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ORIGINAL RESEARCH - PRECLINICAL

Regions of Highly Recurrent Electrogram Morphology With Low Cycle Length Reflect Substrate for Atrial Fibrillation

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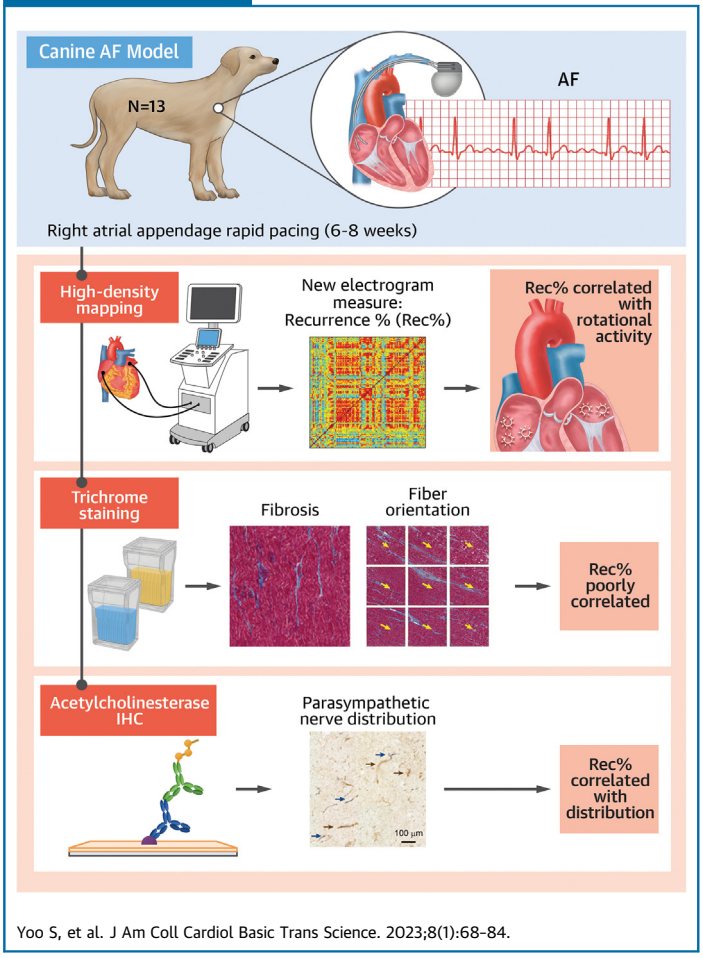
VISUAL ABSTRACT



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HIGHLIGHTS

- **Traditional anatomically guided ablation and attempts in the last decade to perform electrogram guided AF ablation (CFAE, DF, FIRM) have not been shown to be a sufficient treatment for persistent AF.**
- **Using biatrial high density electrophysiological mapping in a canine RAP model of AF, we found that Rec% correlated closely with stability of rotational activity. Unlike other measures, Rec% correlated closely with spatial heterogeneity of parasympathetic nerve fibers; this was reflected in response of Rec% and CL_R to atropine.**
- **Our results suggest that EMR parameters—Rec% and CL_R—may be more reflective of arrhythmogenic substrate for AF than any previously studied electrogram parameter of AF. Further studies are necessary to determine the effectiveness of this novel electrogram therapeutic approach in guiding catheter ablation of persistent AF.**

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SUMMARY

Traditional anatomically guided ablation and attempts to perform electrogram-guided atrial fibrillation (AF) ablation (CFAE, DF, and FIRM) have not been shown to be sufficient treatment for persistent AF. Using biatrial high-density electrophysiologic mapping in a canine rapid atrial pacing model of AF, we systematically investigated the relationship of electrogram morphology recurrence (EMR) (Rec% and CL_R) with established AF electrogram parameters and tissue characteristics. Rec% correlates with stability of rotational activity and with the spatial distribution of parasympathetic nerve fibers. These results have indicated that EMR may therefore be a viable therapeutic target in persistent AF. (J Am Coll Cardiol Basic Trans Science 2023;8:68–84) © 2023 Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation
AI = anisotropy index
CFAE = complex fractionated atrial electrogram
CL_R = cycle length of the most recurrent electrogram morphology
DF = dominant frequency
EGM = electrogram
EMR = electrogram morphology recurrence
FI = fractionation interval
FIRM = focal impulse and rotor mapping
FFT = fast Fourier transform
LAT = local activation time
LAA = left atrial appendage
LAFW = left atrial free wall
OI = organization index
PLA = posterior left atrium
PV = pulmonary vein
RAA = right atrial appendage
RAFW = right atrial free wall
RAP = rapid atrial pacing
Rec% = recurrence percentage
ShEn = Shannon's entropy

Atrial fibrillation (AF) drivers such as rotational and focal activities are thought to sustain AF and are often located near the pulmonary veins (PVs).[1,2] PV isolation has been widely applied to treat patients with AF. However, the suboptimal ablation outcomes in patients with persistent AF (<50%) suggest that other atrial regions besides the PVs may be responsible for sustaining AF activity.[3-5] Multiple electrogram (EGM)-based approaches have been promulgated to help identify regions of interest, including mapping complex fractionated atrial electrograms (CFAEs), dominant frequency (DF), Shannon's entropy (ShEn), voltage, and focal impulse and rotor mapping (FIRM). None have been established as a widely successful strategy. The challenges of using DF analysis for AF EGMs have been shown,[6,7] underscoring the need for a new therapeutic approach.

There are multiple factors that determine atrial EGMs in AF, including characteristics of the recording electrodes, the activation rate, the underlying electrophysiologic properties, and the underlying tissue anatomy/pathology. Analysis of the EGM morphology may provide information on the latter 3 components. Indeed, electrogram morphology recurrence (EGR) analysis can provide important classification of EGMs.[8] We applied EGR analysis in a preliminary clinical study of patients with AF.[9] In that study, multisite mapping of the right and left atrium was performed. At each site, the recurrence percentage (Rec%) of the most frequent EGM morphology was determined, as well as the cycle length of this most frequent EGM morphology (CL_R). None of the patients with shortest CL_R in the right atrium had a successful outcome from left atrial ablation. Rec% provides a measure of the consistency of EGM morphology. This is expected to be high near AF drivers because of the consistency of activation, but may also be high at other sites for anatomic reasons, eg, a narrow isthmus forcing conduction to be uniform. Therefore, the CL_R provides an assessment of the sites with both consistent and rapid activation. Despite the initially introduced concept, the pathophysiologic basis of Rec% and CL_R was not assessed in those studies.

We hypothesized that measures of EGM morphology recurrence—Rec% and/or CL_R—are a more sensitive marker of pathophysiologic substrate for AF than traditional EGM measures of AF. Indeed, even though several investigators have suspected that potential AF mechanisms such as heterogeneous myocyte fiber orientation, ion channel remodeling, structural remodeling (ie, fibrosis),[10-12] and altered parasympathetic nervous system signaling[13] may contribute to AF EGM formation,[14] very few studies have systematically examined the relationship between AF EGM measures and the underlying structural and molecular aspects of AF substrate. The present study was therefore designed to obtain a thorough assessment of the electrophysiologic and structural basis of AF EGMs in a canine model of persistent AF by performing detailed high-resolution epicardial mapping of both atria and assessing both established frequency and complexity

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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parameters and novel recurrence morphology measures. The specific goals of this study were as follows: 1) to assess the regional and subregional distribution of established measures of AF frequency and complexity (DF, organization index [OI], fractionation interval [FI], and ShEn) and recurrence morphology analyses; 2) to determine the precise relationship between these different measures; 3) to determine whether there is a relationship between electrophysiologic substrate for AF—specifically the ability of the atria to harbor rotational (re-entrant) activities—and recurrence morphology; 4) to assess whether myocyte fiber orientation and fibrosis are associated with recurrence morphology; and 5) to determine the relationship between parasympathetic nerve innervation and recurrence morphology.

METHODS

Supplemental material is available online with this article for further details of the materials and methods used.

In 13 dogs subjected to rapid atrial pacing (RAP; 600 beats/min) for 6 to 8 weeks, high-density epicardial mapping (117 electrodes) was performed. AF EGMs were recorded for calculating Rec%, CL_R, DF, OI, FI, and ShEn before and after atropine administration. After in vivo electrophysiologic study, the 6 atrial regions were harvested for Masson's trichrome staining and nerve staining by immunohistochemistry. The myocyte fiber orientation, amount of fibrosis and spatial distribution of the parasympathetic nerve were quantified.

STATISTICS. All data are expressed as mean ± SEM or median (interquartile range) for nonparametric data with the use of SigmaPlot version 11.1 (Systat). When comparing EGM parameters or tissue characteristics between atrial regions, 1-way ANOVA was performed with the Holm-Sidak post hoc method for multiple testing corrections. If the normality test was not passed, Kruskal-Wallis 1-way ANOVA with Tukey's post hoc method for multiple testing correction was performed. The effect of atropine on Rec% and CL_R was compared with the use of linear mixed models with a random intercept for the dog and both a fixed effect and a compound symmetric covariance structure for the electrode. The correlation of the 2 variables was determined with the use of Pearson's product-moment correlation. A P value <0.05 was considered to be statistically significant.

RESULTS

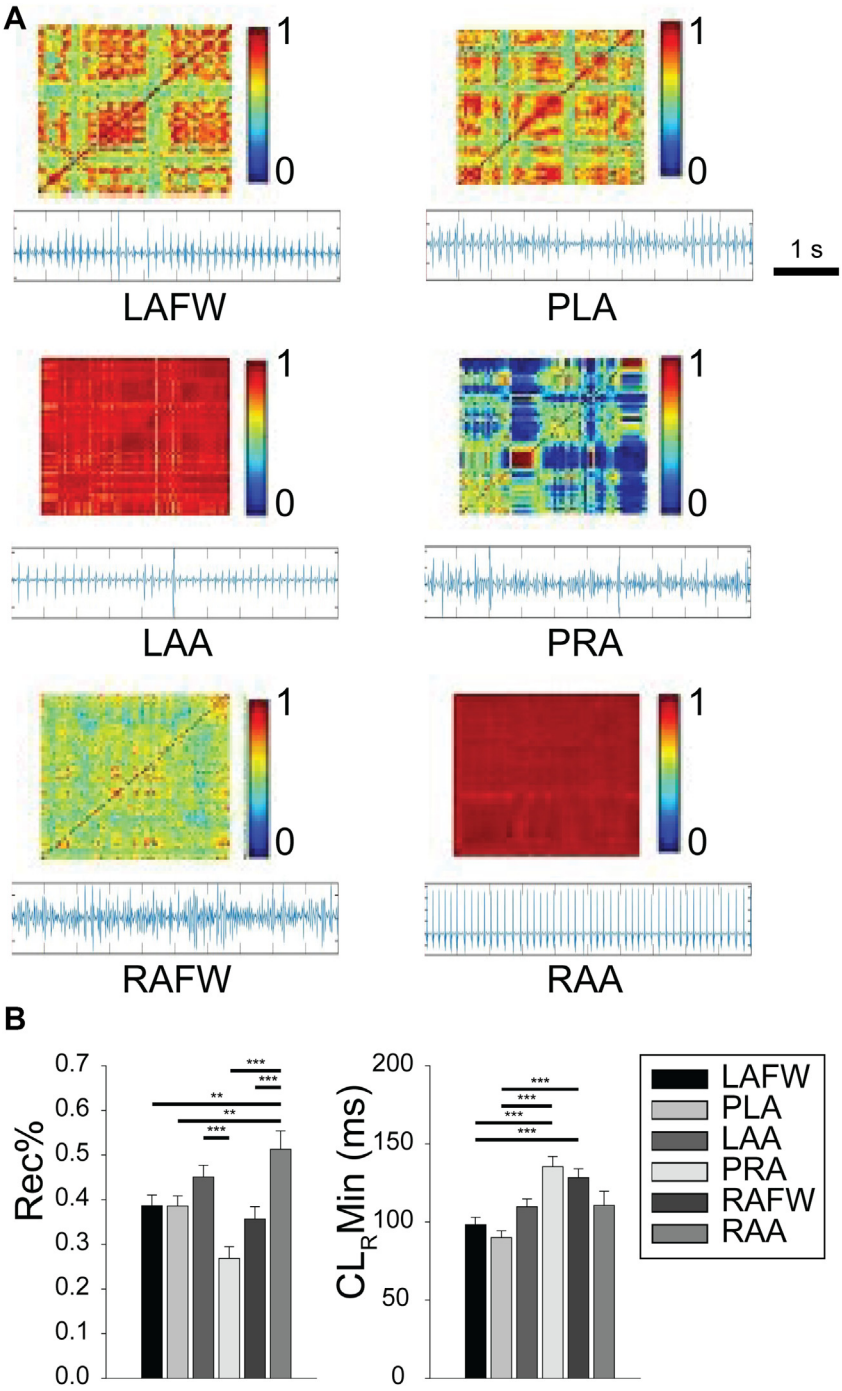
We induced persistent AF in 13 dogs. We analyzed the novel EMR measures—Rec% and CL_R—in 6 different regions in the left and right atrium and compared them with established AF source EGM measures, ie, FI, OI, DF, and ShEn. Next, we assessed whether Rec % and CL_R can help to predict the presence and stability of rotational (reentrant) activity in the fibrillating atrium. We further investigated the effect of degree of fibrosis and myofiber orientation on morphology recurrence. Because parasympathetic nerve remodeling[14-18] has been shown to be an important mechanism underlying AF, we also investigated the effect of parasympathetic innervation—and parasympathetic blockade—on EMR.

EMR ANALYSIS IN CANINE RAP OF MODEL OF AF. We analyzed the EGM morphology in the different atrial regions during AF. Supplemental Figure S1A demonstrates examples of cross-correlation of detected activation waveforms to generate EMR plots. The N × N cross-correlation values are plotted in a 2-dimensional color-coded map, as shown in Supplemental Figure S1B, where N is the number of activations. In this plot, the x-axis and y-axis represent the first and the second activation templates that are cross-correlated. The points in red represent the combination with the highest cross-correlation values near 1 and the points in blue the cross-correlation values near 0. The checkerboard pattern shown in Supplemental Figure S1B suggests that there is a dominant morphology that periodically recurs for the duration of the recording. Supplemental Figures S1C and S1D show the recurrence plots and the spatial distribution of Rec% within an entire plaque. The position of the example in Supplemental Figure S1B is marked by a red box in the Supplemental Figure S1C.

CL_R IS LOWER IN THE POSTERIOR LEFT ATRIUM THAN IN OTHER REGIONS OF THE LEFT AND RIGHT ATRIUM. We quantified Rec% in the different atrial regions of the canine RAP model of AF. We also calculated the mean cycle length of the most recurrent EGM morphology (ie, CL_R) because there may be areas with very recurrent EGM morphology that may be too slow to be likely drivers for AF. For this reason, in addition to quantifying the Rec% at a particular site, CL_R also needs to be determined.

Figure 1A shows examples of recurrence plots and EGMs in each subregion. Distinct checkerboard patterns in the different subregions indicate that the activation patterns have different levels of complexity. High-density mapping data revealed that Rec% and CL_R are regionally variable (**Figure 1B**). The highest overall Rec% was measured in the appendages, and the lowest overall Rec% was observed in the posterior right atrium. CL_R was lower in the left atrium compared with the right atrium, with the lowest CL_R measured in the posterior left atrium

FIGURE 1 **Examples of Recurrence Plots and Electrogram Signals and Regionally Variable Rec% and CL_R**



(A) Illustration of a color-coded cross-correlation matrix of all activations **(top)** and electrogram signals **(bottom)** in 6 atrial regions. **(B)** Regional distribution of Rec% and CL_RMin in 6 atrial regions. Data presented as mean ± SEM; n = 13. $^{**}P < 0.01$; $^{***}P < 0.001$. CL_R = cycle length of the most recurrent electrogram morphology; LAA = left atrial appendage; LAFW = left atrial free wall; OI = organization index; PLA = posterior left atrium; PRA = posterior right atrium; RAA = right atrial appendage; RAFW = right atrial free wall; Rec% = recurrence percentage.

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(PLA). We also assessed the spatial heterogeneity of Rec% and CL_R in each region but found no interregional differences in spatial distribution of Rec% and CL_R (Supplemental Figures S1E and S1F).

REC% AND CL_R ARE MORE CLOSELY CORRELATED WITH FI AND ShEn THAN WITH DF. Next, we examined the correlation between Rec% and each of the rest of the EGM measures at each electrode within each mapped region. The methodology for this correlation analysis is shown in **Figure 2A**. As shown in **Figure 2B**, R was ≥0.5 in nearly all regions for Rec%-FI and Rec%-ShEn, with Rec%-OI being close to 0.5 in several regions. In distinct contrast, Rec%-DF was markedly lower than 0.5 in every region, indicating a poor correlation between Rec% and DF. Although correlations were somewhat weaker for CL_R, the correlation between CL_R-FI and CL_R-ShEn was ≥0.5 in most regions. Taken together, Rec% and CL_R are moderately correlated with fractionation and complexity measures (FI and ShEn) of AF.

To further explore the relationship between Rec% and fractionation, we went on to assess the relationship between Rec% and amplitude of AF EGMs. We looked at 2 different metrics of atrial EGM amplitude, ie, the average amplitude and the maximum-minimum difference. Although average amplitude was not correlated with Rec%, maximum-minimum difference was found to be moderately correlated with Rec% ($R = 0.539$; $P < 0.01$). Taken together, these findings indicate that the relationship between Rec% and fractionation is weak at best. As shown in Supplemental Figure S2, Rec% can be both high and low, whether in the presence of fractionated signals or of unfractionated signals.

REC% STRONGLY REFLECTS STABILITY OF ROTATIONAL ACTIVITIES IN THE FIBRILLATING ATRIUM. Evidence of the presence of rotational activities and their characteristics have previously been suggested to correlate with arrhythmogenic substrate in patients with AF.[19-21] We therefore examined AF sources, showing 360-degree rotations in the local activation time maps in all 6 regions (**Figure 3**). Even though reentry was seen in all regions, the temporal stability of confirmed 360-degree rotations (re-entries) differed significantly among the regions (**Figures 3A and 3B**). The stability of these reentries corresponded closely to that of the regional distribution of Rec%, with the rotations being most stable in the right atrial appendage (RAA), followed by the left atrial appendage (LAA), and then the rest of the left and right atrium (**Figure 3B**). The stability of rotational activities correlated closely with Rec% (**Figure 3C**).

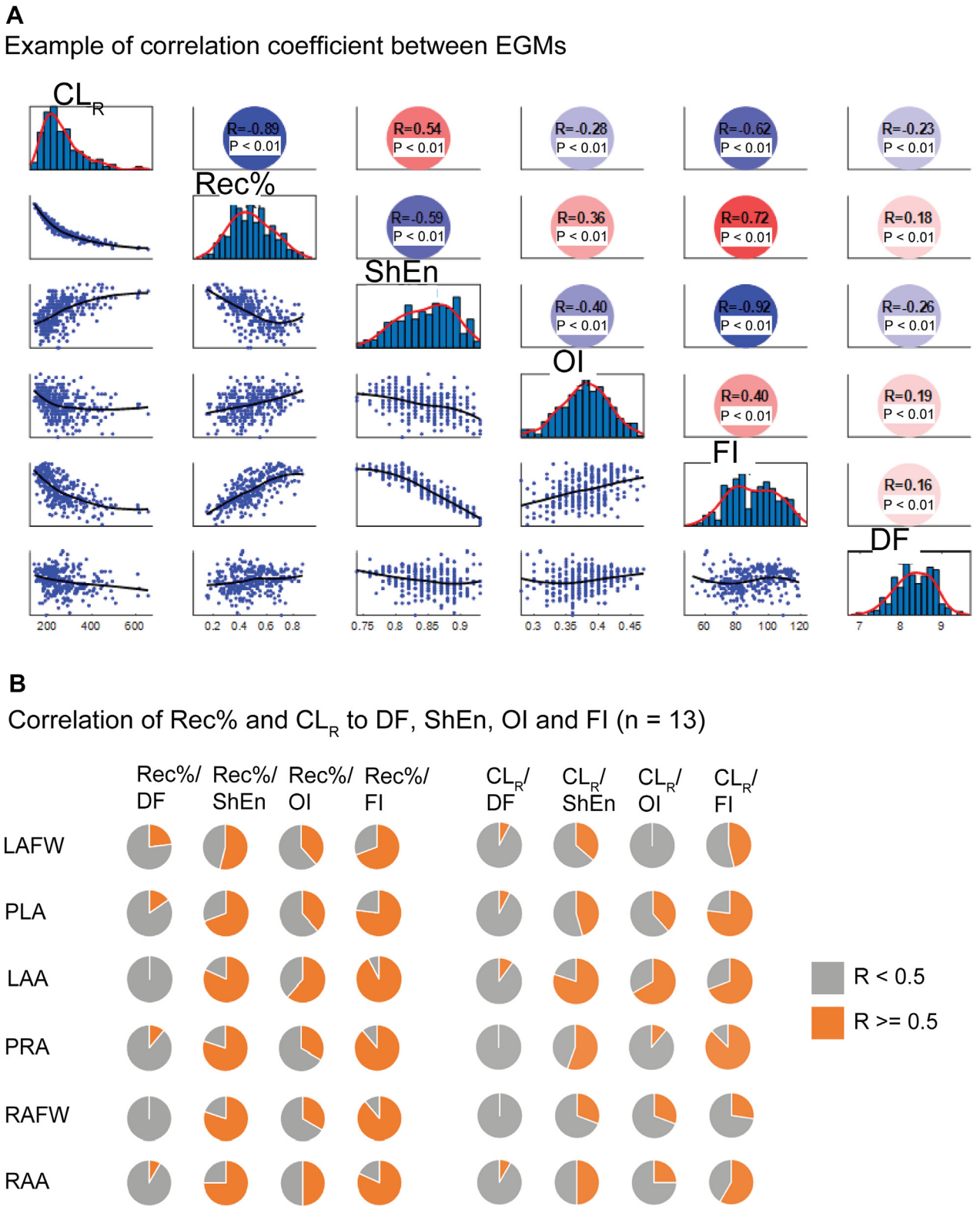
Because Rec% appears to be a highly sensitive marker of stability of reentry, it is likely that CL_R may be highly indicative of sites with potential "drivers" (see Discussion).

REC% AND CL_R ARE POORLY CORRELATED WITH FIBROSIS. A major tissue characteristic thought to affect wave-front directions is fibrosis.[22] Therefore, we quantified the extent of fibrosis in each region and assessed the relationship between fibrosis and AF EGM characteristics. **Figure 4A** shows representative images from Masson's trichrome-stained sections. The amount of fibrosis was regionally variable, being highest in the right atrial free wall (RAFW) and lowest in the 2 appendages (**Figure 4B**). There was a modest inverse correlation between fibrosis and FI and between fibrosis and OI (**Figure 4C**). There was a nonsignificant trend toward a correlation between fibrosis and Rec% (and between fibrosis and CL_R).

REC% AND CL_R ARE NOT CORRELATED WITH MYOFIBER ANISOTROPY. Another tissue characteristic that has been shown to affect electrophysiologic characteristics in atrial and ventricular tissue is myofibril orientation, with nonuniform myofiber orientation (ie, greater anisotropy of fiber orientation) thought to be related to slow and inhomogeneous conduction.[5,23,24] We therefore assessed the uniformity of fiber orientation in all 6 regions of the atria and evaluated the relationship between myofiber anisotropy index (AI) and all EGM measures. **Figure 5A** demonstrates varying degrees of fiber orientation in terms of AI in representative LAFW and LAA tissue sections. AI in the different regions in the atria is shown in **Figure 5B**. The highest AI (ie, most uniform fiber orientation) was seen in the RAFW and the lowest AI was seen in the PLA. This regional variability of AI was different from the regional heterogeneity of Rec% and CL_R described above. Indeed, AI was not found to be correlated with Rec%, CL_R, or other established EGM measures (FI, OI, DF, and Shen) (**Figure 5C**).

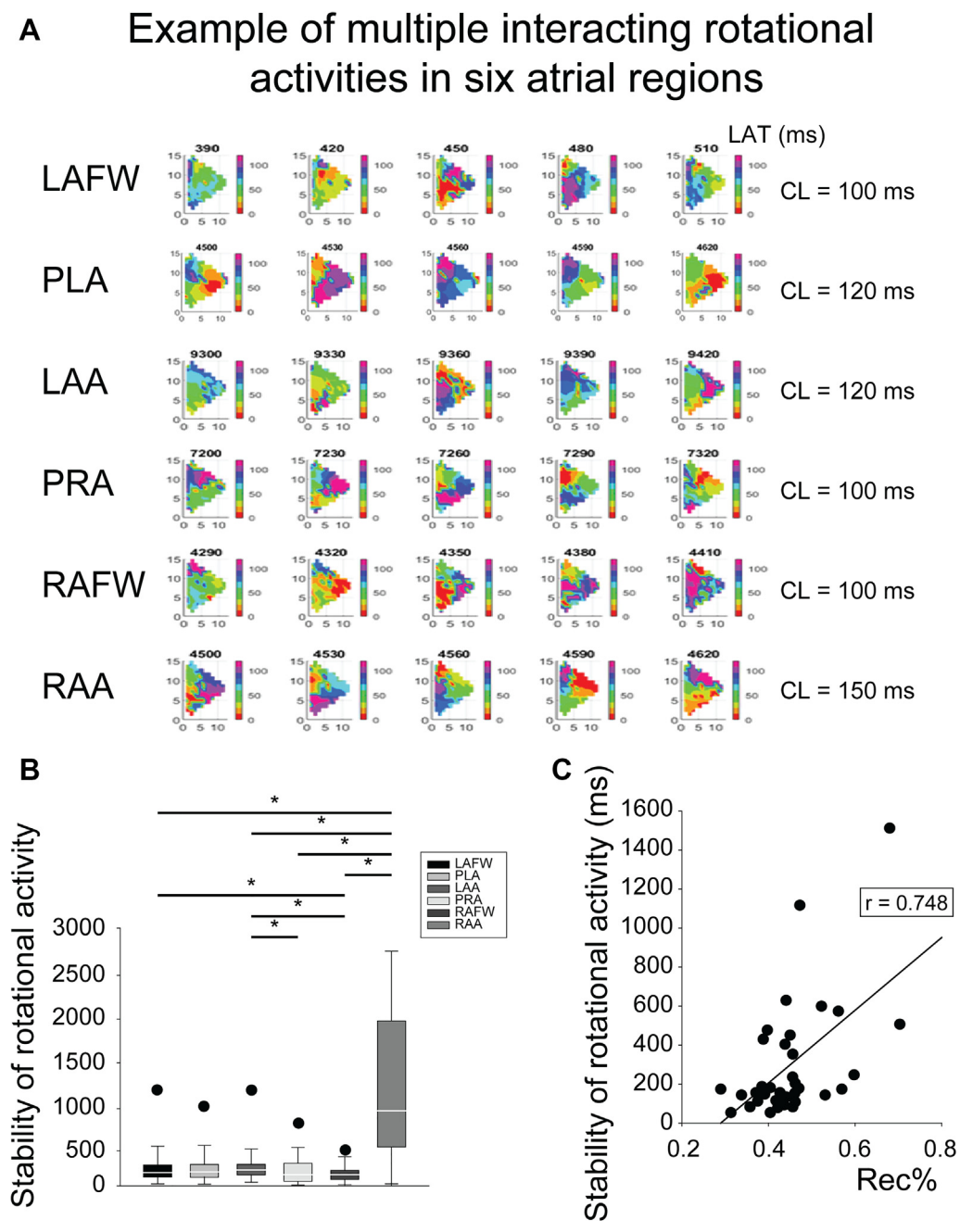
REC% IS CLOSELY RELATED TO THE HETEROGENEITY OF SPATIAL DISTRIBUTION OF PARASYMPATHETIC NERVE FIBERS IN THE ATRIA. Previous studies have shown significant parasympathetic nervous system remodeling in the atria, with the parasympathetic nervous system thought to contribute to the formation of substrate for reentry in the atria.[13] We therefore

FIGURE 2 **Close Correlation of Rec% and CL_R With FI and ShEn But Not With DF**



(A) Example of correlation coefficient between electrograms (EGMs) in the LAA of animal 1. **(B)** Analysis of *R* values of Rec% **(left)** and CL_R **(right)** with other EGMs in 6 atrial regions. The pie charts show proportion of animals with correlation factors $R \geq 0.5$ **(orange)** and $R < 0.5$ **(gray)**; n = 13. DF = dominant frequency; FI = fractional interval; ShEn = Shannon's entropy (ShEn); other abbreviations as in Figure 1.

FIGURE 3 Strong Relation of Rec% With Stability of Rotational Activities

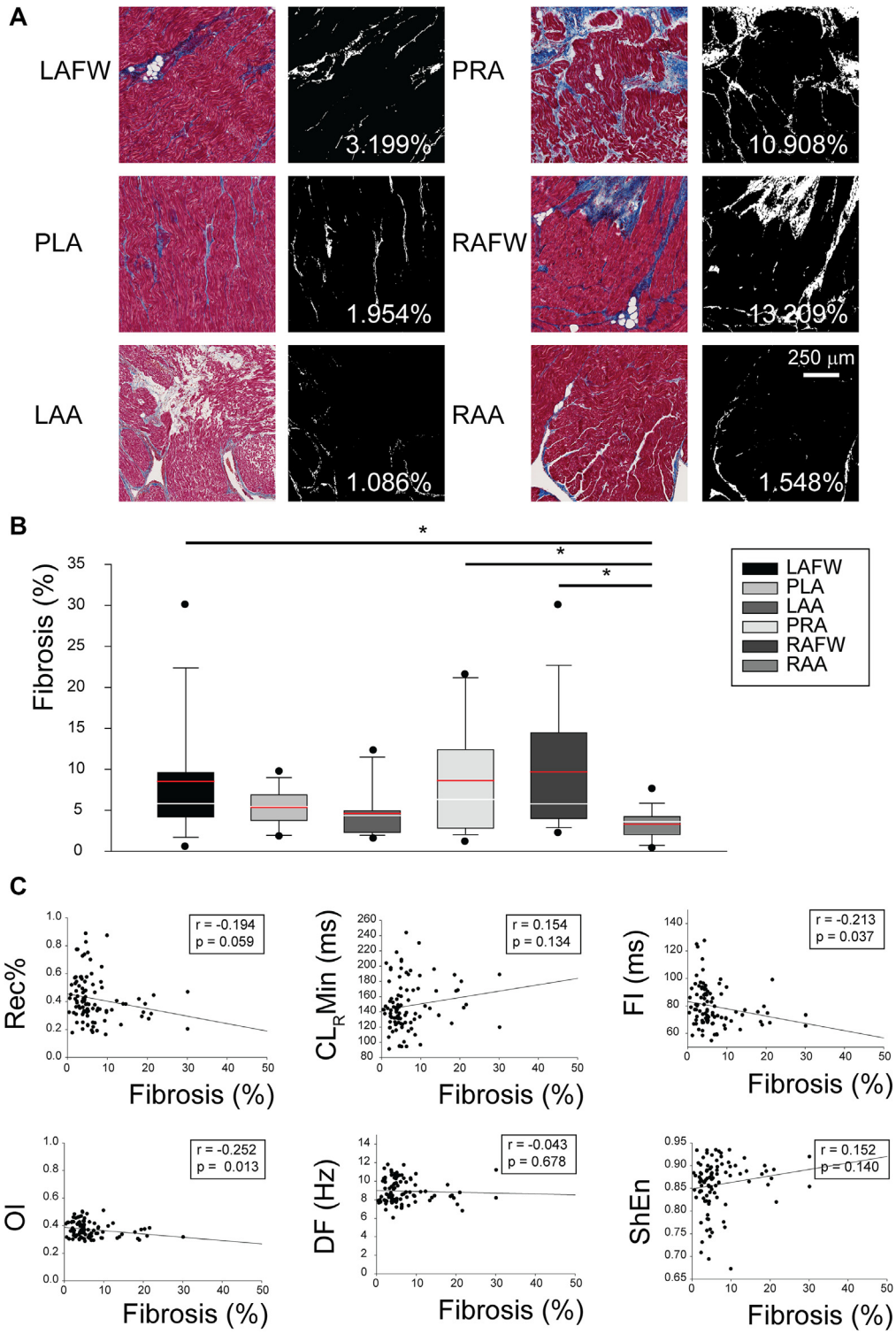


(A) Examples of multiple interacting rotational activities in 6 atrial regions. **(B)** Comparison of stability of rotational activities in 6 atrial regions. Data are presented in box and whiskers plot. Kruskal-Wallis 1-way analysis of variance on ranks with Tukey's post hoc method for all pairwise comparison; n = 13. *$P < 0.05$. **(C)** Correlation of stability of rotational activities with Rec%. Abbreviations as in Figure 1.

examined the spatial relationship between parasympathetic innervation and EMR. We also examined the effects of parasympathetic blockade on EMR in each region of the left and right atrium.

We have previously shown that RAP leads to marked hypertrophy of parent nerve bundles in the PLA, resulting in a global increase in parasympathetic and sympathetic innervation

FIGURE 4 **Poor Correlation of Rec% and CL_R With Fibrosis**



(A) Representative images of Masson's trichrome-stained tissue section and outcome of analysis in 6 atrial regions. **Red** indicates myocardium, and **blue** indicates fibrosis. **(B)** Regional differences in fibrosis. Data are presented in box and whiskers plot. Kruskal-Wallis 1-way analysis of variance on ranks with Tukey's post hoc method for all pairwise comparison; n = 13. *$P < 0.05$. **(C)** Correlation of fibrosis with Rec%, CL_RMin, FI, OI, DF, and ShEn. Abbreviations as in Figure 1.

throughout the left atrium.[18] Parasympathetic fibers were found to be more heterogeneously distributed in the PLA and LAFW compared with the LAA.[18] In the present study, we assessed the relationship between the heterogeneity of spatial distribution of parasympathetic nerve fibers and underlying EMR. **Figure 6A** shows an example of heterogeneous spatial distribution of parasympathetic nerve fibers in the left atrium (ie, high standard deviation); in contrast, **Figure 6B** demonstrates an instance of more homogeneous spatial distribution of parasympathetic fibers (ie, lower standard deviation). We discovered that the heterogeneity of spatial distribution of parasympathetic nerve fibers was closely correlated with the absolute value and the spatial heterogeneity of Rec% (**Figures 6C and 6D**, respectively). Such a correlation was not observed with any other AF EGM parameter (ie, DF, OI, FI, and ShEn) (Supplemental Figure S3). Next, we examined the effect of parasympathetic blockade on AF EGMs. With atropine, there was a significant decrease in Rec% in the LAFW, whereas Rec% was significantly increased in the RAFW and RAA (**Figure 6E**). Atropine led to a significant increase in CL_R in the LAFW, but a significant decrease in CL_R in the RAFW and RAA (**Figure 6F**). These data demonstrate that parasympathetic innervation strongly influences recurrence morphology in the fibrillating atrium, with parasympathetic signaling leading to significant changes in Rec% and CL_R in both atria.

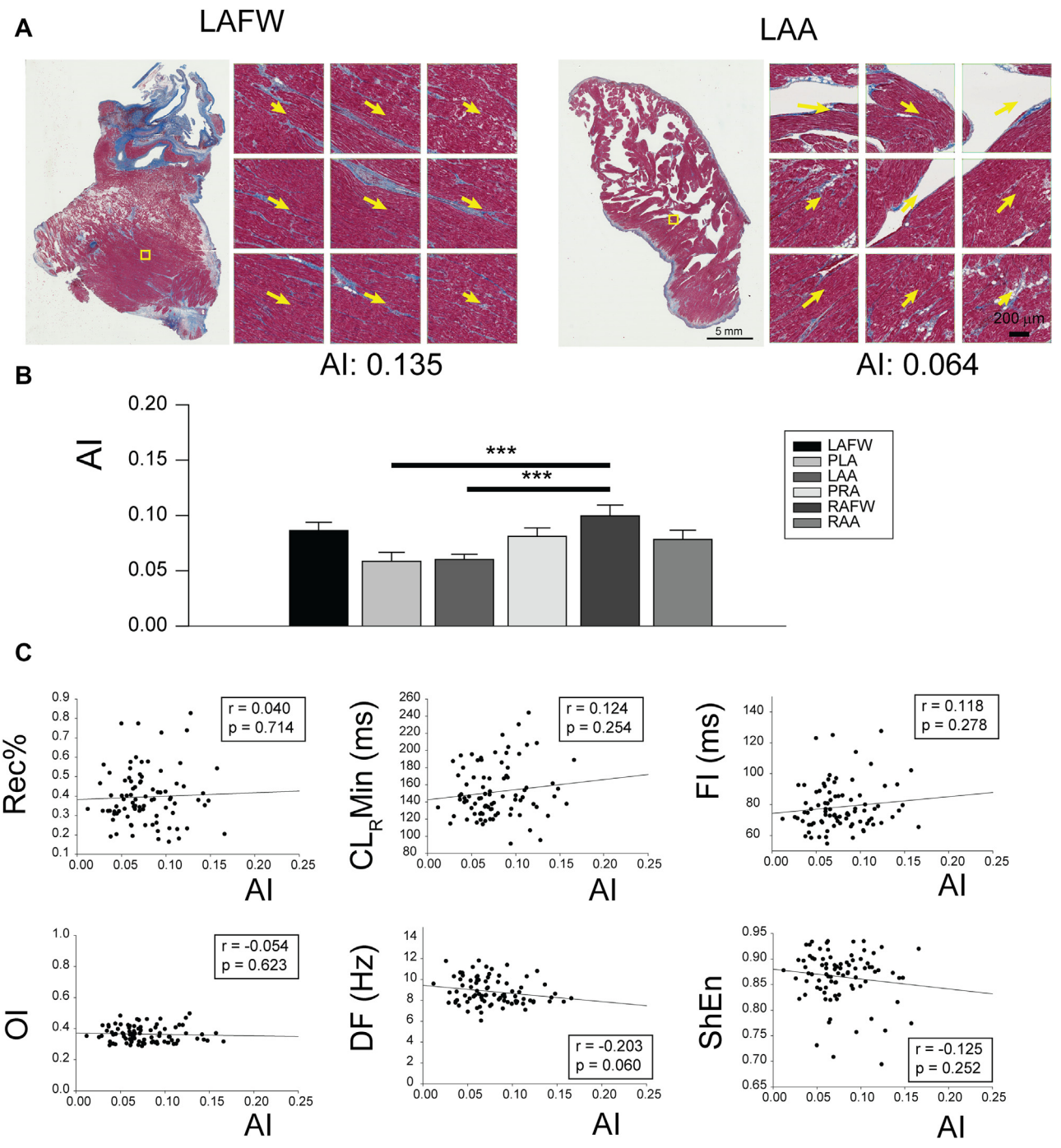
DISCUSSION

In this high-density biatrial epicardial mapping study in a canine model of persistent AF, we evaluated EGM recordings in each of 6 atrial subregions and performed a comprehensive analysis of bipolar atrial EGMs. Our results demonstrated that: 1) the recurrence morphology measures Rec% and CL_R showed significant interregional differences across the different atrial subregions, with Rec% being greatest in the appendages and CL_R being lowest in the PLA; 2) across animals and regions, Rec% and CL_R correlated moderately with measures of AF fractionation (FI) and complexity (ShEn) but less so with DF; 3) Rec% closely reflects stability of rotational activities (arrhythmogenic substrate) in the fibrillating atrium; 4) Rec% and CL_R had no significant correlation with atrial fibrosis and myofibril orientation; and 5) the spatial distribution of Rec% corresponded more closely to the spatial heterogeneity of the parasympathetic nerves, with Rec% and CL_R demonstrating significant responsiveness to parasympathetic blockade.

LIMITATIONS OF PREVIOUS ATTEMPTS TO FIND "ORDER" AMONG COMPLEX AF ACTIVATION PATTERNS WITH THE USE OF AF EGMs. The detection of arrhythmogenic regions during AF is challenging. The dynamics of AF are complex and not clearly understood. EGM morphology is dependent on complex activation wave-fronts during AF.[25] Moe et al[26] hypothesized that multiple simultaneous reentrant depolarization wave-fronts circulate in the atria. However, previous studies from Konings et al[27] and Cox et al[28] during intraoperative studies of AF showed that the process of AF activation in isochronal maps is not random. Further studies from Gerstenfeld et al,[29] Ropella et al,[30] and Wells et al[31] confirmed that wave-front propagation during AF is nonrandom by analyzing similarities between EGM signals and by means of the coherence spectrum. In a related study, Botteron and Smith[32] analyzed the spatial organization of AF and found that the correlation in sequences of activation decreased with the distance between the recordings, with a higher correlation in paroxysmal compared with chronic AF.

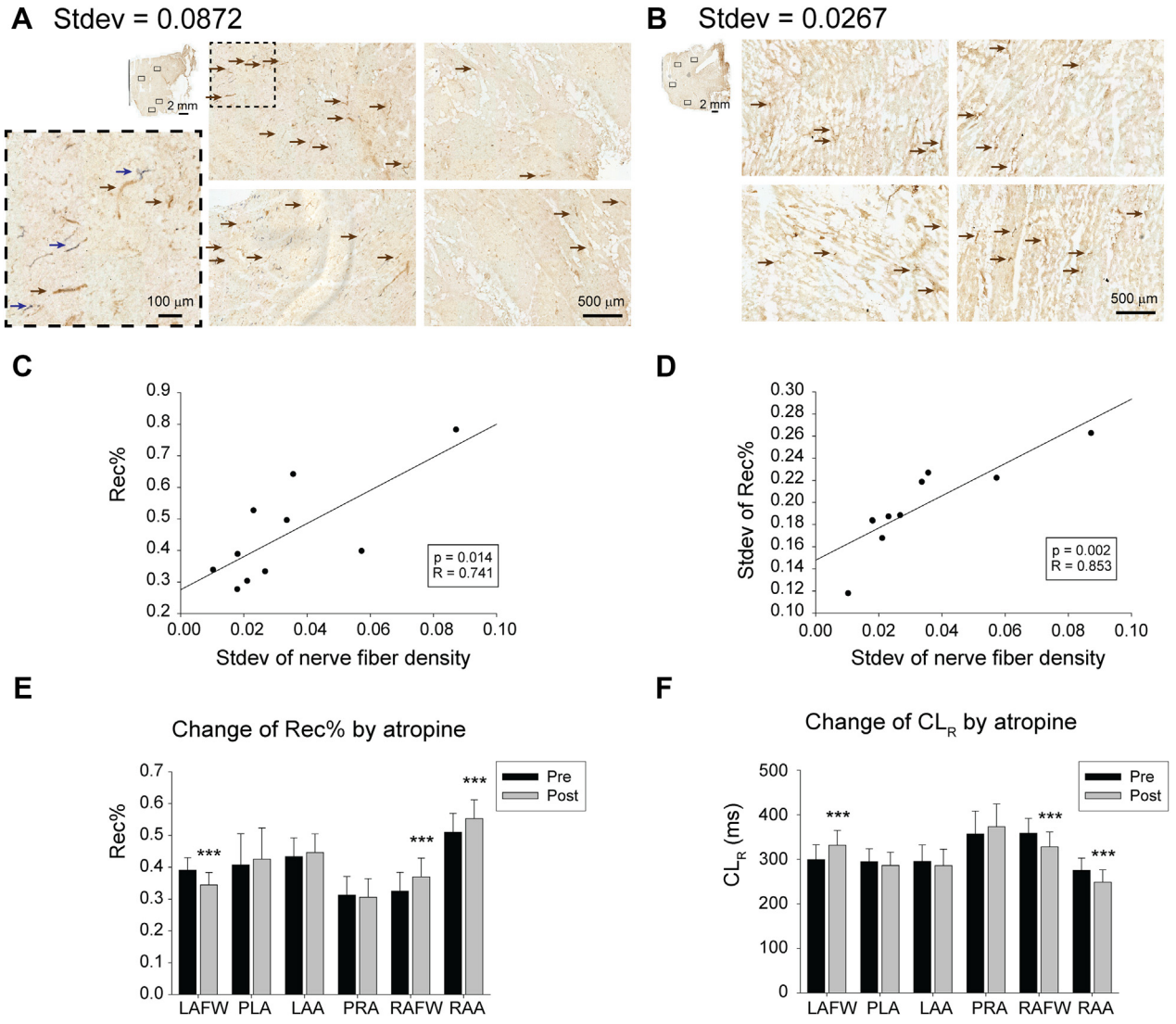
As a result, several investigators have postulated that detailed examination of the frequency, complexity, and, more recently, morphology characteristics of AF EGMs may help determine the presence of potential AF driver sources, as well as yield information on the nature of electrical and structural remodeling in AF.[33,34] However, clinical attempts at using AF EGMs to detect and eliminate arrhythmogenic source regions (by means of ablation) have had mixed results.[35] Nademanee et al[35] demonstrated a high success rate of AF ablation at regions that demonstrated CFAEs. However, similar success rates have not been reproducible, with a recent randomized trial (STAR AF 2 [Substrate and Trigger Ablation for Reduction of Atrial Fibrillation Trial; NCT01203748]) demonstrating no benefit of additional CFAE ablation compared with PV isolation alone in patients with persistent AF.[36] One reason for this seeming failure of CFAE ablation is that the pathologic basis of CFAEs in AF is still not clear. Another possible reason is that there are many definitions and methodologies used to define CFAE. Although numerous studies have demonstrated that progressive atrial remodeling with AF persistence is associated with increasing atrial substrate complexity, including EGM fractionation, those studies used CFAE bipolar algorithms, which were

FIGURE 5 **No Correlation of Rec% and CL_R With Myofiber Anisotropy**



(A) Example of fiber orientation measurements in LAFW and LAA. **(B)** Regional differences in anisotropy index (AI). Data are presented as mean ± SEM; n = 13. ***$P < 0.001$. One-way analysis of variance with Holm-Sidak method for pairwise multiple comparison. **(C)** Correlation of AI with Rec%, CL_RMin, FI, OI, DF, and ShEn. Abbreviations as in Figure 1.

FIGURE 6 Close Relation of Rec% With Spatial Distribution of Parasympathetic Nerve Fiber



Representative micrographs of atrial regions with **(A)** high and **(B)** low standard deviation of parasympathetic nerve fiber density. Location of 4 random micrographs are denoted in mini map. **Blue and brown arrows** designate sympathetic and parasympathetic nerve fibers, respectively. Correlation of **(C)** Rec% and **(D)** SD of Rec% with SD of parasympathetic nerve fiber density. **(E and F)** Change of Rec% and CL_R by atropine in 6 atrial regions. Data are presented as mean ± CI; n = 13. ***$P < 0.001$. Using linear mixed models with a random intercept for dog, and both a fixed effect and a compound symmetric covariance structure for electrode, the effect of atropine was compared in 6 atrial regions.

highly variable.[37] Furthermore, most of those bipolar algorithms were found to correlate poorly with markers of AF substrate complexity such as conduction velocity, number of waves or breakthroughs per AF cycle, and electrical dissociation.[38]

Another EGM measure that has evoked significant interest is cycle length. Regional differences in cycle length were reported in previous studies.[39] These findings increased the interest in the analysis of the frequency spectrum as a measure of the activation

rate in animal models.[34,40-42] Measurements of AF cycle length and frequency domain were used to guide ablation in patients with AF with the use of high-resolution analysis of the Fourier power spectrum with its DF.[43-48] However, no beneficial effect of DF ablation compared with PV isolation alone has been convincingly demonstrated to date.[48,49] One measure of AF frequency that has shown some clinical promise is OI. It has been shown that AF episodes with high OI are more easily terminated with the use of burst pacing and defibrillation.[50] Jarman et al[51] showed that at sites of organized activation, the activation frequency was also significantly more stable over time. This observation is consistent with the existence of focal sources and inconsistent with a purely random activation pattern. Ablation of such regions was associated with organization of AF in remote atrial regions in patients using left atrial noncontact mapping. However, that study approach was limited to focal sources.

Ganesan et al[52] showed that ShEn—a marker of signal amplitude distribution—may be associated with the pivoting zone of rotors in some cases. They showed that ShEn could differentiate the pivot from surrounding peripheral regions and thereby assist in clinical rotor mapping. However, that method was limited to the pivot point of reentry, which might be detected only within 2-3 mm distance to the rotor core.

Most recently, ablation strategies have targeted AF rotors as detected by the FIRM method.[53] Using a basket catheter with 64 electrodes, this method provided a panoramic activation map and initially reported improved ablation outcome compared with conventional ablation alone.[53] However, subsequent studies have not shown significant success with FIRM mapping in patients with persistent AF.[54] Furthermore, it has been thought that the phase map algorithms may lead to possible overdetection of AF sources.[55]

Taken together, nearly all of the EGM-based ablation strategies in AF—several of which have used fast Fourier transform-based analyses of the AF EGM—have had significant shortcomings. We subsequently discuss why novel EGM morphology measures—Rec% and CL_R—may be superior to more established EGM measures of AF at determining the arrhythmogenic substrate for AF.

MEASURES OF EMR—REC% AND CL_R—MAY BE SUPERIOR TO TRADITIONAL AF EGM MEASURES IN DETECTING AF SOURCES. In AF EGMs, the relative timings and morphologies are constantly changing. Indeed, recent studies have argued that the shape (morphology) and the repeatability of the EGM signal over time provide significant information that is not contained in more typical frequency and complexity measures of AF. A recent study showed that a novel frequency analysis algorithm and longer duration of AF EGMs have some promise in the search for temporally stable AF drivers.[56] Ciaccio et al[57] showed that in paroxysmal AF, CFAE repetitiveness is low and randomness is high outside the PVs, particularly the left superior PV, and that in persistent longstanding AF, CFAE repetitiveness becomes more uniformly distributed at disparate sites, possibly signifying an increasing number of drivers remote from PVs. Ciaccio et al[58] further showed that the dominant repetitive EGM morphology of fractionated atrial EGMs has greater temporal stability in persistent compared with paroxysmal AF. Our group recently developed a new EGM measure that analyzes EMR[9] by means of modification of a method by Eckmann et al [59] Rec% describes the percentage of the most common morphology, with CL_R signifying the mean cycle length of activations of the most recurrent morphology.

In the present study, we discovered that discrete morphology patterns exist in AF and can be identified with the novel morphology recurrence plots. Rec% and CL_R are only somewhat correlated with established EGM measures of AF fractionation (FI) and complexity (ShEn), and provide new information in quantifying the degree of repeatability of EGM morphologies. We therefore think that these new measures provide more information about the nature of arrhythmogenic AF substrate than more traditional EGM measures for detecting AF sources. To test this hypothesis, we performed a systematic analysis of the number, stability, and cycle length of 360-degree rotational activity in different regions of the atria. Previous studies have found a strong relationship between the presence and number of these rotational activities and the ability of the atria to sustain AF.[60] In the present study, the stability of rotational activities in different subregions of the atria was found to correlate with Rec%, helping to serve as an important initial validation of our postulate that sites of high recurrence morphology with the shortest cycle lengths—ie, regions of low CL_R—may represent sites of AF drivers. Interestingly though, even this correlation between Rec% and stability of rotational activity is moderate at best,

suggesting that EGM morphology recurrence is influenced by other factors as well (and is perhaps a better indicator of arrhythmogenic substrate than rotational activity alone). Indeed, our results show that while no one AF EGM measure correlates with all of the measures of AF substrate that were systematically examined—myofiber orientation, fibrosis, and autonomic innervation—Rec% and CL_R do reflect arrhythmogenic substrate (especially parasympathetic innervation) better than all previously AF EGM measures. Taken together, we think that EGM morphology recurrence may be a better marker of arrhythmogenic substrate in the atria than traditional measures of AF organization/fractionation as well as measures of rotational activity that rely on frequency characteristics of AF signals. These data support further testing of this hypothesis in large-animal models or human patients with persistent AF by performing targeted ablation at sites of low CL_R.

This experimental study was performed in light of our earlier work in patients with persistent AF where we discovered that failure of left atrial ablation in patients with persistent AF appeared to be greater when CL_R is lowest in the right atrium (ie, high recurrence, low cycle length).[9] Our experimental data provide at least a partial explanation for these findings, with some right atrial regions (eg, RAA) having significantly greater Rec% than any other region in the left or right atrium. Our experimental data show that Rec% may be a marker of stability of rotational activity in the fibrillating atrium, with rotational activity being most stable in the RAA in our study. While these data provide a partial mechanistic basis for sites of high Rec%—and suggest that some right atrial sites with high Rec% may be potential therapeutic targets—the data by no means support targeting of all high-Rec% sites at the time of ablation. Indeed, when put in the context of our prior clinical studies, it is only high-Rec% sites with the shortest cycle length (CL_R) that provide the most prognostic value in patients with persistent AF undergoing AF ablation. In a future ablation study, we postulate that atria demonstrating Rec% above a certain threshold should be closely scrutinized with added assessment of CL_R at these sites. Our ongoing clinical studies in patients with persistent AF (unpublished data) suggest that CL_R assessment at sites with Rec% >80%—which constitute a small minority (<10%) of atrial sites—may provide the greatest prognostic value for success or failure of AF ablation.

PATHOPHYSIOLOGIC BASIS OF SITES OF HIGH MORPHOLOGY RECURRENCE: ROLE OF PARASYMPATHETIC NERVE DISTRIBUTION IN GENESIS OF SITES OF HIGH REC% AND LOW CL_R. Because myofiber orientation and fibrosis are thought to be important contributors to the creation of arrhythmogenic substrate for AF,[24] we systematically assessed the relationship between recurrence morphology measures and underlying myofiber orientation/fibrosis. Previous investigations have suggested that myocyte fiber orientation may affect AF organization.[61] However, we discovered no clear relationship between Rec% and myocyte fiber orientation in the present study, indicating that other mechanisms may underlie the regional predilection of high Rec% for the appendages. In contrast to Rec%, CL_R was lowest in the PLA in the majority of animals. This is consistent with our initial clinical findings in patients with persistent AF,[9] where CL_R was lowest in the PVs or PLA in nearly two-thirds of all patients with AF (see below).

Some previous studies have attempted to relate EGM parameters such as voltage, fractionation, and DF with tissue characteristics like fibrosis. Marrouche et al[62] showed that there is a correlation between atrial fibrosis—as determined by delayed enhancement on MRI—and low-voltage regions. A related study suggested that CFAEs also correlate with regions of atrial fibrosis.[37] However, a limitation of those studies was that they were not performed with high-resolution contact mapping; furthermore, detailed microscopic tissue analyses of fibrosis and anisotropy were not performed. In the present study, we systematically analyzed several EGM measures simultaneously with the use of high-resolution mapping during AF, and then performed detailed tissue correlations with fibrosis. While the amount of fibrosis was discovered to be the least in the appendages, we discovered only a weak correlation between AF EGM measures—both established measures and new morphology measures—and fibrosis.

An important upstream mechanism that is thought to contribute to electrical remodeling is increased activity of the parasympathetic nerve.[13] We and others have shown in recent years that increased parasympathetic nerve sprouting—and a resulting increase in parasympathetic signaling in the atrium—is an important mechanism that contributes to electrical remodeling in the atrium.[18] In a recent publication,[18] we showed that RAP leads to marked hypertrophy of parent autonomic nerve bundles in the PLA, resulting in a global increase in

parasympathetic and sympathetic innervation throughout the LA. Parasympathetic fibers were found to be more heterogeneously distributed in the PLA and LAFW compared with the LAA. The coefficient of variation (SD/mean) of CL_R was also found to be significantly greater in the PLA and LAFW than in the LAA, suggesting that the spatial distribution of parasympathetic nerve fibers likely affects recurrence morphology. In the present study, we assessed the precise relationship between the heterogeneity of spatial distribution of parasympathetic nerve fibers and EMR. We discovered that the spatial distribution of the parasympathetic nerve fibers was more closely related to Rec% than to any other EGM parameter (ie, DF, OI, FI, and ShEn). Furthermore, parasympathetic blockade led to a significant change in Rec% and CL_R in subregions of the right and left atrium, again demonstrating the significant contribution of parasympathetic signaling to EMR. Interestingly, the direction of change of Rec% and CL_R in response to parasympathetic blockade differed between the right and left atria. The mechanisms underlying these regional changes in Rec% and CL_R may reflect differences in the precise pattern of parasympathetic innervation, M_2 receptor, and IK_{Ach} concentrations between the atria.[18,63] Indeed, owing to Rec% differences amongst left atrial subregions being smaller than those observed in the right atrium, a clear relationship between Rec% and parasympathetic nerve fiber distribution became clear only when both atria are taken together. These interatrial differences in parasympathetic innervation and downstream signaling effectors need to be further investigated in future studies.

STUDY LIMITATIONS. Although previous studies showed tachycardia-induced atrial fibrosis[64,65] and our animal model also demonstrated a significant amount of fibrosis (after 4 weeks of RAP),[66] the amount of fibrosis appeared to be <20% in this study, compared with previous attempts that have correlated AF EGMs with underlying atrial fibrosis. It is possible that a stronger correlation between Rec% and fibrosis exists in the presence of greater degrees of fibrosis. Further investigations therefore need to be performed with models incorporating a higher degree of fibrosis (30%-40%). We determined fibrosis percentage only near the epicardial surface. Further studies showing differences of endocardial and epicardial fibrosis and EGM measures need to be conducted. The analysis of the parameter Rec% was calculated based on 10-second EGM recordings in duration and may miss longer-term recurrence patterns. Further investigations of the dependency of Rec% on electrode size and geometry as well as distance to the tissue, near and far-field effects, and endocardial vs epicardial mapping need to be explored. Further investigations of the temporal stability of these new measures in paroxysmal and persistent AF need to be investigated.

Our nerve analysis studies were largely confined to efferent parasympathetic nerve fibers. Afferent nerve fibers were not assessed as part of this study.

We postulate that EMR should help detect "focal" drivers, whether reentrant (ie, rotational) or automatic/triggered in mechanism. Unfortunately, our epicardial mapping plaques did not have the electrode density (resolution) that is required to detect smaller rotational or focal drivers. Nonetheless, the presence of a positive correlation between EMR and larger rotational drivers (as assessed by our epicardial plaques) shows important proof of concept. In ongoing clinical studies in patients with persistent AF, we are performing higher-density mapping with the use of multipolar mapping catheters; these clinical studies will allow us to better examine the relationship between more focal drivers and EMR.

FUTURE DIRECTIONS: POTENTIAL NEW WAYS TO USE EGM MORPHOLOGY RECURRENCE TO GUIDE ABLATION. The apparent increase in efficacy of AF ablation in some studies with concomitant LAA isolation—or with complete exclusion of the entire appendage, eg, with the LARIAT device—supports a role for the LAA in the maintenance of a vulnerable AF substrate in the fibrillating atrium. Our data—which show high Rec% in both appendages, with the LAA also having a fairly short CL_R—suggest that the LAA may indeed harbor arrhythmogenic substrate for AF. If our future clinical studies continue to support a role for slow CL_R in the maintenance of AF, then it is conceivable that a CL_R-based ablation approach in the LAA may be just as beneficial as complete LAA isolation (which is not only procedurally difficult but can be associated with complications such as thrombus generation, etc).

The present study showed that although sites of low CL_R appear to partially correspond to sites of low AI, the actual correlation between these measures is low. What if an EGM measure such CL_R could be

combined with an anatomic marker such as AI? If magnetic resonance imaging techniques with sufficient resolution become readily available to accurately determine myocyte fiber anisotropy, it is tempting of speculate that a combination of CL_R and AI may more accurately determine AF driver sources than either technique alone.

CONCLUSIONS

Traditional anatomically guided ablation and attempts in the past decade to perform EGM-guided AF ablation (CFAE, DF, FIRM) have not been shown to be sufficient treatment for persistent AF. We have extensively studied the mechanistic basis of a new EGM-guided therapeutic approach to AF that combines high morphology recurrence with fast cycle length. Our results suggest that EMR parameters—Rec % and CL_R—may be more reflective of arrhythmogenic substrate for AF than any previously studied EGM parameter of AF. Further studies are necessary to determine the effectiveness of this novel EGM therapeutic approach in guiding catheter ablation of persistent AF.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: AF is the most common heart rhythm disorder and is a major cause of stroke. The detection of arrhythmogenic regions during AF is challenging, and the dynamics of AF are complex and not clearly understood. Though atrial EGMs have been thought to reflect the pathophysiologic substrate for AF, there are currently no sufficient EGM-based ablation strategies (eg, CFAE, DF, and FIRM) for persistent AF. In the present study, using biatrial high-density electrophysiologic mapping in a canine RAP model of AF, we systematically investigated the relationship of EMR (Rec% and CL_R) with established AF EGM parameters and tissue characteristics. We demonstrated that the stability of rotational activities in different subregions of the atria was correlated with Rec%, helping to serve as an important initial validation of our postulate that sites of high recurrence morphology with the shortest cycle lengths—ie, regions of low CL_R—may represent sites of AF drivers. While no one AF EGM measure correlates with all of the measures of AF substrate that were systematically examined, eg, myofiber orientation, fibrosis, and autonomic innervation, Rec% and CL_R reflect arrhythmogenic substrate (especially parasympathetic innervation) better than all previously AF EGM measures.

TRANSLATIONAL OUTLOOK: This study demonstrates that Rec% and CL_R may be superior to more established EGM measures of AF at determining arrhythmogenic substrate for AF. Taken together, these data suggest further testing of our hypothesis in large-animal models or human patients with persistent AF by performing targeted ablation at sites of low CL_R.

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APPENDIX For supplemental material, please see the online version of this paper.