Transseptal Conduction as an Important Determinant for Cardiac Resynchronization Therapy, as Revealed by Extensive Electrical Mapping in the Dyssynchronous Canine Heart

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**Background**—Simple conceptual ideas about cardiac resynchronization therapy assume that biventricular (BiV) pacing results in collision of right and left ventricular (LV) pacing–derived wavefronts. However, this concept is contradicted by the minor reduction in QRS duration usually observed. We investigated the electric mechanisms of cardiac resynchronization therapy by performing detailed electric mapping during extensive pacing protocols in dyssynchronous canine hearts.

**Methods and Results**—Studies were performed in anesthetized dogs with acute left bundle-branch block (LBBB, n=10) and chronic LBBB with tachypacing-induced heart failure (LBBB+HF, n=6). Activation times (AT) were measured using LV endocardial contact and noncontact mapping and epicardial contact mapping. BiV pacing reduced QRS duration by 21±10% in LBBB but only by 5±12% in LBBB+HF hearts. Transseptal impulse conduction was significantly slower in LBBB+HF than in LBBB hearts (67±9 versus 44±16 ms, respectively), and in both groups significantly slower than transmural LV conduction (≈30 ms). In both groups QRS duration and vector and the epicardial AT vector amplitude and angle were significantly different between LV and BiV pacing, whereas the endocardial AT vector was similar. During variation of atrioventricular delay while LV pacing, and ventriculo-ventricular delay while BiV pacing, the optimal hemodynamic effect was achieved when epicardial AT and QRS vectors were minimal and endocardial AT vector indicated LV preexcitation.

**Conclusions**—Due to slow transseptal conduction, the LV electric activation sequence is similar in LV and BiV pacing, especially in failing hearts. Optimal hemodynamic cardiac resynchronization therapy response coincides with minimal epicardial asynchrony and QRS vector and LV preexcitation. 

**Key Words:** cardiac resynchronization therapy ■ electrophysiology ■ heart failure ■ left bundle-branch block ■ pacing

In heart failure patients with abnormal ventricular conduction, cardiac resynchronization therapy (CRT) improves left ventricular (LV) systolic pump function, reverses adverse LV remodeling, and clinical symptoms and outcome. Beneficiary effects of CRT are generally considered to act between single-site LV pacing and BiV pacing. These data indicate that there is still limited understanding of the electrophysiological mechanisms underlying the hemodynamic

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At first sight, this logical concept is, however, violated by multiple observations. First, if during left bundle-branch block (LBBB) LV activation occurs from the septum to the free wall and during biventricular (BiV) pacing from both sides to each other, one would expect a decrease in electric activation of ≈50%. However, BiV pacing reduces QRS duration by less than 20%.

Second, single-site LV pacing at a short atrioventricular (AV) interval is known to increase QRS duration but provides a similar acute and chronic effect to conventional BiV pacing. Also, multiple clinical trials failed to show a different effect on cardiac function, mortality, and hospitalization between single-site LV pacing and BiV pacing. These data indicate that there is still limited understanding of the mechanism of CRT. One of the complicating factors in understanding CRT is the conduction velocity in the various parts of the ventricular wall, which is assumed to be uniform in the above-mentioned concept. In a large group of patients, activation times (AT) across the septum ranged from almost 0 to >100 ms. Septal conduction is difficult to measure and it is not clear how it would influence the effect of the various pacing modes.

The aim of the present study was to investigate the electrophysiological mechanisms underlying the hemodynamic
effects of CRT in canine hearts with LBBB, all or not in combination with heart failure. To this purpose, we related the hemodynamic effects of CRT with detailed electric activation measurements during various pacing modes.

**Methods**

Animal handling was performed according to the Dutch Law on Animal Experimentation and the European Directive for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (86/609/EU). The protocol was approved by the Experimental Animal Committee of Maastricht University.

**Experimental Setup**

The experiments were performed on 16 adult mongrel dogs of either sex and unknown age that had a weight of 29±3 kg. Animals were induced by intravenous pentothal administration and anesthetized by continuous infusion of midazolam (0.25 mg/kg per hour) and sufentanyl (3 µg/kg per hour). During sterile surgery, LBBB was induced by radiofrequency ablation. Two distinct groups were created: dogs with acute LBBB (n=10) and dogs with chronic LBBB and heart failure (LBBB+HF, n=6). Heart failure was induced by 4 weeks of tachypacing (right atrium and RV apex) and monitored by weekly echocardiography. Because the LBBB+HF model was regarded as the closest equivalent to CRT patients, AV/ventriculo-ventricular (VV) optimization was also performed in this group.

A pacing lead was transvenously inserted into the right atrium. After thoracotomy, >100 contact electrodes were placed around the heart and through the myocardial wall (see Figure 1 for experimental setup). An octopolar electrode-catheter (Duig Livewire TC, Minnetonka, MN) was used for septal mapping and RV apical pacing. LV endocardial noncontact mapping (EnSite 3000) was performed in 6 LBBB and 6 LBBB+HF dogs. Noncontact mapping analysis and validation is described in the online-only Data Supplement. Examples of matched noncontact and contact electrograms used for validation are shown in Figure 1. One 7-F catheter-tip manometer (CD-Leycom Zoetermeer, the Netherlands) was used to measure RV pressure, whereas a 7-F combined catheter-tip manometer and conductance catheter was used to measure LV pressure and volume.

**Pacing Protocol**

Recordings of baseline atrial pacing and LV/BiV pacing (DDD, at an AV interval ensuring full capture) included 2 respiratory cycles, were performed at a rate ≈10 beats per minute above the intrinsic sinoatrial rate, and were commenced after ≥20 beats to allow for steady-state measurements. To investigate the behavior of electric propagation wavefronts more extensively, the LV was also stimulated at anterior and posterior regions. CRT optimization was performed in the LBBB+HF group through LV lateral wall pacing at increasing AV intervals (30–250 ms). Sequential BiV pacing was then performed at the optimal AV interval from 90 ms LV preexcitation to 90 ms RV preexcitation.

**Measurements of Vectorcardiography, Electric Activation, and Hemodynamics**

The surface ECG was used to calculate QRS duration and maximal QRS vector amplitude (VAQRS). The VAQRS value halfway from LBBB and LV pacing at a short AV interval has been shown to predict maximal contractility response during AV/VV optimization. For cardiac mapping electrograms, local depolarization times were calculated as the time difference between onset of the Q-wave (during baseline) or ventricular pacing artifact and the time of steepest negative deflection. Using custom MATLAB software (MathWorks, Natick, MA), the depolarization times were plotted on corresponding anatomic locations and on matched interpolated models. AT were defined as the maximum depolarization time difference and were calculated for all electrodes and separately for the endocardial LV (noncontact electrodes, endocardial contact electrodes, and septum-to-free wall contact electrodes). In addition, transseptal conduction was calculated at earliest RV septal depolarization time during LV septal pacing. To express temporal and spatial asynchrony in more detail, AT vectors were calculated in the short-axis direction of the LV endocardium (ATVendo) and LV and RV epicardium (ATVepi). Larger AT vector values indicate a greater electric asynchrony and the angle of the AT vector expresses the main direction of conduction (see the online-only Data Supplement for a detailed description). Mechanical interventricular dysynchrony was determined as the time delay between normalized upslopes of simultaneously recorded LV and RV pressure curves. Hemodynamic data analysis was performed as described previously.

**Statistical Analysis**

Statistical analyses were performed using SPSS software version 18 (SPSS Inc). Continuous data were presented as mean±standard deviation, and discrete variables as counts and percentages. A series of general linear regression models were used to compare pacing modes and experimental models for the several endpoints. To account for intraindividual correlation of measurements (panel data), Huber–White robust standard-errors were calculated. In case of significant overall differences, the level of significance for specific comparisons was calculated using Wilcoxon signed-rank test or Mann–Whitney U test for dependent and independent observations, respectively. No adjustments were made for multiple comparisons. Correlations were evaluated with Pearson correlation coefficient. A P value <0.05 was considered statistically significant.

**Results**

In the LBBB+HF group, LV ejection fraction decreased from 56±6% at baseline to 15±2% after 4 weeks of rapid pacing. LVDp/dtmax was almost 50% lower in the LBBB+HF than in the acute LBBB group (P<0.05 for both comparisons).

**Effects of Pacing on Conduction**

For all LBBB hearts, QRS duration was ≈120 ms, which was significantly increased by LV pacing. BiV pacing reduced QRS duration by 21±10% in the LBBB hearts, but only by 5±12% in the LBBB+HF hearts (P<0.05 for both comparisons; Figure 2). Figure 3 shows examples of electrode depolarization times plotted at corresponding anatomic locations (top) and on interpolated models (bottom). During LBBB (left), the electric wavefront originated in the RV and slowly
propagated through the interventricular septum toward the basolateral wall of the LV. During LV pacing (middle), activation of the RV side of the septum and the RV free wall was delayed, whereas during BiV pacing (right), 2 wavefronts are visible. However, the LV endocardial electric activation pattern was similar during LV and BiV pacing (Figure 3).

The reduction in QRS duration by BiV pacing was mainly attributable to a more homogeneous epicardial depolarization as both contact and noncontact mapping showed that LV endocardial AT did not decrease during BiV pacing (Table I in the online-only Data Supplement). This is further substantiated by LV epicardial depolarization times at the septum and the opposing free wall as shown in Figure 4. During both LV and BiV pacing, the LV septal endocardium was activated consistently later than the LV free wall. This finding is explained by measurements of true transseptal conduction, as measured during LV septal pacing. Transseptal AT was significantly longer than transmural free wall conduction time.

Moreover, in the LBBB+HF group, transseptal conduction was ≈50% more delayed than in the acute LBBB group (67±9 versus 44±16 ms, respectively; P<0.02). This transseptal conduction slowing occurred despite the fact that the septum was significantly thinner in the LBBB+HF than in the LBBB hearts as measured by M-mode echocardiography (0.86±0.12 versus 1.31±0.09 cm; P<0.05). A significant inverse correlation was observed between transseptal conduction time and the percentage reduction in QRS duration (R=0.61, P<0.05).

**Epicardial and Endocardial Activation Vectors**

Typical examples of ATV_epi and ATV_endo are shown in Figure 5. During baseline LBBB, ATV_endo and ATV_epi point away from the early activated septum toward the late activated LV free wall (≈180°), indicating predominant RV-to-LV conduction. By pacing the LV lateral wall, the AT vectors shifted away from this region and pointed toward the RV. During simultaneous BiV pacing, ATV_epi angle and amplitude were in-between those for LBBB and LV pacing. However, ATV_endo angle and amplitude were comparable for BiV and LV pacing. Figure 6 shows that the switch from LV pacing to BiV pacing caused clear changes in the direction of the ATV_epi angles, but not for ATV_endo angles. The latter is also expressed by the high correlation between ATV_endo during LV and BiV pacing (LBBB: R²=0.76; LBBB+HF: R²=0.91; P<0.05 for both correlations).

**Hemodynamic Effects of Pacing**

Both BiV and LV pacing resulted in a significant improvement in LVdP/dt_max compared with baseline atrial pacing (P<0.05; Figure 2). BiV pacing resulted in a larger LVdP/dt_max increase than LV pacing (P<0.05) in the LBBB dogs. In the LBBB+HF group, BiV pacing and LV pacing both resulted in ≈21% increase in LVdP/dt_max (P=N.S. between BiV and LV pacing).
AV and VV Optimization

Figure 7 presents examples of electric maps made during LV pacing at various AV intervals. At an AV interval of 70 ms, the activation was entirely dominated by LV pacing, resulting in large $ATV_{epi}$ and $ATV_{endo}$ amplitudes. At an AV interval of 130 ms, intrinsic activation through the right bundle branch caused synchronous activation of the RV, resulting in a minimal $ATV_{epi}$ amplitude of 9 ms. However, at this AV interval, $ATV_{endo}$ amplitude was not changed yet, because of the lack of transseptal breakthrough. This breakthrough occurred at the AV interval of 170 ms where $ATV_{endo}$ amplitude was minimal. Conversely, at this AV interval, the intrinsic wavefront fully activated the RV and a large part of the anterior and posterior wall of the LV, thereby increasing $ATV_{epi}$ to 38 ms. At an AV interval of 210 ms, LV capture was lost entirely resulting in a LBBB activation pattern.

Figure 8A displays the changes in $ATV_{epi}$, $ATV_{endo}$, and vector amplitude of the QRS complex of the surface ECG ($VA_{QRS}$) during pacing at various AV intervals (during LV-only pacing) and VV intervals (during BiV pacing). The optimal $LVdP/dt_{max}$ consistently occurred at an interval where $ATV_{epi}$ and $VA_{QRS}$ were at values halfway between those during LBBB and LV pacing, supporting data from a previous study. During AV optimization, the average interval offset was $±11$ ms for $VA_{QRS}$ and $−9±24$ ms for $ATV_{epi}$ ($P$=N.S. for both comparisons). During VV optimization, maximal response was predicted with an offset of $−4±9$ ms for $VA_{QRS}$ and $2±8$ ms for $ATV_{epi}$ ($P$=N.S. for both comparisons). In contrast, halfway $ATV_{endo}$ vector amplitudes occurred during longer AV intervals and during RV preexcitation (in VV optimization). Figure 8B shows that for all AV and VV optimizations there was a significant difference between the optimal AV/VV interval and the AV/VV interval resulting in minimal $ATV_{endo}$ amplitude. Although these $ATV_{endo}$ values were derived from noncontact mapping, similar findings were obtained using septum and lateral wall plunge electrodes. Figure II in the online-only Data Supplement shows the values of $LVdP/dt_{max}$, programmed A-LV interval, $ATV_{epi}$, $ATV_{endo}$, and $VA_{QRS}$ during LBBB, LV pacing at short AV interval, optimal AV interval, and optimal VV interval. Optimizing the AV interval during LV pacing resulted in an average $LVdP/dt_{max}$ increase to $≈26$% above baseline LBBB ($P$<0.05 as compared with baseline and LV pacing at short AV interval). Optimizing the VV interval increased...
LVdP/dr max to ≈33% above baseline LBBB (P<0.05 as compared with baseline and simultaneous BiV pacing).

**Discussion**

The findings of the present animal study shed new light on the electric mechanisms of CRT. For the first time it is demonstrated that BiV pacing does not result in collision of pacing-induced wavefronts in between the septum and the LV free wall due to slow transseptal conduction. Septal conduction is slower than LV transmural conduction in hearts with acute LBBB and even more prolonged in failing LBBB hearts. Slow septal conduction is associated with a relatively small reduction in QRS duration by BiV pacing, as seen in clinical CRT trials. Optimal hemodynamic CRT response during LV fusion or sequential BiV pacing is achieved under conditions where epicardial RV+LV asynchrony is minimal, which coincides with a left-to-right activation of most of the LV wall.

**Slow Transseptal Conduction**

Information about transseptal conduction in LBBB hearts in the literature is scarce, because most electric mapping studies measure either the epicardium or the endocardium. Vassallo et al measured transseptal conduction by determining the time from onset of Q-wave to LV breakthrough during catheter mapping, whereas Auricchio et al used BiV contact mapping. These investigators showed a wide range of transseptal conduction times in patients, from almost 0 to ≈100 ms. Interestingly, there seems to be a separation between 30% of this patient population with very short and 70% with long transseptal conduction times. The long transseptal conduction times observed seem to be in line with the delayed septal conduction found in dogs with proximal LBBB. Importantly, we show that conduction in the septum slows down further during 4 weeks of dyssynchronous heart failure. As for the possible cause of this transseptal conduction slowing, factors such as ischemia and hypertrophy are unlikely to play a role in the tachypacing model. A possible explanation for slow septal conduction may be a more vertical orientation of the laminar sheets with myocytes, as demonstrated by Helm et al in the same dyssynchronous heart failure model. Preliminary results from our laboratory show that in 7 dogs with 4 months of LBBB and normal sinus rhythm, endocardial LV

**Figure 6.** Angles of ATV_{epi} (top) and ATV_{endo} (bottom) during biventricular (BiV) pacing (vertical axis) plotted as a function of the corresponding angles during left ventricular (LV) pacing (horizontal axis) in acute left bundle-branch block (LBBB) dogs (left) and chronic LBBB and heart failure (LBBB+HF) dogs (right). ATV indicates activation times vector; endo, endocardium; and epi, epicardium.

**Figure 7.** Examples of 3-dimensional electric depolarization time maps and ATV_{epi} and ATV_{endo} behavior during left ventricular (LV) pacing at increasing atrioventricular (AV) intervals in a chronic left bundle-branch block and heart failure (LBBB+HF) heart.
septum AT during BiV pacing were similarly increased as in the LBBB+HF group. This suggests that slow transseptal conduction may be related with longer-lasting asynchrony, rather than tachypacing-induced dilation. Spach et al\textsuperscript{18} observed that cell-cell delay increases with decreasing cell diameter, a situation that occurs in the early activated regions of dyssynchronous hearts (in our case, the septum)\textsuperscript{19}. Data in the literature on a possible role of remodeling of ion channels, such as connexins and sodium channels, on slowing of septal conduction are scarce. This is primarily due to the fact that only the LV lateral or anterior wall was investigated\textsuperscript{20,21}. An article reporting on RV and LV samples showed no significant change in Cx43 and SCN5A expression in RV-paced rabbits (comparable with LBBB) as compared with control rabbits\textsuperscript{22}.

The present findings on transseptal conduction may explain several hemodynamic aspects of CRT. First of all, several patient studies and data derived from a canine model of dyssynchronous heart failure showed that LV and BiV pacing may have the same hemodynamic benefits\textsuperscript{2,5,6,23}. Findings in this study may also explain the paradoxical findings by Leclercq et al\textsuperscript{23} who showed that in an animal model similar to our LBBB+HF model, LV and BiV pacing results in similar pump function and even regional mechanics despite a dyssynchronous electric activation. The mismatch between their electric and mechanical measurements may be explained by the fact that local strains can be considered as an average of transmural mechanics, whereas electric dyssynchrony was measured exclusively at the epicardium. Slow septal conduction causes, under several conditions, a dissociation between epicardial and endocardial conduction patterns as illustrated by the changes in ATV\textsubscript{epi}, but not ATV\textsubscript{endo}, when comparing LV pacing with BiV pacing. The observation that the changes in ATV\textsubscript{epi} correspond with those in the QRS vector of the surface ECG indicates that the latter mainly represents epicardial electric phenomena.

The fact that LV and BiV pacing at a relatively short AV interval provide a similar increase in pump function does not mean that this is the maximal improvement to be achieved. The present study shows that the largest hemodynamic improvement is achieved during optimal interventricular resynchronization, coinciding with LV preexcitation, which agrees with earlier observation in our canine model and clinical studies\textsuperscript{4,12,24}.

**Potential Clinical Implications**

The main implication of the present study is that it provides a mechanism why contribution of RV pacing in simultaneous BiV pacing is negligible. This has already been suggested during fusion LV pacing, but it may also hold for LV pacing at shorter AV intervals, especially in failing hearts. The present study shows that the BiV epicardium and LV endocardium may contain considerably different electric information. Although detailed LV endocardial mapping unravels similarities between pacing modes, epicardial mapping seems to

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**Figure 8.** A, Typical example of relation between atrioventricular (AV) interval (left) or ventriculo-ventricular (VV) interval (right) and change in LVD\textsubscript{p}d\textsubscript{max}, QRS vector amplitude (VA\textsubscript{QRS}) from the surface ECG, ATV\textsubscript{epi}, and ATV\textsubscript{endo} measured in a chronic left bundle-branch block and heart failure (LBBB+HF) heart. The dashed line represents the optimal AV/VV interval (the setting that resulted in the maximal increase in LVD\textsubscript{p}d\textsubscript{max}). B, Average offset between predicted and observed optima for AV and VV optimization. *\(P<0.05\) compared with observed optimal setting (Wilcoxon signed-rank test). ATV indicates activation times vector; BiV, biventricular; endo, endocardium; epi, epicardium; LV, left ventricular; and RV, right ventricular.
provide more useful information in CRT optimization. This is important in the light of the increasing use of endocardial mapping\textsuperscript{16,25} and ECG imaging for epicardial mapping.\textsuperscript{26,27} In addition, further support is provided that morphology of the QRS complex of the surface ECG is determined primarily by epicardial electric phenomena. This finding is important also for practical reasons because ECG vectorcardiography and ECG imaging are noninvasive, low cost, and widely available.

Limitations

A limitation of this study is that it was performed in canine hearts and, therefore, should be extrapolated to the situation in patients with caution. However, in the decade that this model exists,\textsuperscript{4} several principles of CRT have been proven applicable to patients, such as the remodeling processes both at the tissue\textsuperscript{28} and the molecular\textsuperscript{29} level and optimization of AV and VV delays.\textsuperscript{4} Due to the smaller heart size in dogs, there are quantitative differences between the dog and human heart, but the qualitative effects may well apply to the human heart. This study investigated acute hemodynamic improvement although these effects may not (completely) translate into chronic outcome.\textsuperscript{30} However, the GREATER-EARTH trial reported no differences in reverse remodeling between pacing modalities after 6 months, suggesting that acute similarities may persist into the chronic phase.\textsuperscript{6}

Conclusions

Through simultaneous endocardial and epicardial electric mapping in dysynchronous canine hearts, we established that transseptal conduction in LBBB hearts is slow, especially in failing hearts. Due to this slow transseptal conduction, BiV pacing does not result in collision of RV and LV pacing–induced wavefronts within the LV wall, and the LV activation sequence is not different between LV and BiV pacing. Optimal hemodynamic CRT response is achieved when interventricular asynchrony is minimal, which coincides with LV preexcitation.

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Disclosures

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References


**CLINICAL PERSPECTIVE**

Simple conceptual ideas about cardiac resynchronization therapy assume that biventricular pacing results in collision of right and left ventricular (LV) pacing–derived wavefronts. However, this concept is contradicted by 2 observations from clinical trials: (1) the minor reduction in QRS duration during biventricular pacing, and (2) the comparable effects of biventricular pacing and LV pacing. We performed detailed simultaneous endocardial and epicardial mapping in dys synchronous canine hearts with and without tachypacing-induced heart failure. The data demonstrate that conduction across the septum takes 60% to 75% of total ventricular activation time. As a consequence, (1) biventricular pacing does not result in collision of pacing-induced wavefronts between the septum and the LV free wall, but rather within the septum, (2) LV and biventricular pacing have comparative hemodynamic effects, especially in failing hearts, and (3) endocardial activation maps are similar for LV and biventricular pacing. However, optimal hemodynamic cardiac resynchronization therapy response during LV fusion or sequential biventricular pacing is achieved under conditions where epicardial (but not endocardial) right ventricular+LV dys synchrony is minimal, which coincides with a left-to-right activation of most of the LV wall. These findings may assist in further understanding electrophysiological mechanisms of cardiac resynchronization therapy.
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SUPPLEMENTAL MATERIAL

Methodology of non-contact mapping and validation with direct contact mapping

Non-contact mapping was performed using a multi-electrode array (MEA); a network of 64 polyimide-insulated wires with laser-etched electrodes mounted on a 9 French catheter with inflatable balloon. The MEA was deployed in the LV through a retro-aortic approach. To construct a geometrical map of the endocardium, a standard electropotential (EP) catheter was inserted into the heart chamber under fluoroscopy and moved throughout the chamber whilst the ring electrodes of the MEA localized its distal tip during diastole. The most distant locations of the acquired positions were included, disposing of intracavitary locations. The non-contact mapping system then combined the smoothed endocardial contour and the far-field potentials as measured on the MEA to calculate virtual electrograms as if derived from the endocardium. Subsequently, the virtual electrograms measured by the MEA were ‘drawn’ on the superimposed grid. To enable comparison to direct contact mapping, custom-made plunge electrodes were inserted through the LV myocardium at the apex, lateral apex and at anterior, lateral and posterior sites at the base and mid level. The location of these endocardial electrodes were also localized by the MEA and stored. Baseline (atrial pacing) measurements were followed by simultaneous biventricular pacing (RV apical pacing) and single-site LV pacing. The LV was paced at the epicardium at anterior, lateral and posterior regions of basal and mid LV, apicolateral and apical. The atrioventricular interval was programmed to guarantee complete capture of ventricular activation from the pacing electrodes and atrial pacing occurred at a rate ~10 beats higher than intrinsic heart rate. To avoid possible interference with intraventricular measuring systems, hemodynamic measurements and non-contact mapping were performed separately. For each measurement, 2048 non-contact and 8 contact endocardial electrograms and their locations (respective to the MEA center) were exported. As the EnSite® sampling rate was 1200 Hz; every data point represented 0.833 milliseconds. Using a custom algorithm (Matlab R2007a), the virtual electrograms were converted to 1000 Hz (equal to the epicardial mapping system). The electrograms were imported into standard analyzing software (Microsoft Excel 2007) and the moment of local depolarization was determined by calculating the point of maximum negative deflection (max – dV/dT). For all cardiac mapping electrodes, depolarization times were calculated as the time difference between onset of the Q-wave (during baseline) or ventricular pacing artifact (during pacing) and the time of steepest negative deflection in the electrogram.

The validation study compared endocardial contact and matched non-contact electrodes and consisted of two parts: 1) calculating the depolarization timing difference and 2) cross correlating the electrogram morphologies of ventricular deflections. Depolarization time difference was expressed by average
absolute difference ± S.D. and by the method suggested by Bland and Altman: non-absolute mean difference as 'bias' and upper and lower limits of agreement defined as 'bias ± 1.96 SD'. For the morphological validation, a 120 millisecond timeframe was selected including the ventricular deflection ("QRS" complex) from the contact and non-contact electrogram. A cross-correlation was then performed by shifting signals in time to assure maximum QRS morphology matching.

**Results of Validation Analysis**

Out of the 1920 possible comparisons between contact and non-contact electrodes (10 dogs X 3 pacing modes X 8 LV epicardial pacing sites X 8 electrode pairs), depolarization time difference was calculated for 1766 electrode pairs (92%). Unsuccessful data acquisition was related to problems with contact mapping: unacceptable quality of electrogram or non-endocardial location of plunge electrode at post mortem evaluation.

For all experiments, mean absolute depolarization time difference was 9.66±7.23 ms with a bias of -2.77 ms (limits of agreement ±23.03 ms). The negative bias signified slight earlier estimation of depolarization times with non-contact mapping as compared with contact mapping. There was no significant difference in activation time difference between LBBB or LBBB+HF dogs, pacing modes, LV pacing sites or location of electrode pairs. For all experiments, the morphologic cross correlation between contact and non-contact electrograms was 0.87±0.12. Figure 1 shows typical examples of morphology cross correlation between ventricular deflections of contact electrodes (left panels) and non-contact electrodes (right panels). Also for morphologic cross correlation, there was no significant difference between the models, pacing modes, LV pacing sites or location of electrode pairs.

**Discussion of Validation Analysis**

The depolarization time difference (both absolute, bias and limits of agreement) and morphological cross-correlation results are indicative of good agreement between the methods and suggest that non-contact mapping adequately registers endocardial activation. Morphologic cross correlation between contact and non-contact electrograms from the current study matched well with earlier human validation studies (0.88±0.15 and 0.83±0.16).\(^1\)\(^2\) In the only validation study performed in canine hearts, morphology cross correlation was 0.97.\(^3\) This result is higher than current and older validation studies and might be explained through the limited variation in LV pacing regions used in the study (only anterior and anterolateral regions). Schilling et al calculated a non-absolute average timing difference of −1.94±7.12 ms for distances at <34 mm (from electrode to MEA), −14.16±19.29 ms at >34 mm and for nonequatorial points electrogram timing difference was −8.97±15.75 ms.\(^1\) Even though our results were not grouped as according to the distance to the MEA, overall bias was comparable and also showed
slightly earlier estimation of depolarization of non-contact mapping. Thiagalingam et al performed a validation study comparing thousands of electrograms in paced ovine left ventricles and found a mean absolute activation timing difference of 4.3±3.4 ms which shows higher accuracy compared with the current experiment. This may partly be explained by their use of 50-electrode grids which allows more detailed defining of the LV endocardial contour. In addition, a portion of contact electrograms in the our experiment were subjected to voltage clipping. This introduced possible error in the allocation of depolarization time in the case of possible fractionated electrograms (underestimation).

**Activation Time Vectors**

In addition to calculating activation times, AT vectors were calculated in the short-axis direction to express temporal and spatial asynchrony in more detail. Using the center of the LV as a reference, for each electrode a sub-vector was appointed using the depolarization time as amplitude and anatomical location for direction. The LV and RV epicardial contact sub-vectors were then summed to construct the epicardial AT vector \((\text{ATV}_{\text{epi}})\) and the LV endocardial non-contact sub-vectors were summed for constructing the endocardial AT vector \((\text{ATV}_{\text{endo}})\). AT vectors measure the spatial imbalance indicative of electrical depolarization between opposite sides. Larger AT vector values indicate a greater electrical asynchrony and the angle of the AT vector expresses the main direction of conduction. Angles were measured from the reference vector between the LV free wall \((180^0)\) and RV free wall \((0^0)\) and anterior angles were considered positive.

Supplemental table 1: Electrical and hemodynamic data (means±S.D.) from dogs with LBBB or chronic LBBB+HF during baseline LBBB (atrial pacing), LV pacing and BiV pacing at short AV-interval.

<table>
<thead>
<tr>
<th></th>
<th>acute LBBB (n=10)</th>
<th>LBBB+HF (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline (LBBB)</td>
<td>LV pacing</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>116±17</td>
<td>118±11</td>
</tr>
<tr>
<td>QRS-duration (ms)</td>
<td>118±12</td>
<td>138±13*</td>
</tr>
<tr>
<td>LV endocardial AT (non-contact, ms)</td>
<td>37±7</td>
<td>40±10</td>
</tr>
<tr>
<td>LV endocardial AT (contact, ms)</td>
<td>45±14</td>
<td>43±14</td>
</tr>
<tr>
<td>epicardial AT vector length (ms)</td>
<td>64±5</td>
<td>59±20*</td>
</tr>
<tr>
<td>endocardial AT vector length (ms)</td>
<td>19±11</td>
<td>15±8*</td>
</tr>
<tr>
<td>LV peak-systolic pressure (mmHg)</td>
<td>93±16</td>
<td>88±12</td>
</tr>
<tr>
<td>LV $dP/dt_{max}$ (mmHg/s)</td>
<td>1531±290</td>
<td>1637±377*</td>
</tr>
<tr>
<td>LV $dP/dt_{min}$ (mmHg/s)</td>
<td>-1340±268</td>
<td>-1295±269</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mmHg)</td>
<td>7±3</td>
<td>6±4</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>26±5</td>
<td>30±9*</td>
</tr>
<tr>
<td>SW (mmHg*ml)</td>
<td>2199±500</td>
<td>2433±908</td>
</tr>
<tr>
<td>Mechanical interventricular dyssynchrony (ms)</td>
<td>-28±9</td>
<td>7±22*</td>
</tr>
</tbody>
</table>

BiV, biventricular; LV, left ventricular; AT, activation time; *p<0.05 versus corresponding LBBB baseline. †p<0.05 versus LV pacing.
Supplemental Figure Legends

**Figure 1.** Examples of morphology cross correlation of ventricular deflection ("QRS") between contact (CM) and non-contact (NCM) mapping. The top QRS-complexes show higher cross correlation (0.92 vs. 0.70), but the characteristic shapes can be recognized in both examples. Other examples of electrograms are shown in Figure 1.

**Figure 2.** Average changes in LVdP/dt\textsubscript{max} (%), VA\textsubscript{QRS} (ms), ATV\textsubscript{epi} (ms), ATV\textsubscript{endo} (ms) and PQ duration (ms) during LV-pacing at short AV-interval (AV\textsubscript{short}), optimized AV-interval (AV\textsubscript{opt}), optimized VV-interval (VV\textsubscript{opt}) and LBBB in hearts with LBBB+HF. During the optimal AV/VV-interval, ATV\textsubscript{endo} amplitude was similar to LV pacing at short AV-interval while ATV\textsubscript{epi} and VA\textsubscript{QRS} were halfway between LV pacing and LBBB. *p<0.05 as compared with AV\textsubscript{short}, †<0.05 as compared with LBBB.