Direct Proof of Endo-Epicardial Asynchrony of the Atrial Wall During Atrial Fibrillation in Humans

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Background—The presence of focal fibrillation waves during atrial fibrillation (AF) can, besides ectopic activity, also be explained by asynchronous activation of the atrial endo- and epicardial layer and transmurally propagating fibrillation waves. To provide direct proof of endo-epicardial asynchrony, we performed simultaneous high-resolution mapping of the right atrial endo- and epicardial wall during AF in humans.

Method and Results—Intraoperative mapping of the endo- and epicardial right atrial wall was performed during (induced) AF in 10 patients with AF (paroxysmal: n=3; persistent: n=4; and longstanding persistent: n=3) and 4 patients without a history of AF. A clamp made of 2 rectangular 8x16 electrode arrays (interelectrode distance 2 mm) was inserted into the incision in the right atrial appendage. Recordings of 10 seconds of AF were analyzed to determine the incidence of asynchronous endo-epicardial activation times (≥15 ms of opposite electrodes. Asynchronous endo-epicardial activation ranged between 0.9 and 55.9% without preference for either side. Focal waves appeared equally frequent at endocardium and epicardium (11% versus 13%; P=0.18). Using strict criteria for breakthrough (presence of an opposite wave within 4 mm and ≤14 ms before the origin of the focal wave), the majority (65%) of all focal fibrillation waves could be attributed to endo-epicardial excitation.

Conclusions—We provided the first evidence for asynchronous activation of the endo-epicardial wall during AF in humans. Endo-epicardial asynchrony may play a major role in the pathophysiology of AF and may offer an explanation why in some patients therapy fails. *(Circ Arrhythm Electrophysiol. 2016;9:e003648. DOI: 10.1161/CIRCEP.115.003648.)*

Key Words: atrial fibrillation ■ cardiac conduction defect ■ epicardial mapping ■ heart atria ■ humans

Epicardial high-density mapping in patients with atrial fibrillation (AF) and valvular heart disease has demonstrated that a considerable portion of fibrillation waves showed a focal spread of activation.1 These focal waves were rarely repetitive and mainly appeared as solitary events. They could occur virtually everywhere in the atria, and their coupling interval was often longer than the dominant AF cycle length. In addition, unipolar electrograms recorded at the origin of focal waves, exhibited small R-waves. Based on this indirect evidence, it was postulated that fibrillation waves with a focal pattern of activation could result from endo-epicardial breakthrough.1 Because endo-epicardial breakthroughs can only occur in the presence of electric asynchrony between the endo- and epicardial layer, we hypothesized that the substrate of AF consists of layers of dissociated fibrillation waves that constantly feed each other.1 To demonstrate that endo-epicardial asynchrony (EEA) exists during AF, we performed simultaneous high-resolution, mapping of the endo- and epicardial wall of the right atrium in patients with or without a history of AF undergoing cardiac surgery for coronary artery disease and valvular heart disease.

Methods

Study Population

The study sample consisted of 14 patients (10 men; 67±8.3 years) without a history of AF (n=4) and with a history of AF (n=10). Surgical procedures that were performed included cardiac coronary bypass surgery (n=9), mitral valve surgery (n=7), aortic valve replacement (n=2), and tricuspid valve surgery (n=4). Three patients had paroxysmal AF, 4 persistent AF, and 3 had persistent AF lasting longer than a year. Atrial enlargement was present in 5 patients. Clinical characteristics of the study population are provided in Table 1. The mapping protocol was approved by the institutional ethical committee (MEC2010-054), and written informed consent was
Intraoperative Mapping Procedure
The mapping study was performed immediately after sternotomy. After heparinization and arterial cannulation, a temporary bipolar epicardial pacemaker wire was stitched to the right atrial free wall and served as a temporal reference electrode. The indifferent electrode consisted of a steal wire fixed to subcutaneous tissue of the thoracic cavity.

If patients were in sinus rhythm at the onset of the mapping procedure, AF was induced by fixed rate pacing at the right atrial free wall using an additional temporary bipolar pacing wire. The induction protocol started at a rate of 200 beats per minute. If induction was not successful after 3 burst attempts, the rate was increased by 50 beats per minute, up to maximal 400 beats per minute until AF occurred or atrial refractoriness was reached.

Before commencement to extracorporal circulation, a high-resolution endo-epicardial mapping clamp was introduced through the right atrial incision for the venous cannula and closed with a purse-string suture. The mapping device was positioned toward the crista terminalis and consisted of 2 identical rectangular electrode arrays of 8×16 electrodes (interelectrode distance 2 mm) positioned opposite to each other (Figure 1). The electrode arrays (GS Swiss PCB AG, Küsnacht, Switzerland) consist of an electroless nickel immersion gold–plated electrode array, mounted on a thin, flexible DuPont Pyralux copper-clad (25-μm thickness) polyimide laminate, and coverlay composite (25 μm) film (0.18 mm). As the space constant of the atrial myocardium is ≈2 mm, the effective spatial resolution of 2.0 mm makes it unlikely that narrow fibrillation wavefronts will not be detected.

All recordings were amplified (gain 1000), filtered (bandwidth 0.5–400 Hz), sampled (1 kHz), and analogue to digital converted (12 bits). A calibration signal of 2-mV amplitude and 1000-ms pulse width was stored simultaneously with atrial electrograms on hard disk using a computerized mapping system. After completion of the mapping procedure, AF was terminated by electric cardioversion or sustained until cardioplegia was conducted, depending on the operators' preference. Ten seconds of AF were recorded from every patient.

Table 1. Clinical Characteristics

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</table>

AF indicates atrial fibrillation; AV, aortic valve replacement; CABG, coronary artery bypass grafting; F, female; LA, left atrial; LSPAF, longstanding persistent atrial fibrillation; LVF, left ventricular function; M, male; MVP mitral valve plasty; RA, right atrial; and TVP, tricuspid valve plasty.
all endo- and epicardial recording sites. The combined asynchrony map (Figure 2, lower right panel) shows the longest time delay for every endo-epicardial electrode couple.

Wavemapping was used to identify the individual fibrillation waves. The starting point of the first fibrillation waves was the earliest activated site within the mapping area. Next, the entire mapping area was scanned in steps of 1 ms. For all electrodes activated during every step, the shortest time difference with the 8 neighboring electrodes was calculated. When the time difference was ≤12 ms (17 ms for oblique distances), the electrode site was added to the territory of the surrounding wave. In case of a time difference of >12 ms, the electrode was annotated as the starting point of a new wave. In the wavemap, fibrillation waves are color-coded according to their moment of entrance in the mapping area, and the colors demarcate the area activated by that specific fibrillation wave.4

Wavemaps also identify focal waves at either the endo- or the epicardial surface. Focal fibrillation waves had to meet previously defined criteria. The breakthrough site of the focal fibrillation wave had to be located at least 2 electrodes away from the border of the mapping array and at least 1 reliable activation time should be available between the breakthrough site and the border of the mapping area. The morphology of the electrograms in the breakthrough region should not be distorted by large QRS complexes or artifacts. If this is the case, the wave is excluded from the analysis. The focal wave should at least cover 4 electrodes. The origin of a focal wave had to be activated earlier than all surrounding electrodes. If electrodes adjacent to the origin were activated simultaneously, all electrodes surrounding this area should also be activated later. Shift of the focal activation time to a maximum of 3 ms earlier or later at the earliest activated electrode(s) should not result in disappearance of the focal wave. If a breakthrough site emerged along the border of another fibrillation wave, the time delay between that wave and the origin of the breakthrough had to be at least 40 ms.4 All these criteria were checked manually by 2 independent investigators. A more detailed description of the mapping criteria with examples is provided in the Data Supplement.

To assess whether focal fibrillation waves could originate from endo-epicardial breakthrough, in each case, the opposite layer was examined for the presence of a fibrillation wave that could have served as a source for the focal wave. The presence of an opposite wavefront, within 4-mm distance and <15 ms before the origin of the focal wave, was considered to reflect transmural conduction based on normal atrial conduction properties. In this case, the focal wave could from a theoretically point of view be attributed to endo-epicardial breakthrough.

Statistical Analysis
The Wilcoxon signed-rank test was performed to assess the occurrence of EEA and focal waves between the epi- and the endocardium.

Results
Endo-Epicardial Asynchrony
In the entire study population, the average percentage of missing data caused by poor contact of the mapping array was
7.8 ± 4.9%. The amount of conduction block was similar in the epicardial and endocardial plane with incidences of, respectively, 10.8 ± 5.1% and 10.8 ± 4.6%. Simultaneous epi- and endocardial wavemaps of a single AF cycle recorded in a patient with longstanding persistent AF demonstrating EEA are shown in Figure 3. In this example, marked differences in activation patterns of the endo- and epicardial wall existed; almost all fibrillation waves at the endo- and epicardial surface appeared at different times and propagated in different directions.

In this same patient, the spatio-temporal variation in EEA during 10 seconds of AF is demonstrated in Figure 4. The degree of EEA varied considerably at different locations and at different times. No clear predominance of either the endocardial or the epicardial layer was observed. Examples of unipolar electrogram pairs around the plots illustrate the high spatio-temporal variation in EEA.

As demonstrated in Figure 5, the total degree of EEA in our study population varied widely between 0.9% and 55.9%, and there was no clear difference between endo-epicardial and epicardial asynchrony (7.8 ± 7.7% and 7.2 ± 7.2%, respectively).

### Focal Fibrillation Waves

In total, 1199 focal fibrillation waves were observed: 579 arising at the subendocardium and 620 at the subepicardium. The equal distribution of focal fibrillation waves between both sides is shown in Figure 5C. Applying the previously stated strict criteria for endo-epicardial breakthrough, 784 of all 1199 focal waves (65%) could be attributed to result from endo-epicardial excitation presuming that normal conduction occurs between endo-epicardium (66% of the endocardial and 65% of the epicardial focal waves; Table 2). Examples of pairs of endo-epicardial wavemaps showing focal fibrillation waves originating from endo-epicardial breakthrough of fibrillation waves from the opposite layer are given in Figure 6.

### Discussion

Despite the relatively small number of patients, our data clearly show that a significant degree of EEA is present in the right atrium in patients with AF. Simultaneous endo-epicardial mapping of isolated canine atria, both during sinus rhythm and atrial pacing, only showed small differences in activation times (< 1 ms). However, during atrial tachyarrhythmias, activation of the endo- and epicardial layers has been shown to become more asynchronous (< 25 ms), particularly in the thicker parts of the atria. The concept that endo-epicardial dissociation might play an important role in the maintenance of AF stems from experimental studies in the goat model of persistent AF. These studies showed that the endo- and epicardial layers of the atrial wall became progressively dissociated during the first 6 months of AF. After that time, fibrillation waves in the endo- and epicardial layers often propagated at different speed and in different directions, and endo-epicardial breakthroughs became more abundant.

In this study, the incidence of EEA tended to be higher in patients with AF although we did not observe a clear relation between duration of AF and degree of EEA. This can be explained by the fact that we did not map the left atrium and that if EEA of the left atrial wall also exists, it may play a more important role in the pathophysiology of AF. Also, our study population contained a small number of patients with a variety of cardiac diseases.

We provided additional data that most focal fibrillation waves could be explained by endo-epicardial excitation. Lee et al. also frequently observed focal fibrillation waves without any sustained focal activity in 18 patients with persistent AF. In contrast, low-density mapping studies using a 64-pole basket catheter, have suggested that AF is maintained by a limited number of rapid stable sources (rotors and/or ectopic foci). Body surface mapping during AF elucidated the presence of nonsustained reentries and focal breakthroughs in certain domains of the atria. Recently, we have discussed the discrepancies in the interpretation of high- and low-resolution mapping of AF in detail in a crosstalk articles.

The present study supports the concept that during AF, the endo- and epicardial layers of the atrial wall can be asynchronously activated. The presence of dissociated layers of fibrillation waves will highly stabilize the fibrillatory process because as soon as fibrillation waves die out, they can be replaced by breakthroughs from the opposite side.
In such a substrate, each layer will serve as a multisite generator for the other layer. During 10 seconds of AF, >20 to 30 focal waves appeared at each side of the atrial wall, in an area of only 2.6 cm. Extrapolating this number to the entire atrial surface, the total number of focal breakthrough waves can be estimated to exceed 10,000 per minute. However, we...
like to emphasize that the presence of EEA, of course, does not disprove that also reentry and focal activity may contribute to the maintenance of AF. In different stages of the development of a substrate of AF, the contribution of different mechanisms for perpetuation of AF may vary. We fully acknowledge the large body of evidence that reentrant and focal mechanisms are operative during AF.17–20 In fact, not all focal fibrillation waves in our patients could be attributed to endo-epicardial breakthrough, and sometimes 2 focal waves appeared simultaneously at the endo- and epicardial surface. Equally, our data do not rule out the possibility that some of the endo-epicardial breakthroughs formed a part of a transmural reentrant circuit. However, we venture to suggest that progressive, AF-induced, structural atrial remodeling gradually transforms the atrial wall into multiple layers of narrow dissociated wavelets. With time, more and more focal breakthroughs will be generated, which progressively stabilizes the fibrillatory process. At the end, the main source of fibrillation waves is formed by an abundant number of focal breakthroughs, occurring virtually everywhere in the atria. This is in agreement with the recent finding of Haissaguerre et al14 that the number of driver regions increased with the duration of AF, until after 6 months almost the entire atrial wall acted as a driver (6 of all 7 regions). It also explains why the termination rate of AF by driver ablation sharply declined after 6 months of AF.14

### Limitations

Our study population is presently limited to 14 patients and a larger number of patients is obviously required for a meaningful statistical analysis and to study the relation between persistence of AF and the degree of electric asynchrony. Expanding the study population will also allow for statements about a possible correlation between EEA and breakthroughs. Moreover, the effective spatial resolution of the recordings is

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<th>Epicardium Earlier, %</th>
<th>Endo-Epicardial Activation SD, ms</th>
<th>Endocardiun Fibrillation Waves</th>
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AFCL indicates atrial fibrillation cycle length.

Figure 6. Three examples of endo-epicardial breakthroughs in a patient with longstanding persistent atrial fibrillation (AF). A, A focal wave appeared at the epicardial surface at t=24 (white star). As can be seen from the associated endocardial wavemap, just a couple of milliseconds before an endocardial fibrillation wave (red) had passed that site at t=16 ms. A transmural conduction time of only 8 ms was taken as supportive evidence that the focal wave could be caused by endo-epicardial breakthrough. B, An example of a focal wave arising at the endocardium at t=46. Again a fibrillation wave in the opposed layer (green) had passed that site just 5 ms before (at t=41). C, Two breakthroughs occurred at about the same time, one at the endocardium at t=66 and another at the epicardium at t=73 ms. In both cases, in the opposite layer a fibrillation wave passing the site of origin of the focal waves a few milliseconds earlier at, respectively, t=64 and t=69, indicating that the focal waves could result from endo-epicardial breakthrough.
dependent on the number of electrodes with good tissue contact. Another limitation is, that to date, only a limited part of the atria has been accessible for endo-epicardial mapping (4.2 cm² of the right atrium). To get a full understanding of the role of EEA in the development of the substrate of AF, endo-epicardial mapping of the left atrium is needed as well. However, the left atrium is not standardly opened during cardiac surgery, only during selected procedures. In addition, opening the left atrium before cardiopulmonary bypass is associated with a considerable increase in the risk for air embolism, which may cause brain injury. Therefore, we decided that it was not ethically responsible to perform endo-epicardial mapping of the left atrium for this pilot study.

**Clinical Implications**

Knowledge of the substrate and various mechanisms of perpetuation of human AF is of great importance to understand the natural history of AF. At different stages, the substrate of AF may require different treatment modalities. At an early stage, pulmonary vein isolation alone might be sufficient, whereas in a later stage, also compartmentalization of the atria will be necessary to restore sinus rhythm. Furthermore, when the endo-and epicardial layers of the atria have become electrically dissociated, even extensive ablative therapies may be ineffective and palliative therapy would be a better option. Knowledge of the vulnerable parameter(s) for perpetuation of AF, and the ability to diagnose the stage of development of the substrate of AF, is essential for an individualized and staged therapy of AF.

**Sources of Funding**

Dr de Groot was supported by grants from the Erasmus Medical Center fellowship, Dutch Heart Foundation (2012T0046), LSH-Impulse grant (40-43100-98-008), CoolSingel Foundation (no. 212), and Bayer and Boehringer Ingelheim.

**Disclosures**

None.

**References**


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Circ Arrhythm Electrophysiol. 2016;9:
doi: 10.1161/CIRCEP.115.003648

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SUPPLEMENTAL MATERIAL

Mapping Criteria

Step I. Subtraction of ventricular complexes
Before determination of the local activation time, ventricular complexes were eliminated from the unipolar electrograms using a subtracting technique as previously described in detail by Hoekstra et al.\(^1\) In short, for each fibrillation electrogram an individual template of the ventricular far field was obtained by averaging all time windows of ±70ms around the R-waves detected from surface ECG lead I. Subtraction of these individual QRS templates from the fibrillation electrograms reduces the ventricular far field potentials and results in a more or less ‘clean’ unipolar atrial fibrillation electrograms.

Step II. Determination of the local activation time
Examples of electrograms recorded from both the endo- and epicardium and endo-epicardial (a)synchrony are demonstrated in Figures 1 and 2.

Local activation times were determined by detecting the maximum downslope of the unipolar fibrillation potential, as this coincides with the moment of maximum rate of rise of the transmembrane potential (time differences less than 50 μs).\(^2\) In turn, the maximum rate of rise of the transmembrane potential corresponds with the maximum increase in sodium current and its conductance.\(^3\) The height of the negative slope is measured during a 2 ms period. From the moment of the maximum downslope, time windows both backward and forward in time are scanned to detect the moment of respectively the positive and negative peak of the fibrillation potential. The duration of a non-fractionated potential is then defined as the time difference between the moment of its negative and positive peak. In case of a fractionated potential, the deflection with the steepest down slope was chosen; its duration is defined as the time between the preceding positive and following negative deflection.\(^4\) The duration of a fibrillation potential had to be ≤35ms. The negative slope and amplitude of the unipolar fibrillation potentials depend on numerous variables.\(^5,6\) Hence, cut-off values applied also vary, depending on the signal-to-noise ratios of the recordings and lower limits were set at 0.05 V/sec and 0.2 mV.\(^5,6,7,8\) All fibrillation potentials with slopes <0.05V/sec, amplitudes < 0.2mV and durations >35 ms are thus regarded as either far field or poor contact potentials. After detection of the local activation time, a blanking period of 40 ms was applied in both directions. Though we do not know exactly what the refractory period during AF is, it is estimated to be approximately 50±13ms.\(^9\) Hence, comparable to previous studies, by choosing
a blanking period of 40ms we avoid overestimation of the number of fibrillation waves by marking fibrillation potentials, which are most likely double potentials with interspike intervals between >0 and ≤40ms caused by areas of conduction block.$^{10,11}$

**Step III. WaveMapping**

A wavemapping approach was used to identify individual fibrillation waves. This wavemapping technique has also been described in prior studies.$^{2,3,12,13}$ The starting point of the first fibrillation waves was the earliest activated site within the mapping area. Next, the entire mapping area was scanned in steps of 1ms. For all electrodes activated during every step, the shortest time difference with the 8 neighboring electrodes was calculated. When the time difference was ≤12ms (17ms for oblique distances), the electrode site was added to the territory of the surrounding wave. In case of a time difference >12 ms, the electrode was annotated as the starting point of a new wave. In the wavemap, fibrillation waves are color-coded according to their moment of entrance in the mapping area and the colors demarcate the area activated by that specific fibrillation wave. The cut-off value of >12ms used for separating individual fibrillation waves corresponds for 2 mm inter-electrode distances with an effective conduction velocity of 17 cm/s, which is equivalent to the continuous conduction velocities reported for atrial myocardium of intact hearts.$^{14}$ For separation of the fibrillation waves the requirement of a lower limit CV must be fulfilled along the whole boundary of the wave which of course does not exclude the possibility of slow conduction within parts of the fibrillation waves. Choosing a different cut-off value will lead to a lower or higher number of fibrillation waves. However, this change is very gradual and has no major effects on the measured differences in the number of focal waves. Only at extreme cut-off values our analysis will become useless because it either no longer separates the different fibrillation waves, or results in a very high degree of spatial fractionation, resembling a mosaic-like pattern of numerous small waves that only propagate over very short distances. Based on the origin of the fibrillation wave, three different types of fibrillation waves were distinguished 1) peripheral waves, entering the mapping area from outside the electrode array, 2) epicardial breakthrough, appearing at the epicardial surface inside the mapping area, and 3) discontinuous conduction waves; defined as fibrillation waves starting with a delay of 13 to 40ms from the boundary of another wave.$^{12-13}$ If a fibrillation wave originates along the border of another fibrillation wave it could theoretically be the result of very slow conduction. In order to avoid overestimation of the number of focal waves, we classified these waves as discontinuous fibrillation waves. Applications of this wavemapping technology have been
described previously.\textsuperscript{12-13}

Focal fibrillation waves had to meet several criteria. The breakthrough site of the focal fibrillation wave had to be located at least 2 electrodes away from the border of the mapping array and at least 1 reliable activation time should be available between the breakthrough site and the border of the mapping area in order to exclude propagation from the border of the mapping array. An example is given in Figure 3. The relation between the percentage of endocardial or epicardial focal fibrillation waves and the distance from the origin of the focal wave to the border of the mapping array is shown in Table 1. Next, it is manually checked whether the morphology of fibrillation potentials in the breakthrough region is distorted by large QRS complexes or artifacts due to e.g. movements of the electrodes in order to avoid false positive focal waves; examples are shown in Figure 4.

In case of a fractionated electrogram, marking of one of the other deflections should not result in disappearance of the focal fibrillation wave (Figure 5). In order to include only focal waves which have a more or less considerable impact on endo-epicardial asynchrony, we choose cut-off value of 4 electrodes. Table 2 shows how many focal waves will disappear when a cut-off value of 5 or 6 electrodes would have been chosen. The origin of a focal wave had to be activated earlier than all surrounding electrodes. If electrodes adjacent to the origin were activated simultaneously, all electrodes surrounding this area should also be activated later. Shift of the local activation time to a maximum of 3ms earlier or later at the earliest activated electrode(s) should not result in disappearance of the focal wave. Typical examples of focal waves resulting from these criteria are provided in Figure 6.
Tables

Table 1. Location of the origin of focal fibrillation waves.

<table>
<thead>
<tr>
<th>Row distance to border</th>
<th>Endocardial focal waves</th>
<th>Epicardial focal waves</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>25%</td>
<td>25%</td>
</tr>
<tr>
<td>3</td>
<td>37%</td>
<td>36%</td>
</tr>
<tr>
<td>4</td>
<td>38%</td>
<td>39%</td>
</tr>
</tbody>
</table>

Table 2. Size of the focal waves.

<table>
<thead>
<tr>
<th></th>
<th>Endocardial focal waves</th>
<th>Epicardial focal waves</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 electrodes</td>
<td>5.0%</td>
<td>5.6%</td>
</tr>
<tr>
<td>5 electrodes</td>
<td>4.5%</td>
<td>5.2%</td>
</tr>
<tr>
<td>6 electrodes</td>
<td>3.8%</td>
<td>4.6%</td>
</tr>
</tbody>
</table>

Figures

Figure 1. The total set of endo- and epicardial electrograms of two patients. The position of the electrode numbers in the arrays is illustrated below. The green colored segments in the electrogram tracings indicate the ventricular far field complexes. The superimposed black traces are the signals after application of the ventricular far field subtracting technique as described above. The yellow bands in the marked electrogram figures depict the time window in which the electrodes are activated in that AF beat.
Patient 5 – Epicardial Electrograms
Patient 5 – Endocardial Electrograms
Magnified Epicardial Electrograms

Magnified Endocardial Electrograms
Patient 5 – Marked Epicardial Electrograms
Patient 14 – Epicardial Electrograms
Patient 14 – Magnified Epicardial Electrograms
Patient 14 – Marked Epicardial Electrograms
Patient 14 – Marked Endocardial Electrograms
Figure 2. Examples of two opposite electrogram recordings showing synchronous and asynchronous activation.

**Synchronous Activation**

<table>
<thead>
<tr>
<th>Epicardium</th>
<th>Endocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>[waveform image]</td>
<td>[waveform image]</td>
</tr>
</tbody>
</table>

**Asynchronous Activation**

<table>
<thead>
<tr>
<th>Epicardium</th>
<th>Endocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>[waveform image]</td>
<td>[waveform image]</td>
</tr>
</tbody>
</table>

3.8 seconds
Figure 3. Focal wave originating near the border of the mapping array.

Figure 4. Distortion of the morphology of an atrial fibrillation potential by a far field QRS complex.
**Figure 5.** Fractionated potential next to the origin of a ‘focal’ wave.

![Fractionated potential](image)

**Figure 6.** Wavemaps demonstrating typical examples of focal fibrillation waves.

![Wavemaps](image)
References


