Use of Integrated Optical Clearing and 2-Photon Imaging to Investigate Sex Differences in Macrophage Activation after SNI

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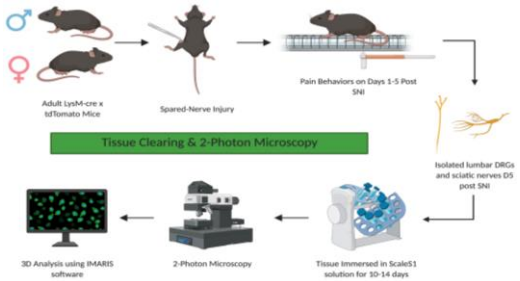
Abstract

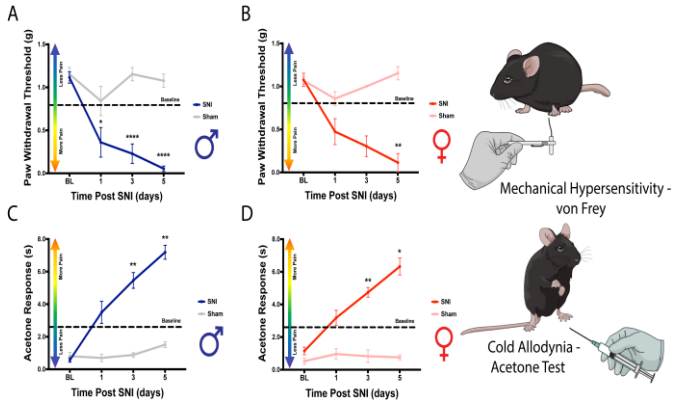
Peripheral nerve injury induces a myriad of immune symptoms that impacts pain and overall quality of life. Recent studies have revealed neuroimmune interactions in various pain states are important to mediate sex differences in their etiology. A described source of these sexual dimorphisms is the innate immune system, which promotes inflammation and pro-nociception through bidirectional signaling with the nervous system. The spatiotemporal interactions between macrophages and sensory neurons could hold the key to explain ascribed differences between sexes. To date, studies have found it difficult to adequately display these interactions. We are poised to answer important questions regarding the recruitment and morphology of peripheral macrophages in key tissues of the pain system: the dorsal root ganglia (DRG) and sciatic nerve (ScN). Our approach utilized ScaleS1, an optical clearing method, to clear whole DRGs and ScNs after peripheral nerve injury. With the concomitant use of 2-photon microscopy and transgenic reporter lines, we visualized macrophage dynamics involved in neuropathic pain development following injury. Male and female mice were sacrificed at the peak of nerve injury-induced pain development and DRGs and ScNs were harvested, processed, and cleared. Whole tissue images were captured via 2-photon microscopy and were processed and analyzed using Imaris imaging software. Macrophage infiltration was increased in the ipsilateral DRGs after nerve injury in males. We also assessed macrophage size and morphology to understand activation states in the context of nervous tissue inflammation. We found sex and injury dependent clustering of macrophage morphology populations in both the DRG and ScN. The altered mechanisms by which the male and female immune systems respond to nerve injury are still topics of further research, however; the continued use of next-generation imaging with advanced whole tissue image analysis remains an important tool in understanding the reciprocal interactions between neuronal and nonneuronal cells.

Materials & Methods



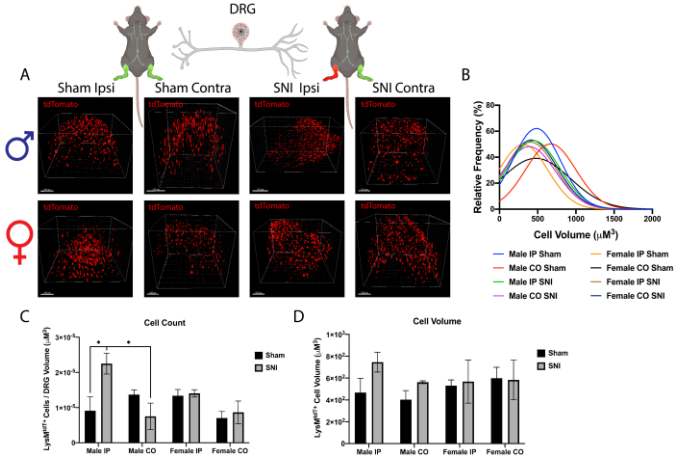
Evoked Pain Behaviors

Both male and female mice exhibit robust mechanical hypersensitivity and cold allodynia starting at D1 post injury.



Macrophage Recruitment to the DRG

LysM+ cell infiltration is increased in the injured DRG in males, but not females. LysM+ cell volumes are higher in the injured DRG of males which is indicative of activation.



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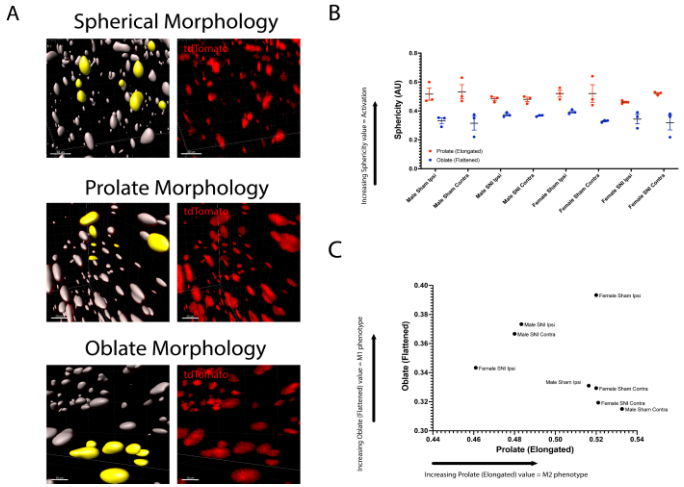
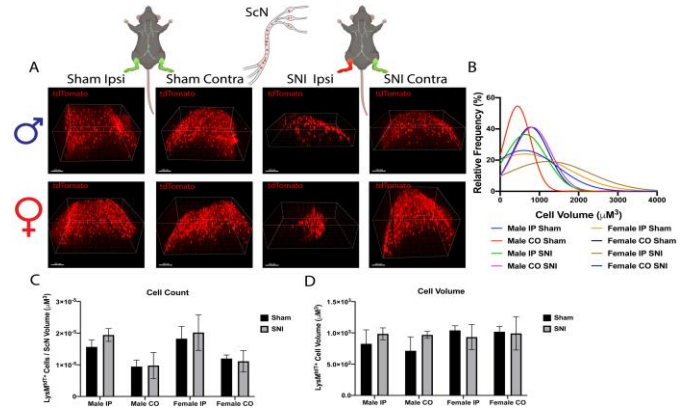
Macrophage Morphology in the DRG

Increased oblate (flattened) LysM+ cell shapes in the injured DRG of both sexes which indicates M1 polarization.



Macrophage Recruitment to the ScN

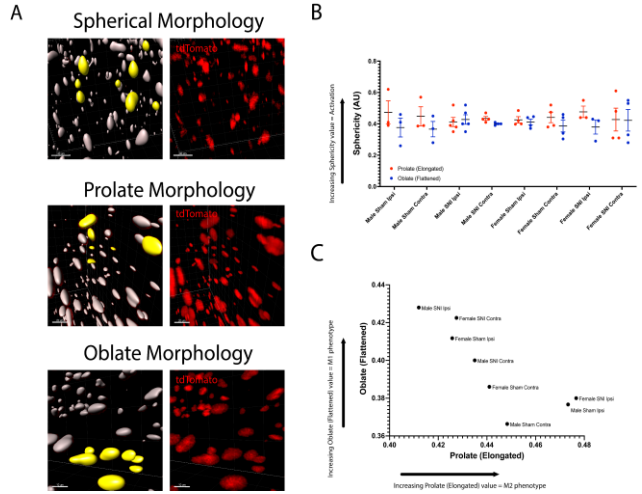
LysM+ cell infiltration is not increased in the injured DRG in males or females. LysM+ cell volumes do not change in response to injury in either sex.



Lab Website https://labs.utdallas.edu/niblab

Macrophage Morphology in the ScN

In the injured ScN, males have increased oblate (flattened) cell shape as opposed to females which are more prolate (elongated). This indicates a sexually dimorphic response to injury where males are more pro-inflammatory (M1) and females are more anti-inflammatory (M2).



Conclusions

In this study, we have presented a useful technique to investigate macrophage morphology in response to nerve injury in peripheral nervous tissues. This work is the foundation for future studies that will further our understanding of the dynamic morphological changes that occur in macrophages during nerve injury.

References

Szabo-Pardi, Thomas A., et al. "In Vivo Two-Color 2-Photon Imaging of Genetically-Tagged Reporter Cells in the Skin." *JoVE (Journal of Visualized Experiments)* 149 (2019): e59647.

McWhorter, Frances Y., et al. "Modulation of macrophage phenotype by cell shape." *Proceedings of the National Academy of Sciences* 110.43 (2013): 17253-17258.

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