Innervation Shifts in Preclinical Models of Knee Osteoarthritis

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INTRODUCTION: Knee osteoarthritis (OA) is a whole-joint disease characterized by progressive structural degradation of cartilage and bone, accompanied by chronic pain and functional impairment. Alterations in sensory innervation are thought to be key drivers of OA-associated pain; however, how the neural landscape is altered with OA remains unclear. In addition, the relationship between neural changes and structural degradation remains poorly understood. Recent advances in the field of advanced microscopy and tissue clearing techniques now enable volumetric imaging of intact joints, enabling such studies. To this end, we leveraged three well-established preclinical models of knee OA (surgical, chemical, and non-surgical) to investigate region-specific alterations in innervation patterns employing established markers of neuronal integrity (neurofilament=NF) and pain signaling (Calcitonin Gene-Related Peptide=CGRP) at the knee and dorsal root ganglion (DRG). We hypothesized that mechanistically distinct models would exhibit both shared and model-specific patterns of neural remodeling at the joint and DRG level. Comparative analysis of neural types and subtypes across different preclinical models of knee OA could help identify those that best replicate features of human OA pain, guiding model selection for mechanistic and translational pain research.

METHODS: Animal procedures were approved by the University of Florida IACUC (Protocol #IACUC202300000492). Male and female Wistar rats (n = 6 per sex per group, 5–7 months old) were used. Knee OA was induced by medial collateral ligament and medial meniscus transection (MMT), intra-articular sodium mono-iodoacetate injection (MIA), or non-invasive anterior cruciate ligament rupture (NIKI), with age-matched naïve animals as controls. Eight weeks post-induction, animals were deeply anesthetized with isoflurane and perfused with phosphate-buffered saline followed by 4% paraformaldehyde (PFA). Knees and DRGs were collected, post-fixed in PFA for 24 h, and processed for histological analysis. Knees were decalcified in Immunocal (StatLab, SKU#1414) on a shaker at room temperature for 7–10 days. For 3D imaging, knees were bisected sagittally, cleared using the polyethylene glycol (PEG)-associated solvent system (PEGASOS) method (1), immunolabeled with neurofilament antibody (anti-NF, 1:400, Abcam, #ab215903) followed by secondary antibody (goat anti-mouse IgG, Dylight, ThermoFisher, cat # 35512), and imaged with a Lightsheet microscope (MesoSPIM). For 2D histology, decalcified knees and DRGs were cryoprotected in sucrose (15% and 30%), embedded in Optimal Cutting Temperature compound, sectioned, and immunostained for NF (1:400, Abcam, cat # ab215903) and CGRP (1:200, ThermoFisher, cat # PA5-114929) followed by secondary antibodies (goat anti-mouse IgG (H+L), Dylight™ 633, ThermoFisher, cat# 35512; goat anti-rabbit IgG, Alexa Fluor™ 488, ThermoFisher, cat # A-11008). Sections were imaged on a Nikon A1R confocal microscope.

RESULTS: Eight weeks post-induction, knee joints and DRGs were analyzed for neural targets (NF, CGRP) using 2D and 3D histology. Bisected rat knees cleared with the PEGASOS protocol were rendered optically transparent (Fig. 1A). Immunostaining with the NF antibody revealed rich innervation of knee joint tissues, including the synovium, fat pad, and meniscus attachment sites (Fig. 1B), across all groups. Qualitative differences in NF+ signal were observed across groups, particularly in the soft tissues of osteoarthritic knees, most notably in the MMT and NIKI groups when compared to naïve animals. While NF signal in soft tissues was robust, antibodies did not efficiently penetrate calcified tissues, limiting the interpretation of 3D datasets to soft tissues only. To investigate innervation changes in calcified regions, we analyzed 2D sections. Confocal images of thin sections (approximately 20 µm) revealed NF+ and CGRP+ signals in both soft tissues (synovium, fat pad, meniscus) and bony tissues (cortical, trabecular, subchondral regions). The presence of positive staining in calcified regions in thin sections further confirmed the lack of antibody penetration in 3D samples. Similar to 3D results, our preliminary data indicate differences in NF signal between osteoarthritic and naïve knee joints in 2D sections. Moreover, we observed differences in CGRP+ neurons at the DRG (L4) level across groups.

DISCUSSION: 2D and 3D imaging modalities enabled high-resolution volumetric reconstructions of nerve structures, revealing distinct differences in neural density and distribution between healthy and arthritic joints. The increased NF signals in the soft tissues of osteoarthritic joints, particularly in the MMT and NIKI groups, across both 2D and 3D histological analyses, suggest a strong association between structural degradation and altered innervation patterns. A key limitation was inconsistent antibody penetration in bone-rich regions, which restricted us to qualitative interpretation of NF+ signal in soft tissues only. Future methodological studies are needed to enable quantitative analysis of innervation shifts using volumetric datasets including improving antibody delivery methods and adopting transgenic labeling approaches targeting specific neuronal types and subtypes in calcified tissues. Future work will focus on quantifying the innervation structures across different joint regions.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings provide a foundation for exploring neural mechanisms of OA pain that are needed to identify potential therapeutic targets to alleviate OA-associated pain.

REFERENCES: (1) Jing *et al.* 2018

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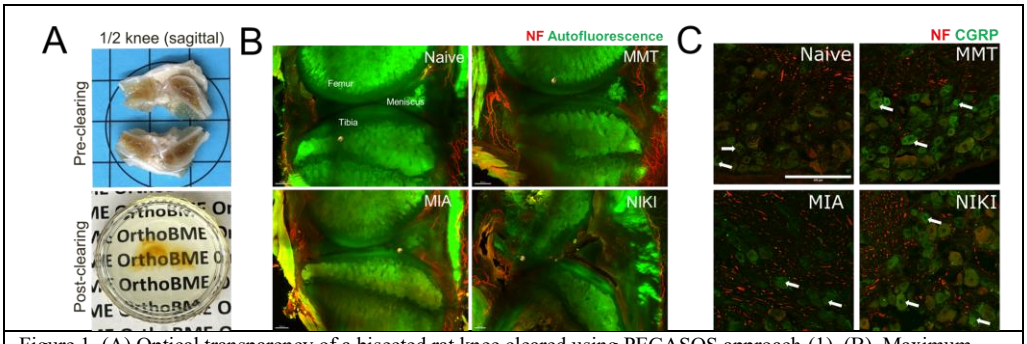

Figure 1. (A) Optical transparency of a bisected rat knee cleared using PEGASOS approach (1). (B). Maximum projected images of 500 µm thick sections (approximately from the loading region of medial half) immunostained with anti-NF (red) and imaged using Lightsheet microscope. While NF+ nerves are apparent in soft tissues (synovium, fat pad, meniscal attachment sites, etc.), no NF signal is observed in the calcified regions in 3D, cleared knees. (C) CGRP+ neurons at the L4 DRG (ipsilateral to injury) across different groups. Scale bars in B = 500 µm and scale bar in C = 200 µm.