INTRODUCTION: Chronic back pain is a major global health challenge, affecting 70–85% of the population; in approximately 40% of cases, intervertebral disc (IVD) degeneration is the main diagnosis, and can be termed as discogenic pain [1–3]. Surgical interventions focusing solely on IVD degeneration, such as spinal fusion, are poorly indicated for chronic low back pain, likely because the IVD is innervated with nociceptive fibers connecting from the dorsal root ganglion to the spinal cord (SC) dorsal horn which can also be the source of pain. Meanwhile, preclinical neuropathic pain studies show enhanced nociception in the SC dorsal horn through sensitization and neuroinflammation. However, there are very few studies particularly investigating time course changes of central and peripheral nerves that are affected by IVD injury in discogenic pain. Therefore, this study aimed to measure the kinetics of SC changes following an IVD injury in a well-characterized rat discogenic pain model exhibiting pain-related behaviors induced from annulus fibrosus (AF) puncture and injection injury [4,5]. We measured changes in the SC dorsal horn at early (acute) and late (chronic) timepoints (i.e., 3 days to 8 weeks) and evaluated the SC using immunohistochemical markers for sensitization with nociceptive neuropeptide substance P (SubP), neuroinflammation with the pro-inflammatory microglia (Iba1), and chronic SC remodeling with the astrocyte marker (GFAP). We hypothesized that AF injury will cause IVD degeneration, acute SC sensitization and neuroinflammation, and chronic astrocyte involvement.

METHODS: All procedures were guided and approved by the IACUC. Thirty-seven skeletonally mature Sprague Dawley rats (5-6 months old) were assigned to Naïve or AF injury groups. AF Injury was induced by puncturing the anterior AF of L3-4, L4-5, and L5-6 IVDs with a 26G needle at a depth of 3mm at the midline as well as left and right lateral areas of the IVD (3 punctures/disc) followed by a singular PBS injection into the midline puncture [5]. Rats in the Naïve group had no surgical interventions. Rats were euthanized at 0 days (d) (Naïve), 3d, 1week (1wk), 2wks, and 8wks post-injury (n=5/group). Lumbar spines and SCs (the corresponding to vertebral level T12-L1) were isolated, fixed, paraffin-embedded, sectioned and stained for spine morphology using Safranin-O/Fast-green/hematoxylin; or SC SubP, Iba1 and GFAP using immunohistochemistry with Nissl as counterstain to visualize general cell morphology. Slides were imaged at 20x and analyzed in ImageJ using a standardized immunoreactivity (ir) threshold to calculate positive pixels as an estimate of numbers of positive cells. The % SubP-ir, Iba1-ir and GFAP-ir relative to SC dorsal horn area (combined left and right dorsal horn of each SC) were identified and compared using one-way ANOVA with Tukey’s post-hoc tests. Statistics were performed with Prism9 (GraphPad Software, Inc.) with α=0.05 as significant.

RESULTS: The AF injured IVDs exhibited severe degenerative changes with less distinct NP-AF boundary, smaller and more fibrous NP, and disrupted annular lamellae at 3d and all subsequent time points compared with Naïve which exhibited healthy IVD morphology with well-defined NP and organized AF lamellae (Fig. 1). The SC was sensitized with SubP-ir levels that significantly increased to 2wks after AF injury and remained elevated until the 8wks timepoint (Fig. 2, A), Iba1-ir also significantly increased after AF injury and peaked at 2wks (Fig. 2, B) then decreased by 8wks returning to Naïve levels, suggesting a wave of microglia-mediated neuroinflammatory processes that peaked ~2wks following AF injury and restored to baseline. GFAP-ir also significantly increased by 1wk following AF injury and gradually and continuously increased reaching highest levels at 8wks (Fig. 2, C) indicating chronically increased astrocyte presence, suggestive of SC matrix remodeling.

DISCUSSION: This study revealed dynamic changes of SubP, Iba1, and GFAP levels in the SC at acute and chronic timepoints following AF injury. The neuropeptide SubP is involved in pain transmission and sensitization [6], and the significant and persistent increased levels of SubP at 2wks and 8wks post-injury are suggestive of pain and neurochemical markers for sensitization with nociceptive neuropeptide sensation. Meanwhile, acute and chronic neuroinflammation in relation to AF injury drives inflammation of the SC with SubP, Iba1 and GFAP levels significantly increased at 2wks after AF injury and remained elevated until the 8wks timepoint (Fig. 2, A), Iba1-ir and GFAP-ir relative to SC dorsal horn area (combined left and right dorsal horn of each SC) were identified and compared using one-way ANOVA with Tukey’s post-hoc tests. Statistics were performed with Prism9 (GraphPad Software, Inc.) with α=0.05 as significant.

SIGNIFICANCE: This study demonstrates temporal patterns of SubP, Iba1, and GFAP expressions at acute and chronic timepoints, suggesting distinct roles of substance P, microglia and astrocytes in the initiation and progression of sensitization, neuroinflammation and chronic plasticity/remodeling of the SC following IVD injuries, providing a framework for identifying molecular targets for acute and chronic therapies for discogenic pain.


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Figure 1. Rat IVD, with safranin-O/Fast-green/Hematoxylin staining indicating severe IVD degeneration changes at 3d post-injury.

Figure 2. Kinetics of SC SubP, Iba1, and GFAP IHC staining with Nissl stain from naive to 8wks post AF injury.

Figure 3. Changes in SubP-, Iba1- and GFAP-ir from naive to 8wks following AF injury. (A) SubP-ir significantly increased at 2wks and maintained until 8wks. (B) Iba1-ir peaked at 2wks and decreased at 8wks. (C) GFAP increased gradually and peaked at 8wks.