Exploring cardiac innervation by 3D light sheet imaging in horses with atrial fibrillation

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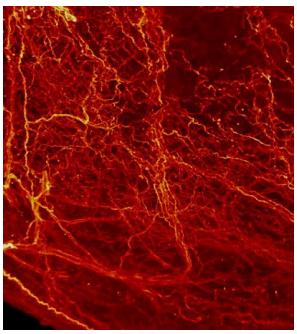
Background incl. aims
Local hyperinnervation plays a pivotal, yet poorly understood role in the initiation and maintenance of atrial fibrillation (AF). Changes in the intricate 3-dimensional (3D) network of nerves are difficult to characterize using traditional histological methods and AF has been challenging to study in preclinical rodent models. Non-destructive imaging techniques capable of visualizing larger tissue samples from large animal models are crucial to understand innervation changes in AF. Here, we aimed to investigate the feasibility of 3D light sheet fluorescence microscopy (LSFM) in equine atrial tissue and characterize the autonomic cardiac remodeling in a horse model of experimentally induced chronic AF.

Methods
Biopsies from the anterior descending ganglionated plexus were harvested from horses after 4-months of induced AF (n = 9), from horses with naturally occurring AF (n = 3) and healthy control horses (n = 3). Immunostaining with two neuronal markers (Tyrosine hydroxylase and Neurofilament) in parallel with a vascular marker (Transgelin) was performed to determine the local density of nerves and vasculature by computational image analysis. Whole-mount immunohistochemistry and clearing was optimized for equine heart samples by testing different depigmentation, permeabilization and imaging protocols.

Results
We present a protocol for 3D imaging of nerves in large equine atrial samples, spanning several centimeters in size. Optimized sample preparation with stepwise chemical and enzymatic extracellular matrix loosening and digestion enabled uniform sample labelling with antibodies against neuronal markers. Customized autofluorescence bleaching, sample clearing and imaging parameters facilitated high resolution imaging of cardiac innervation across the entire tissue sample. Computational analysis of atrial innervation permitted quantitative analysis in the study groups to demonstrate spatial changes occurring in atrial fibrillation.

Conclusion
3D LSFM in large animal models can improve our understanding of the mechanisms of diseases. This newly developed sample preparation protocol is well suited for single-cell resolution imaging in dense cardiac biopsies. We show the applicability of the method in characterizing innervation changes in an equine model of AF.

Graphic:



Keywords:

3D LSFM, AF, equine model

Reference:

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