Mapping and ablation of atrial tachyarrhythmias : from signal to substrate

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chapter

15

General Discussion

Atrial Fibrillation: Men's Most Common Arrhythmia

Atrial fibrillation (AF) is at this moment a major subject of interest for both clinicians and scientists. The interest for AF is partly the result of the fact that AF is the most common arrhythmia.[1] As it is more frequent in the elderly (5% in subjects > 60 years), the incidence of AF will only continue to rise due to ageing of the population.[2] In addition, patients with AF are frequently encountered in clinical practice as AF is a chronic disease associated with a progressive atrial cardiomyopathy.[1]

Subjects with AF represent a spectrum of patients with arrhythmia episodes which vary in duration, number, pattern of onset, triggers and mode of termination. The arrhythmia presentation also changes over time and it occurs both in presence and absence of detectable heart disease.[3;4]

Despite the variable clinical presentation, AF is characterized by an irregular heartbeat, stasis of blood flow in the left atrium which increases the vulnerability to thrombo-embolism and loss of asynchronous atrioventricular contraction which compromises cardiac hemodynamics. Hence, the sequelas of AF necessitate effective treatment.[4]

The AFFIRM study demonstrated the benefit of maintenance of sinus rhythm over AF. In this study, treatment of AF with a rhythm control strategy and a rate control strategy were compared.[5] The study population consisted of 4060 patients (age 69.7 ± 9.0 years) with either hypertension (70.8%) or coronary artery disease (38.2%). The major finding of this study is that rhythm control had no survival advantage over rate control. Yet, the findings of this study do not imply that there is no benefit in maintaining patients in sinus rhythm. Many patients in the rhythm control group were not effectively treated (27.4% of the patients were in AF) and many patients in the rate control group were in sinus rhythm (34.6%). When analyzing data according to the heart rhythm instead of treatment strategy sinus rhythm appeared to be an independent predictor of survival.[6]

Despite extensive research – which is reflected by the huge number of scientific publications in recent years reporting on a diversity of aspects of AF – there is not yet a definite cure for every AF patient.

Ideally, the goal of AF therapy is to 1) abolish AF episodes, 2) restore sinus rhythm, 3) re-establish atrioventricular asynchrony and 4) improve atrial transport function. AF can be treated with anti-arrhythmic drugs aimed at rhythm or ventricular rate control. [7;8] However, the efficacy of anti-arrhythmic drugs is low and most drugs have severe side-effects.[7] Non-pharmacological treatment of AF includes external electrical cardioversion, internal atrial defibrillator, His bundle ablation followed by cardiac pacing and catheter or surgical ablation.[8;9]

Catheter Ablation of Atrial Fibrillation as First Line Treatment Option?

In the past years, intra-operative mapping studies of atrial tachyarrhythmia have not only provided insight into the pathogenesis but they have also facilitated treatment of atrial ar-

rhythmia.[10] The first mapping studies of AF were aimed at identifying an anatomical substrate which could be treated surgically.[11] Conversely, it appeared that AF was maintained by randomly wandering wavelets which was consistent with Moe's Multiple Wavelets Hypothesis.[12] Based on these findings, the surgical Maze procedure was developed.[13] This procedure consisted of creating multiple incisions aimed at interrupting all possible reentrant circuits. The reported success rate of this treatment was even more than 90%.[14]
Endocardial catheter ablation procedures mimicking the surgical Maze were also developed but these catheter-mediated atriotomy procedures were associated with a significant morbidity.[15] The therapeutic approach of AF changed when Haïssaguerre et al. discovered that AF was triggered by (non)-pulmonary vein foci.[16] This finding resulted in development of focal ablation, segmental ablation and isolation of the pulmonary veins as curative treatment modalities for patients with AF.[16-19]
The initial studies demonstrated that *paroxysmal* AF could be eliminated by pulmonary vein ablation. This approach was later also successfully applied in patients with *permanent* AF, suggesting that the pulmonary veins played a role in sustenance of AF as well.[18] Numerous studies have since then reported on elimination of AF by pulmonary vein isolation/ablation performed in patients with and without structural heart diseases, often combined with the construction of linear lesions.
The effectiveness of pulmonary vein ablation in not only patients with *paroxysmal* AF but also in patients with *permanent* AF suggests that there is partly a common pathogenesis though the clinical presentation differs.

Pulmonary Vein Ablation: Why Can this Procedure be Successful?

The success of pulmonary vein ablation in patients with permanent AF has been attributed to 1) modification of the 'arrhythmogenic substrate'/ elimination of venous wavelets, 2) interruption of crucial pathways of conduction, 3) atrial debulking and 4) atrial denervation.

1) *Modification of the 'Arrhythmogenic Substrate'*

Several investigators have provided data supporting the assumption that the arrhythmogenic substrate for AF is located within the pulmonary vein area.
During AF, organized electrograms with relative short cycle lengths have been recorded from the pulmonary veins and posterior left atrial wall during AF. This rapid electrical activity appeared to be originating from focal sources maintaining AF.[16;20;21]
Jais et al. measured the effective and functional refractory period in patients with AF and in patients with no history of AF. [22] In the AF group, the effective and functional refractory period in the pulmonary veins was shorter than in the left atrium. In the control group, on the contrary, the refractory period in the left atrium was shorter than in pulmonary veins. Comparing the control and AF group, the refractory period in the pulmonary veins were

significantly shorter than in the AF patients, and decremental/slow conduction occurred more frequently. These differences may account for pulmonary vein arrhythmogenicity in patients with AF.

Recurrences of either paroxysmal or persistent AF after pulmonary vein ablation tended to be higher if the degree of low-voltage areas encircled after ablation was smaller.[23] Recently, Haissaguerre et al. introduced the "Venous Wave Hypothesis".[24;25] They observed that AFCL progressively increased at a remote site during pulmonary vein isolation, suggesting involvement of pulmonary vein activity in maintenance of AF.

Based on their observation and data from other studies demonstrating reentry in the pulmonary vein-left atrial junction region they proposed the "Venous Wave Hypothesis."

This hypothesis states that in some patients with AF, the pulmonary vein-left atrial junction region is a generator of venous waves maintaining AF.

2) *Interruption of Crucial Pathways of Conduction*

It can also be hypothesized that pulmonary vein isolation disconnects crucial intra-atrial pathways located at the pulmonary vein-left atrium junction essential for sustaining AF. It has been observed that no electrical activity could be recorded from the pulmonary veins after disconnection from the left atrium.[20] This observation implies that initiation of focal activity within the pulmonary veins depends on electrical input from the left atrium.

Recently, Tan et al. performed in canine atria epicardial mapping studies of the left superior pulmonary vein-left atrial junction, including the Marshall bundle.[26] This muscle bundle is located within the vein of Marshall close to the left superior pulmonary vein and connects the coronary sinus muscle sleeve to the left atrium. As the Marshall bundle serves as a source of atrial ectopy giving rise to AF, it has been suggested that the Marshall bundle contributes to the high incidence of focal activation originating from this vein. It was shown that direct connections between the Marshall bundle and the left superior pulmonary vein contributed to the rapid activation rate of the left superior pulmonary vein. When conduction between the pulmonary veins and left atrium was slowed by ablation, focal activation within the left superior pulmonary vein was prevented, despite persistent focal activation originating from the Marshall bundle in some dogs. Hence, the findings of this study emphasize the combined role of the left superior pulmonary vein-left atrium and left superior pulmonary vein- Marshall bundle junction in generating AF. This study also suggests that the Marshall bundle can be regarded as an accessory pathway to the left superior pulmonary vein which may cause failure of electrical isolation of the pulmonary veins. However, evidence from human data supporting this hypothesis is lacking. Also, in most AF ablation procedures the Marshall bundle is already transsected as an additional line between the mitral isthmus and left inferior pulmonary vein which crosses the Marshall bundle is often created.

3) *Atrial Debulking*

"The critical mass of fibrillation" hypothesis states that a certain minimal size of atrial tissue is required for initiation and sustainance of AF. Perpetuation of AF is determined by the number of simultaneously circulating wavelets; a large atrial mass can accomodate more wavelets thereby stabilizing AF. Hence, reduction of atrial mass may be anti-fibrillatory.[27;28] Diminution of atrial mass is achieved by isolation of the pulmonary veins as after this procedure a significant amount of atrial myocardium is replaced by scar tissue.

4) *Atrial Denervation*

There is accumulating evidence that the autonomic nervous system is involved in the pathogenesis of AF.[29-31] Duration and dispersion of refractoriness plays a role in respectively initiation and maintenance of AF. Vagal stimulation increases spatial dispersion in atrial refractoriness, which might be caused by e.g local variability in density of vagal nerve endings/muscarine receptors, discrepancies in the coupling of muscarin receptors to ion-channel effectors, variation in K^+-channel density or variations in the distribution of acetylcholinesterase[30;32-34].

Gonzalez et al. demonstrated in patients with AF a higher vagal tone due a reduced acetylcholinesterase activity.[35] Animal models showed that catheter ablation resulting in partial vagal denervation reduced inducibility of AF.[29;36;37] In humans, Pappone et al. noticed, by analyzing heart rhythm variability, a post-ablation shift of the sympathovagal balance towards parasympathetic predominance.[23] In a subsequent study, vagal denervation was additionally performed in patients (n = 297) with paroxysmal AF referred for isolation of the pulmonary veins. Vagal sites were defined as sites where vagal reflexes (bradycardia, asystole, atrioventricular block, hypotension) occurred during ablation. At such a site, ablation was performed until the vagal reflexes were completely abolished. Vagal reflexes were present in 104 patients; ablation sites were located at the cranial left superior pulmonary vein – left atrium, the septal right superior pulmonary vein– left atrium, the postero-inferior left inferior pulmonary vein – left atrium and the postero-inferior right inferior pulmonary vein – left atrium junction. In patients who have had a vagal denervation, late recurrences were less frequent observed though there was a higher incidence of early recurrences compared to patients without vagal denervation. This finding was explained by destruction of vagal nerve fibers resulting in release of acetylcholine causing early recurrences, followed by a stable vagolytic effect later after ablation which prevented AF recurrences.[32]

Why is Pulmonary Vein Ablation not Effective in Eliminating Permanent Atrial Fibrillation ?

Pulmonary vein ablation in patients with permanent AF may not be as successful as first assumed. Kottkamp et al. reported the long-term outcome of catheter ablation procedures.[38] In 80 patients with paroxysmal AF and 20 patients with persistent AF, circumferential lesions were created around the left and right pulmonary vein and a linear lesion was created between the circular lesions and between the left circular lesion and the mitral valve annulus. During follow up, 7-day electrocardiogram recordings were made before ablation, within one hour after ablation and during outpatient clinic visits 3, 6 and 12 months after ablation. The result of this study are shown in Figure 1. In patients with paroxysmal AF, the freedom of AF increased over time. In patients with persistent AF and recurrences after ablation, the number of AF episodes increased over time and the duration of AF episodes diminished. After a follow-up period of 12 months, only 22% of the patients with permanent AF were free of AF episodes. From these data it was suggested that pulmonary vein trigger elimination did not play a role in abolishing permanent AF and that ablation might have only modified the arrhythmogenic substrate.

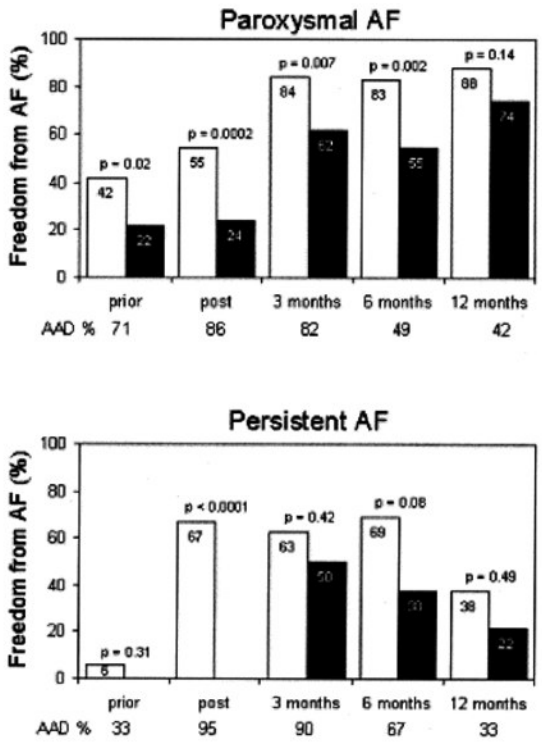

Figure 1.

Freedom from atrial fibrillation in patients with paroxysmal and persistent AF, assessed by 24-h holter electrocardiogram (white bar) and 7-day electrocardiogram (black bar). Modified from Kottkamp et al.[38]

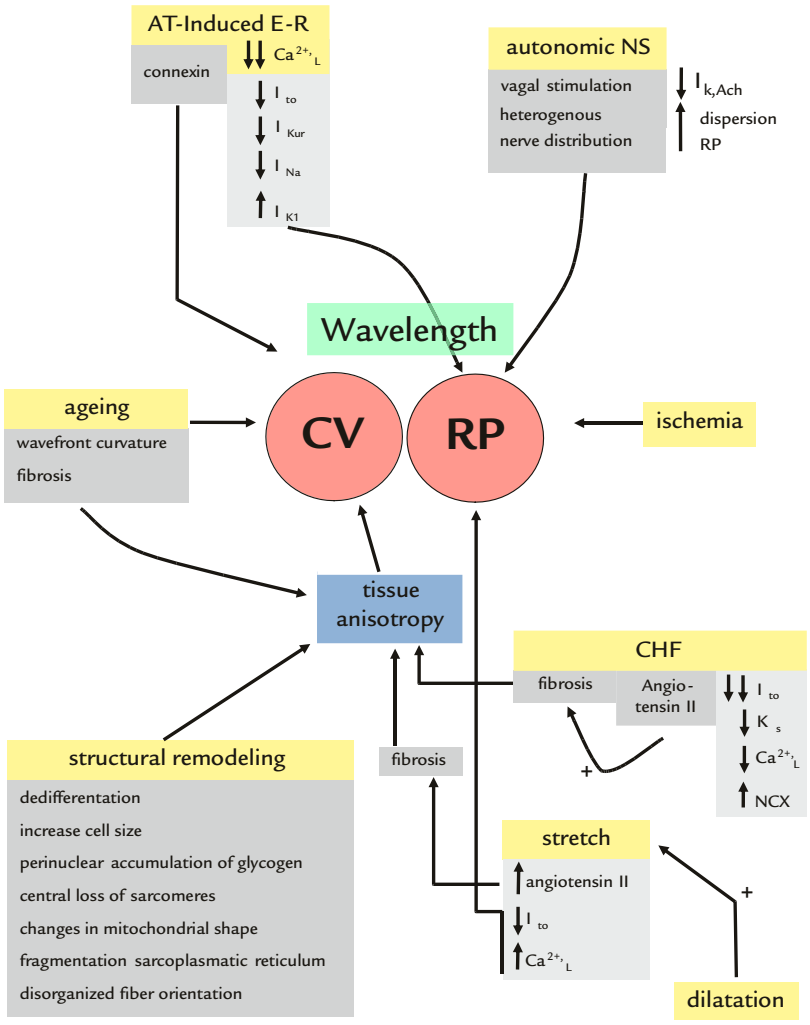

Figure 2.
Schema of the interaction between electrophysiological, structural and neurohumeral alterations and the substrate for AF.

It is in general assumed that in patients with chronic AF, AF has progressed from a trigger-driven to a substrate mediated arrhythmia. In these patients, persistence of AF no longer depends on the presence of a trigger ('*true fibrillation*') but is maintained by an arrhythmogenic substrate. Electrophysiological, structural and neurohormonal alterations creating a vulnerable substrate for AF are summarized in **Figure 2**. Various diseases alter myocardial structure and/or electrical function thereby creating a substrate capable of perpetuating AF. On the other hand, AF itself may also modify the atrial myocardium which in turn stabilizes AF. For example, Wijffels et al. demonstrated in the goat model

of AF that AF resulted in shortening of the atrial effective refractory period and reversion of the physiological rate adaptation (shortening of the atrial refractory period at slower heart rates), facilitating inducibility and stability of AF.[39] Similar findings have been reported in humans. These electrophysiological changes are mediated by changes in ion channel function and differ between patients with normal atria and patients with congestive heart failure (**Figure 2**). In the goat model of AF it has also been demonstrated that longstanding AF is associated with changes in myocardial structure including dedifferentation, increase in cell size, perinuclear accumulation of glycogen, central loss of sarcomeres, changes in mitochondrial shape, fragmentation of the sarcoplasmatic reticulum and disorganization of fiber orientation.[40;41] Studies reporting on histopathological changes in humans with AF are limited. As it is likely that electrical and structural remodeling affects multiple sites of the atria, the arrhythmogenic substrate of AF will not only be confined to the pulmonary vein area. This hypothesis is supported by several clinical studies which provide evidence that there is no relation between encircling the pulmonary veins with continuous lesions and elimination of AF.[23;42] Also, the IRAAF study, (the intraoperative radiofrequency ablation of atrial fibrillation) demonstrated that creation of linear lesions in the left atrium without isolating the pulmonary veins eliminated AF in more 90% of the patients.[43]

Even though a pulmonary vein isolation procedure can be effective in eliminating AF, the long-term outcome may be complicated by development of new atrial tachyarrhythmias, due to either progressive atrial myopathy or incomplete ablative lesions. In addition, serious complications after pulmonary vein isolation have been reported, including pulmonary vein stenosis, embolic stroke, atrio-esophageal fistula, cardiac tamponade, air embolism, phrenic nerve damage and mitral valve damage.[44]A more limited ablation approach which is targeted at either destroying the trigger mechanism or altering the substrate perpetuating AF might therefore be desirable. Such an ablation approach required knowledge of the mechanism of AF. Due to the large variety in disorders associated with AF, it is only logical to assume that there are also inter-individual differences in the mechanism underlying AF. Yet, in the clinical situation is the mechanism underlying AF in the individual patient often not known.

Despite a huge number of studies focusing on modifying ablation approaches of AF, less attention has been paid to the mechanism underlying AF in the individual patient.[10;15;18-20;23;24;44-46] Several questions remain still unanswered, e.g. whether, the severity of AF represents a spectrum ranging from a trigger driven AF to a substrate mediated AF, reentrant circuits and focal activity co-exist, or whether specific cardiac diseases are associated with either trigger driven AF or substrate mediated AF. Even a clear definition of the "arrhythmogenic" substrate is lacking.

Based on the acquired knowledge of the mechanism of AF, four different possible curative catheter ablation approaches for AF have sofar been developed which are currently being evaluated (**Figure 3**).[47]

These treatment modalities are:

1) 'isolation of triggers and perpetuating reentry circuits residing in the pulmonary veins'[16;20]
2) 'disruption of the substrate for perpetuating rotors in the antra of the pulmonary veins or the left atrium'[18]
3) 'ablation of ganglionated autonomic plexus in epicardial fat pads'[36]
4) 'disruption of putative dominant rotors in the left and right atrium as recognized by high-frequency complex fractionated electrograms during mapping of AF'[48]

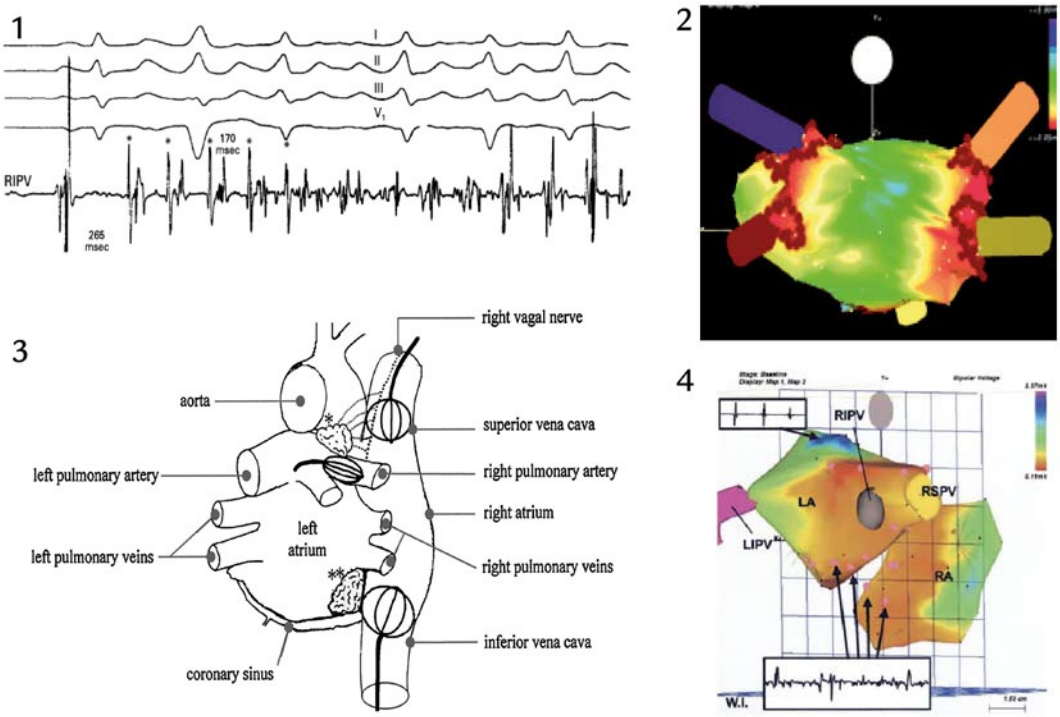

Figure 3.

Four different ablation approaches for AF have sofar been developed, including

1) isolation of triggers and perpetuating reentry circuits residing in the pulmonary veins,
2) disruption of the substrate for perpetuating rotors in the antra of the pulmonary veins or the left atrium,
3) ablation of ganglionated autonomic plexus in epicardial fat pads,
4) disruption of putative dominant rotors in the left and right atrium as recognized by high-frequency complex fractionated electrograms during mapping of AF.

Multi-Site High Density Mapping of Atrial Fibrillation: A New Diagnostic Tool?

High density mapping of AF as presented in our studies was used to compare electrophysiological properties during induced atrial fibrillation in patients with normal atria and during chronic AF in patients with valvular heart disease. The AAF and CAF group noticeably differed in AF cycle length, incidence of fractionation, fractionation delay, patterns of activation and conduction characteristics despite variation within both the AAF and CAF group. These findings indicate that high density mapping could be a suitable tool to distinguish different types of AF.

By performing multi-site high density mapping in patients with chronic AF, our data also showed that there is not only an inter-individual variation but also an intra-atrial variation in the various electrophysiological characteristics.

Theoretically, multi-site high density mapping can be used to localize sources generating AF in patients with trigger-driven AF and to identify areas perpetuating AF in patients with substrate mediated-AF. Based on the premise that AF can be eliminated by ablation of either the trigger or the substrate perpetuating AF it could be hypothesized that multi-site high density mapping is a suitable tool to diagnose AF and subsequent to guide ablation of AF.

Regional Differences in Atrial Fibrillation Cycle Length

Temporal and/or spatial variation in AF cycle length (AFCL) has been studied using a variety of recording techniques such as the surface electrocardiogram, monophasic action potentials and unipolar or bipolar electrograms obtained from either the endocardium or epicardium. The method used for analysis of fibrillation intervals is essential for correct interpretation of the data acquired.[49-51;51;52]

As demonstrated by our studies, analysis of temporal variation in AFCL without knowledge of the corresponding spatial patterns of activation may introduce errors. Series of multiple deflections with relative short fibrillation intervals may merely be the result of local dissociation in conduction and not of circus movement. Prolonged fractionation of fibrillation potentials can be mistaken for short fibrillation intervals and hence interpreted as the presence of focal activity.

Spectral analysis of optical recordings obtained from the right and left atria of isolated sheep heart during acetylcholine induced AF revealed that the highest dominant frequency appeared to be originating from stable microreentrant sources often found at the posterior left atrial wall.[50;53-55] Away from the "driver", there was a frequency dependent breakdown of waves resulting in fibrillatory conduction giving rise to a left-to-right frequency gradient. [56] This driver is therefore assumed to be the fastest and most regular fibrillating spot. Similar findings were observed in humans with AF.[50;57;58]

Thus, analysis of the fibrillatory rate could be useful for localize sources triggering AF.[50;59] Lazer et al. studied left and right atrial frequency gradients in 18 patients with paroxysmal

AF and 13 patients with persistent AF.[60] During paroxysmal AF, there was a frequency gradient from the pulmonary vein region to the coronary sinus and the right atrium. Such a left-to-right frequency gradient was absent in patients with persistent AF due to a relative higher frequency in the right atrium (upper panel **Figure 4**). These finding were interpreted as less dependency of permanent AF on the left atrial posterior wall. Further evidence provided by analysis of the fibrillatory rate demonstrating that the pulmonary vein might not be responsible for maintaining AF is shown by Sanders et al. [50] They analysed the spatial distribution of dominant frequencies during AF in patients with persistent (n = 13) or paroxysmal (n = 19) AF. Endocardial mapping was performed with the 3-dimensional electro-anatomical mapping system (CARTO™). At multiple sites (126 ± 13), bipolar electrograms were recorded during a time period of 5 seconds. Recordings were used to create color coded dominant frequency maps. Highest dominant frequencies sites were defined as sites with a frequency gradient of more than 20% to the surrounding points. In both groups, there was a frequency gradient from the left atrium to the right atrium and to the coronary sinus. Comparing paroxysmal and persistent AF, frequencies were higher during persistent AF. The number of DF sites were not different between the permanent (5.1 ± 1.9) and persistent (5.2 ± 1.8) AF patients.

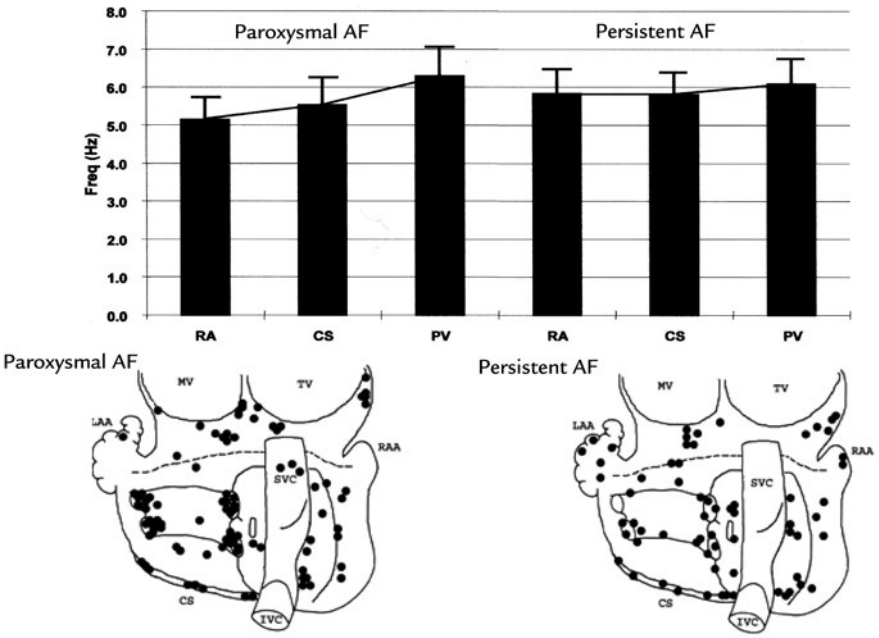

Figure 4.

Upper panel: Comparison of mean dominant frequencies around the pulmonary vein-left atrial junction, coronary sinus and right atrium revealing a significant frequency gradient from the left atrium to coronary sinus to right atrium during paroxysmal AF and not during persistent AF. Modified from Lazar et al. [60]

Lower panel: All dominant frequency sites in patients with paroxysmal (left) and permanent (right) AF. In patients with paroxysmal AF, dominant frequencies were found near the pulmonary veins. Modified from Sanders et al.[50]

In patients with paroxysmal AF, 42% of the dominant frequencies were located within the pulmonary vein/ostial left atrial area; this was even lower for patients with persistent AF (26%). Ablation of the pulmonary veins containing a dominant frequency site resulted in prolongation of AFCL sites whereas ablation of the pulmonary vein area without a dominant frequency site did not change AFCL. Hence, this study shows that high-frequency activity during AF in humans was *not* only confined to the pulmonary vein area (lower panel Figure 4).

Surprisingly, high density mapping of the atria with a fingertip electrode revealed that right atrial fibrillation intervals were shorter than left atrial fibrillation intervals (chapter 8). Also, the longest fibrillation intervals were obtained from the left atrium. The fact that AFCL around the pulmonary veins was not faster than in the remainder of the atria makes it unlikely that a high frequency source in this area was driving AF.

In Search of A Driver

We also studied the spatio-temporal variation in AFCL. At each mapping site, an AFCL histogram was computed from all 60 unipolar electrodes during 10 seconds of AF in order to determine the median AFCL. The median AFCL was then plotted on schematic representation of the mapping sites in order to study the spatial distribution of AFCL. Mapping locations with a dominant AFCL were defined as sites where the increase in AFCL to the surrounding area was ≥ 10 ms. The median AFCL is color-coded ranging from green (shortest AFCL) to red (the longest AFCL); with a color change for every 10 ms. The results for 6 patients are demonstrated in the right panel of Figure 5.

Dominant AFCL at one or more mapping sites were found in all patients. In the entire study population, thirty-seven mapping locations representing 19 areas with a dominant AFCL were identified; 8 sites were located in the left atrium, 9 in the right atrium and 2 at bachmann's bundle. Areas with the shortest AFCL varied from patient to patient and there were no predilection sites. Dominant AFCL were found in the left atrium in three patients and in the right atrium in 4 patients. In 4 patients, dominant AFCL were present in both the right and the left atrium. The shortest AFCL was found in the pulmonary vein area in only three patients. In three patients, isolated areas with a remarkable long AFCL were present.

At each mapping site, the degree of regularity of the AFCL was also determined. For this purpose, a beat-to-beat interval variation histogram was constructed by subtracting each AFCL interval from the preceding interval. The degree of regularity of the AFCL was expressed as the standard deviation of the beat-to-beat interval variation histogram.

The degree of regularity of the AFCL also demonstrated a high variation in spatial distribution (Figure 5). There was no relation between the median AFCL and the degree of regularity. However, at 8 locations, the fastest fibrillating areas were also the most regular sites. Noticeably, a high degree of AFCL regularity was observed in the left atrial appendage of most patients.

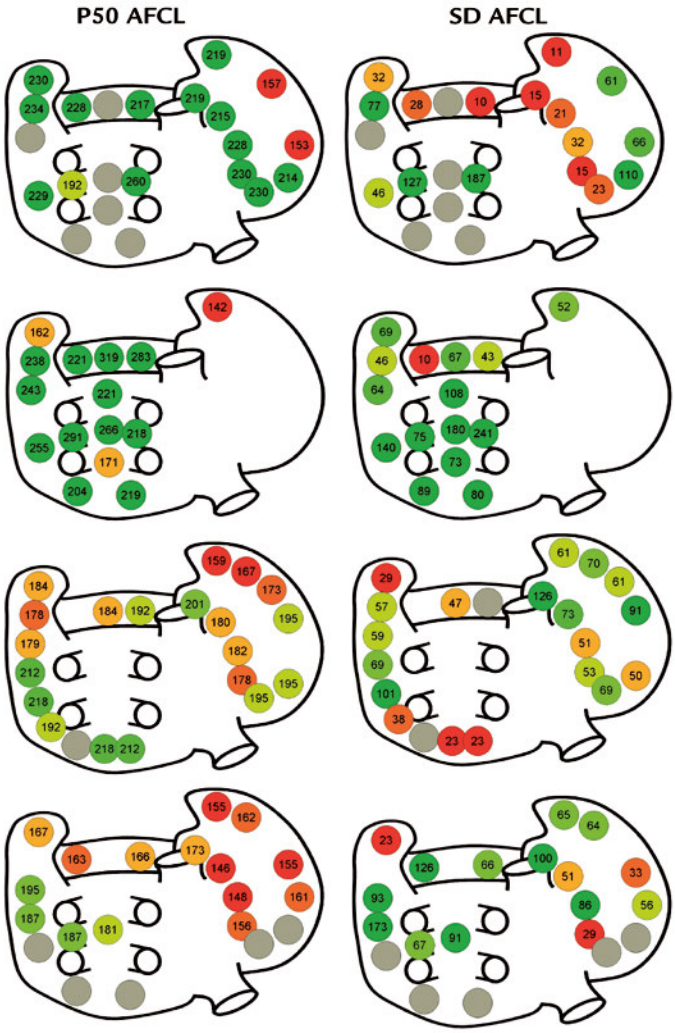

Figure 5.
Left panel: The median AFCL was then plotted on schematic representation of the mapping sites from six different patients. Mapping locations with a dominant AFCL were defined as sites where the increase in AFCL to the surrounding area was ≥ 10 ms. The median AFCL is color-coded ranging from green (shortest AFCL) to red (the longest AFCL); with a color change for every 10 ms. *Right panel:* Regularity of AFCL at each mapping site was expressed as the standard deviation of the beat-to-beat interval variation histogram.

A limitation of the mapping technique used is that the areas were not mapped simultaneously. Although the AFCL remained relatively constant in time, meandering drivers or drivers that are temporarily inactive can not be identified using this method. Hence, only drivers fixed to a certain region can be identified with this mapping approach. Also, certain regions, such as the intra-atrial septum could not be mapped from the epicardial surface.

In summary, the distribution of AFCL in humans with persistent AF and structural heart disease is highly variable, without an anatomical predilection site for the fastest fibrillating areas. The degree of regularity at the majority of sites with a dominant AFCL were not suggestive for the existence of drivers sustaining AF.

Atrial Refractoriness and the Estimated Excitable Gap

In chapter 2, we hypothesized that prolongation of AFCL in case of random reentry of fibrillation waves is due to lengthening of the pathway of fibrillation waves caused by areas of conduction block resulting in a longer waiting time for cells to be activated. Hence, areas of conduction block may give rise to a longer excitable period (P50-P5).

Areas of prolonged atrial refractoriness may be initiators of AF and areas of shortened atrial refractoriness stabilizes AF by decreasing atrial wavelength.[61]

In a preliminary study, we used the fingertip data from 7 patients to study intra-atrial variation in the refractory period and the estimated excitable gap. For this purpose, the P5th was considered an estimator of the refractory period. There were no differences in the estimated averaged refractory periods along the RA- line (137 ± 37 (87-211) ms) and the RAV- line (121 ± 31 (78-203) ms, p = 0.09). The longest estimated refractory periods were measured at the LAV-line (149 ± 28 (93-209) ms) and bachmann's bundle (149 ± 60 (53-252) ms) and the shortest estimated refractory period at the pulmonary vein area (119 ± 26 [103-75] ms, p <0.001). The shortest estimated refractory periods were obtained from the sinus node area (n = 1), along the right atrioventricular groove (n = 2), bachmann's bundle (n = 3), near the sinus transverses (n = 1) and the longest estimated refractory periods from the sinus node area (n = 1), along the right atrioventricular groove (n = 1), bachmann's bundle (n = 4) and between the superior and inferior pulmonary vein (n = 1).

Next, the intra-atrial variation in the estimated temporal excitable gap was determined. The estimated temporal excitable gap was defined as the difference between the median AFCL and the refractory period. There were no differences in the estimated excitable gap along the RA – (56 ± 35 (19-135) ms), RAV (71 ± 32 (16-135) ms), bachmann's bundle (61 ± 36 (6-148) ms) or LAV (64 ± 37 (21-190) ms) -line. However, the estimated excitable gap in the pulmonary vein area was significantly longer (95 ± 37 (24-145) ms, p = 0.001). In all patients in whom the entire right and left atrium were mapped, the *largest* temporal excitable gap was measured at the sinus node area (n = 1), along the right atrioventricular groove (n = 1), bachmann's bundle (n = 1), the left atrioventricular groove (n = 2) and from the pulmonary vein area (n = 2).

Using the standard deviation of the beat-to-beat interval variation histogram to measure variation in fibrillation intervals, the largest variation in AFCL was found in the pulmonary vein area (116 ± 54 (18-241) ms versus RA: 65 ± 37 (15-157) ms, RAV: 68 ± 27 (11-133) ms, bachmann's bundle (60 ± 43 (10-126) ms, P < 0.01).

Thus, data from our preliminary studies show that the pulmonary vein area is characterized by a relative longer median AFCL, a shorter refractory period, a larger variation

in fibrillation intervals and a larger estimated excitable period. The presence of a large estimated excitable period at this site can be explained by an increase in the activation pathway due to the presence of areas of conduction block.

In order to investigate whether conduction block indeed occurred more frequently at the posterior left atrial wall, the degree of dissociation of fibrillation waves at every mapping site was determined. Taking the small size of the mapping area (1.2 x 1.2 cm) into account, each fibrillation map was classified as a '*dissociated*' or *'non-dissociated'* pattern of activation (Figure 6). A dissociated activation pattern was defined as the presence of two or more wavelets separated by areas of conduction block. In all other cases, activation patterns were labeled as non-dissociated.

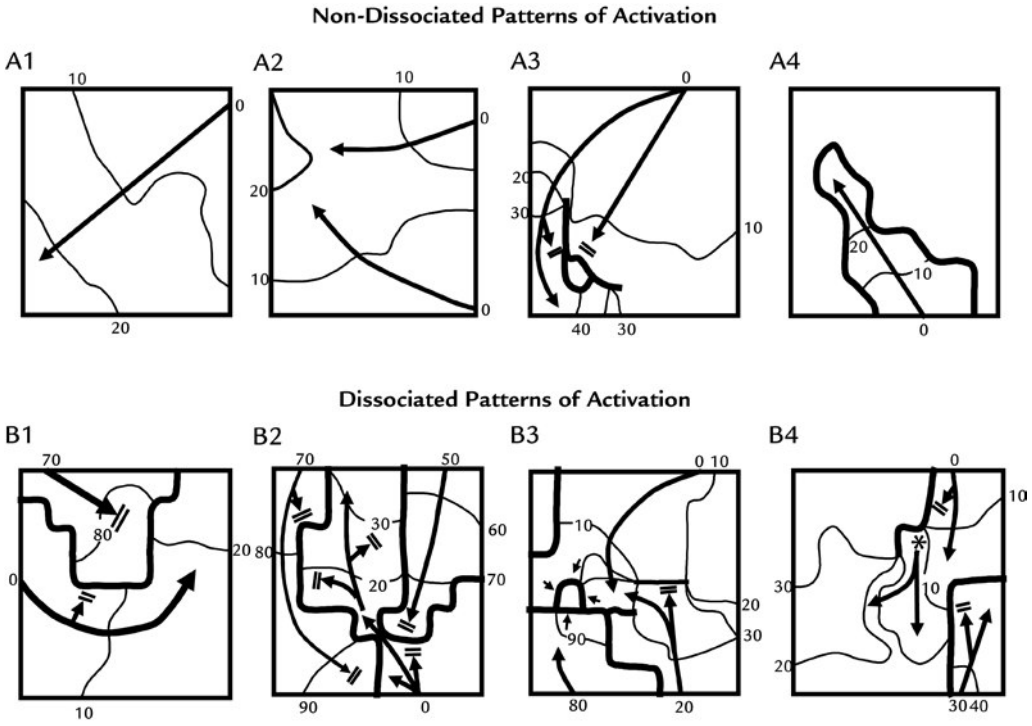

Figure 6.

Examples of dissociated and non-dissociated patterns of activation observed during CAF. Dissociated patterns of activation are defined as the presence of multiple wavefronts separated by areas of conduction block. Isochronal maps were drawn at 10 ms intervals, arrows show main activation direction, thick black lines represent areas of bi-directional conduction block and the asterix indicates a focal pattern of activation. See text for a more detailed explanation.

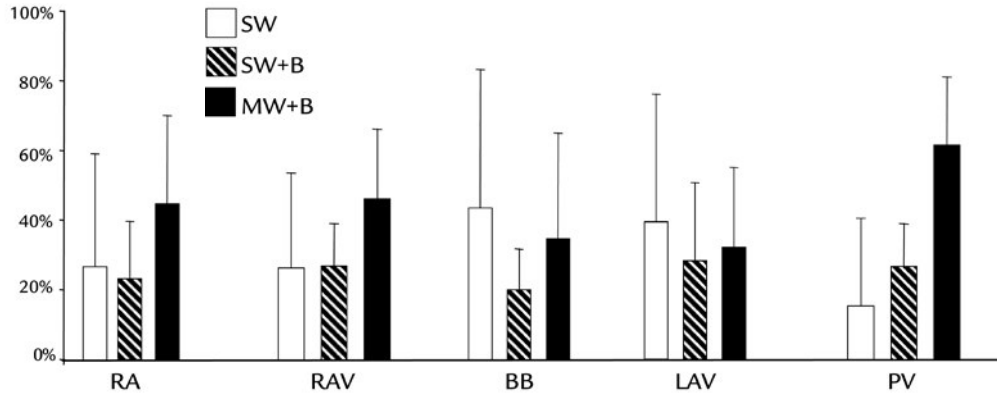

Figure 7.
Incidence of various patterns of activation observed at the RA-, RAV-, BB-, LAV- and PV-line. Most complex patterns of activation were observed at the pulmonary vein region.
SW: single waves, SW+B: single waves and conduction block, MW+B: multiple wavelet and conduction block.

Patterns of Activation

Figure 6 shows examples of dissociated and non-dissociated patterns of activation. In the first two maps, fibrillation waves propagate without encountering areas of conduction block. Map A3 shows a single fibrillation wave propagating across the mapping area which fails to depolarize a small area despite activation of the surrounding tissue *(island of intra-atrial conduction block)*. In map A4, a single fibrillation wave enters a part of the mapping area, fails to elicit a response in all directions and the wave extinguishes.
Examples of dissociated patterns of activation are shown in the lower panel. In map B1, a wavefront enters the mapping area from the left, turns around a line of conduction block. The area at the other site of the line of conduction block is activated by a wavefront entering from another direction (bi-directional conduction block). Map B2 en B3 show multiple wavelets and several areas of bi-directional or omni-directional conduction block. The arisal of a new wavefront in the middle of the mapping area (asterix) is shown in map B4.
Results of categorization of fibrillation maps into dissociated (black) and non-dissociated (whitea and black/white) patterns of activation for all mapping sites for all patients is shown in Figure 7.
Figure 8 shows examples of 5 successive fibrillation maps of the cavotricuspid isthmus (A) and a site along the right atrioventricular groove (B) from the same patient. At each mapping site, there was a clear beat-to-beat change in the complexity of the patterns of activation. Even in a mapping area as small as 1.2X1.2 cm, multiple wavefronts entered from different directions, separated by arcs of conduction block (map A1, A5, B1, B2, B5). Episodes with multiple wavefronts propagating between areas of conduction block

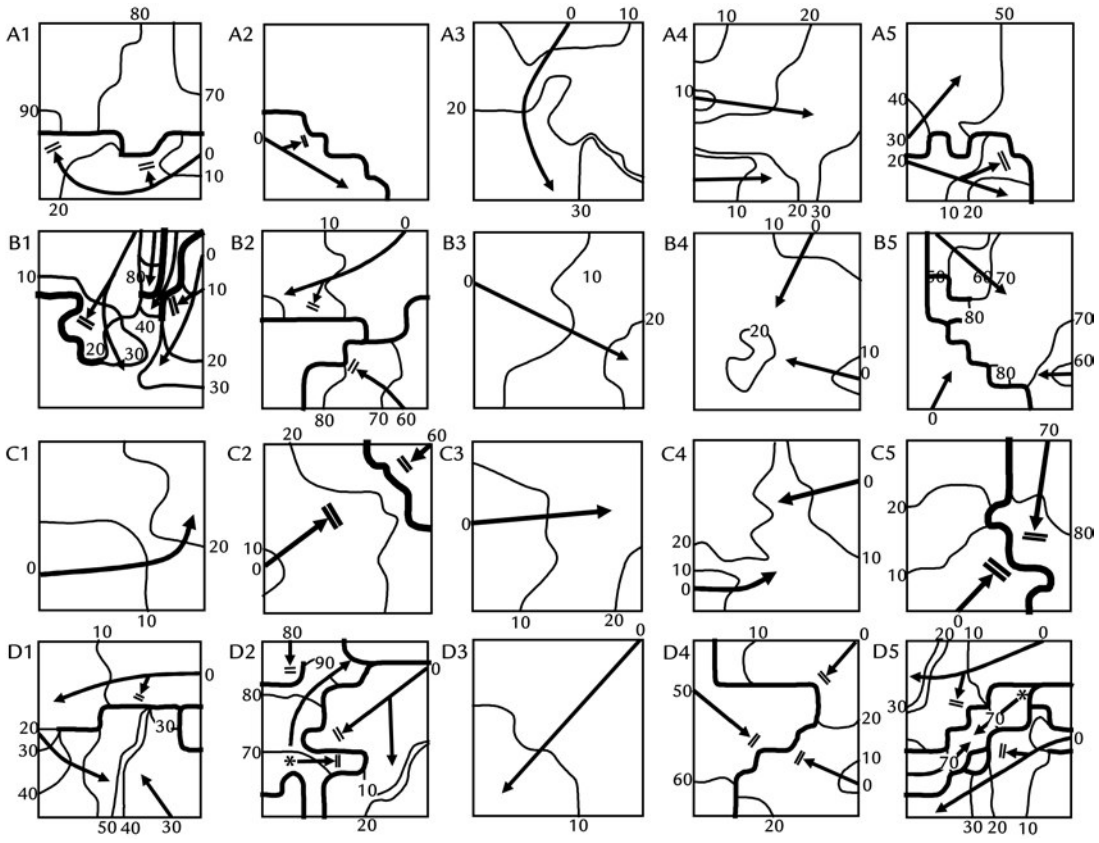

Figure 8.

Successive isochronal maps obtained from the cavotricuspid isthmus (A), right atrioventricular groove (B), left atrioventricular groove (C) and pulmonary vein region (D). At all sites, there is a clear beat-to-beat variation in complexity of the patterns of activation. Even in a mapping area as small as 1.2X1.2 cm were multiple, co-existing wavefronts present. Isochronal maps were drawn at 10 ms intervals, arrows show main activation direction, thick black lines represent areas of bi-directional conduction block.

were interspersed with large wavefronts propagating without conduction delay (map A3, A4, B3, B4). In all patients, 266 ± 85 (175-358) fibrillation maps were constructed from the RA-line and 260 ± 96 (86-372) from the RAV-line. At all right atrial mapping sites, dissociated patterns of activation occurred more frequently (non-dissociated patterns of activation: 26 ± 22 (41-93) %) versus dissociated patterns of activation : 26 ± 23 (0-100) %, $p<0.001$). There was no difference in the incidence of dissociated patterns of activation along the RA- and RAV-line (RA-line: 60 ± 13 (41-93)% versus RAV-line 62 ± 9 (45-80)%, $p = 0.8$).

Mapping in the left atrium resulted in construction of 256 ± 197 (80-583) fibrillation maps along the LAV-line and 366 ± 168 (119-522) maps from the pulmonary vein area. **Figure 8** shows 5 consecutive fibrillation maps of different sites along the left atrioventricular groove (C) and of the pulmonary vein area (D). A large number of fibrillation maps along the left atrioventricular groove demonstrated single waves, fusion of fibrillation waves, extinguishing wavefronts or wavefronts propagating around areas of omni-directional conduction block. The patterns of activation pattern at the pulmonary vein area were dominated by multiple waves separated by areas of conduction block. In some beats, the complexity of the activation pattern was enhanced by the occurrence of focal patterns of activation (asterix in map C2, C5). The incidence of dissociated patterns of activation along the LAV-line was significant lower than in the remainder of the atria (LAV-line: 32 ± 22 (0-74%)%, versus RA-line: 45 ± 25 (0-80%)%, RAV-line 46 ± 19 (0-74%)%, BB-line: 35 ± 30(0-73)%, p = 0.04). In contrast, dissociated patterns of activation were most frequently observed at the pulmonary vein area 61 ± 19 (12-81)%, p = 0.007).

Thus, these findings suggest that areas with relative longer median AFCL, a shorter refractory period and a larger variation in fibrillation intervals are also the sites with the highest incidence of dissociated patterns of activation support the hypothesis that prolongation of AFCL in case of random reentry of fibrillation waves is due to lengthening of the pathway of fibrillation waves caused by areas of conduction block.

Epicardial Breakthroughs

In chapter 4, we demonstrated by comparing fibrillation maps obtained during acute and chronic AF, that epicardial breakthrough occurred more frequently in patients with chronic AF. Epicardial breakthroughs are most likely the result of transmural propagation of fibrillation waves.[62-64] This finding suggests that transition from paroxysmal AF to chronic AF is associated with progression from 2-dimensional to 3-dimensional conduction which could be attributed to electro-pathological alterations of the thin subepicardial layer which directs conduction of fibrillation waves.[62;65] Houben et al. suggested that diversity in RS differences (difference in R and S amplitude divided by the total amplitude) of unipolar fibrillation potentials could partly be accounted for by differences in endocardial to epicardial activation direction. In case of an intact thin epicardial layer, the direction of activation is from the epi- to endocardium resulting in epicardial fibrillation potentials with an rS morphology. If the thin epicardial layer is damaged, the predominance of the S wave may be diminished. **Figure 9** shows the mean RS- differences of single and short double potentials recorded at various mapping sites along the different mapping lines in all patients. Comparing the RA-, RAV-, BB-, LAV-line and the pulmonary vein area, S wave dominance was less pronounced at bachmann's bundle and along the ST- and PV-line (RA-line: -0.04 ± 0.07, RAV-line: -0.03 ± 0.05, LAV-line: -0.03 ± 0.06, versus BB-line: -0.02 ± 0.05, ST: 0.00 ± 0.01 and PV-line: 0.01 ± 0.02, p = 0.002). This finding suggests

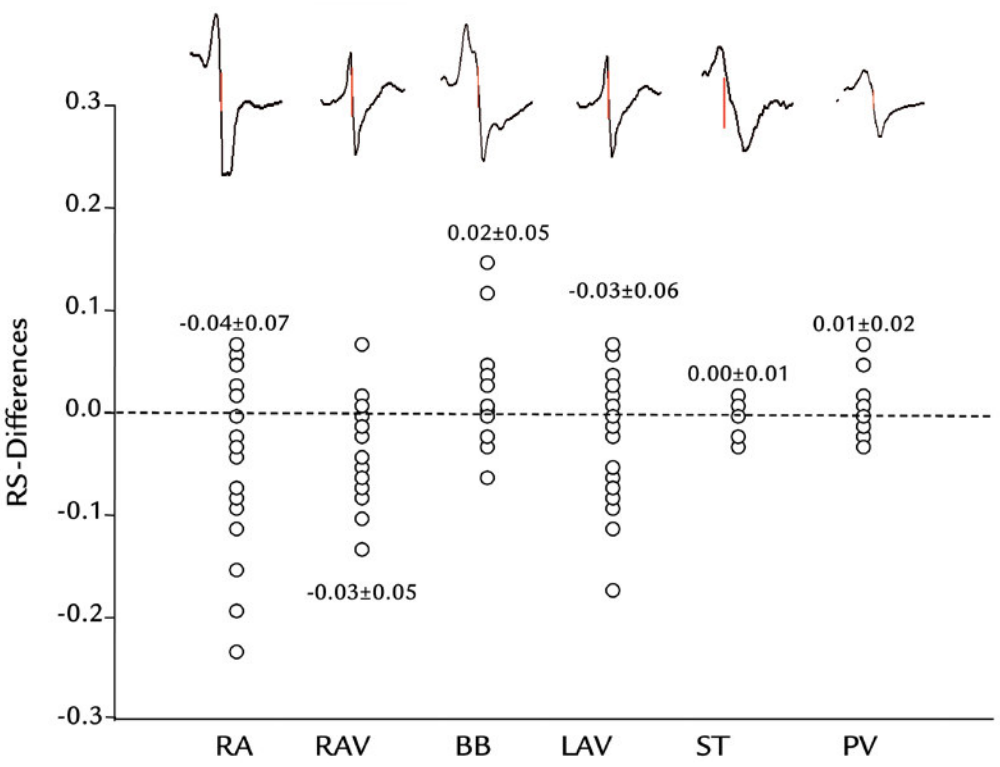

Figure 9.
R-S differences of fibrillation potentials recorded from the various line.

that there are regional differences in electro-pathological alterations of the thin epicardial layer and hence the degree of epi- to endocardial propagation.

If the hypothesis that damage to the thin subepicardial layer directing conduction of fibrillation waves facilitate the occurrence of epicardial breakthrough of fibrillation waves is correct, they can theoretically arise at every site at which the integrity of the atrial structure is lost. To test this hypothesis, we studied the incidence of epicardial breakthrough using the data from the fingertip electrode obtained from six patients. The incidence of epicardial breakthroughs throughout the atria are summarized in **Figure 10**. Hence, these results indicate that epicardial breakthrough can indeed arise at multiple sites throughout the atria. In patients in whom the right and left atria were completely mapped, the incidence of epicardial breakthrough was highest in the pulmonary vein area (29 ± 17 (1-51)% versus RA-line: (15 ± 17 (0-59)%, RAV-line: (10 ± 12 (0-38)% and BB-line: (15 ± 22 (0-61)%, LAV-line: (11 ± 13 (0-38)%, p <0.001).

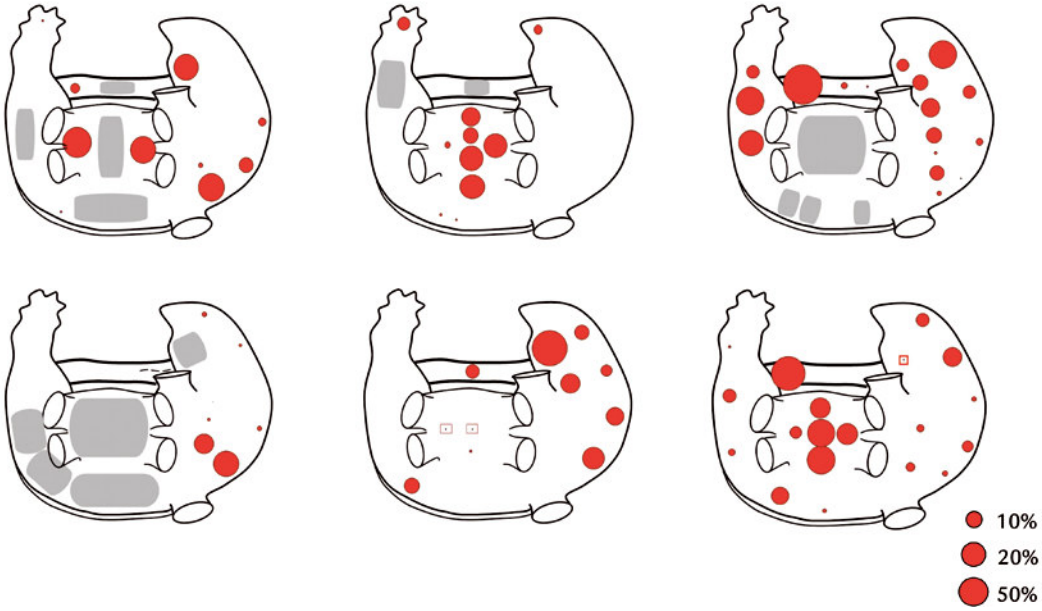

Figure 10.
Incidence of focal patterns of activation throughout the atria. A larger size of the red circle represents a higher incidence of focal patterns of activation.

Despite the fact that epicardial breakthrough were frequently observed in our high density mapping studies in patients with chronic AF, repetitive, rapid organized activation originating from a localized region activating the entire mapping area was never observed in any of the patients.

Selective Ablation of Atrial Fibrillation:
Targeting Fractionated Unipolar Electrograms.

It is assumed that the arrhythmogenic substrate of AF consists of areas of shorter refractoriness, increased dispersion of refractoriness, heterogeneity in conduction or increased conduction anisotropy. Each of these phenomena give rise to local conduction abnormalities (local dissociation in conduction, micro-reentry) and hence fractionation of fibrillation potentials. As areas of conduction block are thought to be the perpetuators of AF, fractionated electrograms could be used to identify the arrhythmogenic substrate of AF. Evidence that fractionated electrograms are markers of the arrhythmogenic substrate perpetuating AF was recently demonstrated by Nademanee et al. who showed that ablation of areas of fractionated electrograms eliminated AF.[48]

In chapter 6, we found differences in the degree of fractionation and fractionation duration of fibrillation potentials recorded during induced AF in patients with normal atria and during chronic AF in patients with mitral valve disease. This finding suggested that fractionation mapping could be used to distinguish different types of AF. In chapter 8, we

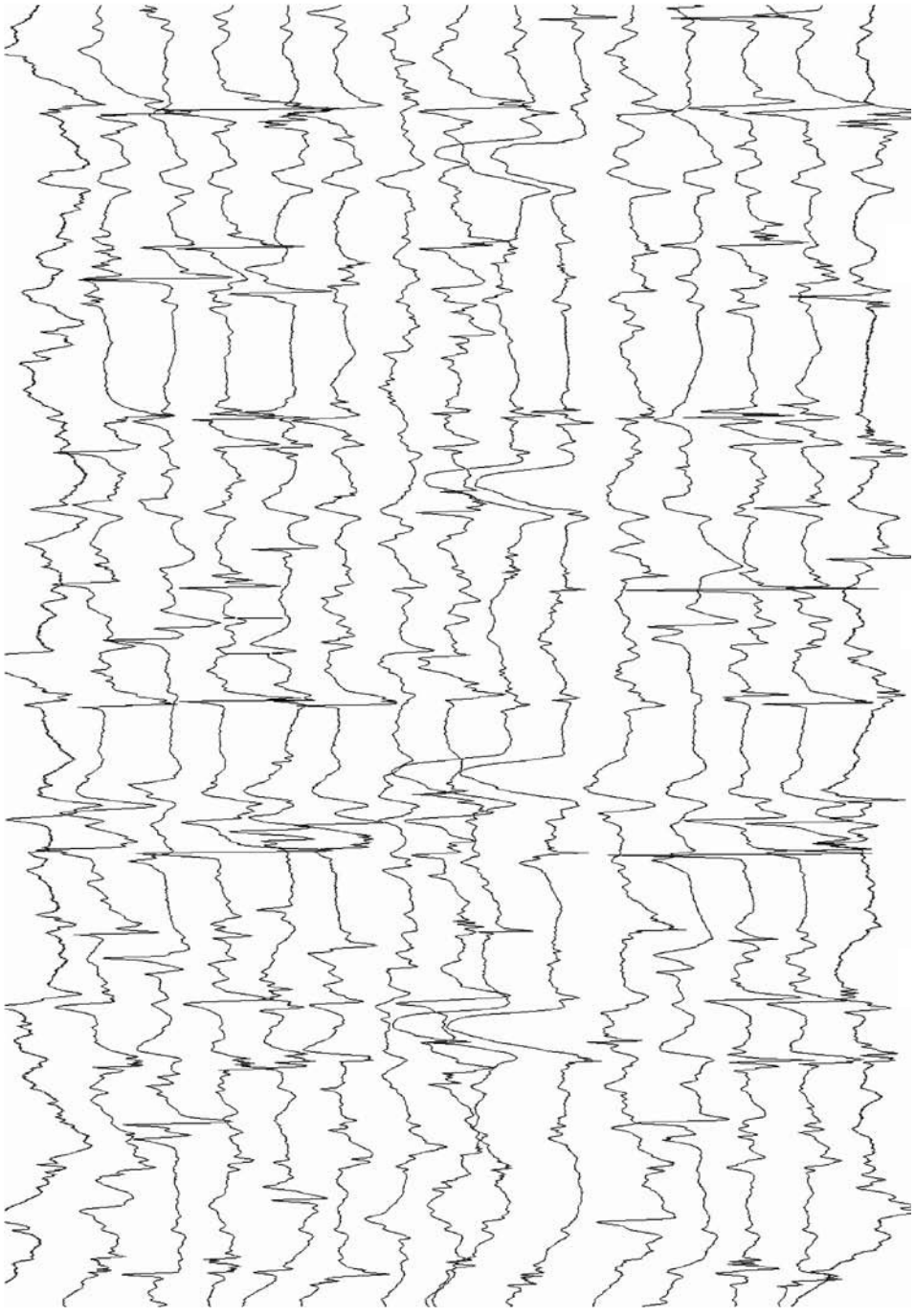

Figure 11.
Unipolar fibrillation potentials recorded from the right atrial free wall obtained from a patient with permanent AF.

assessed intra-atrial differences in fractionation characteristics of fibrillation potentials. Comparing similar mapping sites between patients, there were large inter-individual differences in the degree of fractionation. Also, the degree of fractionation varied considerably over only small distances.

The degree of fractionation assessed with multi-site high density mapping is relative low compared to other mapping studies in patients with AF. In addition, long episodes of continuous electrical activity were not found. This difference might be explained by the recording technique used; as most mapping studies were performed with large bipolar electrodes (tip size: 4 mm, inter-electrode distance 2 mm), "fractionation" is rather the result of integration of different fibrillation waves than true local dissociation in conduction. Up till now, the mapping resolution required for identification of the true arrhythmogenic substrate is unknown.

High Density Mapping of AF: Look Before You Leap

In chapter 8, multi-site high density mapping with a 1 cm^2 electrode revealed large differences in the fibrillatory process over only small distances. When comparing similar mapping sites between different patients there were large differences in various electrophysiological properties. Multiple wavelets separated by areas of conduction block were even observed in an area of only 1 cm^2, indicating the complexity of the fibrillatory process.

As patients with AF have different cardiac diseases, the location and extension of the arrhythmogenic substrate will be different for each individual. Also, there will be most likely regional differences in the degree of electrical and structural remodeling and the severity of remodeling may also vary from patient to patient. Interatrial conduction during AF will show inter-individual variation as interatrial connections vary in presence location and dimension. Multi-site high density mapping of AF will therefore be essential in order to successfully eliminate AF.

As AF is characterized by non-repetitive activation of the atria, there will be a large number of activations per second with a considerable temporal variation of fibrillation potentials at each recording site (**Figure 11**). As demonstrated in chapter 6, multiple consecutive deflections can be the result of either a temporal overlap of fibrillation waves or lines of conduction block. This implies that each fibrillation potential must be analysed in relation to the spatial patterns of activation. Mapping of AF is therefore extremely time-consuming. Up till now, mapping of AF can only reliable performed by semi-automatic marking of fibrillation potentials. However, extensive editing of the marked fibrillation potentials is still required for constructing of isochronal maps. In order to map AF on-line during cardiac surgery, the appropriate software still needs to be developed.

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