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Diverse Fibrosis Architecture and Premature Stimulation Facilitate Initiation of Reentrant Activity Following Chronic Atrial Fibrillation

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Abstract

Introduction—Patients with paroxysmal atrial fibrillation (AF) often transition between sinus rhythm and AF. For AF to initiate there must be both a trigger and a substrate that facilitates reentrant activity. This trigger is often caused by a premature atrial contraction or focal activations within the atrium. We hypothesize that specific architectures of fibrosis alters local conduction to enable AF.

Methods and Results—Control goats (n=13) and goats in chronic AF (for an average of 6 months, n=6) had a high density electrode plaque placed on the LA appendage. Conduction patterns following a premature atrial contraction, caused by an electrical stimulation, were quantified to determine regions of conduction slowing. These regions were compared to architecture, either diffuse fibrosis or regions of obstructive fibrosis, and overall fibrosis levels as determined by histology from the mapped region. The chronic AF goats had more obstructive fibrosis than the controls (17.5±8.0 fibers/mm² vs. 8.6±3.0 fibers/mm²). Conduction velocity of the AF goats was significantly slowed compared to the control goats in the transverse direction (0.40±0.04 m/s vs. 0.53±0.15 m/s) but not in the longitudinal direction (0.70±0.27 m/s vs. 0.76±0.18 m/s).

Conclusions—AF induced atrial remodeling leads to increased obstructive fibrosis and conduction velocity slowing transverse to fiber orientation following premature stimuli. The decrease in conduction velocity causes a decrease in the cardiac wavelength, and increases the likelihood of reentry and AF onset.

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Keywords

atrial fibrillation; conduction; electrophysiological mapping; fibrosis; remodeling

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia, affecting over 2 million people in the United States, and its prevalence is expected to grow in the coming years.¹ AF is a serious health concern and is a high risk factor for stroke¹ and cardiomyopathy². In paroxysmal AF, the pulmonary veins have focal activations that can initiate and drive AF. Ablating around the ostia of pulmonary veins to isolate these focal sources from the rest of the left atrium has shown success in treating paroxysmal AF.³ However, ablation has poor outcomes for treating more extreme forms of AF, persistent and longstanding persistent.³ The current hypothesis for these poor outcomes in treating persistent AF is that the atrial structural remodeling, most notably the development of fibrosis, may cause conduction slowing in regions of the myocardium beyond the pulmonary veins that can initiate and maintain AF.^{4,5} To improve outcomes for persistent AF, it is of utmost importance to study the structural changes associated with this disease as well as the mechanistic links between such changes and reentrant activity.

AF persistence has been shown to increase with arrhythmia duration, due to both structural and electrical remodeling of the atria.^{4,6-8} In animal models electrical remodeling can occur as soon as 6 hours, with a decrease in atrial effective refractory period (AERP).⁹ As AF transitions from paroxysmal to persistent, many structural changes occur that make AF episodes more common and increased in duration, a phenomenon that has come to be known as “AF begets AF”.^{8,10} A major component of atrial structural remodeling is the increase in fibrosis^{4,7,11}, which has been linked to a decrease in endocardial and epicardium synchronization⁷ and an increase in conduction anisotropy¹², both of which facilitate AF maintenance. Furthermore, the amount of fibrosis pre-ablation has been linked to post-ablation outcomes, which indicates that atrial fibrosis is a major component of atrial structural remodeling that stabilizes AF.¹³

Although these studies suggest that AF fibrosis affects global conduction and AF maintenance, the associated mechanisms of initiation of AF are unknown. Patients who have AF often transition between sinus rhythm and AF and this for transition to occur there must be a trigger followed by a transition via reentrant activity into fibrillation. This trigger is often caused by a premature atrial contraction or focal activations within the atrium. We hypothesize that specific fibrosis architectures, i.e., the spatial organization, density, and extent of fibrosis, alter local conduction such that the cardiac tissue is more vulnerable to initiating AF. We have classified atrial fibrosis into two distinct categories and attempted to determine how local fibrosis architecture alters conduction during a triggered event and the subsequent transition between sinus rhythm and AF. We have also been able to determine whether these conduction changes facilitate reentrant activity to initiate AF. Our findings suggest that long continuous strands of fibrosis, termed obstructive fibrosis, but not non-

obstructive diffuse fibrosis alters local conduction to facilitate the initiation and development of sustained atrial fibrillation, but only during a premature simulation.

Methods

We carried out animal studies in accordance with the *Guide for the Care and Use of Laboratory Animals*¹⁴, under a protocol approved by the Institutional Animal Care and Use Committee of the University of Utah. All efforts were made to minimize suffering.

Experimental preparation

Animals—Mixed breed goats (n=6, 34±5 kg) were implanted with pacemakers and chronic AF was induced with rapid atrial pacing (RAP). Control goats (n=13, 37±7 kg) were used for comparison and both groups underwent the same electrophysiological and histological study.

Pacemaker implantation and programming: Procedures for the pacemaker implantation and programming have been described in detail previously.¹¹ In brief, mixed breed goats were fasted 12–24 hours prior to surgery, anesthetized with propofol (5–8 mg/kg iv), and maintained with inhaled isoflurane dosed to effect (1.5–4%) in inspired oxygen. A subcutaneous pocket on the lateral neck was made, and a neurostimulator (Itrel 3 or InterStim, Medtronic, Minneapolis, MN) was implanted to serve as a pacemaker. A pacing lead (Medtronic) with active fixation was introduced into the right atrium through a jugular vein. After at least a 1-week recovery period, pacemakers were programmed to stimulate continuously at 50 Hz with 1 second of stimulation followed by 1 second without stimulation at two to three times the diastolic pacing threshold. Every 1–2 weeks, the ECG was recorded, the rhythm was evaluated, and the pacemaker turned off to determine if AF was sustained for a minimum of 20 minutes. Once AF was sustained, the pacemaker was programmed to stimulate 1 second every minute to reinitiate AF if the heart spontaneously returned to sinus rhythm.

Terminal Study: An open-chest electrophysiological study was performed on control goats that were not paced and chronic AF goats (RAP 6±1 months). Animals received unlimited water but no food for 12–24 hours prior to the experiment. The goats were anesthetized with propofol (5–8 mg/kg iv), intubated, and maintained with inhaled isoflurane dosed to effect (1.5–4%) in inspired O₂ under positive pressure ventilation. An orogastric tube (S-50-HL, 1/2-in. inner diameter, 3/4-in. outer diameter, Tygon Tubing) was advanced into the rumen to evacuate gas and prevent bloat. A medial thoracotomy was performed to expose the heart. Chronic AF goats had their pacemakers turned off, then they were defibrillated with 1–5 50 J biphasic QRS synchronized shocks delivered with paddles placed on either side of the atrium from a defibrillator. Electrophysiological measurements did not begin for at least 10 minutes after cardioversion.

Electrical Mapping Procedure and Analysis

An electrode array consisting of 256 electrodes arranged in a 16-by-16 grid with uniform interelectrode spacing of 1 mm was placed on the LA appendage. The electrograms recorded

from the electrodes were referenced to a remote electrode placed in the left hind leg. In addition to recording unipolar electrograms from the electrode plaque, an ECG, a ventricular electrogram, and a pacing signal were also simultaneously recorded. The ventricular electrogram was recorded with a single, ventricular hook electrode that was referenced to the left hind leg. All signals were band passed filtered between 0.3 and 500 Hz, had a gain of 100, were digitized at 12-bit resolution, and sampled at 8 KHz with a custom build data acquisition system.

The electrophysiological study consisted of three pacing protocols. Monophasic stimuli were used for pacing and were produced using a custom build current controlled stimulator. In all the pacing protocols, stimulation pulses were applied to a pair of electrodes at the center of the plaque. The first protocol was a standard restitution protocol that consisted of a series of 10, 400 ms S1 pulses followed by a premature S2.¹⁵ The S2 cycle length was decremented from 350 ms by 10 ms until loss of atrial capture. After loss of atrial capture at the 10 ms resolution, the S2 cycle length was increased by 5 ms to determine the AERP within 5 ms. The last S2 pulse cycle length that caused atrial capture was considered the AERP. The pulse width for this protocol was 2 ms at a current of twice the diastolic threshold, which was determined as the minimum current for atrial capture at a cycle length of 400 ms.

The second pacing protocol was a dynamic restitution protocol, which consisted of a 30 S1 stimuli for entrainment, followed by 15 seconds of continuous S1 pacing.¹⁵ Atrial capture was determined after the entrainment period. The S1 cycle length was decremented from 350 ms by 10 ms until loss of atrial capture and the last S1 pulse cycle length that caused atrial capture was considered the S1-AERP.

The third protocol was an AF inducibility test, which consisted of burst pacing the atrium at 50Hz for 30-45 seconds at twice the diastolic pacing threshold. If AF persisted for up to 10 minutes it was considered sustained. The pulse width for this protocol was 0.5 ms at a current of twice the diastolic threshold, which was determined as the minimum current for atrial capture at a cycle length of 400 ms.

If any of the protocols induced AF, it was allowed to persist for at least 10 minutes to determine whether the arrhythmia would self-terminate. If AF did not self-terminate after 10 minutes, the heart was defibrillated with 1-5 50 J biphasic QRS synchronized shocks delivered with paddles placed on either side of the atrium from a defibrillator. Care was taken to ensure that the recording plaque did not move during defibrillation. Electrical recordings were then resumed after 10 minutes of sinus rhythm. Arrhythmias were classified as sustained AF, non-sustained AF, or extra beats. Sustained AF was AF lasting at least 10 minutes, non-sustained AF was AF lasting from 5 s to 10 minutes, and short cycle length excitations lasting less than five seconds were classified as extra beats.

Differences between AF initiations were determined by examining the direction of propagation of extra beats across the mapping surface. Only the first extra beat after an S2 was analyzed and the propagation direction spontaneously resulting from the S2 was determined as either longitudinal or transverse (within $\pm 45^\circ$) in relation to the generalized fiber orientation. The longitudinal direction was assessed by determining the direction of

fastest conduction based on isochrone activation maps created during beats paced at 400 ms cycle lengths from the center of the mapped region and was verified with myocardial fiber orientation from histology (Figure 1). The transverse fiber orientation was assumed to be 90 degrees from the longitudinal fiber orientation, this assumed fiber direction agreed well with the fiber direction as determined through histology. Each animal was then grouped into one of two groups based on whether extra beats propagated primarily in the longitude or transverse directions across the recording plaque and a Fischer's exact test was used to determine differences between the AF and control groups.

We measured conduction velocity transverse and longitudinal to the generalized fiber orientation for an S1 of 400 ms, and during an S2 of 160 ms. Conduction velocity was quantified with a previously validated algorithm that can distinguish between transverse and longitudinal conduction.¹⁶ In brief, this algorithm creates an isochrone map based on the activation times from the electrograms on the plaque. Activations were taken as the local minima of the first temporal derivative of the unipolar atrial electrograms. Conduction velocity was calculated by selecting 15-25 activation times within a region of the plaque. Each activation time point contained x and y spatial information that corresponded to the relative distances between the electrodes. From the temporal (activation times) and spatial information (distance between points), a conduction velocity was determined by using a least square fitting of the time and space information to a plane. The conduction velocity and angle of propagation were determined from the fitted parameters as described previously.^{16,17} By this method, two transverse and longitudinal conduction velocities were found for each isochrone activation map. For each animal, an averaged longitudinal and transverse conduction velocity for both S1-400 ms and S2-160 ms, respectively, were found by averaging the results from 2-3 activation maps. An anisotropy ratio was calculated from these conduction velocities, which is the ratio of longitudinal to transverse conduction velocity. A higher anisotropy ratio indicates more anisotropy and a value of 1 indicates isotropic conduction.

Histological Data Acquisition and Analysis

After the electrophysiological study the heart was excised and the LA region that contained the high density recording plaque was removed and placed in a 10% buffered formalin solution. All 6 AF goats and a randomly selected 6 of the control goats had histological tissue samples stained with Masson's trichrome after a one-month fixation period and evaluated for fibrosis. Histology was sectioned parallel to the epicardium at a depth of 500 μ m below the epicardium with a thickness of 4 μ m. Images from the samples were imported into ImageJ software (National Institutes of Health) for fibrosis quantification. The images were segmented into 3 color groups: red for myocytes, blue for collagen/fibrosis, and white for non-fibrotic extracellular content. Each color was manually sampled 7-12 times per image, after which a hidden Markov model was used to automatically segment the entire image into the three colors. The hidden Markov model output the total percentage for each color in the image and the percentage of blue served to estimate the total amount of fibrosis in the tissue sample. The percent fibrosis was then compared with both longitudinal and transverse conduction velocity.

Atrial fibrosis was further quantified and to two groups, non-obstructive and obstructive fibrosis. Obstructive fibrosis was considered fibrosis that was at least the length of a myocyte (100 μm), thus potentially disrupting transverse cell to cell conduction. Non-obstructive fibrosis was fibrosis that was less than 100 μm . This classification was done by examining the maximum length of fibrosis strands in the histology images using a custom MATLAB program. In brief, this program imported the segmented ImageJ images, which were segmented into blue, white and red. These images were then converted into a binary image where the blue was a value of 1 and the red/white had a value of 0. A binary dilate of 5 pixels, 2.52 μm , was then applied to the binary image. An eight neighbor connected components analysis from the MATLAB image processing toolkit was then applied to the binary image to find regions of continuous fibrosis. The maximum distance of each fibrotic connected component for each image was then computed. Strands greater than 100 μm were considered obstructive fibrosis since they could disrupt an entire cell's transverse conduction. Figure 2 shows an example of how obstructive fibrosis was quantified. The local fibrosis densities and architecture of fibrosis in our histological samples were compared with local conduction data, as determined through the electrical mapping studies.

Statistical Analysis

Differences in AERP and S1-AERP between control and chronic AF goats were examined with unpaired *t*-tests. Differences between initiation of sustained AF, non-sustained AF, and extra beats between the control and AF groups were determined with a Fischer's exact test. Extra beat propagation direction between the control and chronic AF goats was examined with a Fischer's exact test. Differences between conduction velocity (average transverse and average longitudinal) between the AF and control groups were determined with an unpaired *t*-test at both 400 ms and the 160 ms cycle length. Differences in percent fibrosis between the AF and control groups were determined with an unpaired *t*-test. Differences in the anisotropy ratio between the AF and control groups were determined with an unpaired *t*-test. Differences in the number of obstructive fibrotic strands per square mm between the AF and control groups were determined with an unpaired *t*-test. Differences in the density of fibrosis predicting the direction of slowest conduction between the AF and control groups were determined using a one-sided Chi-square. Statistical test in which $p < 0.05$ were considered significant.

Results

AERP and AF inducibility

AF was persistent in all RAP goats prior to the electrophysiological procedure. In 2/6 of these chronic AF goats, AF could not be cardioverted to sinus rhythm and the protocol could not be completed. There was a significant shortening of AERP (Controls: 147 ± 21 ms vs. Chronic AF: 118 ± 23 ms, $p < 0.05$) but not the S1-AERP (Controls: 180 ± 53 ms vs. Chronic AF: 169 ± 15 ms, $p > 0.05$) in the chronic AF goats as compared to the controls. The RAP goats had sustained AF more often than those in the control group; however, there was no significant difference in frequency of non-sustained AF or extra beats. All 6 of the chronic AF goats had sustained AF whereas 2/13 of the control goats showed sustained AF during the electrophysiological study. Non-sustained AF occurred in 9/13 of the control goats. A

total of 20 extra beats following the S2 pacing pulse were observed in 6/13 of the control goats. Extra beats (n=22) were observed in 4/6 of the AF goats. The two chronic AF goats that did not have extra beats could not be defibrillated; therefore, the S1-S2 protocols could not be performed on these animals. The extra beats in the hearts of the control goats propagated longitudinally along the fiber (19/20 beats), with 6/6 of the goats having extra beats that propagated primarily in the longitude direction. In the AF goats, the extra beats propagated transversely to the fiber orientation (14/22 beats), with 3/4 of the goats having extra beats that propagated primarily in the transverse direction. Figure 3 shows a comparison of extra beat propagation direction of the AF goats compared to the controls. Table 1 summarizes AF inducibility.

Conduction velocity

Conduction velocity of the AF goats was significantly slowed compared to the control goats in the transverse direction (0.40 ± 0.04 m/s vs. 0.53 ± 0.15 m/s, $p < 0.05$) but not in the longitudinal direction (0.70 ± 0.27 m/s vs. 0.76 ± 0.18 m/s) following a premature atrial contraction, caused by an S2-160 ms. There was no significance difference in either transverse (0.63 ± 0.14 vs. 0.58 ± 0.19 m/s) or longitudinal conduction (0.96 ± 0.21 m/s vs. 0.87 ± 0.18 m/s) at an S1 of 400 ms between the AF and control goats. Figure 4 shows examples of transverse slowing in the AF goats as compared to the controls following S2 stimulation. Figure 5 shows an example of the initiation of extra beats and AF when the conduction velocity restitution becomes steep. Figure 5C shows an increase in the anisotropy for the AF animals as the coupling interval decreases. There was no significance difference in anisotropy ratio at an S1 of 400 ms (1.55 ± 0.42 vs. 1.57 ± 0.33) or an S2 of 160 ms (1.72 ± 0.55 vs. 1.47 ± 0.25) between the AF and control animals.

Histology analysis

The AF goats had a higher density of fibrosis as compared to controls ($5.2 \pm 0.7\%$ vs. $3.5 \pm 1.0\%$, $p < 0.05$) and more diverse fibrosis architecture. The AF animals had more obstructive fibrosis than the controls (17.5 ± 8.0 fibers/mm² vs. 8.6 ± 3.0 fibers/mm², $p < 0.05$). The control group had more non-obstructive, diffuse fibrosis than the AF animals (1109.3 ± 308.5 fibers/mm² vs. 718.1 ± 380.0 fibers/mm², $p = 0.07$, N.S.) however, the result did not meet significance. The organization of this obstructive fibrosis was in strands that were located primary parallel to myocyte bundles, potentially interrupting transverse connections between cells. Figure 6 shows specific examples of the different fibrosis architectures in chronic AF and control animals.

The density of fibrosis in the chronic AF goats, but not the controls, predicted the direction of slowest conduction. Fibrosis density was analyzed in both the longitudinal and transverse directions. These densities were then compared to the conduction velocities in these regions (Figure 7). In 3/4 of the AF animals, the direction of highest fibrosis density predicted the direction of slowest conduction. In 1/6 of the control animals the direction of highest fibrosis predicted the direction of slowest conduction ($p < 0.05$ for AF vs. control).

Discussion

The major findings of this study are as follows: 1) AERP was significantly reduced as a result of chronic AF but S1-AERP is not. 2) Conduction velocity was significantly slower in chronic AF, but only in the transverse direction following a premature atrial contraction, indicating that there is functional slowing in chronic AF but only at short coupling intervals. 3) Extra beats in chronic AF propagated across the mapping region via transverse conduction; however, in the controls the extra beats propagated across the mapping region via longitudinal conduction. 4) Chronic AF goats developed diverse structures of fibrosis (i.e., obstructive fibrosis) that were less common in controls, and this difference was quantitatively defined.

The AERP values we obtained (Controls: 147 ± 21 ms and Chronic AF: 118 ± 23 ms) agree with a study by Schotten et al. that showed that after 5 days of RAP the LA AERP decreased from a baseline of 132 ± 19 ms to 72 ± 7 ms.⁹ The AERP relates to fibrillation initiation and maintenance through restitution. The restitution hypothesis states that an action potential duration restitution slope of > 1 alterations between short and long APD, or alternans, can occur.^{15,18,19} Alterations in action potential durations will cause heterogeneity in repolarization gradients and may result in unidirectional conduction block, which can initiate spiral wave reentry.^{15,20–22} Our data also supports the finding that the initiation of extra beats and AF occurs when the conduction velocity restitution curve became steep. The atrial myocardium of our chronic AF animals could capture at shorter cycle lengths as compared to the controls. This indicates that hearts experiencing chronic AF can, at least transiently, support more rapid activations, which could lead to alterations in APD, thus causing a greater chance of AF initiation. The S1-AERP, obtained from a dynamic restitution protocol, did not show any differences between the AF and control groups. The main difference between dynamic and standard restitution protocols is that dynamic restitution takes into account cardiac memory^{19,23}, a mechanism in which delays in ionic currents, such as calcium and potassium, with slow enough recovery kinetics can accumulate over several cycles. Another possible mechanism is that rapid pacing can induce changes in gene expression.²³ During the S1-AERP protocol the myocardium was in a steady state due to the uniform pacing. Cardiac memory may have a more primary role in determining AERP during uniform activations (steady state) than any structural/functional remodeling as a result of AF. However, the S2 (non-steady state)-AERP did show a difference, suggesting that the structural/functional remodeling as a result of AF does have a major role in determining AERP after a premature atrial contraction. This supports Kawara et al.'s findings that suggested that conduction slowed as a result of a premature stimuli but not at slower pacing cycle lengths.²⁴

AF is also known to alter cardiac conduction anisotropy, which under normal conditions arises because conduction velocity is faster along the direction longitudinal to the myocytes compared to transverse. This anisotropic condition is due to both fiber orientation of the myocytes, as well as the higher density of gap junction connecting the cells in the longitudinal direction. Verheule et al. also showed that atrial fibrosis causes further conduction anisotropy in the longitudinal vs. transverse directions.⁷ Our results agree with the finding that conduction anisotropy increases during atrial remodeling but only after a

premature stimulus. This finding agrees with those from Kawara et al. who suggest that in the ventricles, fibrosis only causes transverse conduction slowing following a premature stimulation.²⁴ Their explanation was that the long strands found in stringy fibrosis cause slowing of conduction. Our results demonstrate that AF induced fibrosis shares similar effects to what has been shown previously only in ventricular preparations with extensive fibrosis.

Extra beats in chronic AF propagated across the mapping region via transverse conduction; however, in the controls the extra beats propagated across the mapping region via longitudinal conduction. This finding was observed by Kawara et al. in which they found that large patches of what they called patchy fibrosis could block longitudinal conduction, thus causing conduction transverse to fiber orientation. There may be large patches of fibrosis outside the mapped region in the AF animals that cause this shift in longitudinal to transverse conduction.

There was more fibrosis in the chronic AF animals than in the control goats but, more importantly, the fibrosis architecture was different. The AF goats had more obstructive fibrosis and less non-obstructive fibrosis than the controls, which demonstrates a difference in the structure of the fibrosis. As AF develops, the strands of fibrosis that are non-obstructive may start to connect, forming obstructive fibrosis that may slow or even block transverse conduction. The organization of this obstructive fibrosis was in strands that were located primary along cells, potentially interrupting transverse connections between cells. This obstructive fibrosis architecture is similar to interstitial fibrosis, which has been described to appear in intermuscular spaces previously devoid of collagen.²⁵ Krul et al. classified LA appendage fibrosis and related the fibrosis to conduction properties in humans. They found that regions of thick interstitial collagen was associated with increased longitudinal conduction velocity and transverse activation delay.²⁶ These long strands of fibrosis located parallel to the myocytes may expand the distance between neighboring cells, reduce the electric conductance, and explain why the transverse conduction velocity was slowed while longitudinal was not.^{7,27,28} Our results are similar to the findings of Krul et al. in humans for transverse conduction slowing. Although we did not observe a significant increase in longitudinal conduction velocity as was found by Krul et al, we did observe the correlation between increased longitudinal conduction velocities with increased levels of fibrosis, which is similar to what they showed in left atrial appendage samples with thick collagen strands. The increase in longitudinal conduction velocity with increased fibrosis may also be explained due to the structure of the fibrosis. The fibrosis appears to be deposited parallel to myocytes, thus obstructing the transverse conduction, and potentially causing a source-sink mismatch. This source-sink mismatch may not only slow transverse conduction, but may also shunt conduction along the longitudinal direction, thus increasing longitudinal conduction velocity. This may explain why many studies show an increase in conduction anisotropy following development of fibrosis in animal models of AF⁷ as well as in human ventricular tissue²⁴ and human atrial tissue²⁸.

The chronic AF goats had higher incidence of sustained AF than the control goats, which may be the result of slowed conduction in the AF animals. Conduction in the AF animals slowed because of two mechanisms. First, conduction slowed transverse to fiber orientation

only during short cycle lengths, which could result from a premature atrial contraction or due to the rapid activation rate that is present during AF. The second mechanism of conduction slowing that we observed was that extra beats propagated transversely to fiber orientation, instead of longitudinally, during AF. Under normal physiological conditions this directional variance alone would slow conduction due to conduction anisotropy. However, in the chronic AF animals this slowing was even further exaggerated, which would result in more AF susceptibility due to two mechanisms. The first, more classical method is that slowed conduction would result in more possible pathways for reentry due to the decreased wave length (wavelength = conduction velocity $\times$ APD). For reentry to occur the wavelength must be shorter than the reentrant path. Therefore a smaller wavelength will allow for more possible reentrant pathways and make AF more likely to be initiated and sustained.^{29,30} The second mechanism of slowed conduction velocity increasing AF susceptibility is due to an increase in rotor stability. Rotors circulate around an unexcitable center known as a singularity point.²³ For the singularity to remain unexcitable, and for the rotor to be sustained, the cardiac tissue must have a so-called source-sink mismatch, which results in slowed conduction velocity and a steep curvature around the singularity point.²¹⁻²³ Debate continues as to whether or not calculating wavelengths has relevance to explaining reentry and fibrillation due to antiarrhythmic drugs that decrease conduction velocity yet still terminate AF.³¹ Slowed conduction has been shown in patients with paroxysmal AF suggesting that it may be critical to sustaining AF,^{32,33} and this may occur either by the classical view of decreasing wavelengths, or the more modern view of setting up steep curvature for rotors.^{21,22,30}

There was no statistical difference in conduction velocity at slow cycle lengths and in S1-AERP between the AF and control goats, indicating that during normal physiological conditions, such as sinus rhythm, the AF affected myocardium and the control myocardium exhibit similar conduction. However, once a premature atrial contraction occurs, the AF cardiac substrate affects the local conduction differently than the non-AF substrate. Such a response suggests that the AF fibrosis may produce a substrate that has little effect during sinus rhythm, but following a premature atrial contraction may display the conduction slowing that could lead to reentry and AF.

Clinical Implications

The clinical relevance of these findings is that reentrant activity observed in patients with chronic AF may be a result from two separate but necessary components. First, reentry is more likely to occur due to slowed conduction that occurs only due to premature contractions. This has been shown in human data, which found that AF could initiate via spiral wave reentry at sites of dynamic conduction slowing.³⁴ Therefore, patients who have more premature atrial contractions or increased activity of focal sources will be more likely to cause this functional slowing as a result of the short coupling interval to initiate and sustain AF. The second component is the architecture of fibrosis. We observed obstructive fibrosis that significantly slowed transverse conduction. Markides et al. reported that patients with paroxysmal AF have an abnormal atrial substrate with regions of slowed conduction, specifically where there is an abrupt change in subendocardial fiber orientation, and under certain pacing parameters these regions of conduction slowing could cause

block.³³ If patients have substantial obstructive fibrosis it will make these regions of abrupt changes in fiber orientation more susceptible to conduction block, thus increasing the likelihood of AF. These findings suggest that both a tendency of frequent premature contractions and specific fibrosis architecture are required for development of sustained AF.

Limitations

This study was limited to AF developed through rapid atrial pacing without the presence of any existing heart disease. Although such a scenario may apply to some of the human patient population, more often AF is accompanied by some form of heart disease that may induce substrate changes in the atrium. In addition, this study was performed in goats and, although goat and human hearts are of roughly similar size, there may be meaningful differences in electrophysiology. Hence, the results of these findings may not directly translate to humans. In spite of these limitations, the chronic rapid atrial paced AF goat model is widely used and well accepted for studying AF.^{6–8,11} Another limitation is we took only a 4 um thick sample of histology 500 μm from the epicardial surface to determine a generalized fiber direction. A remodeled atrium has very complicated atrial structure including a dissociation of epicardium and endocardium.^{6,7} Therefore, we used a generalized fiber direction in our classifications and electrophysiological measurements. Our histology measurements included determination of fibrosis but no measure of alternation within the gap junctions. Several studies have shown that gap junctional remodeling during AF may lead to conduction slowing.^{4,35} A final limitation is that only a region of the left atrial appendage was mapped and our findings may not translate to the rest of the atrium. We made electrophysiological measurements on the left atrial appendage because our mapping protocol required a large continuous surface to calculate accurate conduction velocities.

Conclusion

Structural and electrophysiological remodeling in chronic AF leads to extra beat propagation and conduction velocity slowing transverse to fiber orientation. These two mechanisms combine to decrease global conduction velocity, decrease the cardiac wavelength, and to increase the likelihood of reentry. This sequence may lead to more extra beats and the establishment of stable reentry in tissue with structural and electrical remodeling. The major histological difference between chronic AF and control hearts is the amount and architecture of the fibrosis. Histology from goats with a history of chronic AF had much more obstructive fibrosis, which may lead to the conduction slowing and alteration in conduction pathways to make the tissue more susceptible to AF.

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Figure 1. Dividing mapped region into longitudinal and transverse propagation direction
 Mapped region divided into two transverse and two longitudinal directions. An extra beat is considered either longitudinal or transverse if it is within $\pm 45^\circ$ (within dashed lines). Green box shows the histology sample to confirm that the longitudinal direction as determined with the isochrone map agrees with the myocardial fiber direction as determined through histology.

Figure 2. Determining obstructive fibrosis

A) and B) Raw histology from a control and AF animal. **C) and D)** ImageJ three color segmentation. Red indicates myocytes, blue indicates fibrosis/collagen, and white indicates non-fibrotic extracellular content. **E) and F)** Binary image with white showing fibrosis. The white fibrosis has been binary dilated by 5 pixels (2.52 μm). Yellow has been overlaid onto fibrosis strands that are greater than 100 μm in length, which is considered obstructive fibrosis. E has 4 fibrotic groups and F has 8 fibrotic groups that met the 100 μm threshold to be considered obstructive fibrosis.

Figure 3. Example of extra beat propagation direction of chronic AF vs. control

A) An atrial electrogram that shows the initiation of extra beats following the S2 pacing pulse. The first extra beat at the initiation of extra beats was examined to determine its propagation across the recording plaque. **B)** The S1 column shows isochrone activation maps after an S1 pacing pulse, which was used to determine fiber orientation. The extra beat column shows isochrone activation maps of an extra beat following an S2 induced beat. The arrow shows the direction of extra beat propagation.

Figure 4. Examples of transverse conduction slowing during a premature stimulus

Examples from two AF animals and two control animals are shown. Activation maps were created from an S2 stimulation at 160 ms. The white dashed lines indicate the generalized fiber direction as determined from S1 pacing and confirmed with histology. The two activation maps from the chronic AF group show that the transverse conduction was greatly slowed compared to the controls as a result of a premature. This finding was consistent across all the AF animals.

Figure 5. Examples of conduction velocity restitution

A) Longitudinal conduction velocity restitution curve for a control and chronic AF animal.

B) Transverse conduction velocity restitution curve for a control and chronic AF animal. **C)**

Anisotropy ratio restitution curve for a control and chronic AF animals. Extra beats are indicated with a * and sustained AF is indicated with a #. The control and AF animals began initiated extra beats when restitution started to become steep at an S2 of 140 ms and 160 ms, respectively. When the S2 coupling interval was further reduced the chronic AF animal initiated sustained AF at an S2 of 140 ms. Both chronic AF and controls had nonlinear restitution curves as the coupling interval reduced; however, in the chronic AF animals the anisotropy ratio increased, whereas in the controls it saw little change.

Control Histology

Diffuse Fibrosis

AF Histology

Obstructive Fibrosis Example 1

Obstructive Fibrosis Example 2

Figure 6. Architecture of fibrosis is more diverse in chronic AF as compared to control
The control groups had low levels of diffuse fibrosis (location 1) with few strands of obstructive fibrosis. The chronic AF group had regions of myocardium with obstructive fibrosis (location 2 and location 3) that are indicated by the long, compact groups of fibrotic strands located primarily along the generalized longitudinal cell direction. Examples of obstructive fibrosis are shown with arrows in location 2 and is circled at location 3.

Figure 7. Fibrosis densities and conduction slowing

A) The chronic AF animals had increased longitudinal conduction velocity with increased fibrosis at an S2 of 160 ms (correlation $p = 0.02$) but not at S1 of 400 ms ($p = 0.43$). **B)** The chronic AF animals had no correlation with fibrosis densities and transverse conduction velocity at an S2 of 160 ms or S1 of 400 ms, but was trending towards significance ($p = 0.12$ and 0.10). **C)** Control animals had no correlation with longitudinal conduction velocity at an S2 of 160 ms or S1 of 400 ms ($p = 0.93$ and 0.75). **D)** Control animals had no correlation with transverse conduction velocity at an S2 of 160 ms or S1 of 400 ms ($p = 0.67$ and 0.72).

Table 1
Summary of AF inducibility

Control Data				AF Data			
Goat Number	Sustained AF	Non-Sustained AF	Extra Beats	Goat Number	Sustained AF	Non-Sustained AF	Extra Beats
1	No	No	No	1	Yes	Yes	Yes
2	Yes	Yes	Yes	2	Yes	Yes	Yes
3	No	No	No	3	Yes	Yes	Yes
4	No	Yes	No	4	Yes	Yes	Yes
5	No	Yes	Yes	5	Yes	No *	No *
6	No	Yes	Yes	6	Yes	No *	No *
7	Yes	Yes	Yes	Totals	6/6	4/4	4/4
8	No	No	No				
9	No	Yes	Yes				
10	No	Yes	Yes				
11	No	Yes	No				
12	No	No	No				
13	No	No	No				
Totals	2/13	8/13	6/13				

AF = atrial fibrillation.

* Animals could not be defibrillated.