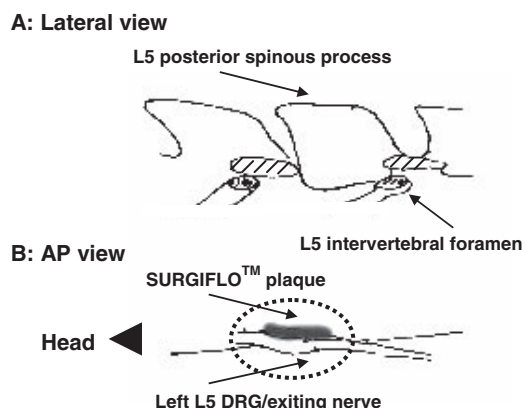


Fig. 1. An illustration of the SURGIFLO™ (Johnson & Johnson, Somerville, NJ) injection site. (A) Lateral view showing the L5 intervertebral foramen. (B) Anterior-posterior (AP) view showing the L5 dorsal root ganglion (DRG)/exiting nerve root and the position of a hardened SURGIFLO™ plaque. See "Materials and Methods" for details of needle insertion and SURGIFLO™ injection.

hind paws but not forepaws after the lidocaine injection. If the catheter were placed intrathecally, sudden respiratory arrest would be observed (high spinal anesthesia) at this dose of lidocaine. We observed a small percentage (approximately 5%) of such cases, and these rats were excluded from the study. During the experimental period, those rats (approximately 5–10%) showing neurologic deficits and/or behavioral abnormalities (poor eating, grooming) were also excluded. Upon completion of each experiment, the actual placement of the epidural catheter (outside the dura) was again confirmed by autopsy.

#### *Drug and Epidural Injection*

Triamcinolone was purchased from Bristol-Myers Squibb (Princeton, NJ) and dissolved in 5% dimethyl sulfoxide diluted with normal saline, which was also used for vehicle treatment. We used this formulation of triamcinolone for more accurate drug dilution for the dose titration, although a depot formulation of triamcinolone is commonly used in clinical practice. Triamcinolone or vehicle was injected epidurally in a 20- $\mu$ l volume, followed by a 20- $\mu$ l saline flush. Experiments were conducted with the experimenter blinded to treatment condition.

#### *Behavioral Testing and Statistical Analysis*

Animals were habituated to the test environment for 3 consecutive days (60 min per day) before baseline testing. Withdrawal threshold to thermal and mechanical stimulation was examined for both ipsilateral and contralateral hind paws. For the measurement of mechanical threshold, a rat was placed into a plastic cage with a wire mesh bottom. Mechanical threshold was measured using a von Frey filament set with a calibrated range of bending force, as described previously.<sup>11,12</sup> A single filament was applied perpendicularly to the plantar surface of a rat's hind paw five times with an interstimulation interval of 5 s. A positive response was defined as at least one clear withdrawal response during these five applications. The threshold force was determined using an up and down approach with different sizes of filament.<sup>11,12</sup>

Thermal hyperalgesia to radiant heat was determined according to a previously described method<sup>13</sup> using a 390 Analgesia Meter (IITC Inc., Woodland Hills, CA). Briefly, a rat was placed individually into a Plexiglas cubicle placed on a transparent glass surface. The light source was from a projection bulb located below the glass and directed at the plantar surface of one hind paw. The withdrawal latency was defined as the time from the onset of radiant heat to withdrawal of the tested hind paw. The radiant heat source was adjusted to result in baseline latencies of approximately 12 s and a cutoff time at 20 s. Two trials with an intertrial interval of 3 min were made for each hind paw, and scores from both

trials were averaged to yield mean withdrawal latency for each hind paw.

#### *Western Blot*

Animals were anesthetized with pentobarbital and decapitated for rapid tissue harvesting. Fresh tissue samples from bilateral L5 DRG and spinal cord dorsal horn were removed after laminectomy. DRG and spinal cord dorsal horn samples were divided into the ipsilateral and contralateral side and homogenized in a sodium dodecyl sulfate sample buffer containing a mixture of proteinase inhibitors (Sigma, St. Louis, MO). The quantification of protein contents was made using the Bradford method. Protein samples (40  $\mu$ g for spinal cord dorsal horn tissues and 15  $\mu$ g for DRG tissues) were separated on 4–20% tris-glycine gels (Invitrogen, Carlsbad, CA) and transferred to Hybond ECL nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ). The filters were blocked with 5% milk and incubated overnight at 4°C with a primary antibody (NR1, 1:1000, mouse monoclonal, Novus Biologic, Littleton, CO;  $\text{I}\kappa\beta$ - $\alpha$ , 1:2000, mouse monoclonal, Cell Signaling Technology, Danvers, MA) and 1 h at room temperature with HRP-conjugated secondary antibody (1:7000; Amersham Biosciences, Arlington Heights, IL). The blots were visualized in ECL solution (DuPont NEN, Boston, MA) for 1 min and exposed to hyperfilms (Amersham Biosciences, Arlington Heights, IL) for 1–10 min. The blots were then incubated in a stripping buffer (67.5 mM Tris, pH 6.8, 2% sodium dodecyl sulfate, 0.7%  $\beta$ -mercaptoethanol) for 30 min at 50°C and reprobed with a polyclonal rabbit anti- $\beta$ -actin antibody (1:25,000; Alpha Diagnostic International, San Antonio, TX) as loading controls. The Western analysis was performed in triplicate.

#### *Experimental Design*

In Experiment 1, we examined the time course of changes in behavioral and biochemical expression after CCD. Withdrawal threshold to thermal and mechanical stimulation was examined over a 6-wk period for both ipsilateral and contralateral hind paws in both CCD ( $n = 12$ ) and sham ( $n = 6$ ) rats. To determine whether CCD would change the expression of the NR1 subunit of the NMDA receptor and  $\text{I}\kappa\beta$ - $\alpha$  in the DRG and spinal cord dorsal horn, we used six groups of rats ( $n = 3$ ) to obtain tissue samples (Western blot) on postoperative day 1, 3, 5, 7, 10, 14, or 21 after behavioral tests. One group each of sham-operated ( $n = 3$ ) or naive rats ( $n = 3$ ) was used as controls, and their DRG and spinal cord dorsal horn samples were removed on postoperative day 21 after the behavioral tests.

In Experiment 2, we studied the effects of epidural triamcinolone on CCD-induced pain behavior. To examine the effect of triamcinolone on CCD-induced pain behavior, epidural triamcinolone was given once on day 3 of CCD. Six groups of rats ( $n = 6$ /group) were used,



which included: (1) CCD + vehicle; (2-4) CCD + triamcinolone (6.25, 50, 100  $\mu\text{g}$ ); (5) sham + vehicle; and (6) sham + triamcinolone (100  $\mu\text{g}$ ). Thermal hyperalgesia and mechanical allodynia were tested on day 0 (baseline) and postoperative days 1, 3, 4, 5, 7, 10, and 12.

### Statistical Analyses

Data from behavioral tests were analyzed by generating a difference score between two hind paws (contralateral minus ipsilateral) such that a higher difference score represents a higher degree of hyperalgesia or allodynia. Difference scores from both thermal hyperalgesia (withdrawal latency in seconds) and mechanical allodynia (threshold bending force in grams) tests were analyzed by using repeated measure two-way ANOVA across testing time points to detect overall differences among treatment groups. Whenever applicable, the data were also examined by using repeated-measure two-way ANOVA among treatment groups to examine overall differences among testing time points (statistical software SPSS; version 10; Chicago, IL). In both cases, when significant main effects were observed, *post hoc* tests were performed to determine the source(s) of differences. Differences were considered to be statistically significant at the level of  $P < 0.05$ .

For the data from the Western blot analysis, the density of specific bands was measured with a computer-assisted imaging analysis system (IPLab software; Scanalytics, Fairfax, VA) and normalized against corresponding loading control bands. Differences were compared using one-way ANOVA followed by *post hoc* tests.

## Results

### CCD-induced Thermal Hyperalgesia and Mechanical Allodynia

Compared with baselines, the difference score (contralateral minus ipsilateral) of thermal withdrawal latencies was significantly increased in the CCD group beginning on postoperative day 1, peaking around days 5-14, and lasting up to day 35 after CCD (fig. 2A) ( $F = 13.5$ ,  $P = 0.0001$ ). The data reported in figure 2 were difference scores between two hind paws (contralateral minus ipsilateral). There were no postoperative changes in withdrawal latencies or threshold force in contralateral hind paws compared with preoperative baselines ( $F = 0.87$ ;  $P = 0.86$ ). No significant changes were found in the sham group before and after the surgical procedure (fig. 2A) ( $F = 0.64$ ,  $P = 0.74$ ), and there were no significant postoperative differences in the withdrawal latency on the contralateral hind paw of CCD rats ( $F = 0.58$ ,  $P = 0.76$ ; data not shown).

Similarly, the difference score of withdrawal threshold to mechanical stimulation (von Frey filament) was also significantly increased in the CCD group beginning on

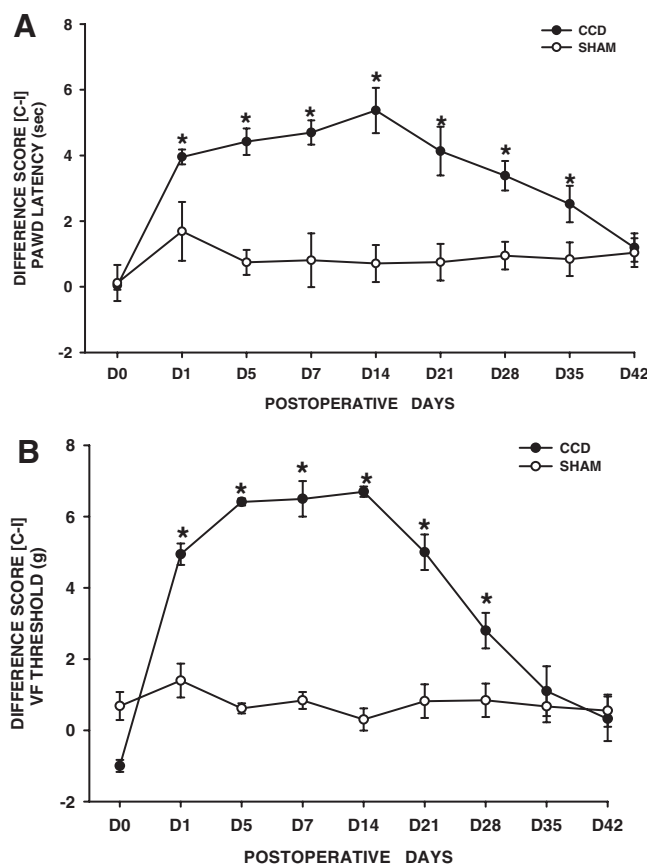
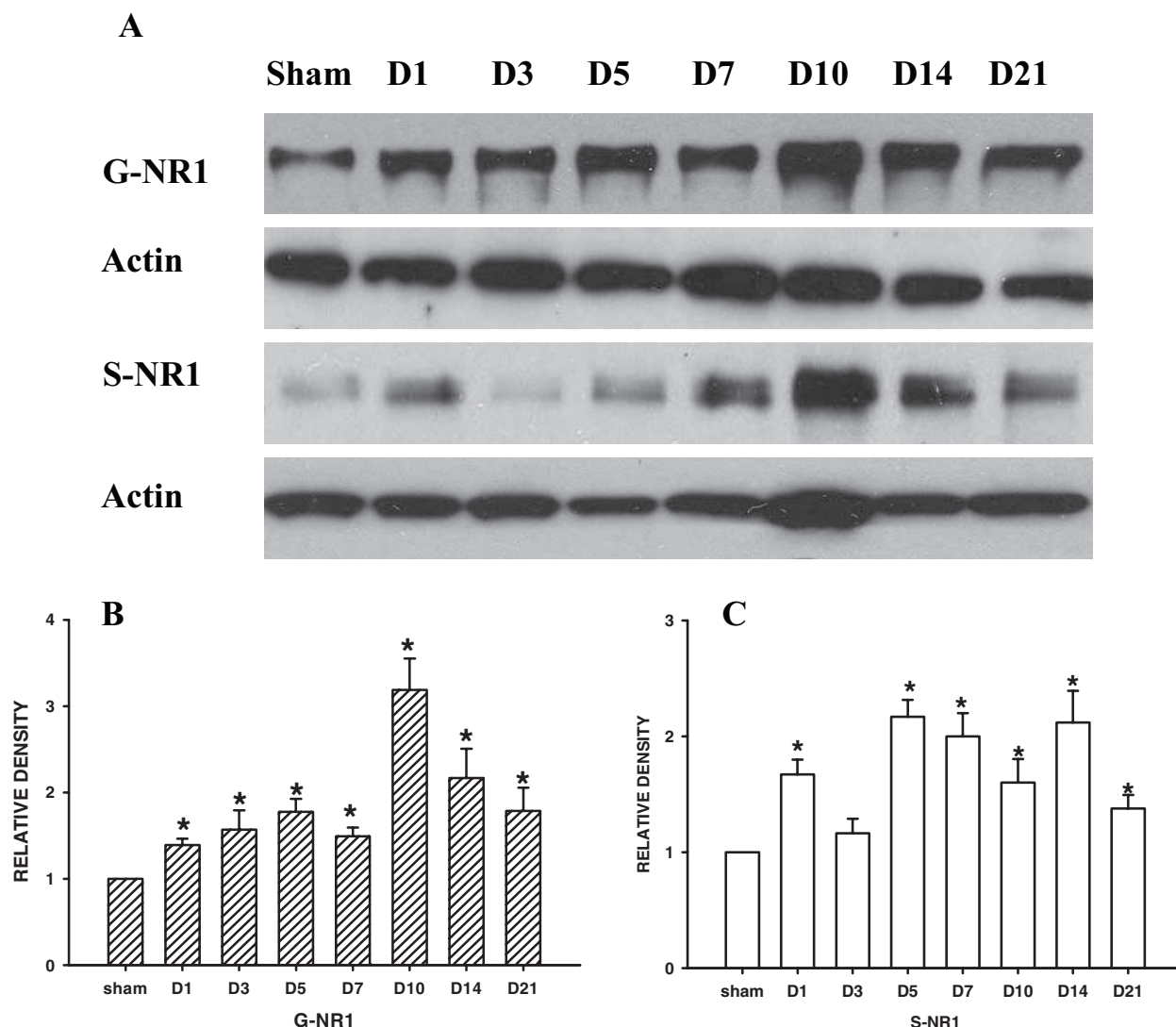


Fig. 2. Time course of changes in foot withdrawal latency to thermal stimulation (A) or threshold bending force to von Frey filament stimulation (B). D0, 1, 5, 7, 14, 21, 28, 35, and 42 indicate days after chronic compression of lumbar dorsal root ganglion (CCD). All data points represent mean  $\pm$  SE ( $n = 6$  for sham rats and  $n = 12$  for CCD rats). \*  $P < 0.05$  compared with sham rats. Difference score refers to differences in paw withdrawal (PAWD) latency or threshold bending force (VF) between the contralateral (C) and ipsilateral (I) hind paws.

day 1, peaking around day 5-14, and lasting up to day 28 after CCD (fig. 2B) ( $F = 32.9$ ;  $P = 0.0001$ ). There were no significant changes in the difference score in the sham group or on the contralateral hind paw in the CCD group (fig. 2B) ( $F = 0.50$ ;  $P = 0.85$ ; data not shown).

In addition, CCD rats showed postural changes as well as shaking and licking of the affected hind paw after thermal or mechanical stimulation, similar to that seen in a previous model induced by inserting metal rods into foramina.<sup>3</sup> In addition, CCD rats were often seen to spontaneously lift the affected hind paw from the floor and hold it in a protective position next to the flank while standing or sitting. When the affected hind paw was touching the floor, it appeared that these rats were slightly leaning toward the contralateral side to reduce the weight bearing. These spontaneous behaviors began on the first postoperative day and were expressed most often during the first 2-3 postoperative weeks. Naive or sham-operated rats showed no abnormal postures.

Correctively, the results demonstrated that chronic compression of the unilateral L5 DRG by infiltrating the



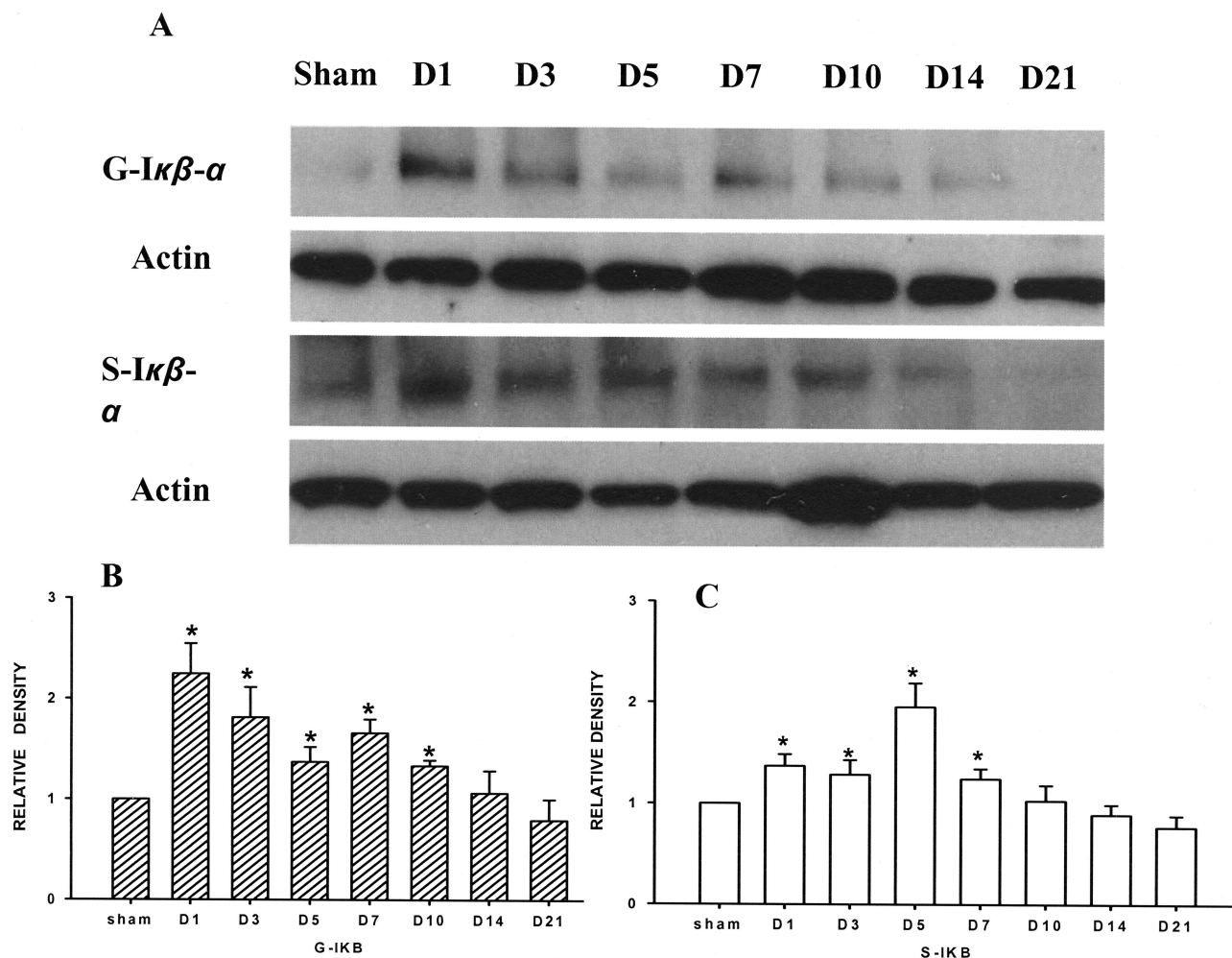
**Fig. 3.** Time course of L5 dorsal root ganglion (DRG) and spinal NR1 subunit changes after chronic compression of lumbar DRG (CCD). CCD induced up-regulation of the NR1 expression within the ipsilateral L5 DRG (G) and spinal cord dorsal horn (S). (A) Bands of Western blot of the NR1 expression (100 kDa).  $\beta$ -actin is a loading control. (B and C) Statistical analysis of relative density of Western blots (B, DRG; C, spinal cord dorsal horn) between CCD and sham rats. D1 to D21 refers to samples taken from CCD rats on postoperative days 1–21. \*  $P < 0.05$  compared with sham rats.

L5 foramen with SURGIFLO™ produced lasting thermal hyperalgesia and mechanical allodynia in the ipsilateral hind paw.

#### *Up-regulation of the NR1 and $\text{I}\kappa\beta$ - $\alpha$ Expression within the Ipsilateral L5 DRG and Spinal Cord Dorsal Horn*

CCD induced a significant increase in the expression of the NR1 subunit of the NMDA receptor within the ipsilateral L5 DRG ( $F = 25.4$ ;  $P = 0.0001$ ) and spinal cord dorsal horn ( $F = 20.7$ ;  $P = 0.0001$ ) as revealed by Western blot (fig. 3). Similarly, the expression of  $\text{I}\kappa\beta$ - $\alpha$  was also increased within the ipsilateral L5 DRG ( $F = 19.3$ ;  $P = 0.0001$ ) and spinal cord dorsal horn ( $F = 22.6$ ;  $P = 0.0001$ ) (fig. 4). There were no significant differences in the NR1 and  $\text{I}\kappa\beta$ - $\alpha$  expression between CCD and sham rats in the contralateral DRG and spinal cord

dorsal horn, and there were no differences in the NR1 and  $\text{I}\kappa\beta$ - $\alpha$  expression between sham and naive rats (data not shown). The NR1 level within the ipsilateral L5 DRG and spinal cord dorsal horn was significantly increased on postoperative day 1 compared with the sham group (fig. 3,  $P < 0.05$ ), an effect that lasted up to at least 21 days of CCD. In contrast, the  $\text{I}\kappa\beta$ - $\alpha$  level within the ipsilateral L5 DRG and spinal cord dorsal horn was significantly increased on postoperative day 1 compared with the sham group (fig. 4,  $P < 0.05$ ), an effect that lasted up to 7 days of CCD. Collectively, the results indicate that CCD produced a significant up-regulation of both NR1 and  $\text{I}\kappa\beta$ - $\alpha$  within the ipsilateral L5 DRG and spinal cord dorsal horn. Moreover, the duration of the NR1 up-regulation outlasted that of the  $\text{I}\kappa\beta$ - $\alpha$  up-regulation, which would be consistent with an early inflammatory process likely to be present in



**Fig. 4.** Time course of L5 dorsal root ganglion (DRG) and spinal I $\kappa\beta$ - $\alpha$  changes after chronic compression of lumbar DRG (CCD). CCD induced up-regulation of the I $\kappa\beta$ - $\alpha$  expression within the ipsilateral L5 DRG (G) and spinal cord dorsal horn (S). (A) Bands of Western blot of the I $\kappa\beta$ - $\alpha$  expression (42 kDa).  $\beta$ -actin is a loading control. (B and C) Statistical analysis of relative density of Western blots (B, DRG; C, spinal cord dorsal horn) between CCD and sham rats. D1 to D21 refers to samples taken from CCD rats on postoperative days 1–21. \*  $P < 0.05$  compared with sham rats.

clinical radicular pain followed by changes involving the NMDA receptor.<sup>1,2</sup>

#### Effect of Epidural Triamcinolone on CCD-induced Pain Behavior

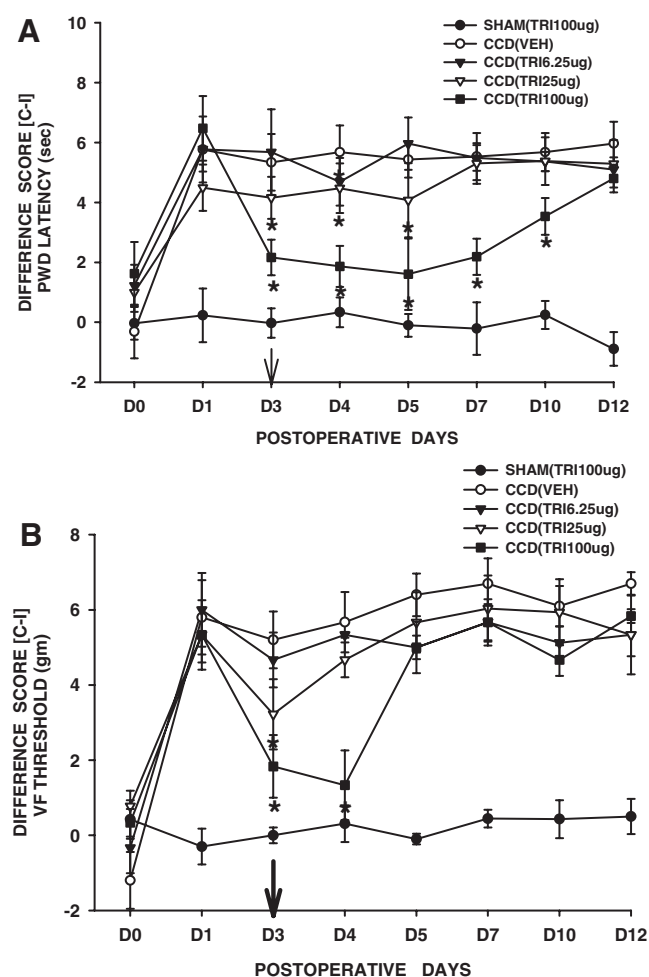
Both thermal hyperalgesia and mechanical allodynia were observable on postoperative day 1 and continued to be present up to at least postoperative day 21 in vehicle-treated CCD rats (fig. 5). Epidural treatment with triamcinolone (100  $\mu\text{g}$  > 25  $\mu\text{g}$  = 6.26  $\mu\text{g}$  = vehicle;  $F = 44.5$ ;  $P = 0.0001$ ), given once on postoperative day 3, significantly reduced thermal hyperalgesia (fig. 5A,  $F = 15.1$ ;  $P = 0.0001$ ) and mechanical allodynia (fig. 5B,  $F = 15.0$ ;  $P = 0.0001$ ) compared with vehicle-treated CCD rats. The effect of triamcinolone on thermal hyperalgesia lasted up to 7 d after a single injection on postoperative day 3. Triamcinolone also significantly reduced mechanical allodynia but for a much shorter duration (24–36 h). Triamcinolone itself had no effect on thermal and mechanical nociceptive threshold in

sham rats, and it did not change the baseline threshold in the contralateral hind paw of CCD rats. These results indicate that the thermal hyperalgesia and mechanical allodynia demonstrated in this animal model were attenuated by epidural treatment with triamcinolone, a glucocorticoid steroid commonly used in the clinical treatment of radicular pain.

#### Discussion

The present data demonstrate that chronic compression of the unilateral L5 DRG and exiting nerve root resulted in: (1) mechanical allodynia and thermal hyperalgesia that lasted up to 4 or 5 postoperative weeks; and (2) up-regulation of both the NMDA receptor and an inflammatory marker (I $\kappa\beta$ - $\alpha$ ) within the ipsilateral L5 DRG and spinal cord dorsal horn with distinctive time courses. In addition, epidural administration of triamcinolone on postoperative day 3 attenuated both thermal





**Fig. 5.** Effect of epidural treatment with triamcinolone (TRI) on thermal hyperalgesia (A) and mechanical allodynia (B) in rats with chronic compression of lumbar dorsal root ganglion (CCD). All injections were given epidurally once on postoperative day 3. All data points represent mean  $\pm$  SEM ( $n = 6$  per group). \*  $P < 0.05$  compared with CCD + vehicle (VEH) in each group. Triamcinolone alone did not affect the behavior response in sham rats (A, B). PWD = paw withdrawal; VF = bending force.

hyperalgesia and mechanical allodynia in CCD rats. Because the material (SURGIFLO™) used to produce CCD differs from metal rods used in a previous rat model of chronic compression of DRG,<sup>3,4</sup> this modified rat CCD model may closely mimic a subset of clinical radicular pain resulting from such conditions as foraminal stenosis, herniated intervertebral disc, and a mass effect from tumor on a DRG/exiting nerve root.

Several technical issues need further clarification. First, SURGIFLO™ is a sterile hemostatic material made from a gelatin matrix, which has a texture similar to that of a herniated disk when it hardens. SURGIFLO™ has been extensively and safely used in surgical operations.<sup>5-7</sup> SURGIFLO™ can be reabsorbed 4–6 weeks after its injection, which is consistent with the time course of behavioral changes in our study and the presence of an almost intact SURGIFLO™ plaque up to 3 or 4 weeks

after its injection as confirmed by autopsy (through laminectomy) in our experiment. Second, the liquid form of SURGIFLO™ is easy to inject and hardens within a minute or so after its injection, so that visual confirmation of the compression site (neuroforaminal/DRG area) can be made during the procedure before the skin closure. Therefore, this CCD condition is highly reproducible in our experiments. Third, based on the data from our pilot experiment using various volumes of SURGIFLO™, we selected 60  $\mu$ l SURGIFLO™ to be the appropriate volume for the injection as discussed in “Materials and Methods.” When injected slowly (within 1 or 2 min), only minor, transient, and infrequent (one or two) twitches were observed, and no rats showed hind paw muscle spasm because of vertebral canal compression induced by too much or too quick an injection. Fourth, we selected L5 DRG to be the compression site because the L5 spinal nerve is a major contributor to the sciatic nerve.<sup>14</sup>

The behavioral change demonstrated in the present model could be related to the mechanical compression and/or inflammatory responses. Although the effect of local tissue inflammation may be contributory to the behavioral change, several pieces of evidence suggest that the local compression would have played a critical role. First, when SURGIFLO™ was injected into the surrounding tissue outside the intended DRG area, these rats did not show behavioral changes indicative of allodynia and hyperalgesia, as demonstrated in our pilot experiment. Second, inflammatory responses were present at both DRG and spinal dorsal horn after the injection as indicated by the  $I\kappa\beta$ - $\alpha$  expression. However, the decline of its expression when the behavioral manifestation of radicular pain remained present suggests that inflammatory responses could not be a major cause of the behavioral change, at least after the first week of CCD. Third, had a systemic effect been responsible for the behavioral change, one would expect to see diffuse hyperalgesia and allodynia possibly involving contralateral hind paws as well. To the contrary, there were no significant changes in thermal and mechanical responses in contralateral hind paws.

Both similarities and differences are present between a previous (metal rods)<sup>3,4</sup> and the present (SURGIFLO™) CCD model. First, both CCD models produce local compression of the affected DRG. Metal rods produce rather rigid compression of the affected DRG, whereas SURGIFLO™, once it hardens, produces a soft form of compression. As such, the former would be more likely to mimic a postlaminectomy radicular pain condition if hardware is implanted during laminectomy and the latter to represent radicular pain conditions resulting from soft tissue compression such as that from herniated or bulging disc, tumor mass effect, and/or foraminal stenosis. Second, SURGIFLO™ is a material used for hemostasis, which would be more biocompatible than metal rods. Third,

technically both CCD models require the exposure of the foramen area, although injecting SURGIFLO™ may require less exposure of the foramen area than inserting metal rods. Fourth, SURGIFLO™ hardens within a minute or so after its injection, and the final position of the hardened SURGIFLO™ plaque can be confirmed before the skin incision is closed, which makes the model more reproducible and predictable.

Previous studies have shown that the NMDA receptor plays a critical role in pathologic pain such as that induced by peripheral nerve injury.<sup>12,15-18</sup> In this model, the expression of the NR1 subunit of the NMDA receptor was up-regulated within the affected L5 DRG and spinal cord dorsal horn, which lasted up to at least 21 days after CCD. However, endogenous inflammatory elements have also been shown to play a role in radicular pain.<sup>19-23</sup> In this regard,  $\text{I}\kappa\beta\text{-}\alpha$  has been proposed to be a reliable marker of inflammatory responses<sup>24-26</sup> because the time course of its expression parallels that of nuclear factor  $\kappa\beta$ , a major player in inflammatory responses.<sup>27-31</sup> Indeed, our results showed an up-regulation of the  $\text{I}\kappa\beta\text{-}\alpha$  expression within the ipsilateral L5 DRG and spinal cord dorsal horn, which lasted for at least 7 days during the early stage of CCD. It should be noted that the lack of a temporal correlation between the expression of  $\text{I}\kappa\beta\text{-}\alpha$  (up to 7 days after CCD) and the persistent behavioral change suggests that inflammatory responses may be contributory to the radicular pain condition primarily during the early stage of CCD, whereas NMDA receptors, which were up-regulated at least 21 days after CCD, may be responsible for both the development and maintenance of the radicular pain condition after CCD.

The present CCD model may be a useful tool for preclinical studies of radicular pain for several reasons. First, our behavioral data showed that difference scores of withdrawal threshold to mechanical or thermal stimulation were significantly increased and lasted up to 4 or 5 wk after CCD. These changes are similar to those observed in a previous model using metal rods.<sup>3</sup> Second, although there were postural changes in CCD rats, no foot deformities were observed in CCD rats, in contrast to peripheral neuropathy models in which animals exhibited persistent foot deformity after nerve injury.<sup>11,15</sup> The lack of foot deformity would be consistent with clinical situations of radicular pain in which most patients do not have significant neurologic deficits.<sup>1</sup> Third, epidural steroid injection is a common interventional procedure used to treat radicular pain,<sup>1,2</sup> and in the present study, epidural administration of triamcinolone given once on post-operative day 3 significantly attenuated both thermal hyperalgesia and mechanical allodynia in CCD rats, although the duration of action from triamcinolone differed between thermal hyperalgesia and mechanical allodynia (see fig. 5). Nonetheless, the positive

response to the epidural triamcinolone treatment in CCD rats supports the usefulness of this rat model to mimic clinical conditions of radicular pain. Therefore, this rat model may also be used as a tool to investigate the cellular mechanisms of radicular pain and to explore new treatment options for the management of clinical radicular pain conditions.

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