Lumbar Intervertebral Disc Injury Induces Pain-Like Behaviors And Disability In A Mouse Discogenic Pain Model

Neharika Bhadouria, Yunsoo Lee, Kaljang Sherpa, Michelle Villagran, Amanda Kopelman, Xueming Hu, Xinqi Guo, Trevonn Gyles,

Andrew C. Hecht, Hongzhen Hu, James C. Iatridis

Neharika.Bhadouria@mssm.edu

Disclosures: None

INTRODUCTION: Back pain is a leading cause of global disability and is strongly associated with intervertebral disc (IVD) degeneration (IVDD). Chronic back pain causes reduced mobility, sagittal imbalance, and anxiety-like behaviors, with no disease modifying treatments available. Mouse models of discogenic pain are needed because genetic modification can identify pain and degeneration mechanisms, because pain-like behavioral assays exist, and because it is a small vertebrate animal model **[1,2]**. However, the small size of mice has resulted in most mouse models of IVDD using mouse coccygeal IVDs which have similar anatomy and a simplified surgical approach, yet coccygeal IVDs differ in their proximity to neural structures and biomechanical loading compared to lumbar IVDs. Few lumbar mouse IVDD models exist and there is a need for repeatable, surgically-induced mouse lumbar IVDD models and this study focuses on deep phenotyping of pain-like behaviors. The aims are to develop an annulus fibrosus puncture-injury induced IVDD model in lumbar spine in female and male mice and to characterize multiple pain-like behaviors (hindpaw mechanical von Frey, axial grip strength, open-field response, and gait abnormality).

METHODS: With IACUC approval, four-month-old female and male C57BL/6J mice underwent anterior spinal surgery under aseptic conditions and were divided into 3 groups: Sham, Mild and Severe Injury (n=3-4/group). A left sided anterior spinal approach was used to expose lumbar IVD (L4-S1), identified by X-ray with femoral neurovascular structures and pelvic rim as landmarks. The lumbar surgery was performed under the microscope with an experienced human spine surgeon. Injuries were created with a 26G needle (0.5 mm depth, ~50% IVD minor diameter) via either a single anterior puncture (Mild) injury or three punctures with twisting (Severe) (**Fig. 1A, B**), similarly to methods previously described **[1]**. Lateral Faxitron X-rays were taken in-vivo under anesthesia from pre-surgery until the 8 week endpoint (data reported here is pre-surgery and Week 8) to determine IVD height, IVD wedge angle and lumbar sagittal Cobb angle. Pain-related behavioral outcomes included: evoked pain, assessed by hindpaw mechanical sensitivity using calibrated von Frey filaments (0.002-2 g); after acclimation, filaments were applied to the plantar surface in ascending order until withdrawal, with 60% thresholds calculated by percent response method **[3]**. Disability was assessed via DigiGait treadmill analysis (3 cm/s) generating digital paw prints for gait dynamics like stance/swing ratio **[4].** The stance/swing ratio denotes the relative time a limb spends in stance (foot on the ground, bearing weight) versus swing (foot off the ground, advancing) within a gait cycle. Additional behavioral assays included open-field testing (50 × 30 cm, 25 min) for voluntary locomotion and fore/hindpaw grip strength to quantify axial discomfort **[4, 5]**. Two-way repeated-measures ANOVA with Tukey's post hoc test was performed to compare pre- and post-surgery (week 8) data across injury groups (**Fig. 2C-E**), and one-way ANOVA assessed differences among sham, mild, and severe injury groups at week 8 (**Fig. 2F**).

RESULT: All animals survived the procedures. The data presented herein are from female mice, as the male cohort was insufficiently powered for conclusive analysis, and the study was initiated with females given clinical evidence that they exhibit greater discogenic pain than age-matched males **[7]**. Mild and severe IVD injury significantly reduced the von Frey thresholds by 92% (p=0.02) and 87% (p=0.05, pre-surgery vs week 8) suggesting mechanical hypersensitivity (**Fig. 2A**). Severe IVD injury significantly reduced grip strength by 28% (p=0.03, pre-surgery vs week 8, **Fig. 2B**), suggesting axial discomfort. Severe IVD injury also significantly increased the stance/swing ratio by 89% (p<0.001, pre-surgery vs week 8), suggesting disability with heightened pain response (**Fig. 2C**), and reduced horizontal distance travelled in the open-field test by 30% (p=0.1, pre-surgery vs week 8) is consistent with pain and/or anxiety-like behaviors (**Fig. 2D**). Severe IVD injury reduced Von Frey thresholds by 77% ($p < 0.001$) and grip strength by 32% ($p < 0.001$) compared with sham **(Fig. 2A, B)**. Pre-surgery and week 8 X-ray images of IVD at L4-L5 exhibited a trend toward reduced IVD height, altered IVD wedge angle/shape **(Fig. 2E, F)** and decreased lumbar lordosis with IVD injury (data not shown). Magnified L4–L5 views at week 8 post-injury qualitatively shows these changes (**Fig. 2E**), and osteophytes at the injury site adjacent to the L4–L5 IVD were observed in some mice (**red arrow, Fig. 2F**).

DISCUSSION: This study developed and comprehensively characterized a surgically induced mouse model of lumbar discogenic pain. Both mild and severe IVD injuries induced similar behavioral and structural changes, with more pronounced effects following severe IVD injury. This study builds on valuable insights from prior IVDD mouse models **[1,2,6]**, and the deep phenotyping of pain and structural changes observed gives a broader context to interpret the variability observed in some outcomes as surgical precision improves with repetition. Pain-like behaviors in mice recapitulated some behaviors observed in human IVDD including pain, anxiety-like responses, impaired mechanical strength, sagittal curvature changes, and altered gait **[7]**. Importantly, the severe IVDD with osteophyte formation, and changes in sagittal balance is a novel measurement in this study that recapitulates several commonly reported spinal conditions linked to chronic pain in humans **[8]**. Ongoing work is increasing sample size to better discern sex differences and mild versus severe IVD injury; as well as microCT vertebral assessments, and histological evaluations of IVDs, spinal cord, and DRGs.

SIGNIFICANCE: Comprehensive characterization of structural, functional, pain-like, and anxiety-like changes following lumbar IVD injury establishes a clinically relevant discogenic pain mouse model that mirrors spine osteoarthritis and its associated pain, providing a platform for genetic manipulation, mechanistic studies, and therapeutic screening to advance translation to human back pain.

REFERENCES:[1]Tang SN+ Front Pain Res.,2022.[2]Tang SN+ Biomaterials.,2024.[3]Deuis JR+ Front Mol Neurosci.,2017.[4]Lai A+JOR., 2015.[5]Gyles TM+ Neuropsychopharmacology., 2024.[6] Millecamps M+ Pain., 2018.[7] Rowland B+ Pain Medicine., 2025.[8]Goode AP+ Curr Rheumatol Rep., 2013.

ACKNOWLEDGEMENTS: We gratefully acknowledge Dr. Devina Purmessur, Khady Dio & Yuzhang Liu, for training on the surgically induced mouse IVDD model. Supported by NIH/NIAMS grants R01AR078857 and R01AR080096.

Figure 1

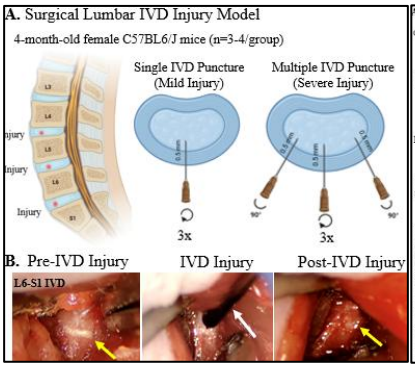

Figure 2

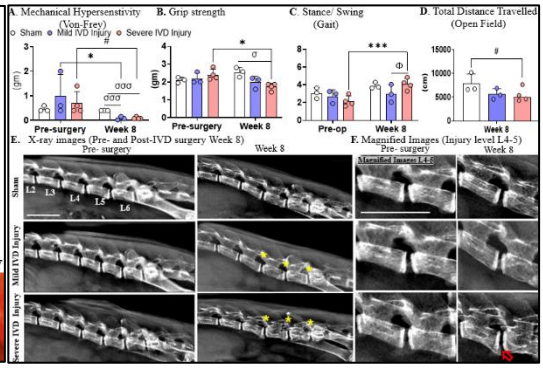

Figure 1. Mouse Lumbar Discogenic Pain Model. A. Lumbar IVD injury (L4–S1): mild injury=single puncture with 3×0.5 mm rotations; severe injury = multiple punctures with rotation and twisting. **B.** In vivo IVD images before, during (white arrow- needle + stopper), and after injury (yellow arrows indicate IVD). **Figure 2. Mild and severe IVD injuries in mouse lumbar spine elicit pain-like behaviors, decreased grip strength, gait abnormalities, anxiety-like behavior, and osteophyte formation. A–D.** Behavioral outcomes pre- and 8 weeks post-injury: (A) Mechanical Hypersenstivity using Von-Frey, (B) Grip strength, (C) Gait Stance/Swing, (D) Open-Field Total Distance Travelled by mouse. **E.** Spine X-rays pre- and post-surgery week 8 (sham, mild injury, severe injury). **F.** Magnified L4–L5 IVD X-ray; red arrow= osteophyte.*, $p < 0.05$; ***, $p < 0.001$ (Pre vs. Week 8); σ, $p < 0.05$ (Sham vs. Mild Injury vs. Severe Injury); #, $0.05 < p \leq 0.1$ (Pre vs. Week 8); Φ, $0.05 < p \leq 0.1$ (Sham vs. Mild Injury vs. Severe Injury)