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Electromechanical Wave Imaging of biologically and electrically paced canine hearts *in vivo*

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Abstract

Ultrasound-based, Electromechanical Wave (EW) Imaging (EWI) can directly and entirely noninvasively map the transmural electromechanical activation in all four cardiac chambers *in vivo*. In this study, we assessed EWI repeatability and reproducibility, as well as its capability in localizing electronic and, for the first time, biological pacemakers in closed-chest, conscious canines. Electromechanical activation was obtained in six conscious animals during normal sinus rhythm (NSR), and idioventricular rhythms occurring in dogs in heart block instrumented with electronic and biological pacemakers (EPM and BPM respectively). After AV 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 transthoracically during NSR, BPM, and EPM pacing, in conscious dogs, using an unfocused transmit sequence at 2000 frames/second. 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 demonstrated that EWI was repeatable and reliable. These findings thus indicate the EWI potential to serve as a simple, noninvasive and direct imaging technology for mapping and characterizing arrhythmias as well as the treatments thereof.

Keywords

Electrical activation sequence; Electromechanical wave imaging; Echocardiography; Imaging; Biological pacemakers

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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 anesthesia, and are limited to epicardial or endocardial activation sequences. They are also time consuming and costly. A major recent advance has been electrocardiographic imaging or ECGI (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 (CT) or magnetic resonance imaging (MRI) scans.

Electromechanical Wave Imaging (EWI) is a non-ionizing, ultrasound-based imaging modality which noninvasively maps electromechanical activity in all four chambers of the heart (Provost et al. 2011a) 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 following local electrical activation. Because the electrical activation lasts for 60 to 100 ms in the heart, tracking the EW requires a temporal resolution of 2–5 ms or less as well as the detection of very small inter-frame strains (0.025 to 1% at 2ms). Our technique estimates displacements and strains using radio-frequency (RF) cross-correlation which has been shown to be 10 times more accurate than standard B-mode images-based method (Hein and O'Brien, W.D. 1993; Walker and Trahey 1995). We previously showed that the EW correlates highly with the underlying electrical activation of the myocardium in normal canine hearts during sinus rhythm and various electronic pacing protocols in vivo (Badke et al. 1980; Provost et al. 2011a; Wyman et al. 1999) and in silico (Provost et al. 2011b). We also demonstrated that EWI is capable of mapping transmural activation (Provost et al. 2011a) and, by introducing new acquisition sequences, we showed that EWI is capable of providing a full-view mapping of small, transient inter-frame strains at very high frame rate ($\sim 0.075\%$ at 2000Hz) in a single heartbeat and during free breathing, both in humans (Provost et al. 2011a) and dogs (Provost et al. 2011c).

In this study, we aim at testing EWI reproducibility and repeatability in closed-chest, conscious canines and, for the first time, EWI feasibility during biological pacing. In order to reach our goal we availed ourselves of a canine heart block model in which one can record sinus rhythm and normal ventricular activation before inducing complete heart block. Post heart block, one can create two different rhythms 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 both types of pacemakers (EPM and BPM). Then, by comparing isochrones obtained from consecutive heart cycles in the same animal during NSR and electronic pacing, we show that EWI is repeatable. Therefore, EWI can transthoracically and noninvasively map the electrical activation of the hearts of conscious, free-breathing canines during a wide array of rhythms, whether of electrical or biological origin, endocardial or epicardial origin, and arising in the atria or either ventricle of the heart, thus suggesting that EWI has the potential to not only be used in longitudinal animal studies, but also to map a wide variety of arrhythmias.

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 (n=6) male mongrels ranging from 25 to 30 kg in weight. Of the six, two (n=2) 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 (n=2) were implanted during open chest surgery with both a BPM in the left ventricular (LV) basal epicardium and an EPM whose lead was located at the RV endocardial apex. The lead implanted was an endocardial CapSureFix MRI SureScan lead (Medtronic, Fridley, MN) and the electronic pacemaker implanted was a single-channel pacemaker (Discovery II, Guidant, Boston Scientific, Natick, MA) set at VVI 35 bpm. During open chest surgery, radiofrequency ablation of the atrioventricular node was performed to create complete heart block and permit the emergence of rhythms initiated by the biological or electronic pacemaker (Bucchi et al. 2006). Electrocardiographic (ECG) monitoring and recording was obtained from standard surface limb leads. EWI was performed on all six dogs pre-surgery (for NSR), and on the four implanted dogs 7 days post-surgery (for BPM and EPM). During data acquisition, the animals were fully conscious and lay on their left side on a standard examination table.

Biological pacemakers

Biological pacemakers aim at overcoming the limitations of their electronic counterparts, such as suboptimal 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 upon 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 biological pacemakers used in this study seek to exploit the pacemaker current I_f in order to induce pacemaker activity in normally silent tissue. To this end we used adenoviral-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 which encode the I_f channel (Plotnikov et al. 2004; Qu et al. 2003). Adenoviral (Ad) constructs incorporating CMV driven expression of green fluorescent protein (GFP) and murine HCN2 were prepared as described previously (Qu et al. 2001). We prepared 3×10^{10} fluorescence focus forming units of one vector and mixed this with an equal amount of the other vector in a total volume of 700 μ L. This mix was subsequently injected subepicardially into three adjacent sites of the anterobasal left ventricular free wall in two (n=2) animals. After ablation of the atrio-ventricular node and implantation of the biological pacemaker, 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 biological pacemaker cannot be externally controlled in the same way they are with electronic pacemakers because biological pacemakers 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 prior to surgery, and 7 days after during biological pacing (resting rhythm) and then during

electronic pacing. An unfocused transmit sequence (Provost et al. 2011c) was developed and used on a Verasonics system (Verasonics, Redmond, WA) in order to acquire RF frames at 2000 fps (Fig. 1 (i)) 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 emitted 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 64 transmits 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 in order to temporally align motion-estimation and anatomical B-mode acquisitions but not for motion estimation unlike previous approaches (Provost et al. 2011a) since no subsectors 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 ECG, thus reducing the amount of data to process. 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 the motion-sampling rates (Provost et al. 2012) were both equal to 2000 fps. We estimated axial displacements using a fast 1D 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. 1 (ii)-c). Axial displacement was comprised between ± 0.0385 mm. At a framerate 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 strain in the axial direction) were estimated using a least-squares estimator (Kallel and Ophir 1997) with a 5mm 1D-kernel (Fig. 1 (iii)-c). We then applied a moving average spatial filter of a 12mm window axially by 10 lines laterally and a temporal low-pass filter with a 125-Hz cut-off frequency to the strain estimates in order 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), i.e., crosses zero. In order to simplify visualization, only the strains corresponding to the electromechanical activation were overlaid onto the corresponding B-mode to create EWI cine-loops (i.e. negative strains in apical views) (Fig. 1 (iii)-a,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) following either the P-wave in the atria or the Q-wave in the ventricles (Provost et al. 2011a) (Fig. 1 (iv)). 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 positive strain) 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%. Subsample 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), both temporally (using ECG) and spatially (using B-mode anatomical landmarks such as the position of the valves and apex) in order to construct bi-planar EWI cine-loops and isochrones (Fig. 1 (v)). 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) and the Matlab parallel processing toolbox (Mathworks, Natick, MA) at a computing speed of 2.4 frames/s. Because only data corresponding to electrical activation (i.e. one P-wave and/or QRS complex which corresponds to 800 frames or 400ms) was processed in each case, processing time for strain estimation and EWI video generation was roughly 5 minutes per data set. In addition, the generation of isochrones was between 2 and 5 minutes depending on how many regions were excluded due to poor strain estimation and how many new points had to be manually selected in order to sufficiently cover the chambers for subsequent interpolation. Thus, the maximum total time for processing one data set was 10 minutes.

Results

In this study, we acquired data in six (n=6) male mongrels ranging from 25 to 30 kg in weight in order to establish EWI reproducibility and repeatability in closed-chest, conscious canines and, for the first time, EWI feasibility during biological pacing. Heart rates during NSR were in the 75 to 90 beats per minute (bpm) range. During EPM pacing, the heart rate depended on the pacing and was either 35 bpm or 60 bpm. During BPM pacing, the heart rate was comprised 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 118–123 ms range and QRS morphologies were typical of left and right bundle-branch block during EPM and BPM pacing respectively.

Normal Sinus Rhythm

Figure 2 shows the bi-planar isochrones of the normal electromechanical activation of the heart during NSR in four dogs before implantation of any pacemaker (Video 1 shows the corresponding EWI ciné-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 towards the left atrium (LA) during the P-wave. During the QRS complex, the EW propagated in the ventricles from multiple locations. Figure 2E and 2F show the electromechanical activation of the atria and the ventricles, respectively, in one animal (Figure 2B) in more details. In the atria (Figure 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 (Figure 2E), which depicts the LA and LV, the EW originated in the anterior wall and propagated toward the posterior wall. Following 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 (Figure 2F).

Biological Pacemakers

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

base of the lateral wall in the LV (Figure 3A–B). In the first case, the EW then traveled along the base of the LV, reached the basal anterior and posterior walls nearly simultaneously, and reached the septum at approximately 70 ms (Figure 3A). In the second case, the EW traveled along the basal region of the LV but the anterior wall was activated ahead of both the posterior wall and the septum. Simultaneously, the EW traveled towards the apex along the LV lateral wall. The time difference between activation of the anterior and posterior walls was 20 ms (Figure 3B). In both cases, the RV presented two earlier sites of activation: at the RV apex, and either in the mid-septum (Figure 3B) or the basal region of the septum (Figure 3A).

Electronic Pacing

Figure 4 depicts the isochrones obtained in four (n=4) dogs during pacing by EPM. Origin of the isochrones corresponds to the onset of the QRS (Video 3 shows the corresponding EWI ciné-loops). The pacing rate was set to override the idioventricular rhythm and ranged from 35 to 60 beats per minute (bpm). In all cases, the EPM lead was implanted at the endocardial RV apex. In three cases the EW originated from the RV apex (Figure 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 traveled towards the mid-lateral wall of the LV via the apical and a small mid region of the anterior wall. The LV mid-lateral wall was reached at 40 to 50 ms. The EW then traveled down to the baso-lateral wall of the LV before eventually reaching all regions of the LV. Total activation of the LV was attained at 120 ms. In figure 4C, the EW originated in the RV apex, traveled towards 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 traveled 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 show EWI repeatability in consecutive cardiac cycles during both normal sinus rhythm (Figure 5) and electronic pacing (Figure 6). Origins of the isochrones correspond to the onset of the P-wave (Figure 5A & 5D) or onset of the QRS (Figure 6A & 6D). Figures 5B, 5E, 6B and 6E show difference maps created by displaying the absolute difference in activation time between two consecutive isochrones and Figures 5C, 5F, 6C and 6F are histograms of the absolute values of the difference in activation time for pixels of the corresponding difference map.

Figure 5A and 5D respectively depict 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 10ms and 80% are below 20ms (Figure 5C). Qualitatively, most regions on the difference map present low differences in

activation time (dark blue color). However a few regions in the difference map show an important difference in activation time (orange-red color): in the mid-lateral wall of the LV, in the apical region of the septum towards the RV, and a small region in the mid-septum. We also observe regions of moderate differences in the RA and basal region of the lateral wall of the RV (green-yellow color). In the two-chamber view, 53% of the differences in activation times are below 10ms, 73% are below 20ms, and 90% below 30ms (Figure 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 (Figure 5E).

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

Discussion

In this study we used EWI to transthoracically image the heart of conscious, free-breathing dogs during normal sinus rhythm, electronic and biological pacing. The objectives were to test reproducibility and show repeatability in numerous closed-chest, conscious dogs and, for the first time, to demonstrate the feasibility of EWI during biological pacing. Testing reproducibility in numerous animals and across a wide array of electrical and biological 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 which occurs in the cardiac muscle following electrical activation after a delay of a few milliseconds (Bers 2002; Cordeiro et al. 2004). In this study, reproducibility was tested during normal sinus rhythm and during biological and electronic 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 (Figure 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 biological pacing from the basal LV epicardium (Figure 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. The variance in activation along the walls may be due to three reasons. First, we hypothesize that the Purkinje network might have been recruited during biological 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 due to the myocardium having to contract against earlier-

activated regions. Third, because we are performing 2D imaging of a 3D propagation pattern, the variance in activation might appear larger than it is in reality.

In figure 4 we characterized the propagation from an electronic pacemaker and, more importantly, confirmed the origins of paced beats. In three of the dogs, the origin was correctly detected at the endocardial RV apex. In the fourth dog, pacing appeared to originate from the paraseptal LV apex. We attribute this to the pacemaker 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 early activation location at the anterior apex. Moreover, the basal lateral and anterior walls of the LV are activated within 40 ms of the origin, suggesting that the biological pacemaker might not have been completely overridden by the electronic 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 2D instead of 3D, 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 normal sinus rhythm and electronic pacing (Figure 5 & 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 20ms for the four-chamber views or below 30ms 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 imperfect incremental strain estimations due to 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 to 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, since 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 generation of the isochrones.

In the past, our group has shown EWI strains to be reliable for a wide array of cardiac conditions and geometries (Provost et al. 2010, 2011a, 2011c, 2012). The novel virtual source imaging sequence (Provost et al. 2011c) we used in this study mitigates the standard strain mapping methods limitation of simultaneously achieving high frame rate and high accuracy in a large field of 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 (NVIDIA, Santa Clara, CA) 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 apex and basal region of the anterior and lateral wall in the four- and two-chamber view, 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 shown that the relationship between the electrical and electromechanical activation was maintained at differentinsonification angles (Provost et al. 2011b). A solution would be to estimate strains in 2-D. 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 2D imaging of a 3D propagation pattern is performed. Indeed, although the electromechanical wave is a 3D phenomenon propagating throughout the heart during the cardiac cycle, imaging through standard 2D 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 2D echocardiographic views, the imaging plane selected might not include the pacing electrode location and, as a result, the 3D propagation of the electromechanical activation may result in earliest activation detected in neighboring locations on the selected echocardiographic view. This limitation could be mitigated by acquiring a higher number of views (for example: two-chamber, four-chamber, three-chamber, and a fourth view located between the two- and four- chamber view) while also performing 3D registration and reconstruction (Provost 2012). 3D 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 rate and SNR 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. Using a similar transmit sequence in 3D would lead to the same energy being spread through a wider volume resulting in EW SNR decrease. However, because of safety concerns, increasing the power is not an option and as a consequence new innovative acquisition sequences need to be developed. Furthermore, in order to achieve reasonable processing time when processing 3D data sets, advanced algorithms fully utilizing parallel processing and GPUs need to be implemented. 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 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. In order to reduce the operator bias, our group is currently developing a new processing algorithm that combines manual initialization followed by full automatization of the isochrone generation.

Previous studies have shown that the delay between electrical activation and the electromechanical activation, defined here as the onset of the axial shortening, may vary throughout the heart, especially in diseased state 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, EW does not directly track electrical activation. However, our group previously showed that there is a strong correlation between electrical activation and the EW in normal dogs as well as during electronic pacing where the electromechanical pattern is a delayed version of the electrical one (Provost et al. 2011a, 2011b, 2011c). Validation of the correlation between the EW and the underlying electrical activation during biological pacing was not performed in this study but is currently being investigated in ongoing studies.

Conclusion

Our study showed that EWI can transthoracically and noninvasively map the electrical activation of the hearts of conscious, free-breathing canines during a wide array of rhythms, whether of electrical or biological origin, endocardial or epicardial origin, and arising in the atria or either ventricle of the heart. We also showed that EWI was reproducible not only within the same heart, but also across different hearts; thus demonstrating that EWI has the potential to not only be used in longitudinal animal studies, but also to map a wide variety of arrhythmias and the treatment thereof.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. EWI flowchart

(i) High frame-rate acquisition (2000 Hz) of standard four (a) and two-chamber (b) RF images with the virtual source sequence **(ii)** Motion maps (a, b) are generated by performing RF cross-correlation (c) in the axial direction **(iii)** Negative axial incremental strains, corresponding to electromechanical activation, estimated using a least-square estimator (c) are overlaid onto the B-mode images to produce the EWI ciné-loop. (a, b) **(iv)** EWI isochrones (a, b) are obtained by mapping the local zero-crossing time of the incremental strains (horizontal red line in (iii)-c) following the onset of the P-wave (in the atria) or the onset of the QRS (in the ventricles). The 2D EWI isochrones (a, b) are subsequently combined to generate a bi-planar isochrone (c) **(v)** EWI ciné-loop bi-plane representations generated by synchronizing and combining both views. The example shown here is from a dog pre-surgery.

Figure 2. EWI isochrones of normal sinus rhythm in four dogs pre-surgery

The origins of all isochrones correspond to the onset of the P-wave. (A–D) Dogs #1, 2, 3, and 4 respectively. Activation in the atria originates from the RA and propagates to the LA. In the ventricles, arrows indicate the sites of early activation. (E–F) Details of EWI isochrones for dog #2 in the two and four-chamber views. (E) In the four-chamber view the atrial activation sequence starts from the RA and propagates in the LA. In the two-chamber view, activation reaches the anterior wall before 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 left ventricle near the base in the anterior wall and near the apex in the posterior wall, and near the apex of the right ventricle.

Figure 3. Ventricular EWI isochrones during biological 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 LV. Early sites of activation are also seen in the septum and at the apex of the RV.

Figure 4. Ventricular EWI isochrones of four dogs during electronic pacing

The origins of the isochrones correspond to the onset of the QRS. (A–C) During EPM pacing early activation is seen near the apex of the right ventricle (dogs #1, 6, and 5 respectively) or (D) at the LV apex near the septum and near the RV lateral wall apex (dog #2).

Figure 5. EWI repeatability during normal sinus rhythm

The origins of the isochrones correspond to the onset of the P-wave. (A) Four-chamber view EWI isochrones for two consecutive heart cycles. (B) Difference Map and (C) histogram of the absolute activation time difference between the two four-chamber isochrones. (D) Two-chamber view EWI isochrones for two consecutive heart cycles. (E) Difference map and (F) histogram of the absolute activation time difference between the two two-chamber isochrones.

Figure 6. EWI repeatability during electronic 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) Difference Map and (C) histogram of the absolute activation time difference between the two four-chamber isochrones. (D) Two-chamber view EWI isochrones for two consecutive heart cycles. (E) Difference map and (F) histogram of the absolute activation time difference between the two two-chamber isochrones.