

# Digitization, tracing, and quantification of CGRP-IR axons in the nociceptive cardiac nervous system in a mouse model

Cody Geoffroy, Kohlton Bendowski, Yuanyuan Zhang, Ariege Bizanti, Maci Heal, Richard Christie, Peter Hunter, Jin Chen, Zixi (Jack) Cheng

Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL 32816

## Abstract

This research is part of a larger NIH initiative called Helping to End Addiction Long-term (HEAL) and attempts to expand the targeted potential of therapeutics outside of drug intervention on the autonomic nervous system. The distribution and morphology of nociceptive axons in the heart have not been completely elucidated within mice utilizing a flat-mount tissue preparation.

C57BL/6J mice were sacrificed and perfused through the left ventricle with 4°C Zamboni's fixative, the heart was then harvested and dissected into the right and left atria and ventricles. The samples were immunohistochemically (IHC) stained for calcitonin gene-related peptide (CGRP). The tissues were then prepared as flat-mounts and scanned using automated color fluorescence and confocal microscopy. The samples that were scanned for CGRP-IR axons – at 488 nm – were digitized and traced using a 3D neuron reconstruction software, and finally quantified using ImageJ.

Within CGRP-IR axons 2D and 3D digitalized axonal tracings have not been reported in the whole mouse heart, as a result, this study sought to expand that limited dataset. Ultimately, the heart-brain atlas is useful in discovering non-pharmacological pain management therapies.

## Materials & Methods

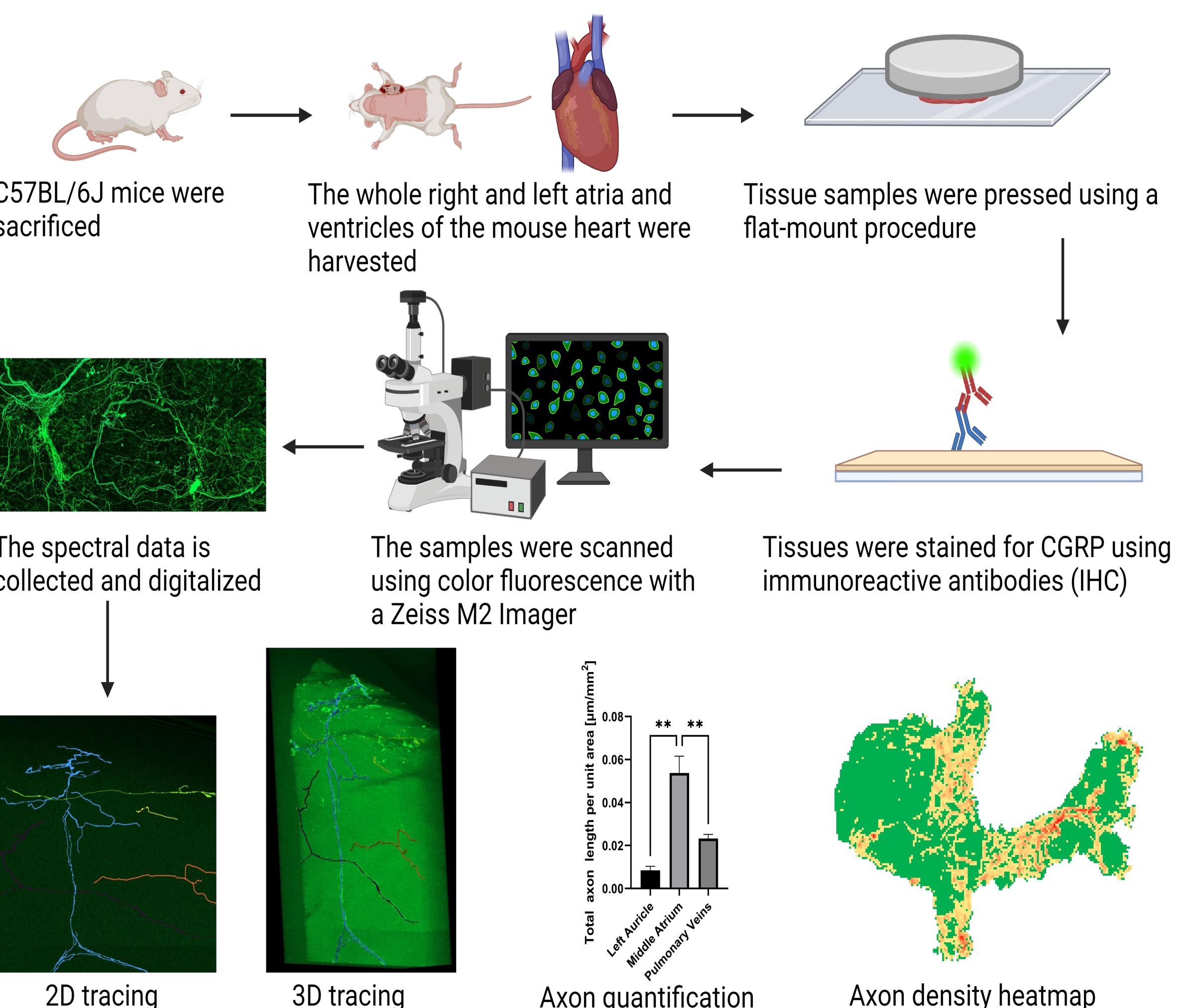


Figure 1: Schematic describing the experimental design workflow. Made in BioRender.

## Results

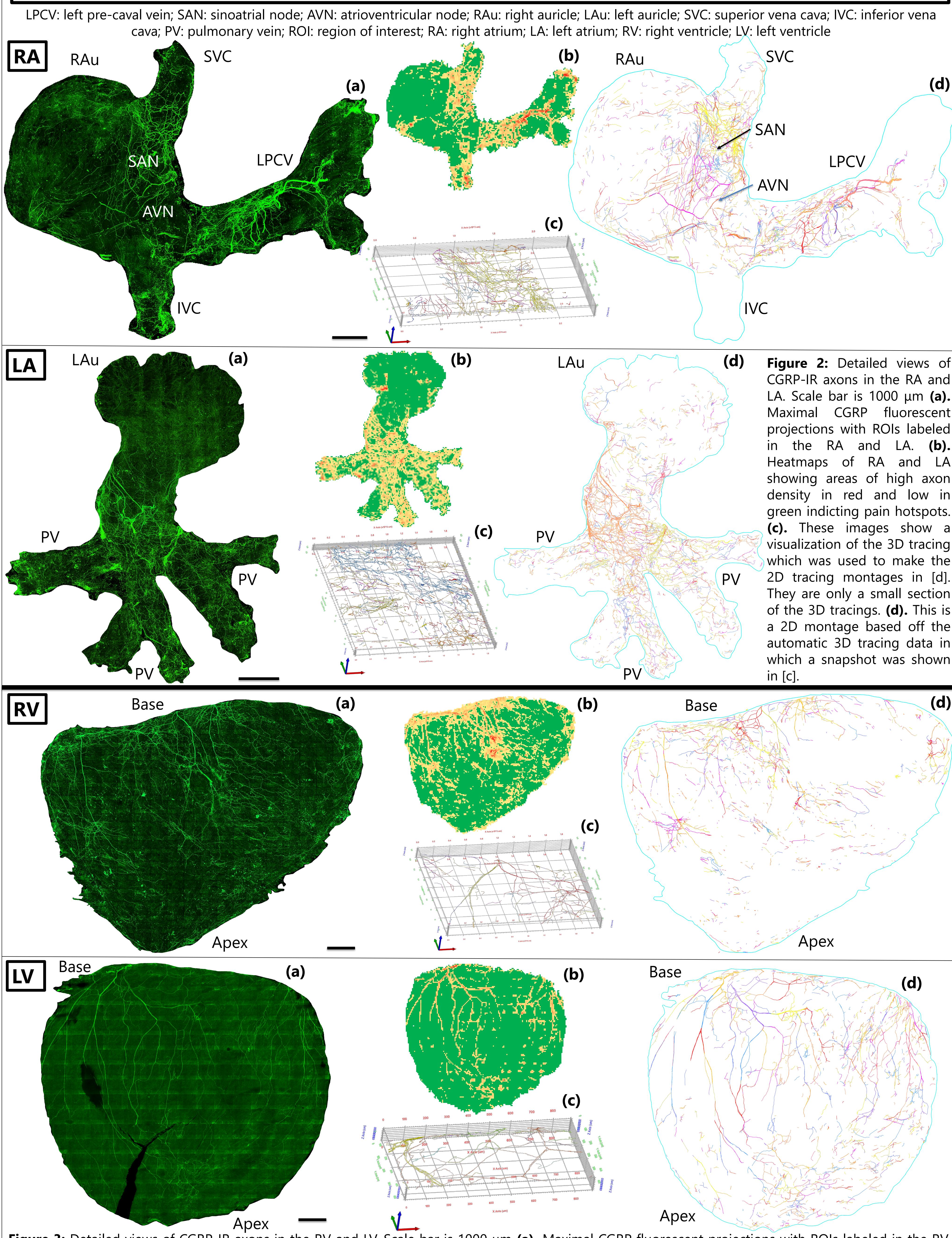


Figure 2: Detailed views of CGRP-IR axons in the RA and LA. Scale bar is 1000 μm (a). Maximal CGRP fluorescent projections with ROIs labeled in the RA and LA. (b). Heatmaps of RA and LA showing areas of high axon density in red and low in green indicating pain hotspots. (c). These images show a visualization of the 3D tracing which was used to make the 2D tracing montages in (d). They are only a small section of the 3D tracings. (d). This is a 2D montage based off the automatic 3D tracing data in which a snapshot was shown in [c].

Figure 3: Detailed views of CGRP-IR axons in the RV and LV. Scale bar is 1000 μm (a). Maximal CGRP fluorescent projections with ROIs labeled in the RV and LV. (b). Heatmaps of RV and LV showing areas of high axon density in red and low in green indicating pain hotspots. (c). These images show a visualization of the 3D tracing which was used to make the 2D tracing montages in (d). They are only a small section of the 3D tracings. (d). This is a 2D montage based off the automatic 3D tracing data in which a snapshot was shown in [c].

## Results

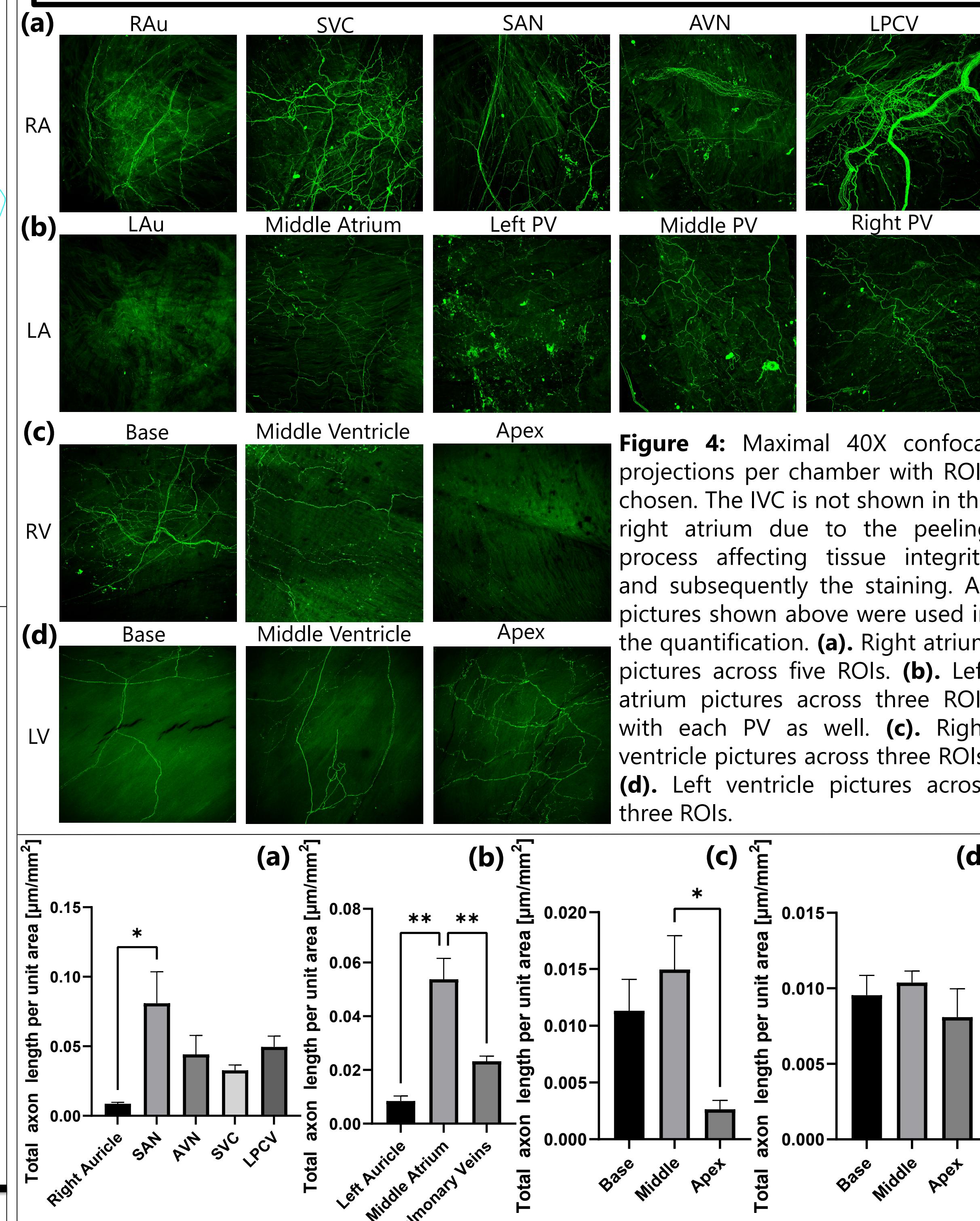


Figure 5: Relative quantification of CGRP-IR axon density in ROIs for each chamber across five mice. A one-way ANOVA was applied, and significances are denoted by the asterisks. Areas where there are no asterisks describes a p-value > 0.05. [\*] denotes a p-value < 0.05 and [\*\*] denotes a p-value < 0.04. (a). RA graph. (b). LA graph. (c). RV graph. (d). LV graph.

## Summary

- This study sought to give significance across ROIs such as the SAN and AVN (which contains pacemaker cells).
- The RAu and LAu tended to contain significantly less CGRP-IR axons than other areas within the RA and LA.
- The apex in the RV contained significantly less CGRP-IR axons than in the middle and base. This was not the case for the LV where the p-value is > 0.05 across all areas.
- Future works include using adult mice to fully elucidate the sex variation and may explain the spread and lack of significance in certain ROIs.
- Moreover, techniques like electrophysiology will be used to functionally test anatomical hotspots (shown in the heatmaps) to determine functional significance in, for example, nociception.

## Acknowledgment

This study was supported by NIH HEAL/SPARC U01 NS113867-01, NIH 1R15HL137143-01A1 [ZJC].