

Mechanical Compression and Nucleus Pulposus Application on Dorsal Root Ganglia Differentially Modify Evoked Neuronal Activity in the Thalamus

Elin Nilsson,¹ Helena Brisby,² Katarina Rask,³ and Ingela Hammar¹

Abstract

A combination of mechanical compression caused by a protruding disc and leakage of nucleus pulposus (NP) from the disc core is presumed to contribute to intervertebral disc hernia-related pain. Experimental models of disc hernia including both components have resulted in changes in neuronal activity at the level of the dorsal root ganglion (DRG) and spinal cord, but changes within the brain have been less well studied. However, acute application of NP to a DRG without mechanical compression rapidly increases neuronal activity in the thalamus, a major brain relay nucleus processing information from sensory pathways including ascending nociceptive tracts. The combination of mechanical compression and NP might therefore result in further increases in central neuronal activity. Using an experimental disc herniation rat model including both mechanical compression and NP the present study aimed to investigate changes in neuronal activity in the contralateral thalamic ventral posterior lateral nucleus *in vivo*. Measurements were obtained while electrically stimulating the ipsilateral sciatic nerve at A δ fiber intensities. The L4 DRG was subjected to light mechanical compression and NP exposure, and acute changes in evoked thalamic responses were recorded for up to 40 min. In order to compare effects in naïve animals with effects following a longer period of NP exposure, animals that were either disc-punctured or sham-operated 24 h previously were also included. In all animals, light mechanical compression of the DRG depressed the number of evoked neuronal responses. Prior NP exposure resulted in less potent changes following mechanical compression (80% of baseline) than that observed in naïve animals (50%). During the subsequent NP application, the number of evoked responses compared to baseline increased in pre-exposed animals (to 87%) as well as in naïve animals (72%) in which the removal of the mechanical compression resulted in a further increase (106%). The contribution of acute DRG compression and disc material leakage to changes in transmission in central neuronal networks is likely to be complex and to involve both short-term and long-term effects. Since a light mechanical compression may reduce transmission in nociceptive pathways, it is possible that the presence or absence of NP is crucial for pain development in the acute phase of disc herniation.

Key words: neuroscience; physiology

Introduction

INTERVERTEBRAL DISC HERNIA was first described by Mixter and Barr,¹ and the ensuing pain has been suggested to arise as a result of the mechanical compression that a ruptured disc exerts on adjacent nerves. While mechanical compression is still presumed to be of major importance,²⁻⁴ leakage of nucleus pulposus (NP) from the disc core onto nervous tissue may also contribute to the experience of pain by causing a local inflammatory response and inducing both structural and functional changes in nerve roots.⁵⁻⁸ Exposing peripheral nerves and dorsal root ganglia (DRGs) to NP has been

reported to result in increased spontaneous neuronal activity in the DRG within hours⁹ that can last for days.^{10,11} Changes in neuronal activity following NP exposure has also been reported in the spinal cord, where wide dynamic range neurons rapidly show increased responses to noxious mechanical stimulation of the innervated skin area.^{12,13}

Experimental models aiming to explore the origin of disc hernia-related pain often include both a mechanical and an inflammatory component. The results have suggested that the presence of both these components increases the effect in terms of behavior^{14,15} and in neuronal activity by resulting in more pronounced changes in dorsal root conduction

¹Department of Physiology, Institute of Neuroscience and Physiology; ²Department of Orthopaedics, Institute of Clinical Sciences, Sahlgrenska University Hospital; ³Center for Physiology and Bio-Imaging; University of Gothenburg, Gothenburg, Sweden.

velocity than when either of these interventions are applied alone.¹⁶ However, very little is known about the effect of experimental models of disc hernia on neuronal activity in the brain and the central processing of nociception. The thalamus is the major brain relay nucleus for sensory information, including transmission in nociceptive pathways from the spinal cord en route to cortical regions where the information is processed further. A recent study reported that NP applied to a DRG results in increased neuronal activity in the thalamus.¹⁷ The neuronal activity recorded in the ventral posterior lateral (VPL) nucleus of the thalamus increased within minutes of application of NP to the L4 DRG and lasted for up to 40 min,¹⁷ suggesting that changes in neuronal networks within the brain are rapidly induced by NP application. However, the questions of whether similar or more potent changes may be induced by the combination of mechanical compression and NP exposure, as suggested by behavior studies,¹⁵ as well as whether exposure to NP prior to the mechanical compression would have resulted in further changes in central neuronal activity were not investigated.

The aim of the present study was to investigate possible changes in neuronal thalamic activity using an acute experimental disc hernia model in the rodent. We included both light mechanical compression of the DRG and exposure to NP, mimicking the clinical situation in which a disc protrusion may be followed by a rupture of the annulus fibrosus with subsequent NP leakage. This model has previously been shown to induce behavioral changes as determined by von Frey withdrawal tests¹⁵ and video analysis of changes in spontaneous behavior¹⁴ as well as morphological changes in the peripheral nervous system, such as changes in the capsule surrounding the DRG.⁷ As a small rift in the annulus fibrosus resulting in NP leakage sometimes precedes a disc protrusion and further leakage, we also aimed to investigate whether any changes in thalamic neuronal activity elicited in the acute model would be increased further by exposing the DRG to small volumes of NP 24 h before an acute compression of the DRG and renewed exposure to NP.

Methods

All experimental procedures were approved by the regional animal ethics committee. Sixty-five adult female Sprague-Dawley rats (weighing 200–250 g) were used. All animals were killed by a lethal dose of pentobarbital sodium (300 mg/kg; APL) administered intravenously at the end of the experiment.

Study design

The aims of the present study were addressed in four different experimental series (Fig. 1). In series 1 the acute effects of continuous light mechanical compression of the L4 DRG were investigated in naïve animals by recording evoked neuronal activity in the VPL nucleus for 30 min ($n=11$). Thereafter, NP was applied to the DRG with the mechanical compression either remaining *in situ* ($n=6$) or being removed ($n=5$) while the sampling of evoked neuronal responses continued for a total of 60 min. In series 2, surgery consisting of either a disc puncture ($n=8$) of the L4–L5 disc or sham surgery ($n=8$) was followed by acute electrophysiological experiments performed 24 h later, in which donor NP was applied to the L4 DRG in order to evaluate whether a previous expo-

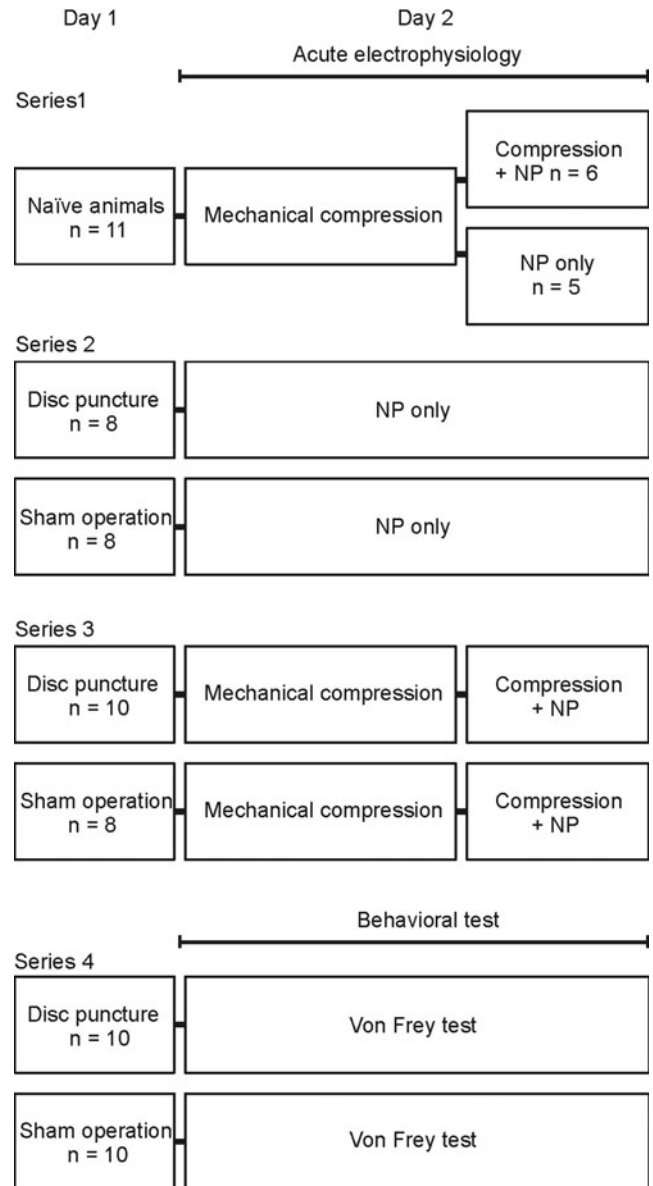


FIG. 1. Study design for series 1–4. The boxes in the left panel indicate the intervention performed on day 1 for each group of animals. An experimental flow chart for the acute electrophysiological and behavioral experiments performed on day 2 is shown on the right.

sure to NP resulted in a more potent response following a renewed NP application. In series 3, the protocol in series 1 including light mechanical compression of the DRG for 30 min followed by application of NP was repeated in animals 24 h after disc puncture ($n=10$) or sham surgery ($n=8$). In series 4, changes in mechanical paw withdrawal thresholds were measured before and 24 h after disc puncture ($n=10$) or sham surgery ($n=10$).

Disc puncture and sham surgery

Anesthesia was induced with isoflurane (Baxter Medical AB). Following a midline incision, the left facet joint between the fourth and fifth lumbar vertebrae (L4–L5) was removed to

expose the L4 DRG. In 28 rats, a syringe connected to a 23-G injection needle was used to incise the disc and inject small volumes of air until NP leaked out and could be applied to the exposed DRG. Twenty-six sham-operated animals were subjected to the same surgical procedures with the exception of the disc incision. Buprenorphine given intramuscularly (Temgesic 60 $\mu\text{g}/\text{kg}$; Shering-Plough) was used as postoperative pain relief.

Acute electrophysiological experiments

Acute electrophysiological experiments were performed in naïve animals (series 1) and 24 h after the experimental disc puncture or sham surgery (series 2–3, see Fig. 1). Anesthesia was induced intraperitoneally with a mixture of fentanyl (Leptanal 272 $\mu\text{g}/\text{kg}$; Janssen-Cilag AB) and medetomidine hydrochloride (DomitorVet 545 $\mu\text{g}/\text{kg}$; Orion Pharma) and maintained by intermittent intravenous administration of α -chloralose (dose 5–30 mg/kg; Rhône-Poulenc Santé). The animals were tracheotomized and attached to a respirator with neuromuscular transmission blocked by intravenous pancuronium bromide (Pavulon, total dose 0.3 mg/kg; Organon). The heart rate was monitored via subcutaneous electrodes and the rectal temperature was maintained at 36°C–38°C by servo-controlled infrared lamps.

The left sciatic nerve was transected at knee level and mounted on a pair of silver-ball stimulating electrodes in a pool of paraffin created by skin flaps. The left L4 DRG was exposed in naïve rats and re-exposed in previously operated animals. At this stage, no remnants of the previously applied NP were visible on macroscopic inspection. Light mechanical compression was induced by gently dislocating the L4 DRG with a 26-G needle and was maintained by inserting the needle in the underlying bone, as previously described by Omarker and Myers.¹⁵ NP from one tail disc in a donor rat killed for other purposes was used for application of NP. A laminectomy was performed at Th11–12, exposing the spinal cord, and used for transdural cord dorsum recordings via a silver-ball electrode in contact with the surface of the spinal cord. A craniotomy was performed, and the dura mater was removed to insert a recording electrode into the VPL nucleus of the thalamus (Fig. 2A).

Stimulation and recording

The sciatic nerve was stimulated 50–100 times at each recording point by a short train of impulses (three stimuli, 0.2-ms duration, 400 Hz) delivered 10–20 times at 2 Hz, at intensities expressed in multiples of threshold (T) for the most sensitive fibers in the sciatic nerve.^{18,19} A glass micropipette (tip diameter 2.5 μm , resistance 1.5–3 M Ω) filled with 2 M NaCl was inserted in the contralateral VPL nucleus. The initial target position (AP –2.5 to –3.5, L 3.0, H –6) was carefully adjusted in small steps so that the final position of the recording micropipette was located in an area where low-intensity sciatic stimulation (2T) activating low threshold afferents only evoked scarce neuronal responses, while higher-intensity stimulation activating high threshold afferents evoked a maximal response (Fig. 2B–D). Recordings from the contralateral VPL nucleus were obtained for stimulation intensities of 20–50T (enough to activate a large proportion of thin myelinated A δ -fibers but excluding higher-threshold and slower-conducting C-fibers).¹⁹ Neuro-

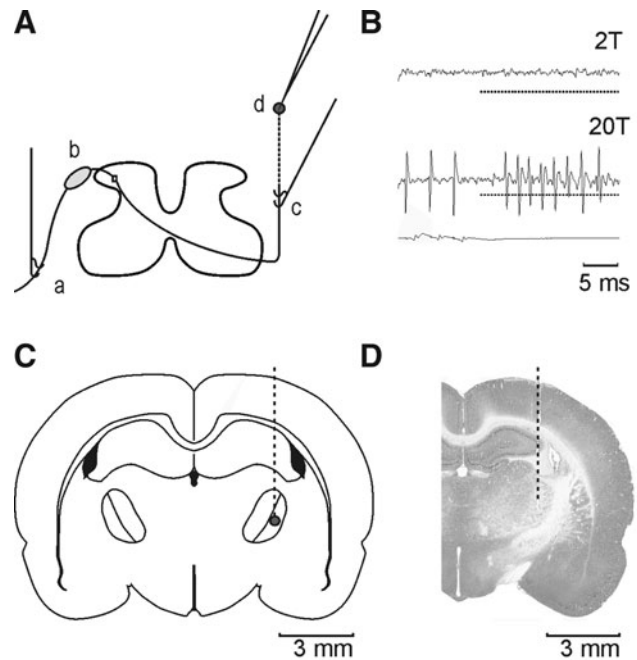


FIG. 2. Experimental set-up with stimulation and recording sites. **(A)** Schematic drawing with a) sciatic nerve and stimulation electrode; b) dorsal root ganglion (DRG) for exposure to nucleus pulposus (NP) and light mechanical compression; c) recording electrode at the surface of the spinal cord; d) recording site in the ventral posterior lateral (VPL) nucleus. **(B)** Examples of evoked activity following stimulation at threshold (T) intensities of 2T and 20T, corresponding to A α/β and A α/β together with A δ fibers, respectively. Upper traces: micropipette recordings (negativity downwards); lower traces: cord dorsum records (negativity upwards). Dotted horizontal lines represent discrimination lines used for spike counting. **(C)** Schematic drawing of the rat brain at Bregma –3mm. The electrode track and recording site corresponding to (d) in the schematic drawing in (A) is indicated by a dotted vertical line and a filled circle. **(D)** Representative cresyl violet-stained histological section of lesion, corresponding to the drawing in (C).

nal responses evoked by sciatic nerve stimulation were sampled every 10 min in a series of 10–20 consecutive stimuli as already described and evaluated within a time window of 25–30 ms.

Behavioral test

One group of animals subjected to the same surgical procedures as in series 2 and 3 were tested for changes in paw withdrawal thresholds (series 4; see Fig. 1). Von Frey filaments (North Coast Medical Inc.) were used to investigate changes in mechanical paw withdrawal response both prior to and 24 h after disc puncture ($n=10$) or sham surgery ($n=10$). The investigator performing the test was blinded with regard to the surgical intervention. Tests were performed on the left and right hind paws on three separate occasions in the week before surgery to establish a baseline, and a final test was performed 24 h after surgery, corresponding in time to the acute electrophysiological experiment in series 2 and 3. The von Frey monofilaments were applied in order of increasing stiffness, starting with 0.4 g and followed by 0.6, 1.0, 1.4, 2.0, 4.0,

6.0, 8.0, 10.0, and 15.0 g, alternating between the left hind paw and the right hind paw. A positive withdrawal was scored when the animal responded to two out of three stimuli presented.

Analysis

Original data records from the electrophysiological experiments were stored online using a software system designed by E. Eide, T. Holmström, and N. Pihlgren (University of Gothenburg). After positioning the recording electrode in the contralateral VPL nucleus a series of baseline records were obtained while stimulating the ipsilateral sciatic nerve. The mean number of evoked responses was set to 100%, and changes in the number of responses during the interventions are given as a percentage mean ± SEM of these initial values. Kruskal–Wallis test was used to compare the number of evoked responses at different time points in between groups, and paired *t*-test was used to compare changes in the number of evoked responses between baseline and each separate time point within the individual groups. Behavioral test data were analyzed with paired-samples *t*-test before and after intervention surgery for both the left hind paw and the right hind paw, and for differences between groups with Mann–Whitney test. *P* values less than 0.05 were considered significant.

Results

Combined light mechanical compression and application of NP in naïve animals (series 1)

Light mechanical compression of the DRG in naïve animals resulted in a reduced number of evoked responses within 10 min to 68% ± 9% of baseline (*p* = 0.009; Fig. 3), and lasted for as long as the mechanical compression alone remained *in situ*. Following the addition of NP an increased number of evoked responses were observed, irrespective of whether the mechanical compression remained or was removed. The

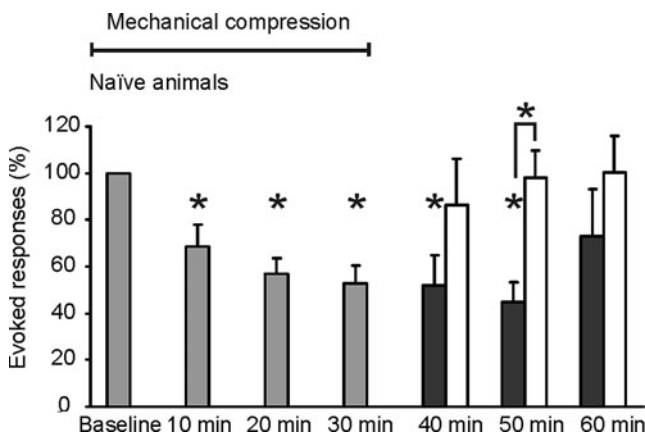


FIG. 3. Effects of mechanical compression and application of NP in naïve animals. The mean number of evoked responses is presented as a percentage of baseline records. Data from all animals are presented together up to 30 min with mechanical compression (*n* = 11, ■). Thereafter, the compression either remained (*n* = 6, ■), or was removed (*n* = 5, □) before application of NP. **p* < 0.05, mean ± SEM.

reversal appeared within minutes in animals in which the mechanical compression was removed, but developed more slowly when it remained, resulting in a significant difference in the number of evoked responses after 20 min of exposure to NP (106 ± 13 and 72 ± 16, respectively, *p* = 0.02).

Application of NP in disc-punctured and sham-operated animals (series 2)

Acute application of NP failed to induce significant changes in evoked responses compared to baseline after 40 min of exposure, in either previously disc-punctured (86% ± 10%) or sham-operated (126% ± 16%) animals (*p* = 0.15 and 0.15, respectively; Fig. 4). However, in sham-operated animals in which the DRG had not been exposed to NP previously, the mean number of evoked responses tended to increase and was significantly different from those in disc-punctured animals after 40 min; *p* = 0.046).

Combined light mechanical compression and application of NP in disc-punctured and sham-operated animals (series 3)

Although the degree and time course differed, acute light mechanical compression of the L4 DRG resulted in a reduced number of evoked responses in the contralateral VPL nucleus in both previously disc-punctured animals (80% ± 7%, *p* = 0.02) and sham-operated animals (68% ± 17%, *p* < 0.05; Fig. 5). In both groups, the onset of the effect was delayed compared to that in naïve animals (see Fig. 3). Similar to the observations in naïve animals, the increase in the number of evoked responses following NP exposure with the mechanical compression remaining *in situ* developed slowly and only appeared after 20–30 min and was preceded by a period of further depression. The increase following NP application was minor in disc-punctured animals (87% ± 8%) and even more so in sham-operated animals (59% ± 18%), in which the depression induced by the mechanical compression was only slightly changed compared to the maximal effect.

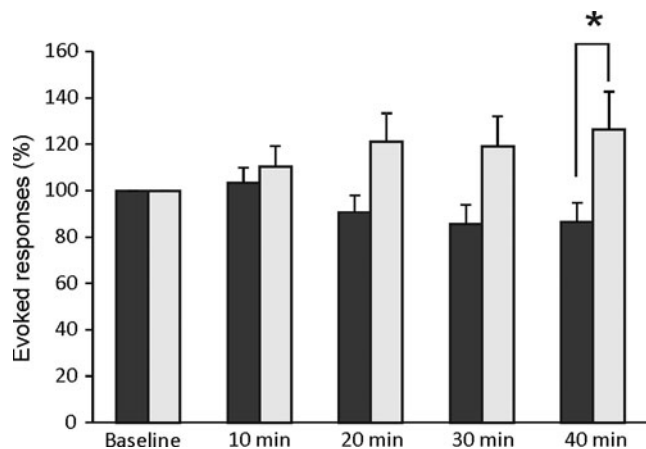


FIG. 4. Effects of application of NP in disc-punctured and sham-operated animals. The mean number of evoked responses is presented as a percentage of baseline records of NP application in previously disc-punctured (*n* = 8, ■) or sham-operated animals (*n* = 8, ■). **p* < 0.05, mean ± SEM.

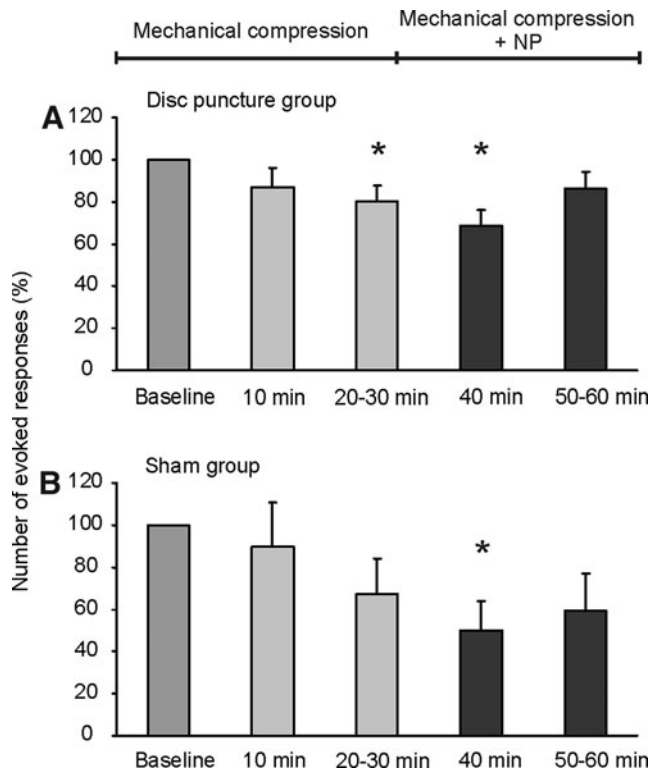


FIG. 5. Effects of mechanical compression and DRG exposure to NP in animals either pre-exposed to NP or sham-operated. The mean number of evoked responses is presented as a percentage of baseline records 24 h after: (A) disc puncture ($n=10$) or (B) sham operation ($n=8$). Baseline records, during mechanical compression (□) and after application of NP with the needle left *in situ* (■) as indicated under each bar. * $p < 0.05$, mean \pm SEM.

Behavioral study (series 4)

No changes were observed in withdrawal thresholds between the left and right hind paws in either disc-punctured or sham-operated animals 24 h after surgery compared to pre-operative thresholds (nonsignificant; data not shown). Furthermore, no differences were found when comparing the disc-punctured and sham-operated animals.

Discussion

Experimental models designed to study the origin of disc hernia-related pain often include both a mechanical and an inflammatory component, and the results obtained have suggested that these two components may mutually enhance each other in terms of effects on behavior^{14,15,20} and neuronal activity.¹⁶ The aim of the present study was to investigate changes in neuronal activity in the brain at the level of the thalamus following mechanical compression and NP exposure of a DRG. For this purpose, we used an experimental model simulating two hypothetical clinical situations, in which the disc protrusion may or may not be preceded by a small continuous leakage of NP from the interior of the disc due to ruptures in the annulus fibrosus. Clinically, leakage of NP might continue for more than 24 h before an acute mechanical compression to the DRG occurs. In the present series of experiments, the presence of NP for 24 h was considered

sufficient for events such as induced changes in neuronal excitability due to altered protein expression to occur in the DRG. Since disc herniation surgery includes a mechanical decompression while the possible chemical effect of the disc material may remain, series 1 included subgroups in which the effects of applied NP were investigated at the level of the thalamus with and without remaining compression.

The results suggest that in the acute phase, light mechanical compression results in reduced evoked neuronal activity in the thalamus that is rapidly reversed following decompression. When the compression remains, the application of NP may to some degree counteract the reduction in evoked responses and result in an increase but less so than when NP is applied to noncompressed DRGs in naïve animals.¹⁷ The changes in neuronal activity induced by mechanical compression are more pronounced in naïve animals and, although present, develop more slowly and are less potent in pre-operated animals. The similar degree and time course following mechanical compression in animals pre-exposed to NP or sham-operated as illustrated in Figure 5 may therefore indicate that mechanical compression, at least for the first 30 min, reduces central neuronal activity in the thalamus and that this effect is independent of whether the DRG has been exposed to NP previously or not.

In the present study the effects of a light mechanical compression of the DRG were not investigated for more than 30 min. Thus, it cannot be determined whether the recovery observed in series 1 and 3 at later time points and in the presence of NP only reflects the natural time course of changes in central neuronal activity following DRG compression *per se* and is due to a recovery of nerve function, or whether the recovery is at least in part due to the application of NP. However, the consistent finding of a reversal of the number of evoked responses following NP application together with the previously demonstrated facilitatory effect of NP in naïve animals¹⁷ could be taken to suggest that it is in fact related to the presence of NP. In addition, nerve compression has been demonstrated to reduce or completely abolish neuronal firing and more often results in paresthesia and muscle weakness than in pain.²¹⁻²⁵

Since morphological and functional changes including Schwann cell damage,⁵ changes in blood flow,⁸ and up-regulation of the sodium-selective acid-sensing ion channel 3 (ASIC3)²⁶ have been reported to occur within hours to days of application of NP to nerve roots, it might be expected that the presence of small volumes of NP for 24 h would result in altered sensitivity to light mechanical compression and renewed exposure to NP. The lack of significant changes in evoked neuronal activity in the thalamus irrespective of whether the animals were previously disc-punctured or sham-operated, together with the similar lack of changes in mechanical hind paw withdrawal thresholds examined at the same time point as the electrophysiological experiments were performed, suggest that if any functional changes were induced by the presence of NP for 24 h they are not detected in terms of neuronal activity in the thalamus or withdrawal behavior at this time point. Taken together, these results may therefore indicate that a single episode of NP leakage does not result in lasting changes in neuronal activity, but that any such effects are restricted to acute and short-lasting events. However, the significant difference found between the disc-puncture and sham-surgery experimental

groups in series 2 after 40 min of renewed NP exposure on day 2 (Fig. 4), although lacking when compared to baseline records, may suggest that changes in neuronal excitability lasting at least 24 h might nonetheless have been induced by the presence of NP but might only be revealed at later time points.

The cellular mechanisms behind the observed effects of light mechanical compression and NP exposure are not addressed in this study. Cytokines may be released from NP,^{27,28} and are potential candidates for playing an important role in the increased evoked activity observed following application of NP in the present study because cytokines such as tumor necrosis factor (TNF)- α may alter Na⁺ currents^{29,30} and thereby affect neuronal excitability. Mechanical compression may cause a block in action potentials propagating toward the spinal cord, resulting in a reduced number of evoked responses,^{23–25} which would be consistent with the findings in the present study. Importantly, the observed decrease was not likely to be due to irreversible fiber damage because the addition of NP not only reversed the decrease in neuronal activity but also resulted in an increased activity while the mechanical compression still remained. In addition, removal of the compression resulted in immediate recovery, further suggesting that the decrease induced by light mechanical compression is transient in the acute phase.

It cannot be ruled out that a methodological error is introduced when setting the baseline records to 100% in all acute electrophysiological experiments, thereby assuming that baselines between groups are the same and therefore comparable. The model used in the present work, however, includes extensive surgery and therefore does not allow recordings *in vivo* to be taken other than at the terminal experiment. It therefore cannot be excluded that the NP leakage onto the DRG introduced on day 1 might already have altered the activity before baseline records were obtained on day 2. However, a statistical comparison of the raw data obtained as baseline records failed to reveal any significant differences between the experimental groups (Kruskal–Wallis test; non-significant).

Several ascending tracts carrying nociceptive information from the spinal cord to the brain are relayed in the thalamus^{31–33} and changes in evoked neuronal responses in the VPL nucleus may thus reflect changes in activity in a number of nociceptive pathways. Graded nociceptive stimuli to the rat plantar surface were recently reported to result in increases in firing rate and in the number of activated neurons in the VPL nucleus and to be associated with increased neuronal activity in cortical areas involved in nociceptive processing.³⁴ While alterations in evoked thalamic activity in the VPL nucleus cannot be directly related to the presence or absence of pain perception, they suggest that the processing of nociceptive transmission at supraspinal levels may be modified following light mechanical compression or exposure to NP at the level of the DRGs.

Taken together, the findings in the present study using an experimental acute disc hernia model including light mechanical compression and application of NP to the DRG suggest that any ensuing changes in transmission in central neuronal networks are likely to be complex. As a light mechanical compression may reduce transmission in nociceptive pathways it is possible that the presence or absence of NP is crucial for the development of pain development in the acute phase of disc herniation.

Acknowledgments

We thank Jytte Grännsjö (Department of Physiology, Sahlgrenska Academy) for excellent technical assistance and Valter Sundh (EpiStat, Sahlgrenska Academy) for statistical support. This study was supported by grants from the Dr. Felix Neubergh Foundation, the Gothenburg Medical Association, ALF Västra Götaland, the Foundation in Memory of Sigurd and Elsa Golje, the Magnus Bergvall Foundation, and the Wilhelm and Martina Lundgren Foundation.

Author Disclosure Statement

No competing financial interests exist.

References

- Mixter WJ, Barr JS. Rupture of the intervertebral disc with involvement of the spinal canal. *N Engl J Med*. 1934;211:210–215.
- Hu SJ, Xing JL. An experimental model for chronic compression of dorsal root ganglion produced by intervertebral foramen stenosis in the rat. *Pain*. 1998;77:15–23.
- Rydevik B, Brown MD, Lundborg G. Pathoanatomy and pathophysiology of nerve root compression. *Spine (Phila Pa 1976)*. 1984;9:7–15.
- Smyth MJ, Wright V. Sciatica and the intervertebral disc; an experimental study. *J Bone Joint Surg Am*. 1958;40-A(6):1401–1418.
- Byröd G, Rydevik B, Nordborg C, et al. Early effects of nucleus pulposus application on spinal nerve root morphology and function. *Eur Spine J*. 1998;7:445–449.
- McCarron RF, Wimpee MW, Hudkins PG, et al. The inflammatory effect of nucleus pulposus. A possible element in the pathogenesis of low-back pain. *Spine (Phila Pa 1976)*. 1987;12:760–764.
- Murata Y, Rydevik B, Takahashi K, et al. Incision of the intervertebral disc induces disintegration and increases permeability of the dorsal root ganglion capsule. *Spine (Phila Pa 1976)*. 2005;30:1712–1716.
- Yabuki S, Kikuchi S, Olmarker K, et al. Acute effects of nucleus pulposus on blood flow and endoneurial fluid pressure in rat dorsal root ganglia. *Spine (Phila Pa 1976)*. 1998;23:2517–2523.
- Takebayashi T, Cavanaugh JM, Cüneyt Ozaktay A, et al. Effect of nucleus pulposus on the neural activity of dorsal root ganglion. *Spine (Phila Pa 1976)*. 2001;26:940–945.
- Chen C, Cavanaugh JM, Song Z, et al. Effects of nucleus pulposus on nerve root neural activity, mechanosensitivity, axonal morphology, and sodium channel expression. *Spine (Phila Pa 1976)*. 2004;29:17–25.
- Kallakuri S, Takebayashi T, Ozaktay AC, et al. The effects of epidural application of allografted nucleus pulposus in rats on cytokine expression, limb withdrawal and nerve root discharge. *Eur Spine J*. 2005;14:956–964.
- Anzai H, Hamba M, Onda A, et al. Epidural application of nucleus pulposus enhances nociceptive responses of rat dorsal horn neurons. *Spine (Phila Pa 1976)*. 2002;27:E50–55.
- Cuellar JM, Montesano PX, Antognini JF, et al. Application of nucleus pulposus to L5 dorsal root ganglion in rats enhances nociceptive dorsal horn neuronal windup. *J Neurophysiol*. 2005;94:35–48.
- Olmarker K, Störkson R, Berge OG. Pathogenesis of sciatic pain: a study of spontaneous behavior in rats exposed to experimental disc herniation. *Spine (Phila Pa 1976)*. 2002;27:1312–1317.
- Olmarker K, Myers RR. Pathogenesis of sciatic pain: role of herniated nucleus pulposus and deformation of spinal

- nerve root and dorsal root ganglion. *Pain*. 1998;78:99–105.
16. Takahashi N, Yabuki S, Aoki Y, et al. Pathomechanisms of nerve root injury caused by disc herniation: an experimental study of mechanical compression and chemical irritation. *Spine (Phila Pa 1976)*. 2003;28:435–441.
 17. Brisby H, Hammar I. Thalamic activation in a disc herniation model. *Spine (Phila Pa 1976)*. 2007;32:2846–2852.
 18. Jack JJB. Some methods for selective activation of muscle afferent fibres. In: Porter R (ed.) *Studies in Neurophysiology*. University Press: Cambridge; 1978; pp. 155–176.
 19. Schomburg ED, Steffens H, Dibaj P, et al. Major contribution of A δ -fibres to increased reflex transmission in the feline spinal cord during acute muscle inflammation. *Neurosci Res*. 2012;72:155–162.
 20. Olmarker K, Iwabuchi M, Larsson K, et al. Walking analysis of rats subjected to experimental disc herniation. *Eur Spine J*. 1998;7:394–399.
 21. Fern R, Harrison PJ. The effects of compression upon conduction in myelinated axons of the isolated frog sciatic nerve. *J Physiol*. 1991;432:111–122.
 22. Fern R, Harrison PJ. The contribution of ischaemia and deformation to the conduction block generated by compression of the cat sciatic nerve. *Exp Physiol*. 1994;79:583–592.
 23. Han SE, Lin CS, Boland RA, et al. Changes in human sensory axonal excitability induced by focal nerve compression. *J Physiol*. 2010;588(Pt 10):1737–1745.
 24. Howe JF, Loeser JD, Calvin WH. Mechanosensitivity of dorsal root ganglia and chronically injured axons: a physiological basis for the radicular pain of nerve root compression. *Pain*. 1977;3:25–41.
 25. Yayama T, Kobayashi S, Nakanishi Y, et al. Effects of graded mechanical compression of rabbit sciatic nerve on nerve blood flow and electrophysiological properties. *J Clin Neurosci*. 2010;17:501–505.
 26. Ohtori S, Inoue G, Koshi T, et al. Up-regulation of acid-sensing ion channel 3 in dorsal root ganglion neurons following application of nucleus pulposus on nerve root in rats. *Spine (Phila Pa 1976)*. 2006;31:2048–2052.
 27. Takahashi H, Suguro T, Okazima Y, et al. Inflammatory cytokines in the herniated disc of the lumbar spine. *Spine (Phila Pa 1976)*. 1996;21:218–224.
 28. Yoshida M, Nakamura T, Sei A, et al. Intervertebral disc cells produce tumor necrosis factor alpha, interleukin-1beta, and monocyte chemoattractant protein-1 immediately after herniation: an experimental study using a new hernia model. *Spine (Phila Pa 1976)*. 2005;30:55–61.
 29. Chen X, Pang RP, Shen KF, et al. TNF-alpha enhances the currents of voltage gated sodium channels in uninjured dorsal root ganglion neurons following motor nerve injury. *Exp Neurol*. 2011;227:279–286.
 30. Jin X, Gereau RW 4th. Acute p38-mediated modulation of tetrodotoxin-resistant sodium channels in mouse sensory neurons by tumor necrosis factor-alpha. *J Neurosci*. 2006;26:246–255.
 31. Davidson N. The projection of afferent pathways on the thalamus of the rat. *J Comp Neurol*. 1965;124:377–390.
 32. Giesler GJ Jr, Björkeland M, Xu Q, et al. Organization of the spinocervicothalamic pathway in the rat. *J Comp Neurol*. 1988;268:223–233.
 33. Peschanski M, Besson JM. A spino-reticulo-thalamic pathway in the rat: an anatomical study with reference to pain transmission. *Neuroscience*. 1984;12:165–178.
 34. Zhang Y, Wang N, Wang JY, et al. Ensemble encoding of nociceptive stimulus intensity in the rat medial and lateral pain systems. *Mol Pain*. 2011;7:64.

Address correspondence to:

Ingela Hammar, MD, PhD
 Department of Physiology
 Institute of Neuroscience and Physiology
 University of Gothenburg
 P.O. Box 432
 SE-405 30 Gothenburg
 Sweden

E-mail: ingela.hammar@physiol.gu.se