**Original Contribution**

**ELECTROMECHANICAL WAVE IMAGING OF BIOLOGICALLY AND ELECTRICALLY PACED CANINE HEARTS IN VIVO**

ALEXANDRE COSTET,* JEAN PROVOST,* ALOK GAMBHIR, YEVGENY BOBKOV, PETER DANILO, JR., GERARD J. J. BOINK, MICHAEL R. ROSEN, and ELISA E. KONOFAGOU*†

*Department of Biomedical Engineering, Columbia University, New York, New York, USA; †Department of Medicine—Cardiology, Columbia University, New York, New York, USA; ‡Department of Pharmacology, Columbia University, New York, New York, USA; §Interuniversity Cardiology Institute of the Netherlands (ICIN), Utrecht, The Netherlands; ¶Heart Failure Research Center, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; and ‡Department of Radiology, Columbia University, New York, New York, USA

(Received 17 April 2013; revised 20 August 2013; in final form 26 August 2013)

Abstract—Electromechanical Wave Imaging (EWI) has been show capable of directly and entirely non-invasively mapping the trans mural electromechanical activation in all four cardiac chambers in vivo. In this study, we assessed EWI repeatability and reproducibility, as well as its capability of localizing electronic and, for the first time, biological pacing locations in closed-chest, conscious canines. Electromechanical activation was obtained in six conscious animals during normal sinus rhythm (NSR) and idioventricular rhythms occurring in dogs with complete heart block instrumented with electronic and biologic pacemakers (EPM and BPM respectively). After atrioventricular node ablation, dogs were implanted with an EPM in the right ventricular (RV) endocardial apex (n = 4) and two additionally received a BPM at the left ventricular (LV) epicardial base (n = 2). EWI was performed trans thoracically during NSR, BPM and EPM pacing, in conscious dogs, using an unfocused transmit sequence at 2000 frames/s. During NSR, the EW originated at the right atrium (RA), propagated to the left atrium (LA) and emerged from multiple sources in both ventricles. During EPM, the EW originated at the RV apex and propagated throughout both ventricles. During BPM, the EW originated from the LV basal lateral wall and subsequently propagated throughout the ventricles. EWI differentiated BPM from EPM and NSR and identified the distinct pacing origins. Isochrone comparison indicated that EWI was repeatable and reliable. These findings thus indicate the potential for EWI to serve as a simple, non-invasive and direct imaging technology for mapping and characterizing arrhythmias as well as the treatments thereof. (E-mail: ek2191@columbia.edu) © 2014 World Federation for Ultrasound in Medicine & Biology.

Key Words: Electrical activation sequence, Electromechanical wave imaging, Echocardiography, Imaging, Biological pacemakers.

**INTRODUCTION**

Safe and real-time non-invasive imaging of cardiac electrical activation has been a long-term goal of clinicians and laboratory investigators. Standard methods currently employed in the clinic are all catheter-based, resulting in ionizing exposure and prolonged sedation or anesthesia, and are limited to epicardial or endocardial activation sequences. They are also time consuming and costly. A major recent advance is electrocardiographic imaging (Ramanathan et al. 2004; Zhang et al. 2005), which reconstructs action potentials based on body surface potential measurements, but is limited to the epicardium and is dependent on patient-specific modeling derived from X-ray computed tomography or magnetic resonance imaging scans.

Electromechanical wave imaging (EWI) is a non-ionizing, ultrasound-based imaging modality that non-invasively maps the electromechanical activity of the heart, in all four chambers (Provost et al. 2011b) at very high spatial and temporal resolution (~0.5–3.0 ms) (Wang et al. 2008) and with real-time feedback capabilities (Luo and Konofagou 2010; Provost et al. 2012). EWI tracks the electromechanical wave (EW), which denotes the spatial and temporal progression of the transient deformations of the myocardium after local electrical activation. Because the electrical activation lasts 60 to 100 ms in the heart, tracking the EW requires...
a temporal resolution of 2–5 ms or less. Imaging the heart at such high frame rates also allows, perhaps more importantly, a 5-fold improvement in the signal-to-noise ratio of cardiac motion and deformation mapping (Provost et al. 2012) when using RF cross-correlation. We previously found that the EW correlates highly with (Provost et al. 2012) when using RF cross-correlation. The underlying electrical activation of the myocardium in normal canine hearts during sinus rhythm and various electronic pacing protocols in vivo (Provost et al. 2011b) and in silico (Provost et al. 2011a) in accordance with earlier studies based on invasive methods (Badke et al. 1980; Wyman et al. 1999). We also reported that EWI is capable of mapping trans mural activation (Provost et al. 2011b), and by introducing novel acquisition sequences, we found that EWI is capable of providing full-view mapping of small, transient inter-frame strains at a very high frame rate (~0.075% at 2000 Hz) in a single heartbeat and during free breathing, both in humans (Provost et al. 2011b) and in dogs (Provost et al. 2011c).

In this study, our aim was to quantify the reproducibility and repeatability of EWI in closed-chest, conscious canines and, for the first time, to demonstrate the feasibility of EWI during biological pacing. To reach our goal, we availed ourselves of a canine heart-block model in which sinus rhythm and normal ventricular activation can be recorded before inducing complete heart block. During heart block, two different rhythms can be created in the ventricle via implantation of a right ventricular endocardial electronic pacemaker (EPM) and a left ventricular epicardial biological pacemaker (BPM). During normal sinus rhythm (NSR), we show that the electrical activation sequence depicted by EWI follows the expected electrical activation. During pacing, we show that EWI correctly detects the pacing origins of the expected electrical activation. During diastolic depolarization and autonomic modulation of cardiac rhythm (Barbuti et al. 2007). This inward current slowly activates on hyperpolarization and, thus, facilitates activation during diastole. In addition, \( I_f \) is directly modulated by cAMP, making it sensitive to adrenergic and muscarinic modulation, leading to a respective acceleration or deceleration in rate (DiFrancesco and Tromba 1988; DiFrancesco et al. 1986).

**Biological pacemakers**

BPMs aim at overcoming the limitations of their electronic counterparts, such as sub-optimal sensitivity to autonomic modulation, and have the potential to cure rather than merely palliate (Rosen 2005; Rosen et al. 2004). The pacemaker current (or “funny” current \( I_f \)) contributes to diastolic depolarization and autonomic modulation of cardiac rhythm (Barbuti et al. 2007). This inward current slowly activates on hyperpolarization and, thus, facilitates activation during diastole. In addition, \( I_f \) is directly modulated by cAMP, making it sensitive to adrenergic and muscarinic modulation, leading to a respective acceleration or deceleration in rate (DiFrancesco and Tromba 1988; DiFrancesco et al. 1986).

The BPMs used in this study seek to exploit the pacemaker current \( I_f \) to induce pacemaker activity in normally silent tissue. To this end, we used adenovirus-mediated transduction to overexpress HCN2 in normally quiescent ventricular myocytes. The HCN2 gene is part of the HCN (hyperpolarization-activated, cyclic nucleotide-gated) family of genes that encodes the \( I_f \) channel (Plotnikov et al. 2004; Qu et al. 2003). Adenoviral constructs incorporating cytomegalovirus-driven expression of green fluorescent protein and murine HCN2 were prepared as described previously (Qu et al. 2001). We prepared \( 3 \times 10^{10} \) fluorescence-encoding units of one vector and mixed this with an equal amount of the other vector in a total volume of 700 µL. This mixture was subsequently injected sub-epicardially

**METHODS**

**Experimental protocol**

This study conformed to the Public Health Service Policy on Humane Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of Columbia University. We performed data acquisition on six male mongrels ranging from 25 to 30 kg in weight. Of the six, two were implanted during open-chest surgery with an EPM whose lead was located at the endocardial apex of the right ventricle (RV); and another two were implanted during open-chest surgery with both a BPM in the left ventricle (LV) basal epicardium and an EPM whose lead was located at the RV endocardial apex. The lead implanted was an endocardial CapSureFix magnetic resonance imaging SureScan lead (Medtronic, Fridley, MN, USA), and the electronic pacemaker implanted was a single-channel single chamber ventricular pacemaker (Discovery II, Guidant, Boston Scientific, Natick, MA, USA) set at 35 bpm. During open-chest surgery, radiofrequency ablation of the atrio-ventricular node was performed to induce complete heart block and permit the emergence of rhythms initiated by the biologic or electronic pacemaker (Bucchi et al. 2006). Electrocardiography (ECG) monitoring and recording were obtained from standard surface limb leads. EWI was performed on all six dogs pre-surgery (for NSR) and on the four implanted dogs 7 d post-surgery (for BPM and EPM). During data acquisition, the animals were fully conscious and were positioned on their left side on a standard examination table.

**Biological pacemakers**

BPMs aim at overcoming the limitations of their electronic counterparts, such as sub-optimal sensitivity to autonomic modulation, and have the potential to cure rather than merely palliate (Rosen 2005; Rosen et al. 2004). The pacemaker current (or “funny” current \( I_f \)) contributes to diastolic depolarization and autonomic modulation of cardiac rhythm (Barbuti et al. 2007). This inward current slowly activates on hyperpolarization and, thus, facilitates activation during diastole. In addition, \( I_f \) is directly modulated by cAMP, making it sensitive to adrenergic and muscarinic modulation, leading to a respective acceleration or deceleration in rate (DiFrancesco and Tromba 1988; DiFrancesco et al. 1986).

The BPMs used in this study seek to exploit the pacemaker current \( I_f \) to induce pacemaker activity in normally silent tissue. To this end, we used adenovirus-mediated transduction to overexpress HCN2 in normally quiescent ventricular myocytes. The HCN2 gene is part of the HCN (hyperpolarization-activated, cyclic nucleotide-gated) family of genes that encodes the \( I_f \) channel (Plotnikov et al. 2004; Qu et al. 2003). Adenoviral constructs incorporating cytomegalovirus-driven expression of green fluorescent protein and murine HCN2 were prepared as described previously (Qu et al. 2001). We prepared \( 3 \times 10^{10} \) fluorescence-encoding units of one vector and mixed this with an equal amount of the other vector in a total volume of 700 µL. This mixture was subsequently injected sub-epicardially
into three adjacent sites of the anterobasal left ventricular free wall in two \( n = 2 \) animals. After ablation of the atrioventricular node and implantation of the BPM, the ventricles are driven solely by the newly implanted pacemaker as it overrides the intrinsic ventricular rhythm of the heart. As a result, the rates of the BPM cannot be externally controlled in the same way the rates of EPMs are controlled, because BPMs operate by facilitating depolarization and only respond to the autonomic nervous system.

**Electromechanical wave imaging**

EWI was performed in four- and two-chamber echocardiographic views during NSR, before surgery and 7 d after, during biological pacing (resting rhythm) and then during electronic pacing. An unfocused transmit sequence (Provost et al. 2011c) was developed and implemented on a Verasonics system (Verasonics, Redmond, WA, USA) to acquire RF frames at 2000 fps (Fig. 1i) using a 2.5-MHz ATL P4-2 phased array.

The acquisition sequence allowed for very high frame rates by emitting unfocused circular ultrasound waves from a virtual source located 10.2 mm behind the probe. Raw signals from each element were recorded, and beamforming was performed during post-processing, allowing the reconstruction of one RF frame per transmit—unlike conventional sequences requiring, typically, 64 transmits or more to generate one frame. As a result, the complete acquisition sequence consisted of a motion-estimation acquisition sequence using one unfocused transmit per frame performed during 2 s at 2000 fps (4000 frames acquired), which was immediately followed by an anatomical imaging sequence consisting of a standard 64-line B-mode acquisition performed during 1.5 s at 30 fps. Retrospective ECG gating was used to temporally align motion estimation and anatomical B-mode acquisitions, but not for motion estimation,
Unlike previous approaches (Provost et al. 2011b) as no sub-sectors were used. One cardiac cycle was considered for each data set and, more specifically, the data corresponding to electrical activation. This was done by selecting one P wave and QRS complex on the electrocardiogram. Data segmentation was then performed on the aligned B-mode images. Beamforming was conducted using a delay-and-sum algorithm to reconstruct the RF frames for the motion-estimation sequence by generating 128 lines in the direction orthogonal to the ultrasound wavefront propagation with a reconstructed sampling frequency of 20 MHz (axial sampling of 0.0385 mm) (Provost et al. 2011c). The motion-estimation and motion-sampling rates (Provost et al. 2012) were both equal to 2000 fps. We estimated axial displacements using a fast 1-D cross-correlation algorithm (Luo and Konofagou 2010) with overlapping 9.2 mm axial windows (15 wavelengths) and a 0.385 mm window shift (95.8% overlap) (Fig. 1ii–c). Axial displacement was within ±0.0385 mm. At a frame rate of 2000 fps, this corresponds to velocities of ±7.7 cm/s, which are consistent with previous studies using tissue Doppler (Uematsu et al. 1995; Urheim et al. 2000). The axial incremental strains (i.e., inter-frame strains in the axial direction) were estimated using a least-squares estimator (Kallel and Ophir 1997) with a 5-mm 1-D kernel (Fig. 1iii–c). We then applied a moving-average spatial filter of a 12-mm window axially by 10 lines laterally and a temporal low-pass filter with a 125-Hz cut-off frequency to the strain estimates to filter out the high-frequency noise and ensure continuity of the strains. Because we used overlapping windows, the axial sampling for both displacement and strain was determined by the window shift, in this case 0.385 mm (Righetti et al. 2002). The myocardium was manually segmented on the first anatomical B-mode frame, and the myocardial contour was then tracked throughout the cardiac cycle using an automated contour tracking technique based on the estimated displacements (Luo and Konofagou 2008). Electromechanical activation was defined as the time at which the incremental strain value changes from positive (lengthening in the axial direction) to negative (shortening or contraction in the axial direction), that is, crosses zero. To simplify visualization, only the strains corresponding to the electromechanical activation were overlaid onto the corresponding B-mode images to generate EWI cine loops (i.e., negative strains in apical views) (Fig. 1iiia, b). Based on this definition of the electromechanical activation, we subsequently generated isochrones by mapping the first occurrence of the incremental strain crossing zero (positive to negative values, i.e., onset of contraction) after either the P wave in the atria or the Q wave in the ventricles (Provost et al. 2011b) (Fig. 1iv). The zero-crossing timings were manually obtained in up to a hundred randomly and automatically selected regions, and regions with noisy strain curves where positive-to-negative zero-crossing times could not be determined were excluded. Criteria for exclusion of noisy strain curves included: only negative-to-positive zero crossing, no zero crossing discernible (all negative or all positive strains) or discontinuities in the incremental strain curves. Noisy strain curves were mostly located at the border of the segmented regions, where points can go in and out of the mask. Typically, when the segmentation is performed accurately, the prevalence of poor-quality strain curves is less than 5%. Sub-sample resolution was obtained through cubic spline interpolation, and smooth continuous isochronal maps were then generated through Delaunay triangulation-based cubic interpolation. Finally, four-chamber and two-chamber views were co-registered in Amira 4.1 (Visage Imaging, Chelmsford, MA, USA), both temporally (using ECG) and spatially (using B-mode anatomical landmarks such as the position of the valves and apex), to construct bi-planar EWI cine loops and isochrones (Fig. 1v). Beamforming, motion estimation, strain estimation, spatial moving-average of the strains, temporal moving-average filtering of strains and the automated contour tracking technique were performed off-line, on a Tesla GPU (NVIDIA, Santa Clara, CA, USA) and the MATLAB parallel processing toolbox (The Mathworks, Natick, MA, USA) at a computing speed of 2.4 fps. Because only data corresponding to electrical activation (i.e., one P wave and/or QRS complex, which corresponds to 800 frames or 400 ms) were processed in each case, processing time for strain estimation and EWI video generation was roughly 5 min per data set. In addition, the isochrone computation time varied between 2 and 5 min depending on how many regions were excluded because of poor strain estimation and how many new points had to be manually selected to sufficiently cover the chambers for subsequent interpolation. Thus, the maximum total time for processing one data set was 10 min.

RESULTS

In this study, acquisition in six (n = 6) male mongrels ranging from 25 to 30 kg in weight to quantify the reproducibility and repeatability of EWI in closed-chest, conscious canines and, for the first time, to demonstrate the feasibility of EWI during biological pacing. Heart rates during NSR were in the range 75 to 90 bpm. During EPM pacing, the heart rate depended on the pacing and was either 35 or 60 bpm. During BPM pacing, the heart rate was between 30 and 45 bpm. ECG QRS durations during NSR varied between 60 and 100 ms. During EPM and BPM pacing, the QRS duration stayed within the range 118–123 ms, and QRS morphologies
were typical of left and right bundle-branch block during EPM and BPM pacing, respectively.

Normal sinus rhythm

The bi-planar isochrones of the normal electromechanical activation of the heart during NSR in four dogs, before implantation of a pacemaker, are shown in Figure 2 (Supplementary Video 1 shows the corresponding EWI cine loops). The origin of the isochrones corresponds to the onset of the P wave. In all four (n = 4) dogs, the EW originated from the right atrium (RA) and propagated toward the left atrium (LA) during the P wave. During the QRS complex, the EW propagated in the ventricles from multiple locations. Figure 2(e and f) illustrates the electromechanical activation of the atria and the ventricles, respectively, in one animal (Fig. 2b) in more detail. In the atria (Fig. 2e), the EW originated in the RA near the atrial septum and propagated toward the lateral wall of the RA and the LA. The origin of the EW in the atria is in accordance with the expected location of the sino-atrial node. In the two-chamber view (Fig. 2e), which depicts the LA and LV, the EW originated in the anterior wall and propagated toward the posterior wall. After a delay similar to the PR segment, the ventricles were activated from four main origins located near the apex in the lateral wall of the RV, in the apical region of the septum, near the apex in the posterior wall of the LV and near the base of the anterior wall of the LV (Fig. 2f).

Biological pacemakers

Figure 3 depicts the isochrones obtained in two dogs during biologic pacing from the anterobasal epicardial LV (Supplementary Video 2 shows the corresponding EWI cine loops). In all isochrones, 0 ms corresponds to the onset of the QRS. In both dogs, the EW originated

---

**Fig. 2.** Electromechanical wave imaging (EWI) isochrones obtained from four dogs before surgery during NSR. The origins of all isochrones correspond to the onset of the P wave. (a–d) Dogs 1–4 respectively. Activation in the atria originated from the RA and propagated to the LA. In the ventricles, arrows indicate the sites of early activation. (e, f) Time magnification of EWI isochrones for dog 2 in the two- and four-chamber views. (e) In the four-chamber view, the atrial activation sequence started from the RA and propagated in the LA. In the two-chamber view, activation reached the anterior wall prior to the posterior wall. (f) In the ventricles, arrows indicate the sites of early activation, which are located in the septum near the apex; in the LV near the base in the anterior wall and near the apex in the posterior wall; and near the apex of the RV. LA = left atrium, LV = left ventricle, RA = right atrium, RV = right ventricle.
at the base of the lateral wall in the LV (Fig. 3a, b). In the first case, the EW then propagated near the base of the LV, reached the basal anterior and posterior walls nearly simultaneously and reached the septum at approximately 70 ms (Fig. 3a). In the second case, the EW propagated in the basal region of the LV, but the anterior wall was activated prior to both the posterior wall and the septum. Simultaneously, the EW propagated toward the apex along the LV lateral wall. The time difference between activation of the anterior wall and activation of the posterior wall was 20 ms (Fig. 3b). In both cases, the RV presented two earlier sites of activation: at the RV apex, and either in the mid-septum (Fig. 3b) or the basal region of the septum (Fig. 3a).

Electronic pacing

Figure 4 depicts the isochrones obtained in four dogs during pacing by the EPM. The origin of the isochrones corresponds to the onset of the QRS (Supplementary Video 3 shows the corresponding EWI cine loops). The pacing rate was set to override the idioventricular rhythm and ranged from 35 to 60 bpm. In all cases, the EPM lead was implanted at the endocardial RV apex. In three cases, the EW originated from the RV apex (Fig. 4a–c), whereas in one case, the origin of the EW was located near the septum at the LV apex. Figure 4a shows the EW propagating from the RV apical septum to the RV apical lateral wall, the basal septum and the mid-posterior wall of the LV before reaching the mid-lateral wall of the LV at 40 ms. The EW then propagated toward the basal anterior, posterior and lateral walls of the LV, which were activated at 60 to 70 ms. Finally, at 120 ms, the EW reached the RV lateral wall as well as the posterior and anterior walls of the LV. In Figure 4b, electromechanical activation started at the RV apical septum and traveled down both the septum and the RV lateral wall. The EW then propagated toward the mid-lateral wall of the LV via the apical region and a small mid-region of the anterior wall. The LV mid-lateral wall was reached at 40 to 50 ms. The EW then propagated downward toward the basolateral wall of the LV before eventually reaching all regions of the LV. Total activation of the LV was observed at 120 ms. In Figure 4c, the EW originated in the RV apex, propagated toward the apical and basal anterior and posterior walls and reached the LV mid-lateral wall at 40 to 50 ms. The remaining regions of the RV (mid- and basal regions of the septum and lateral walls) were activated next, at approximately 70 ms. Finally, the mid-anterior and posterior walls, as well as the rest of the lateral wall of the LV, were activated at 110 to 120 ms. In Figure 4d, the EW appeared to originate from the LV apical septum region. Activation of the mid- to basal anterior and lateral LV walls, corresponding to the site of implantation of the BPM, followed shortly after at 40 to 50 ms. The RV was then activated as the EW propagated from the anterior part of the LV back to the RV (80 to 90 ms after initial activation) and eventually reached the RV apex as well as the posterior wall of the LV at 120 ms.

Repeatability

Figures 5 and 6 illustrate EWI repeatability in consecutive cardiac cycles during both NSR (Fig. 5) and EPM pacing (Fig. 6). Origins of the isochrones correspond to the onset of the P wave (Fig. 5a, d) or onset of the QRS (Fig. 6a, d). Figures 5(b, e) and 6(b, e) are difference maps created by displaying the absolute difference in activation time between two consecutive isochrones, and Figures 5(c, f) and 6(c, f) are histograms of the absolute values of the difference in activation time for pixels of the corresponding difference map.

Fig. 3. Ventricular electromechanical wave imaging (EWI) isochrones during BPM pacing. The origins of the isochrones correspond to the onset of the QRS. (a, b) Dogs 2 and 5, respectively. During BPM pacing, early activation is seen in the basal region of the lateral wall in the left ventricle LV. Early sites of activation are also seen in the septum and at the apex of the right ventricle RV. BPM = biological pacemaker, LV = left ventricle, RV = right ventricle.
Figure 5(a and d, respectively) depicts the four-chamber and two-chamber isochrones generated from data acquired from consecutive cardiac cycles during NSR. For the four-chamber view, we show that 70% of the differences in activation time between the isochrones from consecutive cardiac cycles are below 10 ms, and 80% are below 20 ms (Fig. 5c). Qualitatively, most regions on the difference map present small differences in activation time (dark blue). However a few regions in the difference map show an important difference in activation time (orange red): in the mid-lateral wall of the LV, in the apical region of the septum toward the RV and in a small region in the mid-septum. We also observed regions of moderate difference in the RA and basal region of the lateral wall of the RV (green yellow). In the two-chamber view, 53% of the differences in activation times are below 10 ms, 73% are below 20 ms and 90% are below 30 ms (Fig. 5f). On the difference map, a small region near the epicardium in the RA, as well as a region near the apex, present important differences in activation time (Fig. 5e).

During electronic pacing (Fig. 6), the four-chamber view presents 57% of the differences in activation time between the isochrones from consecutive cardiac cycles below 10 ms and 82% below 20 ms (Fig. 6c). On the difference map, regions of important difference between activation times (red) are detected at the apex and on the lateral wall of the LV near the base. Moderate differences (light blue) are depicted near the base of the lateral wall of the RV and in the septum, as well as in the mid-region of the lateral wall of the LV (Fig. 6b). In the two-chamber view, 33% of the differences in activation time are below 10 ms, 68% are below 20 ms and 82% are below 30 ms (Fig. 6f). On the difference map, regions of moderate difference (light blue) can be seen near the apex toward the anterior wall, as well as in the posterior wall near the base and in the mid-region (Fig. 6e).

**DISCUSSION**

In this study, we used EWI to trans thoracically image the heart of conscious, free-breathing dogs during NSR, EPM and BPM pacing. The objectives were to assess reproducibility and quantify repeatability in numerous closed-chest, conscious dogs and, for the first time, to demonstrate the feasibility of EWI during biologic pacing. Testing reproducibility in numerous animals and across a wide array of electrical and biologic rhythms establishes the potential of EWI for longitudinal...
animal studies, as well for mapping a wide variety of arrhythmias.

EWI depicts propagation of the electromechanical wavefront that occurs in the cardiac muscle following electrical activation following a delay of a few milliseconds (Bers 2002; Cordeiro et al. 2004). In this study, reproducibility was assessed during NSR and during BPM and EPM pacing. Because we performed EWI on the heart of six different animals, identical isochrones were not expected. Indeed, each heart presents its own electromechanical characteristics and, for those that underwent surgery, its own remodeling processes.

We confirmed that during NSR (Fig. 2), the EW propagated in both the atria (during the P wave) and the ventricles (during the QRS complex) following an expected pattern described in previous literature (Durrer et al. 1970; Lewis and Rothschild 1915; Lewis et al. 1914; Scher and Young 1956).

During biologic pacing from the basal LV epicardium (Fig. 3), EWI mapped the electrical activation and determined a pacing origin in the basolateral LV wall. The location is within the site of implantation of the BPM. There are three possible reasons for the variance in activation along the walls. First, we hypothesize that the Purkinje network might have been recruited during biologic pacing, resulting in early activated points in the walls at the mid-septum and near the apex of the RV. Second, tethering may be responsible for the variance in activation: indeed there might be a longer lag in some regions between electrical and electromechanical activation because of the myocardium having to contract against earlier-activated regions. Third, because we are performing 2-D imaging of a 3-D propagation pattern, the variance in activation might appear larger than it is in reality.

In Figure 4 we characterized the propagation from an EPM and, more importantly, confirmed the origins of paced beats. In three of the dogs, the origin was correctly detected at the endocardial RV apex. The location is within the site of implantation of the BPM. There are three possible reasons for the variance in activation along the walls. First, we hypothesize that the Purkinje network might have been recruited during biologic pacing, resulting in early activated points in the walls at the mid-septum and near the apex of the RV. Second, tethering may be responsible for the variance in activation: indeed there might be a longer lag in some regions between electrical and electromechanical activation because of the myocardium having to contract against earlier-activated regions. Third, because we are performing 2-D imaging of a 3-D propagation pattern, the variance in activation might appear larger than it is in reality.

In Figure 4 we characterized the propagation from an EPM and, more importantly, confirmed the origins of paced beats. In three of the dogs, the origin was correctly detected at the endocardial RV apex. The location is within the site of implantation of the BPM. There are three possible reasons for the variance in activation along the walls. First, we hypothesize that the Purkinje network might have been recruited during biologic pacing, resulting in early activated points in the walls at the mid-septum and near the apex of the RV. Second, tethering may be responsible for the variance in activation: indeed there might be a longer lag in some regions between electrical and electromechanical activation because of the myocardium having to contract against earlier-activated regions. Third, because we are performing 2-D imaging of a 3-D propagation pattern, the variance in activation might appear larger than it is in reality.
lead being located anteriorly to the four-chamber-view imaging plane. This is confirmed by the bi-planar view, in which the two-chamber view shows an early activation location at the anterior apex. In addition, the basal lateral and anterior walls of the LV are activated within 40 ms of the origin, suggesting that the BPM might not have been completely overridden by the EPM pacing. The differences in activation patterns observed in this figure may be attributed to inherent differences in anatomy and electrical activation between dogs. Furthermore, because EWI is currently performed in two dimensions instead of three dimensions, the isochrones presented here might not correspond to the exact same plane in all of the dogs.

We demonstrated EWI repeatability within a single heart for consecutive cardiac cycles during both NSR and EPM pacing (Figs. 5 and 6). According to the difference maps and the histograms of the difference in activation times, more than 80% of the pixels have a difference in activation time below 20 ms for the four-chamber views or below 30 ms for the two-chamber views. The regions depicted on the difference map with higher differences in activation times (in orange red) are small and centered on a few specific locations. The high values found at or near the apex might be the result of suboptimal incremental strain estimations caused by the limited contraction of the apex as well as its fiber orientation, which differs from the orientation in the rest of the myocardium. Indeed, the apex has a more complex myocardial fiber architecture (Greenbaum et al. 1981) that could result in the discrepancy found. Other discrepancies near the base and at the junction between atria and ventricles could be explained by tethering of the myocardium during the P wave and/or the QRS. Finally, because the regions of high differences are mostly limited in space, errors might also be the result of occasional errors in the manual selection of the hundreds of regions necessary to generate the isochrones.

In the past, our group has reported EWI strains to be reliable for a wide array of cardiac conditions and geometries (Provost et al. 2010, 2011b, 2011c, 2012). The novel unfocused transmit sequence (Provost et al. 2011c) we used in this study mitigates the standard strain mapping method limitation of simultaneously achieving high frame rate and high accuracy in a large field of

---

Fig. 6. Electromechanical wave imaging (EWI) repeatability during EPM pacing. The origins of the isochrones correspond to the onset of the QRS. (a) Four-chamber view EWI isochrones for two consecutive heart cycles. (b, c) Difference map (b) and histogram (c) of the absolute activation time difference between the two four-chamber isochrones. (d) Two-chamber view EWI isochrones for two consecutive heart cycles. (e, f) Difference map (e) and histogram (f) of the absolute activation time difference between the two two-chamber isochrones. EPM = electronic pacemaker, LV = left ventricle, RV = right ventricle.
view. Indeed, EWI non-invasively mapped the electromechanical activation at high frame rates (2000 Hz) and high accuracy (0.385-mm axial resolution) in a full view of the heart and using ultrasound systems similar to those available in cardiology suites. The processing of the EWI data (RF reconstruction, displacement and strain estimation, EWI cine loop and isochrone generation), as described in Figure 1, can be performed within minutes of acquisition with the Tesla GPU and suggests that EWI could be readily used in real time in a clinical setting.

There are several limitations to this study. A limitation of the current implementation of EWI is that it estimates strain in only one direction. As a result, in regions of the heart where the ultrasound beam is not clearly aligned with the cardiac longitudinal direction, interpretation of the axial incremental strains becomes more complex because the assumption that the axial direction matches or has the largest projection component in the longitudinal direction may not hold. In the apical views, such regions include apical and basal regions of the anterior and lateral walls in the four- and two-chamber views, respectively. Because EWI does not rely on the magnitude of the strains to detect electromechanical activation, as long as the ultrasound beam is oriented such that the largest projection of the desired strain component is in the axial direction, the zero crossings of the incremental strain correspond to a change in mechanical function from relaxation to contraction. Indeed, previous studies have indicated that the relationship between electrical and electromechanical activation is maintained at different insinification angles (Provost et al. 2011a). A solution would be to estimate strains in two dimensions. Such estimation can be more sensitive to noise and is the subject of ongoing studies (Lee et al. 2007; Luo and Konofagou 2009; Okrasinski et al. 2012).

A main limitation lies in the fact that 2-D imaging of a 3-D propagation pattern is performed. Indeed, although the electromechanical wave is a 3-D phenomenon propagating throughout the heart during the cardiac cycle, imaging through standard 2-D echocardiography apical imaging planes was performed in this study. We believe this limitation explains the discrepancy between detected and expected pacing origins in the EPM case. Because we use 2-D echocardiographic views, the imaging plane selected might not include the pacing electrode location, and as a result, the 3-D propagation of the electromechanical activation may result in the earliest activation being detected in neighboring locations on the selected echocardiographic view. This limitation could be mitigated by acquiring a larger number of views (e.g., two-chamber, four-chamber, three-chamber and a fourth view located between the two- and four-chamber views) while also performing 3-D registration and reconstruction (Provost 2012). Three-dimensional ultrasound in a single cardiac cycle would also be advantageous and is theoretically possible, but new hardware and processing methods need to be developed. For example, high frame rates and high signal-to-noise ratios are essential for reliable displacement and strain estimates and, in our case, were achieved by developing a new acquisition method involving the emission of one unfocused circular wave per frame. This constitutes a topic of ongoing research by our group.

Another limitation lies with the semi-automated generation of isochrones. Indeed, in order to discard noisy strain curves characterized by either incremental axial strain negative-to-positive zero crossings, no strain zero crossing (all negative or all positive strains) or discontinuities in the incremental strain curves, manual selection of zero-crossing timings for up to a hundred randomly and automatically selected regions is performed. To reduce operator bias, our group is currently developing a new processing algorithm that combines manual initialization followed by full automatization of isochrone generation.

Previous studies have indicated that the delay between electrical activation and electromechanical activation, defined here as the onset of axial shortening, may vary throughout the heart, especially in diseased states such as left bundle-branch block (Faris et al. 2003; Gurev et al. 2010; Russell et al. 2011). Because the EW results from myocardial electromechanical coupling, EWI does not directly track electrical activation. However, our group previously reported that there is a strong correlation between electrical activation and the EW in normal dogs, as well as during EPM pacing, where the electromechanical pattern is a delayed version of the electrical one (Provost et al. 2011a, 2011b, 2011c). The correlation between the EW and the underlying electrical activation during BPM pacing was not validated in this study, but is currently being investigated in ongoing studies.

CONCLUSION

Our study found that EWI can trans thoracically and non-invasively map the electrical activation of the hearts of conscious, free-breathing canines during a wide array of rhythms, whether of electrical or biological, endocardial or epicardial origin, and arising in the atria or either ventricle of the heart. It also found that EWI is reproducible not only within the same heart, but also across different hearts, thus indicating that EWI has the potential to not only be used in longitudinal animal studies, but also to map a wide variety of arrhythmias and their treatment.

Acknowledgments—This study was supported in part by the National Institutes of Health (R01 EB006042, R01 HL114358 and R01- HL67101). Gerard Boink received grant support from the Netherlands
Electromechanical wave imaging of biologically paced canine hearts ● A. Coster et al. 187

Foundation for Cardiovascular Excellence (NFCVE), the Netherlands Heart Foundation, the Dr. Saal van Zwanenberg Foundation and the Interuniversity Cardiology Institute of the Netherlands. The authors want to thank Stanley J. Okrasinski and Ethan Bunting in the group for the helpful discussions and their assistance with data acquisition; Eugene A. Sosunov for his help during data acquisition; and Ira S. Cohen and Richard B. Robinson for their help in making the biologic pacemakers used in this paper.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.ultrasmedbio.2013.08.019.

REFERENCES


