# Transcriptional and Functional changes in Dorsal Root Ganglion neurons from Male and Female mice with injury induced Discogenic Back Pain

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**INTRODUCTION**: Low back pain (LBP) is a prevalent condition affecting up to 84% individuals worldwide and contributing significantly to financial and societal burdens [1][2]. According to epidemiological studies, around 40% of cases are associated with degeneration of the intervertebral discs (IVD) [3], while the relationship between degeneration and pain is not well studied. The dorsal root ganglion (DRG) is a cluster of sensory neuron cell bodies connecting the peripheral and central nervous systems, playing a key role in sensory signaling and pain perception. Some evidence suggests that genetic loci within DRGs are associated with chronic pain, implicating pathways involved in nociceptor sensitization and immune activation [4]. However, how the transcriptional alterations in DRGs contribute to discogenic LBP remains unclear. Thus, the objective of this study was to characterize both the transcriptome and functional changes of DRG neurons in our established mouse model of disc injury and discogenic back pain [5]. We hypothesized that there will be distinct gene expression profiles between both conditions, including differences between male and female mice, and we will be able to identify novel genes that are associated with painful disc degeneration, which could be potential targets for developing therapeutic approaches.

**METHODS**: Animal experiments were approved by IACUC (2016A00000074-R2) at The Ohio State University. Three levels of IVDs (L4/L5, L5/L6, and L6/S1) were punctured and injected with 2 µL of sterile medical-grade saline using a 30-gauge needle to induce degeneration in mice at 15-week-old (injury group, N=8, male/female). The same disc levels were surgically exposed but left uninjured in mice of the same age (sham group, N=8, male/female). Seven weeks after surgery, DRGs adjacent to the T12/T13 through L6/S1 IVDs were bilaterally harvested. **(1) RNA-seq**: RNA was extracted from DRG tissue and then sequenced on Illumina platform following library preparation and quantification (Novogene). Reads were aligned, gene-level expression was quantified and differentially expressed genes between groups were determined using the tools available in the Galaxy platform (The Galaxy Community, 2024). Functional enrichment and network analyses were performed using Ingenuity Pathways Analysis (IPA). Statistical analyses were conducted using R (v 4.4.2) **(2) Immunofluorescence (IF)**: The top three genes that were differentially expressed in our RNA sequencing analysis were interrogated as pain-associated markers (injury group, N=10, male/female; sham group, N=10, male/female). Primary antibodies included mouse anti-ATF3 (1:300), rabbit anti-Itpr1 (1:250), rabbit anti-NTS (1:200). Images were captured with Nikon Eclipse Ji Inverted Microscope with a 60X lens. **(3) Electrophysiology**: DRGs were harvested (injury group, N=10, male/female; sham group, N=9, 5 male/4 female) and neurons were isolated for whole-cell patch-clamp recordings with a multiclamp 700B amplifier (Molecular devices). Neurons larger than 20 µm in diameter were selected. Action potentials were evoked by a series of 20 pA current injections (from 0 to 200 pA), and spikes were counted for 1 second per step. Data were acquired and analyzed using Clampfit 11 (Molecular Devices). Statistical analysis was conducted via two-way ANOVA and Kruskal–Wallis test of variance at α=0.05.

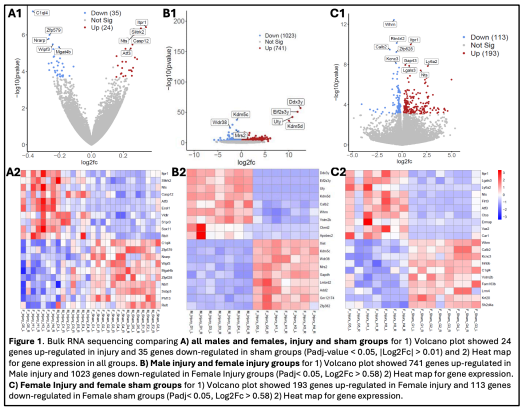


**Figure 1.** Bulk RNA sequencing comparing **A) all males and females, injury and sham groups** for 1) Volcano plot showed 24 genes up-regulated in injury and 35 genes down-regulated in sham groups (Padj-value < 0.05, |Log2Fc| > 0.01) and 2) Heat map for gene expression in all groups. **B) Male injury and female injury groups** for 1) Volcano plot showed 741 genes up-regulated in Male injury and 1023 genes down-regulated in Female injury groups (Padj< 0.05, Log2Fc > 0.58) 2) Heat map for gene expression. **C) Female injury and female sham groups** for 1) Volcano plot showed 193 genes up-regulated in Female injury and 113 genes down-regulated in Female sham groups (Padj< 0.05, Log2Fc > 0.58) 2) Heat map for gene expression.

**RESULTS**: **(1) RNA-seq**: In the combined analysis of **males and females mouse (injury vs sham (Fig. 1A)),** the volcano plot shows 24 genes up-regulated in injury and 35 genes down-regulated in sham groups (Padj-value < 0.05, |Log2Fc| > 0.01). In the corresponding heatmap, up or down regulation of differentially expressed genes wasn't homogeneous across the combined injury and sham groups. The top three differentially expressed relevant genes were **Itpr1, NTS, and ATF3**. Comparing **female and male in injury groups (Fig. 1B)**, the volcano plot shows 741 genes up-regulated in male injury and 1023 genes down-regulated in the female injury group (Padj< 0.05, Log2Fc > 0.58). The heatmap shows a similar expression across samples of the same group. There was a predicted activation on CR1L, HAND2, TBX5, MEF2C, MYOCD and calcium signaling in the male injury model, and inflammatory response related pathways and pro-inflammatory cytokines like IL1B, IFNG, IL18, IL13, IL4, IL3, TNF in the female injury model from IPA. Comparing **injury and sham in female groups (Fig. 1C)**, the volcano plot shows 193 up regulated and 113 down regulated genes (Padj<0.05, Log2Fc > 0.58). There was an activation of inflammation related pathways along with a predicted activation of inflammatory cytokines like IL6, TNF, IL1A, IL1B from IPA. **(2) IF**: Representative male samples showed slightly higher immunofluorescent signals for Itpr1, NTS, and ATF3 protein in the injury group compared to controls **(Fig. 2)**. Further work is ongoing to complete quantification across all samples. **(3) Electrophysiology**: Evoked spikes were significantly decreased, and rheobase was significantly increased in both male and female injury groups compared to sham. **(Fig. 3)**

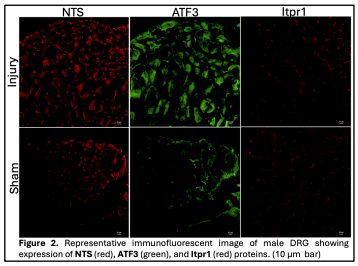


**Figure 2.** Representative immunofluorescent image of male DRG showing expression of **NTS** (red), **ATF3** (green), and **Itpr1** (red) proteins. (10 µm bar)

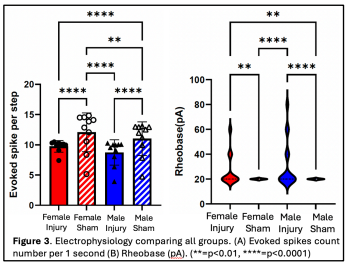


**Figure 3.** Electrophysiology comparing all groups. (A) Evoked spikes count number per 1 second (B) Rheobase (pA). (**=p<0.01, ****=p<0.0001)

**DISCUSSION**: Our study revealed distinct transcriptional signatures and functional changes between injury and sham groups, with prominent sex-specific differences influencing these changes. These findings highlight the involvement of sex-dependent molecular mechanisms in discogenic back pain and provide a foundational dataset for identifying potential DRG markers that can be interrogated for therapeutic studies. We identified 3 key pain-associated markers Itpr1, NTS, and ATF3 that were also validated at the protein level via IF. The female model of lumbar disc puncture demonstrated a greater inflammatory response to injury compared to the male model, but further validation is required and on-going. Interestingly, lower evoked spike counts and higher rheobase were observed in DRG neurons in injury groups which may indicate that our disc puncture is causing negative effects on neuronal function and possible leakage of the nucleus pulposus tissue as observed in disc herniation. This injury might induce inflammation, alterations in ion channel activities, and neuropathic pain, which aligns with our previous mouse behavioral [5] and canine model [6] results. DRG neurons for electrophysiology analysis were selected based on size without specific markers. DRG subtypes may exhibit distinct firing patterns, which cannot be differentiated in this study [7].

**SIGNIFICANCE/CLINICAL RELEVANCE**: This is the first study to characterize DRG transcriptional and functional changes in a mouse disc puncture model comparing male and female. Our findings help to uncover novel molecular targets associated with discogenic pain and demonstrate potential to guide the development of new therapeutic strategies.

**REFERENCES**:[1] Balagué et al. 2011 [2] Ferreira et al. 2023 [3] Luoma et al. 2000 [4] Parisien et al. 2017 [5] Tang et al. 2024 [6] Heimann et al. 2025 [7] Wu et al. 2021

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