Dorsal Root Ganglion Neuro-Glial Sensitization to Mechanical Flow Contributes to Osteoarthritis Pain

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INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease that affects more than 500 million people worldwide[1], severely limiting mobility and causing chronic pain that undermines quality of life. Despite its prevalence, OA lacks curative therapies, underscoring the urgent need for integrated translational, clinical, and basic science research efforts. Studies indicate that persistent osteoarthritis pain appears to involve a two-step process characterized by altered joint innervation, such as neo-innervation of the articular cartilage, followed by sensitization at the levels of the joint, dorsal root ganglion (DRG), and central nervous system[2]. Emerging evidence identifies the neurotrophic factor nerve growth factor (NGF) as a central mediator of OA pain through its effects on joint innervation and sensory neuron sensitization. Sustained activation of these NGF-sensitized DRG neurons induces central sensitization within the spinal dorsal horn, amplifying nociceptive transmission and contributing to chronic OA pain, thereby establishing the pivotal role of DRG in OA pain pathogenesis[2,3]. However, the pathophysiology underlying OA pain production is not completely known. Mechanical compression of the DRG, as seen in neuropathy or disc herniation, can directly activate neurons[4-6]. Likewise, physiological interstitial fluid flow (IFF) generated by vascular pulsatility, movement, or local tissue compression is predicted to stimulate both neurons and satellite glial cells (SGCs) [7,8]. Thus, we hypothesize that OA-induced joint pathology triggers a distinct interstitial flow-driven neuro-inflammatory signature in the DRG driving chronic pain and functional impairment.

METHODS: To investigate the mechanisms underlying OA pain and movement impairment, we used a mouse OA model induced by Monoiodoacetate (MIA; MedChemExpress) [9]. Five-month-old adult male and female C57BL/6J mice (n = 6-8 per group) received single intra-articular injections of MIA (50 mg/mL, in 15-20µL of saline solution) or an equivalent volume of saline into one knee joint. The contralateral knee joint serves as an untreated control. Behavioral assessments, including open field testing (Noldus) and mouse grimace scoring, were performed weekly from 1 to 8 weeks post-injection to evaluate spontaneous pain-related behaviors. Pain sensitivity was assessed using tactile hypersensitivity measurements on the plantar surfaces of the ipsilateral and contralateral hindlimbs (Von Frey test). At 8 weeks post-injection, knee joints were harvested for histological assessment and OARSI scoring of bone and cartilage damage. Dorsal root ganglia (DRG; L2-L6) corresponding to each knee were collected for qPCR analysis of pain biomarkers including tumor necrosis factor alpha (*Tnfa*), interleukin-1 beta (*il1b*), calcitonin gene-related peptide (*Cgrp*), components of the NLRP3 inflammasome (*Nlrp3, ASC*) and pain mediators Piezo1 (*Piezo1*), Panx1 (*Panx1*), and P2X7R (*p2rx7*). Intracellular Ca^{2+} imaging of DRG neurons and satellite glial cells (SGCs) in response to interstitial flow (IFF) was performed using the ratiometric Ca^{2+} indicator Fura-2AM (Invitrogen). IFF (stepwise laminar flow, τ = 0.7, 2.2, 4, 5.5, 7 dyne/cm^2 for 30 s duration with 200 s resting intervals) was applied using the fluid flow setup consisting of µ–slide VI$^{0.4}$ chamber (ibidi GmbH), Legato 200 syringe pump (KD Scientific) and a syringe containing extracellular solution (NaCl, KCl, $CaCl_2$, $MgCl_2$, HEPES, D-Glucose). All experiments were conducted under IACUC approval.

RESULTS SECTION: Open field behavioral testing showed that MIA-induced OA mice were less active, exhibiting significant reductions in travel velocity and distance moved as early as one-week post-injection, which remained significantly reduced throughout the eight-week period. Expression levels of pain biomarkers (*Tnfa, Il1b, Cgrp*), inflammasome components (*Nlrp3, ASC*) and mediators (*Piezo1, Panx1, p2rx7*) in DRGs innervating MIA-injected knees was significantly upregulated when compared to that of DRGs innervating saline-injected or untreated knees, indicating enhanced pain sensitivity following MIA injection in adult mice. Ca^{2+} imaging experiments using ratiometric Ca^{2+} indicators revealed that DRG neurons and SGCs under OA conditions were more sensitive to low levels of IFF than were untreated controls (Fig. 1). Moreover, the differences in Ca^{2+} response between OA and untreated conditions appeared to be greater in SGCs than in neurons.

DISCUSSION: Our study demonstrates that MIA-induced OA in adult mice causes persistent reductions in locomotor activity accompanied by upregulation of inflammatory cytokines, inflammasome components, and mechanosensitive channels in DRGs. Notably, DRG neurons and SGCs became more responsive to low-level interstitial fluid flow, suggesting that joint pathology sensitizes sensory ganglia to physiological mechanical stimuli. These findings support a model in which OA pain arises from peripheral neuro-inflammatory changes that amplify nociceptive signaling, highlighting DRG neurons and flow-sensitive pathways as potential therapeutic targets. By elucidating the roles of Piezo1, Panx1, and P2X7R in DRG neuron-SGC communication during OA, this work provides mechanistic insight linking joint pathology to chronic pain.

SIGNIFICANCE: This study reveals that OA-induced joint pathology drives chronic pain in part by sensitizing DRG neurons and SGCs to physiological interstitial fluid flow. These findings highlight DRG neuro-glial signaling as a potential therapeutic target, offering new avenues to improve pain management and mobility in OA patients.

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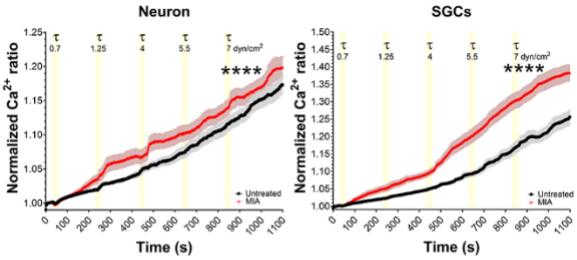

Figure 1. Ca^{2+} signaling in DRG neurons and satellite glial cells (SGCs) from 7-month-old mice under control (untreated; black trace) and MIA-induced OA (red trace) conditions in response to interstitial fluid flow (IFF; stepwise laminar flow). **** $P < 0.0001$, unpaired t-test. Data are presented as mean ± SEM. Neurons: n=64 - 96 per group, SGCs: n= 128 - 160 per group.