

Non-invasive Visualization of the Complete Cardiac Conduction System using MR Microscopy

M-S. Hwang^{1,2}, K. E. Odening³, B-R. Choi³, G. Koren³, S. J. Blackband^{1,2}, and J. R. Forder^{1,4}

¹McKnight Brain Institute, Gainesville, FL, United States, ²Neuroscience, University of Florida, Gainesville, FL, United States, ³Cardiovascular Research Center, The Rhode Island Hospital, Alpert Medical School of Brown University, Providence, RI, United States, ⁴Radiology, University of Florida, Gainesville, FL, United States

Introduction

The impulse-conducting system that coordinates the cardiac cycle requires a well-orchestrated interplay of its multiple components for both synchronous and reliable contractions [1]. Thus, detailed insight into the anatomy of the conduction system is essential to understand the coordinated contractile behavior arising from atrial depolarization. Furthermore, this information will be useful in investigations of cardiac function and morphology in both normal and abnormal (pathophysiologic) states [2, 3]. To date, the techniques most commonly used to study the conduction system are invasive, and use intrinsically destructive procedures. MR imaging has previously been used to visualize free-running Purkinje fibers [3, 4], but not those in the myocardium, and thus only provide a partial account of the conduction system. In this study, we demonstrate that MRI at microscopic resolutions, i.e. MR microscopy (MRM), combined with high angular resolution diffusion microscopy (HARDM), can describe non-invasively the complete cardiac conduction system and anatomical features in isolated rabbit hearts, as a precursor to developing a mathematical model of depolarization in the heart. The combined investigative technique of MRM and HARDM is observed to be an effective method of monitoring morphological changes occurring in the cardiac conduction system.

Materials and Methods:

Isolated heart preparation: Isolated perfused hearts ($n = 5$) of New Zealand White male rabbits ($3 \sim 5$ kg) were prepared according to the approved animal protocol. The isolated hearts were fixed *in situ* during an intravascular formalin-perfusion fixation procedure that was carefully standardized. And the fixed hearts were kept in a refrigerator until MR imaging experiments.

MRI: MR experiments of the isolated hearts were performed on a 17.6 T / 89 mm vertical wide-bore magnet (Bruker Instrument spectrometer and console, Billerica, MA). The RF coil used for the *in vitro* imaging was an Alderman-Grant birdcage coil, diameter = 25 mm, length = 35 cm. The temperature in the magnet was maintained at 19 - 20°C. Three dimensional high resolution MR image data were collected using a fast gradient echo pulse sequence, achieving a voxel resolution of $35.2 \mu\text{m} \times 35.2 \mu\text{m} \times 82 \mu\text{m}$ with a matrix size = $710 \times 710 \times 256$ in the field of view of $25 \text{ mm} \times 25 \text{ mm} \times 21 \text{ mm}$. Imaging parameters implementing relatively T2*-weighting were TR = 150 ms, TE = 18.5 ms, 1 average, sampling bandwidth = 20 k.

For HARDM data sets, diffusion-weighted imaging in 21 non-collinear directions was conducted using a standard pulsed gradient spin echo (PGSE) pulse sequence, achieving an isotropic in-plane resolution of $60 \mu\text{m} \times 60 \mu\text{m} \times 600 \mu\text{m}$, b -values = 1000 s/mm^2 (using $\Delta = 13.4 \text{ ms}$ and $\delta = 1.8 \text{ ms}$), diffusion time (τ) = 12.8 ms, TR = 3000 ms, TE = 25.1 ms, bandwidth = 15k, and 1 average. The pilot images with three orthogonal planes were collected to check whether or not the isolated heart imbedded in the dense FC-43 solution moved during the relatively long scans. For data analysis, volume rendering of the 3D MR data sets was performed using ImageJ (ver. 1.31, <http://rsbweb.nih.gov/ij/>). The tensor processing of HARDM data sets was conducted using fanDTasia™ [5].

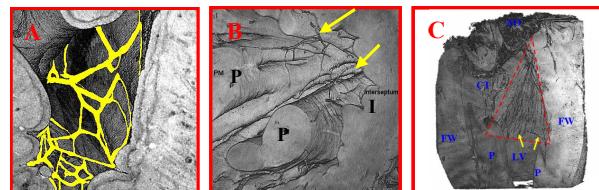


Fig. 2 Demonstration of the conducting fibers in isolated hearts. A: polygonal Purkinje fiber network in the LV (segmented in yellow), B: the Purkinje fibers attaching into the conical masses of papillary (yellow arrows) muscles. C: fan-like division of the left bundle (red triangle).

Conclusions: The MR volume rendered images and high angular resolution diffusion microscopy visualize non-invasively the conduction paths observed in previous histological studies. Further, this modality appears to efficiently demonstrate detailed or integrative features that are difficult to visualize, even using traditional destructive techniques. When taking into account the labor and time required for sectioning of the entire heart, the use of MRM and HARDM as efficient and integrative tools is very attractive. These results will help to elucidate the anatomical-functional relationship, and will be used to develop a working model of electrical conduction in the heart.

References: [1] A. Katz and P. Katz Circulation 1989; 79: 712-7. [2] A. Ansari et al. Anat Rec 1999; 254: 92-7. [3] C. Goodyer et al. Conf Proc IEEE Eng Med Biol Soc 2007: 147-51. [4] F. Vadakkumpadan et al. J Electrocardiol 2009; 42:157.e1-10. [5] A. Barmoutis and B. C. Vemuri Conf Proc IEEE ISBI 2010: 1385-88.

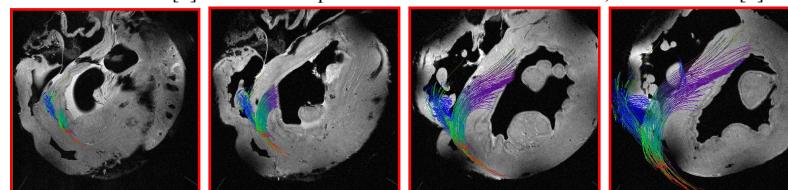


Fig. 3 Representative data set from rabbit heart showing fiber tracking of the conduction network from base to midwall (A-D). The conducting network originates behind the non-coronary leaflet at the base (A) and quickly bifurcates (B) into fibers that proceed down the right ventricular subendocardium (shown in blue) and a branch that continues along the septum for a short distance before continuing down the left ventricular subendocardium (C, D) in a fan-like structure (shown in purple). The fan-like structure terminates in free-running Purkinje fibers in the left ventricle. Data from conducting fibers that are more apical has been omitted for clarity.

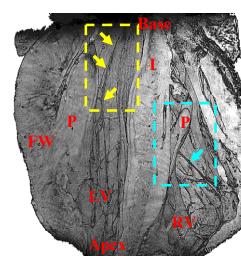


Fig. 1 A representative volume rendered 3D MR image to visualize the conduction paths and anatomical features in an isolated heart. Note the free-running Purkinje fibers in the left ventricular cavities (LV), the left bundle(s) in ventricular inner wall (yellow arrows), a branch from the right bundle (cyan arrow). RV: right ventricular cavity, FW: free wall, P: papillary muscle, I: ventricular interseptum

Results and Discussion

The volume rendered image from the original 3D MR data, generating an adequate T2*-weighted image contrast, made it possible to non-invasively and reproducibly trace the conduction paths in both ventricles, as well as to describe the micro-anatomical features of the heart (Fig. 1). The tendinous free-running Purkinje fibers form a reticular polygonal net in the ventricular cavities (Fig. 2, A), with multiple attachment points to the papillary muscles and the ventricular subendocardium (Fig. 2, B). In addition, the fan-like left bundle branch originating from the connecting bundle were visualised (Fig. 2, C). Fiber tracking from the HARDM data sets may represent the conducting pathways from the connecting bundle to the left and right bundles (Fig. 3). Since the tendinous fiber-like cords observed in the ventricular cavities contain conducting fibers, called the free-running Purkinje fibers, the primary eigenvector of the cords visible in the transverse HARDM data set may correspond to the neurofilament stained Purkinje fibers (Fig. 4). Histology is currently underway to validate these findings.

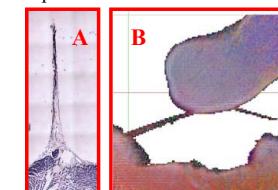


Fig. 4 Representative images showing myocardial attachment of the free-running Purkinje fibers. A: neurofilament stained free-running Purkinje fiber in dark. B: the primary eigenvector map of free-running Purkinje fibers.

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