Females have increased susceptibility to develop ongoing pain in a mouse model of knee joint osteoarthritis

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Background

Osteoarthritis (OA) is a **leading cause of chronic pain** and disability in the US, characterized by progressive loss of articular cartilage with bone remodeling. OA patients also develop **pain**, **loss of joint function**, and a **diminished quality of life**. OA patients develop different pain phenotypes that are characteristic of either mid-stage or advanced OA. Patients with mid-stage OA report pain during joint use that subsides during rest and can be managed with non-steroidal anti-inflammatory agents (NSAIDs). In contrast, patients with advanced OA report ongoing joint pain that is nonresponsive to NSAID treatments. Clinical reports have demonstrated that **females are more susceptible to develop OA pain** and report more severe joint pain compared to males. The mechanisms underlying differences between pain phenotypes and the sex differences in pain experienced are unknown.

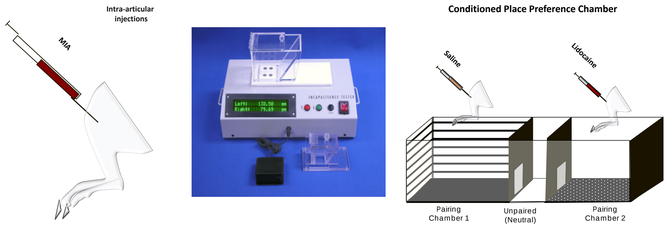
HYPOTHESIS

Changes in joint pathology and innervation underlie sex differences in the emergence of knee joint OA pain phenotypes.

METHODS

Modeling OA pain in rodents

Intra-articular injections of monosodium iodoacetate into left knee joint are used to induce mid-stage and advanced OA pain phenotypes in preclinical rodent models of knee joint OA. Behavioral assays including conditioned place preference (CPP) and weight asymmetry were used to assess ongoing pain and joint discomfort, respectively.



Immunohistochemistry

Tissue collection occurred 14 days post-MIA injection. Samples were sectioned into 20 µm-thick sections that underwent immunohistochemistry for Beta III tubulin, a pan-neuronal marker, to determine innervation of the tibialis anterior. Knee joint sections were stained with Hematoxylin and Eosin (H&E) and toluidine blue to evaluate joint pathology and cartilage degradation.

Joint Pathology Ratings

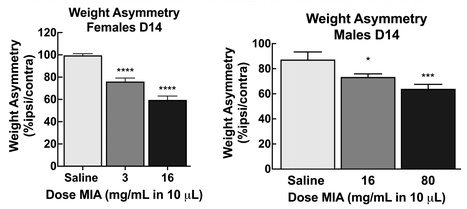
Joint pathology was scored by an experimenter blinded to treatment conditions using a Modified-Mankin scale to evaluate the structure of the tibia and femur, cellular abnormalities in the articular cartilage, changes in the meniscus, and the development of osteophytes.

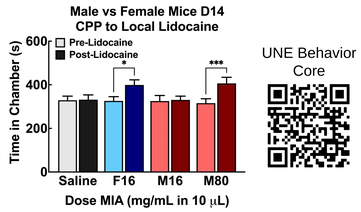
Quantitative PCR

Real-time quantitative PCR (RT-qPCR) was used to quantify the expression of a marker for neuronal damage, activating transcription factor 3 (ATF3), in lumbar dorsal root ganglia (DRG). Relative fold-changes in ATF3 were calculated using the $\Delta\Delta C_T$ method using GAPDH as the stable internal reference gene.

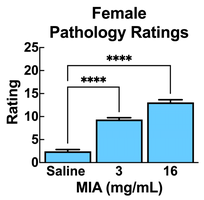
CHARACTERIZATION OF OA PAIN PHENOTYPES

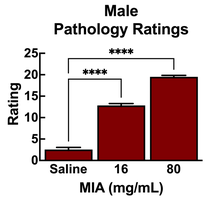
Females develop advanced OA pain at a MIA concentration 5-fold lower than males.



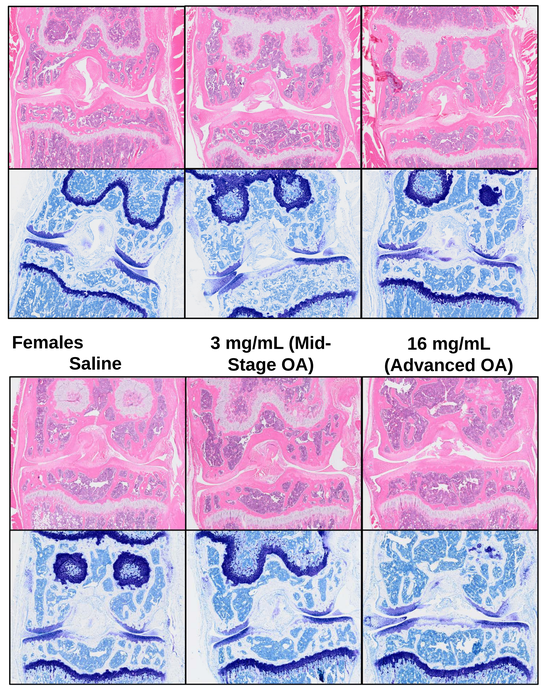


UNE Behavior Core

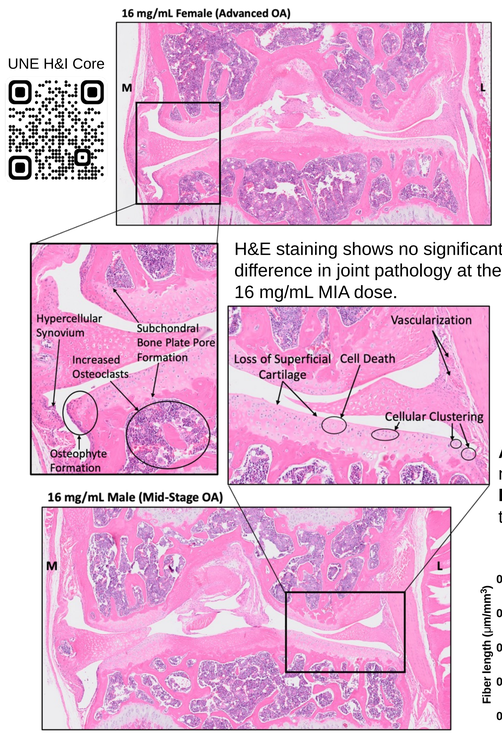




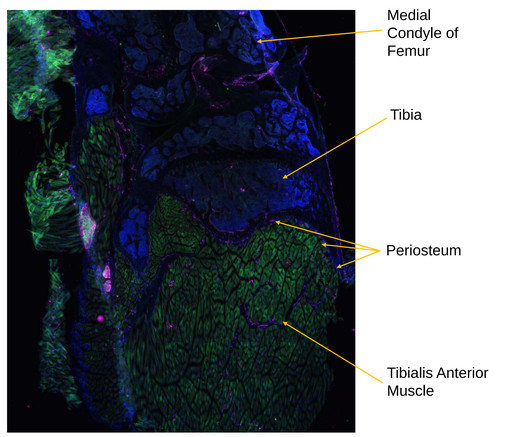
MIA injections induce dose-dependent joint pathology in both males and females.



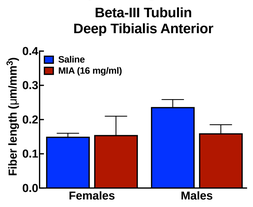
JOINT PATHOLOGY AND INNERVATION

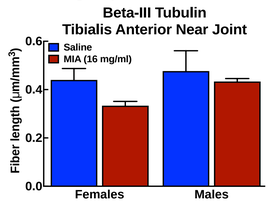


H&E staining shows no significant difference in joint pathology at the 16 mg/mL MIA dose.



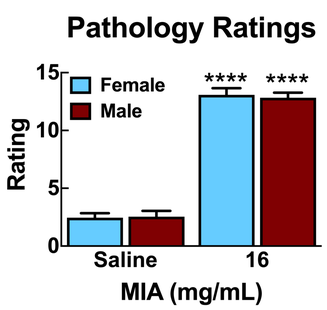
Above: DAPI (blue) and beta-III tubulin (magenta) stain nuclei and nerve fibers, respectively.
Below: No significant differences in fiber lengths were found within the analyzed regions between treatment groups or between sexes.

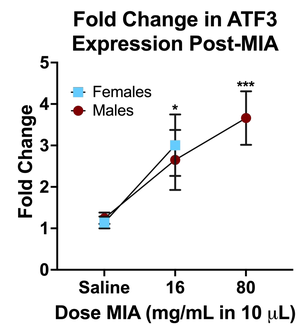


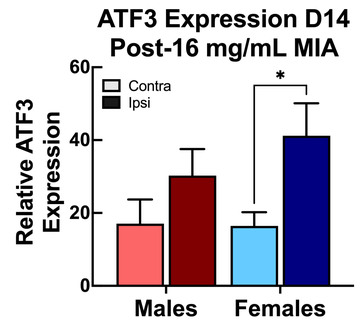


SENSORY NERVE DAMAGE

No differences were observed in joint pathology, muscle innervation, or ATF3 expression at the 16 mg/mL MIA dose where females presented with advanced OA pain and males presented with mid-stage OA pain.







SUMMARY

- Male mice with advanced OA pain show a high degree of joint pathology, increased innervation in the tibialis anterior muscle, and elevated ATF3 expression in lumbar DRG associated with knee joint innervation.
- Females show an increased susceptibility to develop advanced OA pain.
- Males and females do not show any significant differences in joint pathology, innervation, or ATF3 expression at the 16 mg/mL MIA concentration.

CONCLUSIONS

- Analysis of joint pathology, muscle innervation, and a marker for nerve damage in sensory afferent neurons provides **converging lines of evidence against our hypothesis.**
- This evidence suggests that mechanisms other than knee joint pathology mediate the observed sex differences in the development of advanced OA joint pain.
- These observations suggest that sex differences in OA pain could be due to central modulation of pain.

ACKNOWLEDGEMENTS

This research was supported by the NIH National Institute of General Medical Sciences COBRE grant P20-GM-103642 supporting the UNE Histology and Imaging Core and UNE Behavior Core, as well as a National Institute of Health Grant 1R01NS121533-01A1: PI, B. Harrison. Researchers on this team were supported by the Peter Morgane Research Fellowship (Sian) and Kahn Family Foundation Fellowship (Vesey).