Fibrillatory Conduction in the Atrial Free Walls of Goats in Persistent and Permanent Atrial Fibrillation

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Background—Over a time course of months, the stability of atrial fibrillation (AF) gradually increases and the efficacy of pharmacological cardioversion declines both in humans and in animal models. Changes in fibrillatory conduction over this period largely are unexplored.

Methods and Result—Goats were instrumented with an atrial endocardial pacemaker lead and a burst pacemaker. AF was maintained for 3 weeks (short-term AF [ST], n=10) or 6 months (long-term AF [LT], n=7). AF could be cardioverted pharmacologically at the early time point (persistent AF), but not at the later time point (permanent AF). At follow-up, a high-resolution mapping electrode was used to record epicardial conduction patterns in the free walls of the right atrium (RA) and left atrium (LA). A new method for mapping of fibrillation waves was used to describe AF conduction patterns.

Wavefronts propagated uniformly during slow pacing in both groups, although conduction velocity was significantly lower in the LT group (LA, 93±14 versus 72±10 cm/s; RA, 94±8 versus 78±8 cm/s). Median AF cycle length (AFCL) was not significantly different between the groups. However, the LT group showed highly complex activation patterns during AF, with an increased number of simultaneously propagating waves (LT group RA, 8.4±3.0 waves/AFCL; LA, 12.8±2.4 waves/AFCL; versus ST group RA, 4.3±2.2 waves/AFCL; LA, 4.5±2.5 waves/AFCL). Fibrillation waves in the LT group showed pronounced dissociation with large activation time differences. The incidence of waves newly appearing within the recording area also was increased in both atria. These alterations in conduction were accompanied by myocyte hypertrophy and increased endomysial fibrosis.

Conclusions—Long-term AF in goats leads to dissociated conduction in the atrial free walls that may contribute to increased AF stability. (Circ Arrhythm Electrophysiol. 2010;3:590-599.)

Key Words: atrium  ■  fibrillation  ■  conduction  ■  tissue  ■  structure

In patients with paroxysmal atrial fibrillation (AF), the pulmonary veins often harbor high-frequency sources that can initiate and maintain AF episodes. In patients with chronic AF, however, sites with a relatively high activation frequency are more widespread throughout the atria, and isolation of the pulmonary vein region has a substantially lower efficacy in restoring sinus rhythm. This indicates that the relative contribution of the pulmonary vein region to AF stability is smaller in chronic AF and that the atrial myocardium outside this region has become a substrate for AF. There is evidence that atrial conduction patterns are indeed more complex in patients with chronic AF than in those with acute AF. However, patients with AF often have advanced age or an underlying heart disease, such as valvular disease and heart failure, which are known to affect the AF substrate. Here, we have studied the process of AF substrate formation due to the arrhythmia itself in a goat model of “lone AF.”

Clinical Perspective on p 599

As in many patients, the stability of AF in goats gradually increases with time. After 2 days, the atrial refractory period and AF cycle length (AFCL) are markedly shortened (electric remodeling), and AF episode duration has increased to seconds or minutes. After 2 to 3 weeks, AF does not terminate spontaneously anymore, but it can still be converted pharmacologically (persistent AF). Over a period of weeks to months, structural alterations gradually increase. In parallel, the success rate of pharmacological cardioversion decreases until it is no longer possible to cardiovert AF in most goats (permanent AF). To investigate the transition from persistent to permanent AF, we have studied goats after 3 weeks of maintained AF (short-term AF [ST]) and goats after 6 months of AF (long-term AF [LT]). We characterized conduction patterns in the atrial free walls during pacing and AF using high-resolution epicardial mapping and a new method to...
describe fibrillation patterns. Compared to the ST group, the LT group showed more complex, dissociated activation patterns, that is, the free walls were activated by higher numbers of simultaneously propagating wavefronts.

Methods

Instrumentation

Ten ST and 7 LT goats were included in this study (weight ST, 50±3 kg; weight LT, 49±4 kg). All animal procedures were in accordance with national and institutional guidelines. Anesthesia was induced with 10 mg/kg pentobarbital and maintained with 2% isoflurane. A bipolar screw-in J-lead (Medtronic; Minneapolis, Minn) was inserted through the jugular vein into the RA. The lead was connected with an Itrel pacemaker (Medtronic) in a subcutaneous pocket in the neck. AF was maintained by applying a 1-second burst of 50 Hz every 2 seconds for 3 weeks (ST) or 6 months (LT). In the LT goats, a second RA lead was connected to a Kappa pacemaker (Medtronic) to record an RA electrogram.

Monitoring of AF Stability

In the LT group, AF stability was evaluated by a monthly pharmacological cardioversion experiment. An I_{Kur}/I_{to}-blocker, AVE0118 (Sanofi-Aventis; Bridgewater, NJ), was used in combination with an I_{Kr}-blocker, dofetilide. For determination of the AFCL from an RA electrogram, the Kappa pacemaker was interrogated telemetrically.

A continuous infusion of 10 mg/kg per hour AVE0118, a novel wave mapping algorithm was used, which is detailed in the online supplement.

Histology

Endomysial fibrosis and myocyte diameter were determined from thin sections of plastic-embedded atria from 4 ST and 5 LT goats. See the online supplement for details.

Statistical Analysis

Results are expressed as mean±SEM. Statistical analysis was performed using 1-way ANOVA with Bonferroni correction. P<0.05 was considered statistically significant.

Results

Progressive Stabilization of AF

In the LT group, AF stability was evaluated monthly in awake goats. Figure 1A shows the success rate of pharmacological cardioversion by AVE118 (I_{Kur}/I_{to}-blocker) and dofetilide (I_{Kr}-blocker) at different time points. After 1 month of AF, sinus rhythm could be restored in 80% of the goats, but cardioversion efficacy gradually decreased to 50% after 3 months of AF and to 0% after 4 months of AF. Over the 6-month monitoring period, the baseline AFCL calculated from the RA electrogram did not decrease significantly (Figure 1B). The AFCL measured 100±9 ms after 1 month of AF and 92±6 ms after 6 months of AF. Thus, failure of pharmacological cardioversion was not attributable to a further progression of electric remodeling.
In Figure 1C, the effect of AVE0118 and dofetilide on the AFCL is provided for a representative goat after 1, 3, and 6 months of AF. After 1 month of AF, 14 minutes of AVE0118 administration increased AFCL from 96 ms at baseline to 128 ms before cardioversion (total dose, 140 mg/kg AVE0118). After 3 months of AF, AFCL increased from 82 ms at baseline to 130 ms after 30 minutes of AVE0118 and to 140 ms after 14 minutes of combined infusion of AVE0118 and dofetilide before cardioversion (total dose, 440 mg/kg AVE0118 and 4.7 μg/kg dofetilide). After 6 months of AF, AFCL increased from 86 to 129 ms with AVE0118 alone and to 140 ms after 14 minutes of combined infusion of AVE0118 and dofetilide before cardioversion (total dose, 440 mg/kg AVE0118 and 4.7 μg/kg dofetilide). Despite exceeding the AFCL before cardioversion at the earlier time points, sinus rhythm was not restored. After 4 months of AF, pharmacological cardioversion failed in all LT goats. In ST goats, no spontaneous cardioversions were observed during the day before follow-up experiments as determined by monitoring the surface ECG after the pacemaker was turned off. Therefore, goats in the ST and LT groups were considered to be in persistent and permanent AF, respectively, at the time of euthanasia experiments.

Conduction Velocity and Fibrillation Intervals

After 3 weeks (ST group, n=10) or 6 months (LT group, n=7), an open-chest euthanasia experiment was performed. LT goats had a preserved cardiac output (42.1±9.3 versus 50.0±8.2 mL/min per kg in ST goats; P not significant) and did not show any clinical signs of heart failure. During sinus rhythm and slow pacing, both groups showed uniform atrial propagation. However, conduction velocity during slow pacing (basic CL, 400 ms) was decreased by 30% in both atria in the LT group compared to the ST group (Figure 2A and 2B).

During AF, the average of the median AFCLs in the ST group (136±38 and 141±31 ms in the RA and LA, respectively) was not significantly different from the LT group (103±23 and 112±17 ms for the RA and LA, respectively) (Figure 2C). In addition, the beat-to-beat variability in AFCL was not significantly different between the 2 groups for either atrium (Figure 2D).

Conduction Heterogeneity and Block

To quantify differences between ST and LT goats, the distribution of conduction times during AF was evaluated. The distribution in LT goats had a more pronounced tail toward long conduction times, as illustrated in Figure 3A. Compared to the ST group, the average of the median conduction times in the LT group was significantly increased in the LA but not in the RA (Figure 3B). The incidence of conduction block (defined as a conduction time >12 ms) was significantly higher in the LT atria (Figure 3C).
Activation Patterns During AF
To investigate the increase in AF stability, we studied fibrillatory conduction patterns. During AF, activation patterns were more complex in the LT goats than in the ST goats (see supplemental videos). In Figure 4, a representative example of conduction patterns during consecutive beats of AF in the LA of an ST goat are shown. During these beats, the LA was activated by 3 to 5 waves. In the first beat, 2 waves

Figure 4. Activation patterns during AF in the LA of an ST goat. Representative example of isochronal maps recorded from the LA free wall during 4 consecutive beats of AF. In these beats, the recording area is activated by 3 to 5 waves, colored different shades of gray. Thin dashed lines indicate the boundaries of individual waves. White numbers indicate the starting time point of the waves. Peripheral waves are indicated by arrows. Asterisks denote epicardial breakthroughs. Discontinuous waves are indicated by a zigzag line (beat 4). Isochronal lines are separated by 5 ms.

Figure 3. Conduction times. A, Representative examples of conduction time distributions during AF in an ST (left) and an LT goat (right). In the LT goat, the distribution had a longer tail, indicating an increased incidence of long conduction times. B, The average of the median conduction times (P50 of the distribution) was significantly increased in the LT group compared to the ST group in the LA but not in the RA. C, The incidence of conduction block (conduction times >12 ms) was significantly increased in the LT LA compared to the ST LA and significantly larger in the LT RA compared to the ST RA. *P<0.05. ***P<0.001.
entered the recording area from the side of the array, followed shortly by an epicardial breakthrough wave (asterisk) near the middle of the array. Three peripheral waves activated the area during the second beat, and 4 peripheral waves and a breakthrough wave were observed during the third beat. In the fourth beat, a broad wave activated most of the area from the periphery, with notable areas of slow conduction, followed by 2 late, discontinuous waves after a considerable delay (zigzag lines). As illustrated in a representative example for the LA in Figure 5, more-complex activation patterns were observed in the LT goats. In the 4 consecutive beats of AF shown, the LA free wall was activated by 10 to 16 waves per beat. For example, during the second beat, 7 peripheral waves entered the recording area at various time points. In addition, 3 epicardial breakthroughs waves originated away from the periphery. Finally, 5 discontinuous waves originated after a delay at the boundary of previous waves. The other beats show comparable, highly complex, and irregular patterns. Within the areas activated by a wave, regions of slow conduction often were observed. Overall, compared to ST goats, AF in LT goats was characterized by a larger number of simultaneously propagating wavefronts that often followed a meandering trajectory. The high degree of dissociation of fibrillation waves together with numerous epicardial breakthroughs and discontinuities led to a greatly increased complexity of fibrillatory conduction.

Figure 5. Activation patterns during AF in the LA of an LT goat. Representative example of isochronal maps recorded from the LA free wall during 4 consecutive beats of AF is shown. In these beats, the recording area is activated by 10 to 16 waves. Some discontinuous waves, indicated by white zigzag lines, are conducted from a wave in the previous beat window (eg, the wave in the upper left quadrant in beat 2, starting at 40 ms). By contrast, a black zigzag line indicates discontinuous conduction from 1 wave to another within the beat shown. Other annotations as in Figure 3.

Figure 6 shows an example of the dissociated conduction patterns that were far more prominent in the LT group. Both the unipolar electrograms and the derived isochronal map show that neighboring electrodes can be part of different dissociated, narrow fibrillation waves with large activation time differences. Figure 6 also illustrates an example of epicardial breakthroughs in the RA of an LT goat. The isochronal map shows 3 epicardial breakthroughs with the resulting fibrillation waves spreading away from their sites of origin and colliding near the middle of the array.

Number and Characteristics of Fibrillation Waves

The activation maps in Figures 3 and 4 indicate that the number of fibrillation waves per cycle is higher in the LT group than in the ST group. Indeed, as shown in Figure 7A, the total number of waves was significantly increased in both atria. Comparing the ST and LT groups, the average number of waves per AFCL increased from 4.3±0.7 to 8.4±1.1 in the RA and from 4.5±0.8 to 12.8±0.9 in the LA. Thus, the number of waves per AFCL was increased by factors of 2 and 3 in the RA and LA, respectively.

To further delineate the alterations in conduction pattern, the source of these fibrillation waves was investigated. We distinguished between peripheral waves entering the recording area from the edge of the array and nonperipheral waves newly appearing within the recording area away from the outer edge. Compared to the ST group, the number of
peripheral waves per AFCL was significantly increased in the LT group for both atria (Figure 7B). The number of nonperipheral waves also was significantly increased in both atria of the LT group (Figure 7C). However, the ratio between nonperipheral and peripheral waves was not significantly different between the ST and the LT groups in either atrium (Figure 7D).

Structural Changes
Histological changes were assessed in the epicardial layer of 4 ST and 5 LT goats. No significant difference in the separation of myocyte bundles (perimysial fibrosis) or in the occurrence of larger areas of (replacement) fibrosis was observed between the groups (data not shown). However, a significantly larger degree of endomysial fibrosis was present in both atria of LT goats (Figure 8A and 8B), leading to increased transverse myocyte-to-myocyte distances (Figure 8C). In addition, myocyte diameter was significantly larger in the atria of LT goats, reflecting myocyte hypertrophy due to chronic AF (Figure 8D).

Discussion
In this study, we have investigated conduction patterns at 2 stages in the progressive stabilization of AF. We demonstrate a greatly increased complexity of fibrillatory conduction in the atrial free walls after 6 months of AF, indicating that these regions contribute more to the AF substrate. Based on our previous studies, the ST group has complete electric remodeling without significant structural changes, whereas the LT group has both complete electric remodeling and significant structural remodeling. In the goat model, electric remodeling is complete after 2 to 3 days of AF. Here, we found that the AFCL was not significantly different between ST and LT goats during follow-up experiments, although there was a trend toward a lower median AFCL in LT goats during anesthesia. However, median AFCL did not change over a time course of 6 months in awake LT goats. Thus, the difference in AF stability between the groups cannot be attributed to a progression of electric remodeling. A much slower process of structural remodeling has been proposed to explain the progressive AF stabilization after the first days of AF. These slow structural changes include cellular hypertro-
phy and dedifferentiation, myolysis, an increased extracellular matrix volume per myocyte, and heterogeneous connexin40 distribution.12,15

**Time Course of AF Stabilization**

We have shown previously that AF stability gradually increases over a time course of months. Eijsbouts et al13 showed that the cardioversion efficacy of flecainide gradually decreases from 60% after 1 month to 17% after 4 months of AF. In another study, we reported a 100% success rate of cardioversion with a combination of AVE0118 and dofetilide in goats after 46 days of AF.14 Here, we show that cardioversion efficacy of this drug combination also gradually declined to 0% after 4 months of AF. This loss of cardioversion efficacy indicates an increased AF stability, reflecting the transition from persistent to permanent AF.

**Changes in Fibrillatory Conduction**

The increased AF stability after 6 months of AF was associated with changes in the activation pattern. Overall, fibrillation patterns in the LT group were more complex, particularly in the LA. In summary, we observed the following proarrhythmic alterations in the LT group: (1) a decrease in conduction velocity, (2) increased conduction heterogeneity and block during AF, (3) an increase in the number of simultaneously propagating fibrillation waves, and (4) an increased incidence of newly appearing waves.

During slow pacing, macroscopic conduction velocity was 30% lower in both atria in the LT group compared to the ST group.

**Figure 7.** Number and types of fibrillation waves. A, The total number of fibrillation waves per AFCL was significantly higher in the LT group than in the ST group for both atria and significantly higher in the LA than in the RA. B, The number of peripheral waves was significantly increased in both atria of LT goats compared to ST goats and significantly larger in the LA than in the RA. C, The number of nonperipheral waves (i.e., waves appearing within the outer edge of the recording area away from the outer edge of the array) was significantly increased in both atria of the LT group. D, The ratio between nonperipheral and peripheral waves was not significantly different between the ST and the LT groups in either atrium. *P<0.05. **P<0.01. ***P<0.001.

**Figure 8.** Endomysial fibrosis and myocyte hypertrophy. Examples of the epicardial side of toluidine blue stained sections of an ST goat (A), showing tightly packed myocytes, and an LT goat (B), showing increased myocyte width and endomysial fibrosis. There were significant increases in average myocyte-to-myocyte distance within bundles (C) and myocyte diameter (D) (n=4 for ST LA and RA, n=5 for LT LA and RA). Original magnification, 200×.
group. In other large-animal models of AF, such as canine models of congestive heart failure and chronic LA dilatation, no change in conduction velocity was observed. In these models, marked structural alterations have been observed, including relatively large areas of replacement fibrosis. In our goat model of AF, we have not observed such large fibrotic areas. However, we have previously reported a heterogeneous decrease in the expression of the gap junction protein connexin40, which is associated with increased complexity of fibrillatory conduction in patients with chronic AF patients, and an increase in the amount of extracellular matrix per myocyte. The latter observation is confirmed in this study by the increased transverse intermyocyte distances within bundles (endomysial fibrosis), resembling the pattern of interstitial fibrosis (microfibrosis) reported for human atrial trabeculae during normal aging. Alterations in cellular connectivity due to microfibrosis can produce large delays during conduction perpendicular to the main fiber orientation. Thus, this pattern of fibrosis may have a pronounced effect on epicardial conduction, especially during transverse conduction.

We expect the impairment of epicardial conduction in the LT group to lead to an increased conduction heterogeneity during AF. Indeed, 6 months of AF resulted in an increased incidence of long conduction times and conduction block. In detailed activation maps, the decrease in epicardial conduction velocity also was reflected by areas of slow conduction within waves in the LT group (examples in Figure 5). Furthermore, there was marked beat-to-beat variability in the location of conduction block between waves and areas of slow conduction within waves. This indicates that structural remodeling does not lead to fixed transmural obstacles to propagation but rather to a decrease in conduction reserve due to the ubiquitous occurrence of myocyte hypertrophy and endomysial fibrosis throughout the epicardial layer. Because these changes are expected to compromise transverse conduction in particular, conduction disturbances may depend on the coupling interval and propagation direction.

Using a computer model, Moe and Abildskov demonstrated that AF can be maintained by multiple randomly propagating wavelets. In a canine model of cholinergic AF, Allessie et al showed that AF was maintained as long as a critical minimal number of 4 to 6 fibrillation waves were simultaneously present. In the present study, we have found that a 10-cm² area of the atrial free wall in the ST group displayed an average of 4 to 5 fibrillation waves per AFCL. The observed fibrillation patterns were similar to those in humans during acute AF. In LT goats, the number of waves within this 10-cm² area was a factor of 2 to 3 higher. In addition, the atria dilate due to chronic AF, which will further increase the number of simultaneously propagating waves in LT goats compared to ST goats. The large number of fibrillation waves may explain why AF is sustained in the ST group and why AF stability is further increased in the LT group.

The number of fibrillation waves appearing on the epicardial surface within the central region of the array was increased in both atria of the LT group. We have further classified these fibrillation waves into epicardial break-throughs and discontinuous waves. Although there may be a degree of contamination between the 2 types of nonperipheral waves, this approach allows us to distinguish diverging patterns of newly appearing waves, which may have different underlying mechanisms. Epicardial breakthroughs may result either from transmural propagation from the endocardial trabecular network or from local generation of a new wave due to cellular proarrhythmic mechanisms. Discontinuous waves may result from a delay in conduction between adjacent electrodes in the epicardial plane. Spach et al showed that myocyte hypertrophy and increased interstitial fibrosis, similar to that observed in this study, can lead to activation delays during transverse conduction. A major advantage of our method of analysis is that it allows a detailed reconstruction of activation patterns during AF. Using this analysis, we have not observed sites with rapid repetitive activation patterns acting as AF drivers in the regions investigated here. Quantitatively, both the number of peripheral and the number of nonperipheral waves (epicardial breakthroughs and discontinuous waves) was higher in LT than in ST atria. However, the ratio between nonperipheral and peripheral waves was not different between the groups. Thus, the number of waves entering from the periphery and new waves appearing within the recording area increase in the same proportion. This finding indicates that the additional complexity generated within the area under observation is at least as large as that generated outside the field of view, supporting an anarchical (substrate-based) rather than a hierarchical (driver with or without a substrate) mechanism. Interestingly, a recent study by Everett et al using simultaneous endocardial and epicardial mapping indicated that fibrillation patterns in dogs after 6 to 8 weeks of rapid atrial pacing could be explained by multiple wavelet reentry. However, our results are also compatible with high-frequency sources outside the field of view, with an increased propensity for waves to break throughout the atrial free walls. In either case, the altered conduction properties of the free walls in LT goats are likely to increase AF stability.

In most parameters investigated in this study, the LA was more severely affected than the RA. During AF, the LA may face a larger hemodynamic load and degree of stretch. However, in earlier studies we did not find systematic differences between the atria in cellular hypertrophy, extracellular matrix volume, and connexin distribution. Alternatively, differences in conduction during AF may reflect the interaction between structural remodeling and differences in anatomy between the atria. The overall anatomy and endocardial trabecular network differ between the atria, and this is likely to influence conduction pathways during AF.

Overall, the factors discussed here contribute to a higher degree of complexity of fibrillatory conduction in the LT group, which is likely to increase AF stability. Our results are consistent with the assumption that structural remodeling causes a decrease in conduction reserve, leading to dissociated conduction of fibrillation waves.

Study Limitations

The substrate for AF in this goat model of lone AF may not be representative for all patients with AF. In many patients,
underlying heart disease or advanced age may lead to atrial structural changes that create a preexisting substrate for AF. In our goat model, we have studied alterations in fibrillation patterns due to AF itself. We show differences in propagation patterns associated with the transition from persistent to permanent AF in the absence of a preexisting substrate. Whether a preexisting substrate would affect propagation during AF and the time course of AF stabilization requires further investigation.

We did not observe high-frequency sources driving AF. It is conceivable that such sources were located outside our recording locations, which spanned the major part of the anterior surface of the free walls. In patients with paroxysmal AF, the pulmonary vein region can display rapid ectopic activity.1 In a sheep model of cholinergic AF26 and a dog model of lone AF,72 activation frequencies were higher in the LA than in the RA. In the present model, the difference in AFCL between RA and LA was small, and there was no consistent gradient in activation frequency: The RA had a smaller median AFCL in 5 out of 10 ST goats and in 5 out of 7 LT goats. Nevertheless, conduction patterns in LT goats were more complex in the LA than in the RA as evidenced by the larger number of simultaneous waves. Thus, the median AFCL does not accurately reflect the complexity of fibrillatory conduction. Overall, the complexity of conduction patterns in the free walls was markedly increased after 6 months of AF. Irrespective of the possible contribution of areas outside these recorded areas, the free walls have at that point become a substrate that is likely to increase AF stability.

Conclusions
Comparison of fibrillation pattern between goats with persistent and permanent AF shows an increased dissociation of epicardial conduction, leading to an increased number of simultaneously propagating fibrillation waves. Thus, over a time course of months, AF itself causes changes in fibrillatory conduction that are likely to contribute to increased AF stability. The widespread changes in behavior of the atrial free walls may contribute significantly to the substrate of chronic AF and may help to explain the limited efficacy of targeted ablation strategies in patients with chronic AF.

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Disclosures
None.

References
CLINICAL PERSPECTIVE

Clinical studies have demonstrated that paroxysmal AF often can be treated by ablation of localized sources, predominantly occurring in the pulmonary vein region. In patients with chronic AF, however, such ablation strategies have a substantially lower efficacy in restoring sinus rhythm, indicating that in chronic AF, areas outside the pulmonary vein region make a higher relative contribution to AF stability. We have studied the development of the substrate for AF in a goat model of lone AF by comparing fibrillatory conduction patterns in the atrial free walls of goats after 3 weeks of AF (complete electrical remodeling without significant structural changes) and 6 months (complete electrical remodeling and significant structural remodeling) of AF. Pharmacological cardioversion was still effective in the 3-week group (persistent AF) but not in the 6-month group (permanent AF). Compared to the 3-week group, goats in the 6-month group showed an increased dissociation of epicardial fibrillation patterns, with a larger number of simultaneously propagating fibrillation waves. Thus, over a time course of months, AF itself leads to an increase in the complexity in fibrillatory conduction in the atrial free walls that is likely to contribute to increased AF stability. Histologically, these changes were accompanied by myocyte hypertrophy and endomysial fibrosis. These widespread alterations in the behavior of atrial myocardium may contribute significantly to the AF substrate and may help to explain the limited efficacy of targeted ablation strategies in patients with chronic AF.
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SUPPLEMENTAL MATERIAL

Methods: Analysis of fibrillation patterns

Using custom software, time points of maximum negative dV/dt were determined for each electrode to determine local activation times and construct activation time maps. Conduction velocity (CV) during pacing at a basic cycle length (BCL) of 400 ms was calculated as the average of local CVs determined by a vector-based algorithm.

For each recording location, 4-5 seconds of AF was analyzed in detail. AF cycle length (AFCL) was determined by measuring the time between consecutive activations at each electrode. The beat-to-beat variation in AFCL of successive beats were determined for each electrode. To quantify the width of the distribution of beat-to-beat differences in AFCL, the range containing 60% of the AFCL variations within this distribution was denoted as the 'AFCL irregularity'. The distribution of conduction times was assessed by calculating the difference in activation time between each electrode and its neighbours. Conduction block was defined as a conduction time between adjacent electrodes >12 ms.

For quantitative analysis of fibrillation patterns, activation time points were grouped into separate fibrillation waves. First, sites of earliest activation were identified that did not have preceding activation times in the immediately adjacent electrodes within 12 ms. From such a starting points of a wave, neighbouring electrodes were ascribed to that wave using an iterative procedure, by finding conduction times between adjacent electrodes ≤12 ms. With the interelectrode distance of 2.4 mm, this corresponds to a CV >20 cm/s. This limit of 12 ms was chosen because it represents the average value of the 95th percentile of the local
conduction time histogram in normal goats. Therefore, this value lies at the lower end of what can be considered activation by normal conduction. A starting point for a new wave was marked when its activation time was not preceded within 12 ms with ANY of its neighbors, making activation through normal conduction unlikely. Waves were defined as areas surrounded by lines of block (interelectrode conduction times >12 ms) or fusion (interelectrode conduction times ≤12 ms) everywhere along their boundaries within the recording array. Even with the complex fibrillation patterns observed in LT goats, waves were still comprised of multiple electrode positions, indicating that the interelectrode distance of 2.4 mm was sufficient to resolve individual waves. Wave maps were generated automatically by a computer algorithm applying these criteria and then independently checked and edited by two researchers. Depending on their origin, different types of fibrillation waves were distinguished: 'Peripheral waves' entering the mapping area from the outer edge of the array and 'non-peripheral waves' appearing at the epicardial surface inside the mapping area, excluding the outer edge. The non-peripheral waves were further subdivided in 'epicardial breakthroughs' and 'discontinuous waves'. An epicardial breakthrough was defined as a non-peripheral wave with a site of origin either not immediately adjacent to a previous wave or immediately adjacent to a previous waves, but with a delay larger than 50 ms. Thus, epicardial breakthroughs were characterized as the appearance of a wave in the recording area that could not be explained by propagating fibrillation waves present in the epicardial plane.\textsuperscript{1} Discontinuous waves were defined as waves originating at the boundary of an earlier wave with a delay between 12 and 50 ms. The delay of >12 ms to the starting point of a discontinuous wave qualified as conduction block. For that reason, these events were qualified as separate fibrillation waves. Thus, discontinuous waves can be seen as a type of 'breakthrough', but one
that is closely linked in space and time to a previous epicardial wave. The incidence of all types of waves was normalized to the median AFCL of that AF episode. Examples of comparisons between conventional activation movies and 'waves movies' can be seen in the online supplemental movies. From such movies, consecutive time windows were selected and represented as 'wave maps', illustrating the starting points of waves within the recording array and the boundaries of these waves. In the areas within a wave boundary, activation time isochrones were drawn manually to illustrate propagation patterns of individual fibrillation waves (figures 3-5).

**Methods: Histology**

After electrophysiological studies, hearts were excised and fixed in zinc acetate buffered formalin solution (4%, pH 5.5). The fixated atria were embedded in plastic resin (Technovit 7100, Heraeus Kulzer). Transverse sections (5µm) were stained with toluidine blue. For quantitative analysis, photographs were taken at 200x magnification. The diameter of the atrial myocytes was measured in myocytes with the nucleus in plane of the section. Endomysial fibrosis was defined as connective tissue separating individual myocytes within bundles. The width of endomysial tissue septa was quantified as the distance between myocytes within bundles.

**Legend to Supplemental Movies.**

Movies showing LA fibrillation patterns in a representative ST and LT goat. The left panels show a normal activation movie, the right panels show the corresponding 'wave map'. Fibrillatory conduction was substantially more dissociated
in the LT LA than in the ST LA. Gray circles indicate peripheral waves, white stars represent epicardial breakthroughs and white arrows denote discontinuous waves.

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