

Bone hypertrophy and reduced quality after nucleus pulposus aspiration induces low back pain-like behavior and neuronal hyperexcitability

Eui Ho Park^{a,*} and Junesun Kim^{b,c,*}

Objective There has been less explanation for whether lumbar disc injury, particularly through puncture and nucleus pulposus (NP) aspiration, can influence chronic low back pain (LBP). We aim to investigate whether intradiscal injury modifies spine structure and contributes to behavioral alteration and peripheral neuronal hyperexcitability in a rat model.

Methods Male Sprague–Dawley rats ($n = 50$) were subjected to lumbar disc (L4/5 and L5/6) puncture with nucleus pulposus aspiration (PUNCT) or sham surgery. Nociceptive processing was investigated through behavioral tests [dynamic weight bearing (DWB) and hindpaw withdrawal threshold], electrophysiological recordings of mechanosensitive single afferent nerves (MSAN), and calcium imaging of Dil-labeled dorsal root ganglion (DRG) neurons in response to capsaicin. Expression levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in the disc and subchondral bone were quantified, and bone structure was assessed using ex vivo micro-computed tomography (μ CT).

Results The PUNCT group displayed significant behavioral changes, including increased forelimb dependency in DWB and decreased hindpaw withdrawal thresholds. Electrophysiological data indicated MSAN hyperexcitability with a reduced threshold to intradiscal pressure, and calcium imaging revealed heightened

capsaicin ($1 \mu\text{M}$)-induced calcium influx in Dil-labeled DRG neurons from the PUNCT group. NGF and BDNF expression significantly increased in both the disc and subchondral bone of the PUNCT group. μ CT analysis revealed hypertrophic bone volume, diminished trabecular bone quality, and localized bone erosion in the PUNCT group.

Conclusion Intradiscal injury caused by puncture and NP aspiration induces spinal structural remodeling and peripheral neuronal sensitization, contributing to chronic LBP. *NeuroReport* 37: 86–94 Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved.

NeuroReport 2026, 37:86–94

Keywords: aspiration, brain-derived neurotrophic factor, intervertebral disc, low back pain, micro-computed tomography, nerve growth factor, nucleus pulposus

^aDepartment of Physiology, College of Medicine and Neuroscience Research Institute, ^bDepartment of Health and Environmental Science, Undergraduate School, College of Health Science and ^cRehabilitation Science Program, Department of Health Sciences, Graduate School, Korea University, Seoul, South Korea

Correspondence to Eui Ho Park, PhD, Department of Physiology, College of Medicine and Neuroscience Research Institute, Korea University, Seoul 02841, South Korea
E-mail: euiho@korea.ac.kr

*Eui Ho Park and Junesun Kim contributed equally to this work as corresponding author.

Received 28 March 2025 Accepted 30 November 2025.

Introduction

Low back pain (LBP) is a prevalent musculoskeletal disorder globally. Among its etiologies, degenerative changes in the intervertebral discs (IVDs) are recognized as a primary contributor to LBP [1]. Disc degeneration can lead to disc bulging or herniation, which mechanically compresses adjacent spinal nerves or spinal cord, thereby generating pain signals. Moreover, extrusion of the nucleus pulposus (NP) onto surrounding tissues triggers immune cells infiltration and elevates levels of cytokines and neurotrophins. This inflammatory cascade activates nociceptive nerve fibers and promotes their ingrowth into the degenerated disc, amplifying pain transmission.

Disc degeneration using needle puncture has been utilized to understand the pathological process underlying LBP. The absence of NP appears to exacerbate lumbar spine instability and triggers a pathological interplay between disc and subchondral bone. In-vitro studies in humans demonstrated that NP removal alters spinal biomechanics by decreasing its ability to resist axial and shear forces [2,3]. In rodent models, NP removal leads to severe disc degeneration, accompanied by osteophyte formation, increased inflammatory mediators, and robust LBP-like behavior [4]. Micro-computed tomography (μ CT) imaging has further reported that trabecular bone quality deteriorates and the subchondral endplate becomes sclerotic as disc degeneration progresses [5,6]. Therefore, these structural changes of lumbar disc abnormally redistribute mechanical loads, potentially driving pathological bone remodeling – characterized by sclerosis

Related digital media are available in the full-text version of the article at www.neuroreport.com.

and aberrant sensory innervation [7] – that exacerbates pain.

LBP is strongly attributed to abnormal neuronal ingrowth and sensitization in degenerated spinal tissues. Nerve growth factor (NGF) plays a pivotal role in peripheral sensitization by activating nociceptors at nerve terminals or dorsal root ganglion (DRG) cell bodies, particularly in inflammatory or degenerative states. In addition, intraperitoneal and intrathecal administration of NGF induced brain-derived neurotrophic factor (BDNF) expression in TrkA-expressing DRG neurons in rats [8]. Patients with severe disc degeneration exhibit upregulated NGF-TrkA and BDNF-TrkB receptor expression, along with increased angiogenesis and neuronal sprouting within disc tissue [9,10]. These NGF-/BDNF-dependent molecular changes underscore the transition from acute to persistent LBP.

Nevertheless, the precise relationship between severe disc degeneration, subchondral bone pathology, and their combined contribution to LBP remains inadequately understood. Here, we aim to investigate how the absence of NP in the puncture with nucleus pulposus aspiration (PUNCT) model influences the degenerative process in the lumbar disc and subchondral bone, and whether these morphometric alterations contribute to LBP. Our previous findings revealed that afferent nerve fibers innervating the rat lumbar disc become hypersensitive to intradiscal pressure following PUNCT [11]. In the current study, we intend to elucidate the degenerated spine structure underlying chronic LBP using a combined evaluation of behavioral assessments, μ CT imaging, western blotting, *in vivo* electrophysiology, and calcium imaging.

Method

Animals

Male Sprague–Dawley rats (Orient Bio, South Korea; 10 weeks, 350–400 g) were housed in a temperature- and light-controlled room (22–25 °C, 12 hours light/dark cycle). Food and water were available *ad libitum*. All animal use procedures were conducted according to the guidelines of Institutional Animal Care and Use Committee at Korea University (KOREA-2021-0012-C2).

Animal model with disc puncture/nucleus pulposus aspiration

Rats (total $n = 50$, Table 1) were anesthetized with a mixture of alfaxalone (10 mg/kg) and medetomidine (0.5 mg/kg), and a midline ventral longitudinal incision was made. For the mechanical injury model with complete removal of NP, two-level lumbar 4/5 (L4/5) and 5/6 (L5/6) discs were punctured, and the nucleus pulposus was aspirated (PUNCT) through a 22-gauge needle and tubing connected to a suction pump (CW-300, CHANGWOO, South Korea), which generated negative pressure (–760 mmHg). Muscles and skin margins were

sutured with 6-0 and 5-0 silk. Sham-surgery group underwent the same surgical procedures, except for the disc puncture step.

Experimental design

Our experimental design is included in Table 1.

Ex vivo micro-computed tomography

For scan and reconstruction, the animals were scanned before and 14–28–42 days after the surgery. The field of view included both surgery sites: L4/5 and L5/6 disc. SkyScan1176 μ CT (Bruker, Kontich, Belgium) was used. The prepared spine samples and the phantom set (8 mm diameter) were scanned. The image pixel size was 18 μ m, the filter option was copper 0.1 mm, and the applied voltage was 90 kV. The scanned projection image datasets were reconstructed in NRecon (Bruker). The dynamic range was 0–0.025, the ring artifact correction was 9, and the beam hardening correction was 30%. The reconstructed cross-section datasets were reoriented and analyzed in Dataviewer and CT Analyzer (Bruker).

For ex-vivo data analysis, Volume of interest for the trabecular bone of L5 spine was determined. Ten parameters, including bone mineral density (BMD), tissue volume (TV), bone volume (BV), percent bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular pattern factor (Tb.Pf), trabecular separation (Tb.Sp), connectivity (Conn), and connectivity density (Conn.Dn), were measured in the volume of interest [12].

Western blotting

Lumbar discs (4/5 and 5/6) and subchondral bones (L4-6) were frozen using liquid nitrogen, ground into a powder, and homogenized in radioimmunoprecipitation assay buffer supplemented with a 50 $\times$ protease inhibitor cocktail. The supernatant was collected after incubation in lysis buffer with vortexing every 10 min for 30 min and centrifugation at 13 000 rpm for 10 min at 4 °C. Protein concentration was determined using the Bicinchoninic acid Assay Kit (BioVision, Milpitas, California, USA). Each protein sample was denatured by boiling at 100 °C for 5 min and separated by 10% SDS-PAGE. Proteins were transferred to a nitrocellulose membrane (Millipore, Burlington, Massachusetts, USA) for 90 min using a constant 80 V and then blocked in tris-buffered saline with tween 20 with 5% skim milk solution for 1 hour at room temperature. After blocking, the membranes were incubated with antibodies against NGF (1 : 500, Abcam, Cambridge, Massachusetts, USA), BDNF (1 : 500, Invitrogen, California, USA), or β -actin (1 : 1,000, Abcam) for overnight. The relative amounts of proteins were normalized to β -actin in each sample.

Table 1 Experimental design

Groups	Number of animals	Number of tissues	Number of nerves	Number of DRG neurons	Experiments	Postsurgery days								Figures		
						-7 days	Pre	7 days	14 days	21 days	28 days	35 days	42 days		44 days	
Sham	6	6			Behavior (dynamic weight bearing & Withdrawal threshold)										Figure 1 Figure 3 Figure 2a-e	
PUNCT	8	8			Ex-vivo µCT imaging (bone)									✓		Figure 2f-h Figure 4
Sham	8		8		In vivo electrophysiology (intradiscal pressure)						✓					
PUNCT	8		8													
Dil*+Sham	5			Capsaicin (55)	Calcium imaging										Figure 2f-h Figure 4	
Dil*+PUNCT	5			Capsaicin (37)												
Sham	5	5			Western blot (NGF & BDNF)						✓					
PUNCT	5	5														
Total	50															

This table outlines the experimental design and time schedules for different groups and measurements.
√ indicates when measurements were taken.
DiI* indicates administration was performed 7 days before surgery. The total number of animals across all experiments is 50.
BDNF, brain-derived neurotrophic factor; DRG, dorsal root ganglion; NGF, nerve growth factor; PUNCT, puncture and nucleus pulposus aspiration.

Dil-labeling from lumbar intervertebral discs

Rats were anesthetized with a mixture of alfaxalone (10 mg/kg) and medetomidine (0.5 mg/kg), and a mid-line ventral longitudinal incision was made in the supine position. The L4/5 and L5/6 discs were exposed under a microscope, and intradiscal administration of DiI (2 µL) was cautiously performed.

Intracellular Ca2+ imaging

On 28 days after the surgery (35 days after DiI injection), rats were anesthetized with urethane (250 mg/kg; Sigma-Aldrich, St. Louis, Missouri, USA), and left and right lumbar DRG (L1-4) were rapidly sampled. The dissociated neurons of DRG were suspended onto 12 mm circular glass coverslips pre-coated with Poly-D-lysine and stabilized in the Dulbecco's modified eagle medium culture media (2 hours at 37 °C). After being loaded with Fura-2 AM (1 µL/1 mL, Invitrogen; 30 min), DRG neurons were monitored by sequential dual excitation (340 nm and 380 nm; every 5 s), and DiI (Invitrogen)-labeling was also identified (540/25 nm of excitation, 605/55 nm of emission). DRG neurons were exposed to a baseline period (70 s), 1 µM capsaicin (Sigma-Aldrich, St. Louis, USA; 20 s), an interval period (105 s), and 50 mM potassium chloride (KCl; 20 s). Only small-sized DRG neurons (diameter of less than 20 µm) were analyzed within 10 hours after processes of dissociation and stabilization.

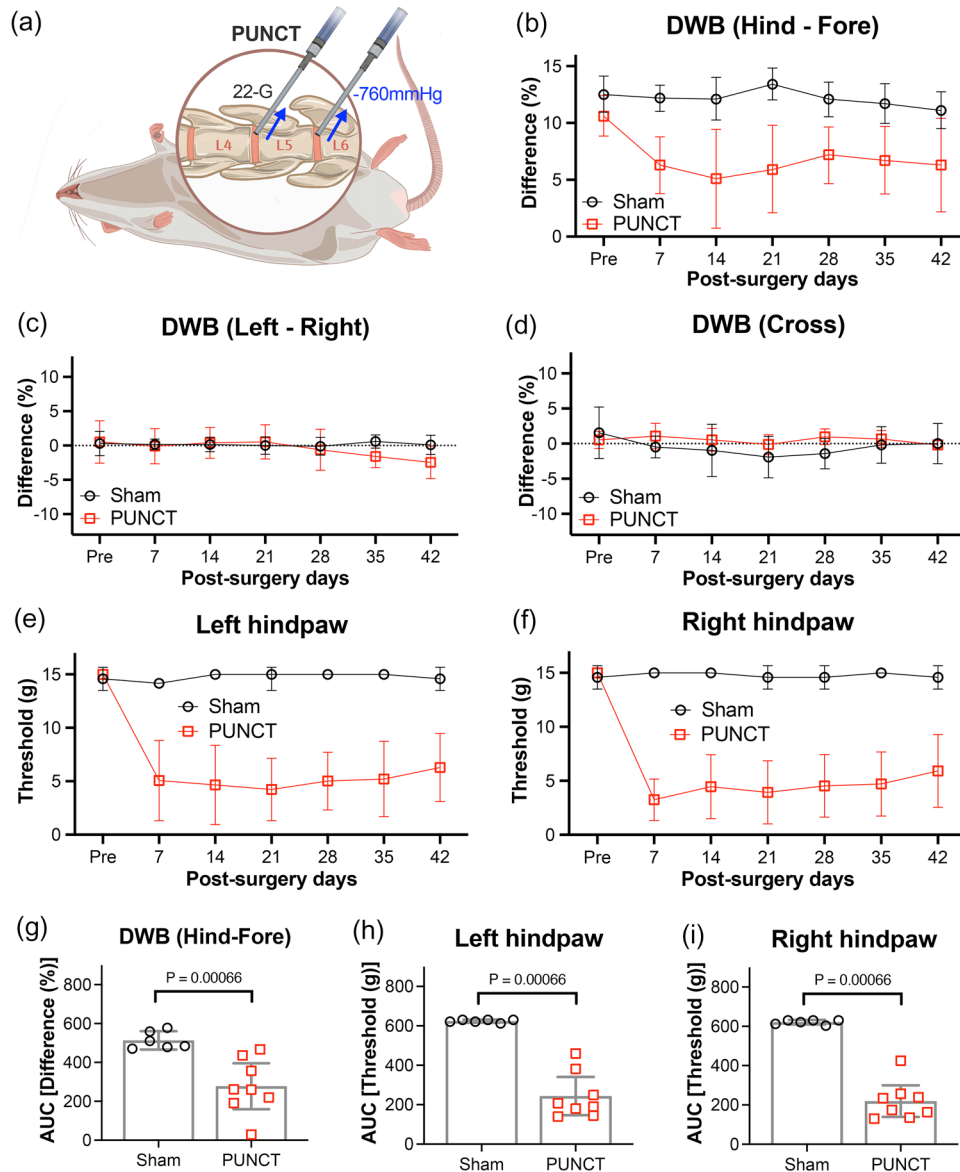
In vivo single nerve recording

Autonomic afferent nerve fibers innervating lumbar discs of rats under anesthesia (urethane, 250 mg/kg) were teased into a single fiber for recording neuronal activity. The firing frequency (maximal spikes/s) of the mechanosensitive single afferent nerve (MSAN) was recorded and compiled into a poststimulus time histogram (PSTH, bin width of 1 s). The MSAN threshold was determined as at least two firings over a 2-s stimulation. Intradiscal pressure (100-500-1000-1500-2000-2500 mmHg, 70-s interval) was generated through expansion of volumes and applied to lumbar 4/5 disc via tubing with a 23-gauge needle.

Dynamic weight bearing and hindpaw withdrawal threshold

Rats were habituated to experimenter handling (30-min per rat) and behavioral equipment (30-min per rat) 1 day before baseline (pre) measurement. For dynamic weight bearing (DWB), the weight load difference between the averaged hind and fore limbs (hind-fore), averaged right and left limbs (left-right), and averaged crossing limbs (cross) of rats was analyzed [11]. To measure the mechanical threshold for hindpaw withdrawal, a series of von Frey filaments (0.41–15.10g, Stoelting, Wood Dale, Illinois, USA) was applied. The 50% withdrawal threshold was determined using the up-down procedure, and stimuli were presented at intervals of several seconds.

Fig. 1



Low back pain-like behavior after PUNCT of lumbar disc. (a) Schematic illustration of the PUNCT procedure targeting lumbar discs at L4-5 and L5-6 using a 22-gauge needle to disc degeneration. (b-d) Time-course of DWB difference over 42 days post-PUNCT, comparing hindlimbs vs. forelimbs (Hind-Fore), left limbs vs. right limbs (Left-Right), and diagonal limbs (Cross) between PUNCT ($n = 8$) and Sham ($n = 6$) groups. (e,f) Time-course changes of withdrawal thresholds in left and right hindpaws over 42 days post-PUNCT (g-i) AUC analyses for DWB parameters (Hind-Fore, Left-Right, and Cross) and hindpaw mechanical withdrawal thresholds, quantifying cumulative differences over 42 days. Data represent mean $\pm$ 95% CI, and P values were determined using Mann-Whitney U test. AUC, Area under the curve; CI, confidence interval; DWB, dynamic weight bearing; PUNCT, puncture and nucleus pulposus aspiration.

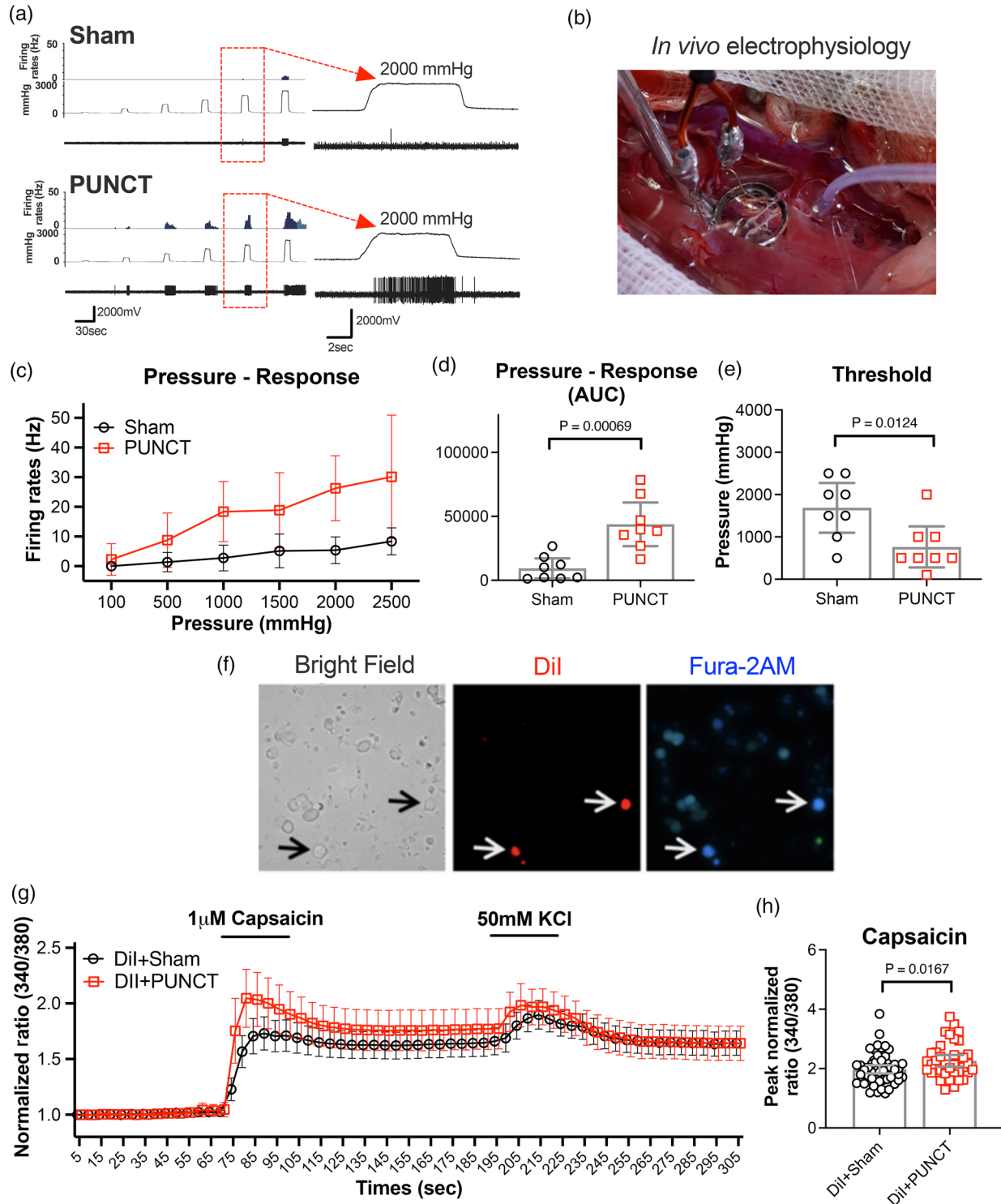
Statistical analysis

Data were expressed as means with 95% confidence interval (CI) or median with interquartile range (IQR) and evaluated using SPSS software version 26 (IBM corp., Armonk, New York, USA). Parametric or nonparametric statistics analysis of data was used depending on the pass of normal distribution testing. Differences between experimental conditions were considered statistically significant when $P < 0.05$.

Results

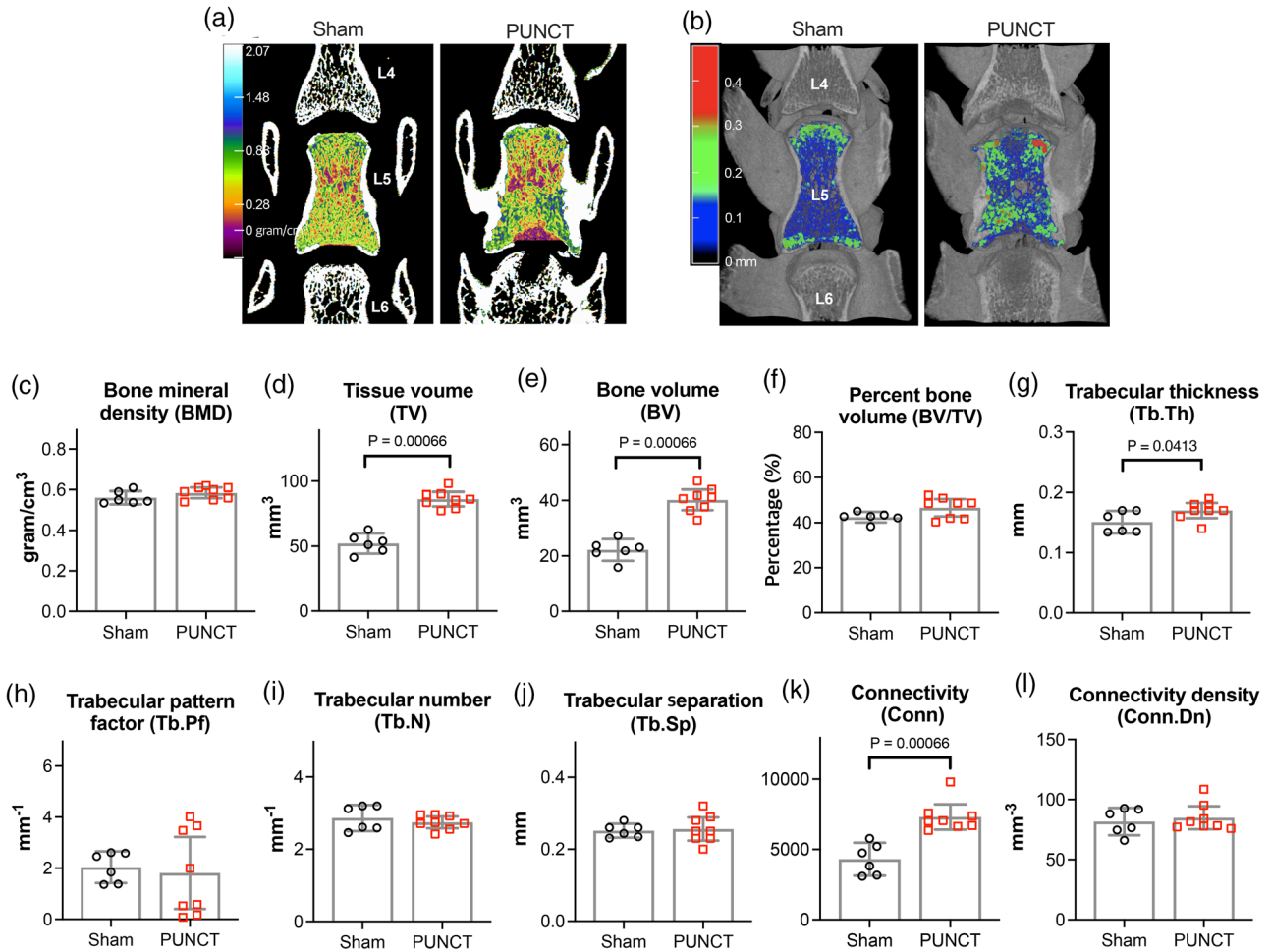
PUNCT of the lumbar disc induces pain-like behavior. Figure 1a shows the establishment of the PUNCT model. DWB and hindpaw withdrawal threshold were evaluated from presurgery to 42 days post-PUNCT. Figure 1b-f shows the time-course analysis of behavioral alterations, indicating a shift towards forelimb-dependent weight bearing and reduced hindpaw withdrawal thresholds following PUNCT-induced disc degeneration.

Fig. 2



Hyperexcitabilities of MSAN and enhanced TrpV1-mediated calcium influx of DRG neurons after PUNCT. (a) Representative electrophysiological recordings of single MSAN firings response to graded intradiscal pressures (100–2500 mmHg) 28 days post-PUNCT. (b) In vivo electrophysiology experimental setup. (c,d) Pressure-response curve and corresponding AUC of peak firing rates for MSAN in PUNCT (nerve/animal; $n = 8/8$) and sham ($n = 8/8$) group. (e) Pressure thresholds to elicit MSAN firings. (f) Representative images of bright field microscopy, Dil-labeling (red), and Fura-2AM calcium imaging (blue/green). (g,h) Normalized calcium influx traces (340/380 ratio) and peak values in response to 1 μ M capsaicin application in Dil-labeled DRG neurons from Sham (neuron/animal; $n = 55/5$) and PUNCT ($n = 37/5$) groups. Data represent mean + 95% CI, and P values were determined using Mann–Whitney U test. AUC, area under the curve; DRG, dorsal root ganglia; MSAN, mechanosensitive afferent nerve; TrpV1, transient receptor potential vanilloid 1; PUNCT, puncture with nucleus pulposus aspiration.

Fig. 3



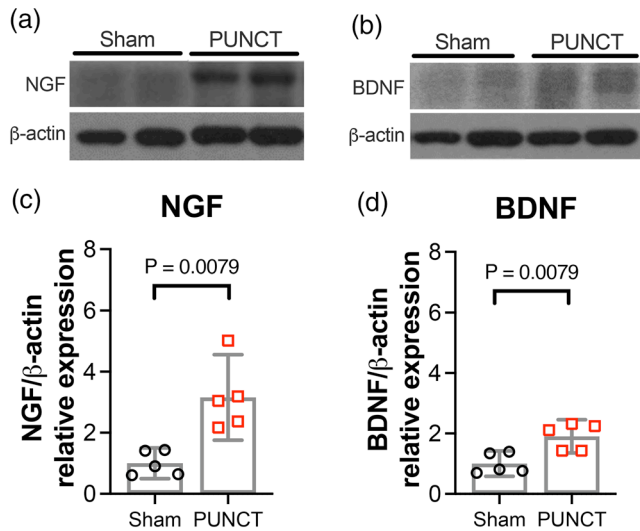
Morphometric alterations of bone structure following PUNCT. (a,b) Representative ex-vivo μ CT images of lumbar 5 vertebrae, displaying BMD (a) and trabecular thickness (Tb.Th; B) 42 days post-PUNCT. (c-l) Quantitative μ CT analysis of BMD, tissue volume (TV), bone volume (BV), percent bone volume (BV/TV), Tb.Th, trabecular pattern factor (Tb.Pf), trabecular number (Tb.N), trabecular separation (Tb.Sp), connectivity (Conn), and connectivity density (Conn.Dn) between PUNCT ($n=8$) and sham ($n=6$). Data represent mean + 95% CI, and P values were determined using Mann-Whitney U test. BMD, bone mineral density; CI, confidence interval; PUNCT, puncture with nucleus pulposus aspiration; μ CT, micro-computed tomography.

Typically, rats walk with a hindlimb-dependent weight load (Hind-Fore) of 10–12%. Following PUNCT, a forelimb-dependent load (Hind-Fore: 4–5%) is expressed, accompanied by a decrease in hindpaw withdrawal thresholds. Figure 1g shows a significant reduction of area under the curve (AUC) for hind-fore ($P = 0.00066$) in the PUNCT group compared to the sham group, but no significant changes in left-right or cross, indicating chronic LBP-like behavior. In addition, Figure 1h,i elicits significant decreases in both left and right hindpaw withdrawal (AUC, all $P = 0.00066$) of PUNCT compared with the sham group, indicating increased sensitivity to mechanical stimuli.

PUNCT induces hyperexcitability of mechanosensitive afferent nerve fibers and increases in transient receptor potential vanilloid 1 (TrpV1)-mediated calcium influx of

DRG neurons. We examined the mechanosensitivity of afferent nerve fibers innervating disc tissue in response to intradiscal pressure 28 days post-PUNCT (Fig. 2). Figure 2a provides representative electrophysiological data, illustrating that intradiscal pressures evoked firing of MSAN. Figure 2c shows hyperexcitability in these nerve fibers of the PUNCT group in response to intradiscal pressure (100–2500 mmHg). Compared with the sham group, the PUNCT group exhibited a significant increase in peak MSAN firing frequency (AUC, $P = 0.00066$; Fig. 2d) and a significant decrease in firing threshold [mmHg, 763 (CI, 277–1248) vs. 1688 (CI, 1099–2276), $P = 0.0124$; Fig. 2e].

To further validate the sensitization of afferent neurons innervating the degenerated disc, we examined calcium imaging. Figure 2f illustrates the experimental setup for

Fig. 4

The upregulation of NGF and BDNF expression after PUNCT. (a,b) Representative western blotting images of NGF and BDNF protein expression in lumbar disc (L4/5 and 5/6) and subchondral bone (L4-6) 28 days post-PUNCT. (c,d) Quantification analysis of NGF expression levels in PUNCT ($n = 5$) and sham ($n = 5$) groups. Data represent mean ± 95% CI, and P values were determined using Mann-Whitney U test. BDNF, brain-derived neurotrophic factor; CI, confidence interval; NGF, nerve growth factor; PUNCT, puncture with nucleus pulposus aspiration.

assessing calcium influx in DiI-labeled DRG neurons 28 days post-PUNCT surgery (or 35 days post-DiI injection). Figure 2g shows the normalized ratio (340/380) of capsaicin (a TrpV1 agonist)-induced calcium influx in DRG neurons. Figure 2h shows the enhanced calcium influx pattern of the PUNCT group following capsaicin application. In detail, a significant increase in 1 μ M capsaicin-evoked calcium influx was expressed in the DiI-PUNCT group compared with DiI-labeled sham groups [the peak normalized ratio, 2.26 (CI, 2.06–2.46) vs. 1.98 (CI, 1.84–2.11), $P = 0.0167$].

PUNCT causes distinct morphometric alterations of bone. As shown in Figure 3, the lumbar IVD and vertebrae structure were analyzed using ex vivo μ CT imaging 42 days post-PUNCT surgery. Figure 3a,b displays representative images of BMD and Tb.Th. The PUNCT group displayed remarkable bone erosion coupled with hypertrophic trabecular bone. Figure 3d, e, g, k reveal that the PUNCT group had significantly increased TV [mm^3 , 86.25 (CI, 80.68–91.82) vs. 52.07 (CI, 44.22–59.91), $P = 0.00066$], BV [mm^3 , 40.18 (CI, 46.46–43.89) vs. 22.17 (CI, 18.23–26.10), $P = 0.00066$], Tb.Th [mm, 0.17 (CI, 0.16–0.18) vs. 0.15 (CI, 0.13–0.17), $P = 0.0413$], and Conn [7310 (CI, 6408–8212) vs. 4305 (CI, 3138–5473), $P = 0.00066$] compared with the sham group. However, Fig. 3c,f–j,l indicates that parameters of BMD, TV, BV, BV/TV, Tb.Pf, Tb.N, and Conn.Dn in the PUNCT group were not statistically significant compared with the sham group.

PUNCT upregulates NGF and brain-derived neurotrophic factor (BDNF) expression. We evaluated neurotrophin expression in the disc (L4/5 and 5/6) and subchondral bone (L4-6) 28 days post-PUNCT. Figure 4a and B show representative images of NGF and BDNF. Figure 4c and D present the upregulated expression of these neurotrophins in the PUNCT group. In detail, the PUNCT group showed significant increases in both NGF [3.16 (CI, 1.76–4.56) vs. 1.00 (CI, 0.49–1.50), $P = 0.0079$] and BDNF [1.91 (CI, 1.36–2.45) vs. 1.00 (CI, 0.58–1.42), $P = 0.0079$] compared with the sham group.

Discussion

The present study provides compelling evidence that intradiscal injury induces significant morphometric alterations in the lumbar IVD and adjacent bone structures. Specifically, the puncture with NP aspiration (PUNCT) model resulted in marked structural disruptions, including endplate erosion and hypertrophic remodeling of the trabecular bone. These morphological changes were closely correlated with heightened nociceptive processing, as demonstrated by behavioral hypersensitivity, upregulation of NGF and BDNF, and hyperexcitability in mechanosensitive afferent neurons and DRG neurons.

Disc puncture models have been widely used to elucidate the interplay between the disc and surrounding bone. In our PUNCT model, nucleus pulposus removal appeared to rapidly destabilize the structural integrity of the low back structure, particularly impacting the vertebral endplates and subchondral bone. Quantitative μ CT analysis revealed significant increases in TV and BV, indicative of bone hypertrophy as a compensatory response to the injury. Concurrent increases in Tb.Th and Conn indicated a reduction in trabecular bone quality. Erosion was observed at the center of the endplates and subchondral bone, with affected regions filled with soft callus tissue. These observations are consistent with previous studies linking increased BV/TV and Tb.Th in the endplate-subchondral bone, alongside reduced endplate vascularity, to advanced disc degeneration [13]. Interestingly, a puncture model without NP aspiration exhibited minimal bone hypertrophy, with BV/TV differences of less than 5% compared with the sham group, underscoring the pivotal role of NP removal in initiating bone remodeling processes [14]. Therefore, it is feasible that the absence of NP alters biomechanical properties of the lumbar spine or compromises endplate integrity, which can initiate a cascade of pathological processes, including inflammation, callus formation, endochondral ossification, and hypertrophic remodeling.

Our study demonstrated that morphometric alterations in degenerative disc and bone are strongly associated with the severity of nociceptive processing. Specifically, the activation of nociceptive nerve endings and DRG

neurons innervating these degenerated tissues appears to be a critical factor for LBP. In our PUNCT model, *in vivo* electrophysiological data showed hyperexcitability of mechanosensitive afferent neurons, characterized by significantly lowered thresholds in response to intradiscal pressure. Previous immunohistological and molecular studies identified the presence of TrpV1 channels in disc-related DRG neurons [15–17]. A puncture model using NP removal showed that structural alteration and inflammatory cytokines in the lumbar spine are associated with upregulated TrpV1, calcitonin gene-related peptide, nerve growth factor/tropomyosin receptor kinase A, voltage-gated sodium channel 1.7, and voltage-gated sodium channel 1.8, within DRG neurons following injury [4]. Consistent with these findings, we provided robust functional evidence that TrpV1-mediated signaling in DRG neurons plays a key role in peripheral sensitization induced by a degenerated spine. It appears that intradiscal injury fosters an intensive inflammatory milieu in disc and bone, thereby lowering activation thresholds and amplifying firing frequency via nociceptors such as TrpV1. These findings highlight a strong association of structural degeneration and inflammation in the pathogenesis of LBP.

To confirm these neurophysiological findings, we conducted a behavioral assessment of pain responses to mechanical load or movement in our animal model. The findings showed that reducing hindlimb loading—by redistributing more weight to the forelimbs—may represent a behavioral strategy to alleviate LBP. This observation aligns with previous reports that disc injury results in prolonged standing time and shortened stride gait during locomotion [18,19], suggesting that compensatory postural adjustments may mitigate mechanical stress on the degenerated spine. However, discrepancies persist in some studies regarding reduced hindlimb withdrawal thresholds as an indicator of secondary hyperalgesia in disc degeneration models [11,20–22].

The actions of NGF and BDNF within the degenerative disc can promote both peripheral sensitization and tissue remodeling. Multiple human and animal studies have supported that the upregulation of NGF and BDNF within degenerative disc and bone drive inflammation, vascularization, and nerve ingrowth [23]. In addition to roles in nociception, these neurotrophins have been implicated in bone metabolism by acting synergically with vascularization and tissue repair [24]. In support of these findings, our current findings revealed the increase of NGF and BDNF in the hypertrophic structure of disc-endplate-subchondral bone. In the peripheral nerve system, a high population of DRG neurons – including myelinated A δ fibers and peptidergic unmyelinated C fibers – express TrkA receptor, a high-affinity binding site for NGF. Upregulated neurotrophins of lumbar disc and bone likely contribute to the sensitization of nociceptors

expressed in low back tissues or lumbar DRG [25]. Therefore, this neurotrophin-mediated sensitization represents a direct response to low back tissue injury, potentially promoting nociceptive effects. Beyond neurogenic activity, NGF and BDNF have emerged as key regulators of bone metabolism in a close association with osteoblast or osteoclast. NGF expression in osteoblasts is enhanced during mechanical loading and indirectly stimulates bone formation of mice through Wnt/ β -catenin signaling [26]. During fracture healing of mice, levels of NGF protein or mRNA peaked between 2 and 3 days postfracture, followed by increased vascularization (day 7), callus ossification (day 14), and mineralization (day 56) [27,28]. Combined application of NGF with bone morphogenic protein-9 and vascular endothelial growth factor produced faster healing of bone structures in rabbit mandible fracture [29]. The role of NGF in bone remodeling is supported by showing that anti-NGF reduces the numbers of TRAP-positive osteoclasts of the subchondral bone through the receptor activator of NF- κ B ligand (RANKL)-independent mechanism [30]. Likewise, the local application of BDNF was found to enhance bone remodeling and induce sclerotic changes after cortical osteotomy in rat mandibles, with long-term osteoblasts activation observed 28 days post-BDNF [31]. Despite these regenerative effects, clinical use of anti-NGF or anti-TrkA antibodies has been associated with adverse structural outcomes, including rapid joint space narrowing and chondrolysis [32]. These complications, referred to as rapidly progressive osteoarthritis (RPOA), underscore the importance of optimizing therapeutic efficacy with tissue repair strategies when targeting neurotrophic pathways.

A limitation of this study is that structural changes alone are insufficient to fully explain the induction or progression of pain signals. Further studies are required to clarify correlation evaluations or underlying mechanisms between spine morphology and nociceptive processing in the context of anti-NGF or anti-BDNF therapeutic treatments.

Acknowledgements

This work was supported by the Basic Science Research Program (RS-2023-NR076883, RS-2024-00347913, RS-2025-02303227) through the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT (MSIT) and Korea University Research Fellowship grant program. All authors have made substantial contributions to (1) study concept and design (Eui Ho Park), (2) data acquisition and assembly (Eui Ho Park), (3) data analysis and interpretation (Eui Ho Park and Junesun Kim), and (4) final drafting of the article (Eui Ho Park and Junesun Kim).

Conflicts of interest

There are no conflicts of interest.

References

- Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nat Rev Rheumatol* 2014; **10**:44–56.
- Meakin JR, Redpath TW, Hukins DW. The effect of partial removal of the nucleus pulposus from the intervertebral disc on the response of the human annulus fibrosus to compression. *Clin Biomech (Bristol)* 2001; **16**:121–128.
- O'Connell GD, Malhotra NR, Vresilovic EJ, Elliott DM. The effect of nucleotomy and the dependence of degeneration of human intervertebral disc strain in axial compression. *Spine (Phila Pa 1976)* 2011; **36**:1765–1771.
- Shi C, Das V, Li X, Kc R, Qiu S, *et al.* Development of an in vivo mouse model of discogenic low back pain. *J Cell Physiol* 2018; **233**:6589–6602.
- Choi H, Tessier S, Silagi ES, Kyada R, Yousefi F, Pleshko N, *et al.* A novel mouse model of intervertebral disc degeneration shows altered cell fate and matrix homeostasis. *Matrix Biol* 2018; **70**:102–122.
- Rutges JP, Jagt van der OP, Oner FC, Verbout AJ, Castelein RJ, Kummer JA, *et al.* Micro-CT quantification of subchondral endplate changes in intervertebral disc degeneration. *Osteoarthritis Cartilage* 2011; **19**:89–95.
- Teboul-Coré S, Roux C, Borderie D, Kolta S, Lefèvre-Colau MM, Poiraudéau S, *et al.* Bone mineral density and bone remodeling markers in chronic low back pain patients with active discopathy: a case-control exploratory study. *PLoS One* 2018; **13**:e0196536.
- Michael GJ, Averill S, Nitkunan A, Rattray M, Bennett DL, Yan Q, Priestley JV. Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord. *J Neurosci* 1997; **17**:8476–8490.
- Freemont AJ, Watkins A, Le Maitre C, Baird P, Jeziorska M, Knight MT, *et al.* Nerve growth factor expression and innervation of the painful intervertebral disc. *J Pathol* 2002; **197**:286–292.
- Binch AL, Cole AA, Breakwell LM, Michael AL, Chiverton N, Cross AK, Le Maitre CL. Expression and regulation of neurotrophic and angiogenic factors during human intervertebral disc degeneration. *Arthritis Res Ther* 2014; **16**:416.
- Park EH, Moon SW, Suh HR, Hochman S, Lee MG, Kim YI, *et al.* Disc degeneration induces a mechano-sensitization of disc afferent nerve fibers that associates with low back pain. *Osteoarthritis Cartilage* 2019; **27**:1608–1617.
- Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *J Bone Miner Res* 2010; **25**: 1468–1486.
- Ashinsky BG, Bonnevill ED, Mandalapu SA, Pickup S, Wang C, Han L, *et al.* Intervertebral disc degeneration is associated with aberrant endplate remodeling and reduced small molecule transport. *J Bone Miner Res* 2020; **35**:1572–1581.
- Maerz T, Newton M, Marek AA, Planalp M, Baker K. Dynamic adaptation of vertebral endplate and trabecular bone following annular injury in a rat model of degenerative disc disease. *Spine J* 2018; **18**:2091–2101.
- Leimer EM, Gayoso MG, Jing L, Tang SY, Gupta MC, Setton LA. Behavioral compensations and neuronal remodeling in a rodent Model of chronic intervertebral disc degeneration. *Sci Rep* 2019; **9**:3759.
- Ohtori S, Takahashi K, Moriya H. Existence of brain-derived neurotrophic factor and vanilloid receptor subtype 1 immunoreactive sensory DRG neurons innervating L5/6 intervertebral discs in rats. *J Orthop Sci* 2003; **8**:84–87.
- Aoki Y, Ohtori S, Takahashi K, Ino H, Douya H, Ozawa T, *et al.* Expression and co-expression of VR1, CGRP, and IB4-binding glycoprotein in dorsal root ganglion neurons in rats: differences between the disc afferents and the cutaneous afferents. *Spine (Phila Pa 1976)* 2005; **30**:1496–1500.
- Miyagi M, Ishikawa T, Kamoda H, Suzuki M, Sakuma Y, Orita S, *et al.* Assessment of pain behavior in a rat model of intervertebral disc injury using the CatWalk gait analysis system. *Spine (Phila Pa 1976)* 2013; **38**:1459–1465.
- Lai A, Moon A, Purmessur D, Skovrlj B, Winkelstein BA, Cho SK, *et al.* Assessment of functional and behavioral changes sensitive to painful disc degeneration. *J Orthop Res* 2015; **33**:755–764.
- Suh HR, Cho HY, Han HC. Development of a novel model of intervertebral disc degeneration by the intradiscal application of monosodium iodoacetate (MIA) in rat. *Spine J* 2022; **22**:183–192.
- Huang Y, Lei L, Zhu J, Zheng J, Li Z, Wang H, *et al.* Pain behavior and phenotype in a modified anterior lumbar disc puncture mouse model. *JOR Spine* 2024; **7**:e1284.
- Hou SX, Tang JG, Chen HS, Chen J. Chronic inflammation and compression of the dorsal root contribute to sciatica induced by the intervertebral disc herniation in rats. *Pain* 2003; **105**:255–264.
- Wise BL, Seidel MF, Lane NE. The evolution of nerve growth factor inhibition in clinical medicine. *Nat Rev Rheumatol* 2021; **17**:34–46.
- Su YW, Zhou XF, Foster BK, Grills BL, Xu J, Xian CJ. Roles of neurotrophins in skeletal tissue formation and healing. *J Cell Physiol* 2018; **233**:2133–2145.
- Schmelz M, Mantyh P, Malfait AM, Farrar J, Yaksh T, Tive L, Viktrup L. Nerve growth factor antibody for the treatment of osteoarthritis pain and chronic low-back pain: mechanism of action in the context of efficacy and safety. *Pain* 2019; **160**:2210–2220.
- Tomlinson RE, Li Z, Li Z, Minichiello L, Riddle RC, Venkatesan A, Clemens TL. NGF-TrkA signaling in sensory nerves is required for skeletal adaptation to mechanical loads in mice. *Proc Natl Acad Sci U S A* 2017; **114**:E3632–E3641.
- Li Z, Meyers CA, Chang L, Lee S, Li Z, Tomlinson R, *et al.* Fracture repair requires TrkA signaling by skeletal sensory nerves. *J Clin Invest* 2019; **129**:5137–5150.
- Asami K, Nakanishi T, Asahara H, Inoue H, Takigawa M. Expression of neurotrophins and their receptors (TRK) during fracture healing. *Bone* 2000; **26**:625–633.
- Yang S, Cheng J, Man C, Jiang L, Long G, Zhao W, Zheng D. Effects of exogenous nerve growth factor on the expression of BMP-9 and VEGF in the healing of rabbit mandible fracture with local nerve injury. *J Orthop Surg Res* 2021; **16**:74.
- Xu L, Nwosu LN, Burston JJ, Millns PJ, Sagar DR, Mapp PI, *et al.* The anti-NGF antibody muMab 911 both prevents and reverses pain behaviour and subchondral osteoclast numbers in a rat model of osteoarthritis pain. *Osteoarthritis Cartilage* 2016; **24**:1587–1595.
- Ida-Yonemochi H, Yamada Y, Yoshikawa H, Seo K. Locally produced BDNF promotes sclerotic change in alveolar bone after nerve injury. *PLoS One* 2017; **12**:e0169201.
- Gonçalves S, Gowler PRW, Woodhams SG, Turnbull J, Hathway G, Chapman V. The challenges of treating osteoarthritis pain and opportunities for novel peripherally directed therapeutic strategies. *Neuropharmacology* 2022; **213**:109075.

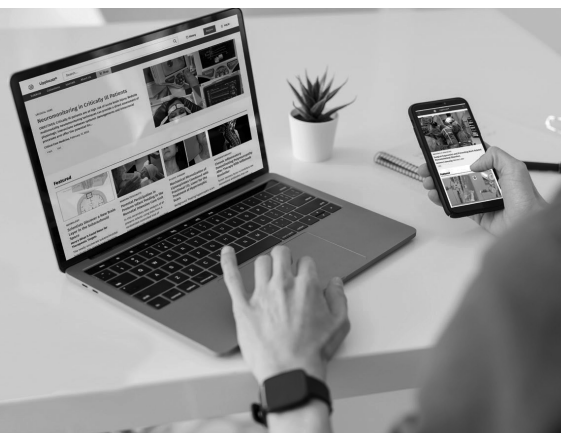
Lippincott Journals on Ovid



To stay ahead of the increasing rate of scientific and medical research output today, you need one destination where you can conduct all your online research in order to do your work efficiently and effectively. Search and discover current full-text Lippincott® journals and books, as well as bibliographic information, multimedia, and Open Access; plus manage your results and all of your research documents — all within the Ovid® platform.

Contact your Ovid Representative to learn more or email sales@ovid.com.

How do I get online access to my personal Lippincott® journal subscription?



Register

- 1) Go to **lww.com**
- 2) In the upper right corner, click **REGISTER** for your **FREE** account.
- 3) Complete the form. All fields are required, including the End User License Agreement and the Privacy Policy.

Be sure to verify your user account in the next 24 hours, by checking your email and clicking the “verify” link!

Activate

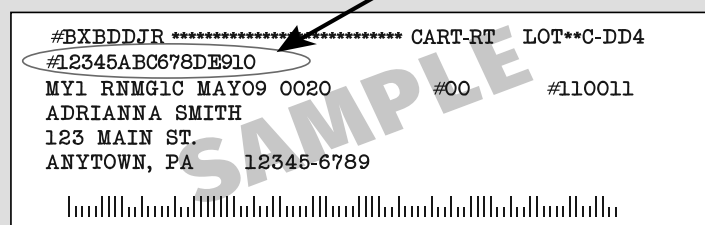
- 1) Once logged in, navigate to the **JOURNALS** section in top navigation bar and search for your subscribed title. Click on the title to be redirected to the journal’s website.
- 2) Your name should be listed as logged in, in the top left corner of the page. Click the drop-down menu and select **Activate Subscription**.
- 3) Enter your **12-Digit account/subscriber ID** number including zeros: (ex: 000123456789) and click **Activate Subscription**.

If you need assistance,
please contact:

customerservice@lww.com

1-800-638-3030 (US)
1-301-223-2300 (Int)

Monday to Friday,
8:30 AM to 7:00 PM EST



Where can I find my Subscriber ID or Society Member ID?

Your ID is located on the mailing label of the journal (Arrow shown in adjacent figure).