Intermittent Atrial Tachycardia Promotes Repolarization Alternans and Conduction Slowing During Rapid Rates, and Increases Susceptibility to Atrial Fibrillation in a Free-Behaving Sheep Model

JOANNA MONIGATTI-TENKORANG, M.D.,* FLORIAN JOUSSET, PH.D.,† PATRIZIO PASCALE, M.D.,* JEAN-MARC VESIN, PH.D.,† PATRICK RUCHAT, M.D.,‡ MARTIN FROMER, M.D.,* SANJIV M. NARAYAN, M.D., PH.D.,§ and ETIENNE PRUVOT, M.D.*

From the *Department of Cardiology, Lausanne University Hospital, Lausanne, Switzerland; †Applied Signal Processing Group, Swiss Federal Institute of Technology, Lausanne, Switzerland; ‡Department of Cardiovascular Surgery, Lausanne University Hospital, Lausanne, Switzerland; and §University of California, San Diego, California, USA

Repolarization Alternans and Atrial Remodeling. ***Introduction:*** **Paroxysmal atrial fibrillation (AF) may be triggered by intermittent atrial tachycardia, and ultimately lead to persistent AF. However, the mechanisms by which intermittent atrial tachycardia promotes sustained AF are not well understood.**

Methods and Results: **Eight sheep were chronically implanted with 2 pacemakers for the recording of broadband right atrial unipolar electrograms, and for the delivery of electrophysiological stimulation protocols and intermittent right atrial tachycardia. Right atrial kinetics of activation recovery interval (ARI) as a surrogate for action potential duration, of conduction time and velocity, and of repolarization alternans were analyzed at incremental pacing rates during the remodeling process induced by weeks of intermittent atrial tachycardia until the development of sustained AF. Intermittent atrial tachycardia decreased ARI and blunted its rate adaptation, facilitated atrial capture, and slowed conduction at high rates, and increased susceptibility to pacing-induced AF. In spite of blunted ARI rate adaptation, right atrial repolarization alternans was maintained during remodeling, and further increased in magnitude just before rapid pacing-induced AF.**

Conclusion: **This study suggests that weeks of intermittent right atrial tachycardia result in a gradual electrical remodeling favorable for wavebreaks and reentry that may facilitate fibrillation.** *(J Cardiovasc Electrophysiol, Vol. 25, pp. 418-427, April 2014)*

alternans, atrial fibrillation, atrial tachycardia, electrophysiology, remodeling

Introduction

Several models of atrial fibrillation (AF) have been developed in the last 2 decades based on a phenotype of atrial electrical remodeling by burst pacing[1-7] or on a phenotype of heart failure induced by rapid ventricular stimulation[8,9] or slow ventricular rate following atrioventricular block.[8,10,11] Atrial burst pacing consistently causes electrical remodeling, although interspecies differences exist in the development of fibrosis.[2,8,12] Atrial burst pacing alters repolarization that includes shortening of effective refractory period (ERP), blunting of ERP rate adaptation and increased dispersion of ERP.[14,6,7,10] Assessment of atrial repolarization, however, has been limited to the measurement of ERP and monophasic action potential recordings, and their rate adaptation.[1,4,6,7,10,13]

We recently showed that atrial repolarization alternans (Re-ALT) and its kinetics are measurable *in vivo* in a free behaving atrial burst pacing sheep model.[14,15] Re-ALT and complex action potential duration (APD) oscillations can dynamically promote dispersion of repolarization during rapid pacing leading to wavebreak and reentry,[16,17] and have been implicated in transitions from rapid pacing to AF.[14,17-19] However, the electrophysiological substrates enabling transitions from paroxysmal to sustained AF remain unclear. We hypothesized that intermittent but progressive right atrial tachycardia (AT), to model tachycardia-induced AF, increases the susceptibility to sustained AF by shortening atrial repolarization over time, blunting its rate adaptation, promoting atrial Re-ALT and slowing of conduction, and tested this in a freely moving ovine model.

Methods

Animal Experimental Setup and Data Acquisition

Two pacemakers, each with a single lead screwed into the right atrium (RA), were implanted in 8 male sheep (80 ±

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Address for correspondence: Etienne Pruvot, M.D., CHUV, Department of Cardiology, Rue du Bugnon 46, 1011 Lausanne, Switzerland. Fax: +41-21-314-51-96; E-mail: etienne.pruvot@chuv.ch

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17 kg) under general anesthesia: a Vitatron™ T70 for the recording of broadband atrial unipolar electrograms (EGM, sampling rate 800 Hz, 0.4 Hz high pass filter), and a Vitatron™ Prevent AF to deliver customized electrophysiological pacing (EP) protocols.[14,15] Both leads were positioned on the lateral wall of the RA half way from the posterior wall and the tricuspid annulus verified in right and left anterior oblique (RAO, LAO) projections. Interelectrode distance was measured radiographically at time of pacemaker implantation and at the end of the study. Atrial EGM and subcutaneous ECG recorded with a wireless Holter device (TMSi™, B_Holter Recordings) were acquired 1 month following lead implantation during light sedation (xylazine 0.2 mg/kg). Transthoracic left ventricular ejection fraction (LVEF) was assessed by 2-dimensional echocardiography under general anesthesia at the time of pacemaker implantation and at sacrifice (following 1 week of sustained AF). Experiments were carried out in accordance with the European convention for the protection of vertebrate animals used for scientific purposes.

Electrophysiology Protocols

We measured the kinetics of RA Re-ALT amplitude and conduction time (CT) as a function of pacing cycle length (PCL). Study protocols consisted of right atrial (RA) pacing for 400 beats starting at PCL 400 ms with 10 ms decrements until stable 2:1 capture was achieved. The amplitude of stimulation (2.3 ± 0.6 V, 0.5 ms) was twice diastolic threshold and remained constant throughout the study.

Intermittent Atrial Tachycardia Pacing Protocol

One month after implantation, the Vitatron™ Prevent AF pacemaker was activated to deliver intermittent right atrial AT, comprising a programmed period of AT (2–5 seconds) alternating with a period of sinus rhythm (2–5 seconds). The PCL of burst pacing was set 20 ms longer than the longest PCL at which 2:1 atrial capture occurred. Sheep were studied weekly on Monday morning in the fasting state. The intermittent AT protocol was temporarily withheld during weekly recordings, which comprised pacing for 400 beats at decreasing PCL that took an average of 1 hour. Following data acquisition, the intermittent AT pacing protocol resumed until 1 week later. The burst pacing period was initially set at 2 seconds and the sinus rhythm period at 5 seconds. Both were gradually altered in the weeks following the onset of pacing to cause progressive atrial remodeling. The burst pacing duration was then prolonged by 1 second and the sinus rhythm duration was reduced by 1 second every other week (until sustained AF occurred). In some experiments, this progression of burst pacing slowed if the recording protocol triggered nonsustained AF in early remodeling.

Sustained AF was defined as AF lasting at least 1 week. For animals in sustained AF at the time of the study protocol after pacing, AF was required to continue throughout the data acquisition period (1 hour). The intermittent AT protocol then resumed. The following week, sheep that were still in AF were considered to be in sustained AF. The intermittent AT protocol was then continued until sacrifice 2 weeks later, i.e., 2 weeks after the first observation of sustained AF. Before sacrifice, the ECG was rechecked to confirm sustained AF, and then the animal was sacrificed.

Study Pacing Protocol

The Vitatron™ Prevent AF pacemaker was used to deliver EP protocols of 400-beat duration on a weekly basis until the week preceding onset of sustained AF. Three time points were defined: 1. Before Remodeling (BR, i.e., before activation of intermittent AT), 2. During Remodeling (DR, i.e., halfway between activation of intermittent AT and the week before onset of sustained AF), and 3. After Remodeling (AR, i.e., the week before sustained AF). Cumulative 2:1 capture as a percentage of the entire 400-beat recording was determined for the 8 sheep. The ERP was defined as the longest PCL at which stable 2:1 capture occurred.[14] For PCLs $<$ ERP, the percentage of 2:1 capture was deemed equal to that at ERP. Complex oscillations of RA repolarization, defined as variations in T_a magnitude of >50 μV that do not alternate, have been previously reported in this model.[14] In each remodeling stage, mean CT, mean Re-ALT and mean beat-to-beat difference in T_a magnitude for complex oscillations were determined for the last 9 beats preceding onset of rapid pacing-induced AF during the EP protocols.[14] Heart rate values during periods of intermittent AT and sinus rhythm were measured and compared before (BR) and after (AR) remodeling.

Signal Analysis

The approach to filtering, ventricular cancellation, identification, and error in time and amplitude of the T-wave apex (T_a) of atrial unipolar signals have been described.[14] Briefly, Re-ALT sequences were determined from time series of beat-to-beat differences of atrial T_a amplitude >50 μV.[14,20] The conduction time (CT; panel A of Fig. 1) of unipolar EGM was defined as the time interval between the pacemaker stimulus (S_1) and the maximum of the atrial depolarization wave (R_a). Conduction velocity (CV, cm/s) was calculated as the ratio of interelectrode distance and CT. Activation recovery intervals (ARI, Fig. 1) as a surrogate for APD[21-23] was measured from R_a to the following T_a because of the occurrence of the next atrial depolarization on the terminal part of the atrial repolarization wave during rapid pacing.[14] ARIs at PCLs $<$ 150 ms could not be determined reliably because the next atrial depolarization wave impinged on the preceding atrial T-wave. The diastolic interval (DI) was defined as the time interval between the preceding T_a and the following R_a. Importantly, during remodeling stages episodes of nonsustained AF occurred mainly at short PCL ($\leq$ 150 ms). For the limited number of nonsustained AF episodes occurring during the 400-beat protocol ($n = 17$), the analysis of the parameters was limited to the beats preceding but not following the episodes.

Statistical Analysis

Statistical tests were performed using StatView for Windows version 5 (SAS Institute Inc.). Time series of T_a magnitude were used to determine Re-ALT sequences, which were considered significant when the 2 following conditions were fulfilled: 1) T_a was alternating for $\geq$5 consecutive beats, 2) even and odd T_a distributions were statistically different based on a Student's *t*-test ($P < 0.01$, 2 sided) as recently published.[14] Comparisons of continuous variables between groups were performed using Student's *t*-test. Multiple

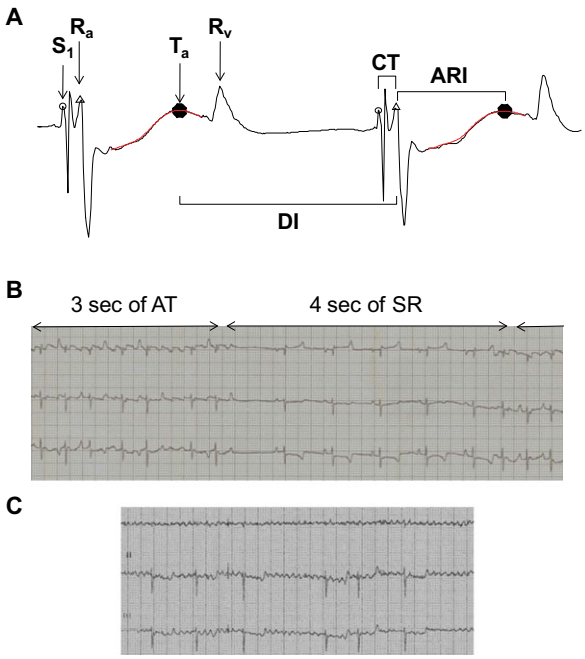

Figure 1. *RA unipolar EGM and subcutaneous ECG recordings. Panel A: representative example of right atrial (RA) unipolar EGM. Circles denote pacemaker stimulus (S_1), triangles the maximum of the atrial depolarization wave (R_a), dots the atrial T-wave apex (T_a), and R_v the right ventricular farfield potential. RA repolarization waves are highlighted by bold red curves. Panel B: subcutaneous ECG recording with intermittent atrial tachycardia (AT) and sinus rhythm (SR). Panel C: example of sustained AF after 6 weeks of intermittent AT.*

1-way ANOVA was used to test differences in ARI, Re-ALT amplitude, and CT as a function of PCL and remodeling stages. A $P < 0.05$ was considered significant.

Results

The 8 sheep were successfully paced into sustained AF using an intermittent right atrial AT (mean duration of 4.3 ± 1.1 seconds for the entire study) at a mean PCL of 170 ± 18 ms, alternating with a SR period (mean duration of 2.9 ± 1.1 seconds for the entire study) for an average study duration of 8.0 ± 3.3 weeks. The average times from before remodeling (BR) to during remodeling (DR) was 3.3 ± 2.1 weeks, and from before remodeling (BR) to after remodeling (AR) was 6.8 ± 3.6 weeks.

Panel B of Figure 1 shows a representative subcutaneous ECG recording of intermittent AT (PCL 150 ms) for 3 seconds alternating with sinus rhythm (4 seconds). Panel C of Figure 1 shows an example of sustained AF induced after 6 weeks of intermittent AT. Heart rates (HR) values during alternating periods of intermittent AT and SR were compared before (BR) and after (AR) remodeling. HR was significantly higher during intermittent AT compared to sinus rhythm before and after remodeling (BR: 106 ± 38 vs 69 ± 8 bpm; AR: 99 ± 28 vs 74 ± 13 bpm, $P < 0.05$ for both comparison), but was comparable for sinus rhythm or pacing between stages of remodeling (SR period, BR vs AR $P = 0.36$; intermittent AT period, BR vs AR $P = 0.63$).

Left ventricular ejection fraction (LVEF) did not decrease significantly between implantation and sacrifice (63 ± 5 vs 57 ± 14%, $P = 0.17$), thus minimizing the impact of significant LV systolic dysfunction as a confounder. In tandem, a nonsignificant increase ($P = 0.11$) in inter-electrode distance was also observed after remodeling (BR, 25.6 ± 10.4 mm to AR, 28.6 ± 11.0 mm, Δ = 3.0 ± 3.5 mm).

Rate Dependence of ARI, CT, and CV

The RA ERP and the rate-dependence of ARI, CT, and CV values were affected by weeks of intermittent AT. A nonsignificant decrease in mean ERP (BR vs AR, $P = 0.13$) was noticed from before (BR, 138 ± 29 ms) to during (DR, 126 ± 11 ms) to after (AR, 118 ± 12 ms) remodeling.

Panel A of Figure 2 shows representative RA EGMs before (BR, left) and after (AR, right) remodeling induced by 2 weeks of intermittent AT. Before remodeling, ARI decreased from 131 ms at PCL 400 ms to 100 ms at PCL 180 ms, while at the same time CT prolonged from 23 ms to 33 ms. After remodeling, ARI was shorter (89 ms at PCL 400 ms) and lost its rate adaptation (89 ms at PCL 180 ms) compared to BR values, while CT was prolonged and increased with rate (from 26 ms at PCL 400 ms to 33 ms at PCL 180 ms). Panel B shows summary data (n = 8, mean ± SE) of ARI (left), and CT and CV (right) values as a function of PCL. Inter-electrode distance was only available at time of pacemaker implantation and sacrifice; both time points were used as surrogates for BR and AR, respectively. At BR, ARI decreased significantly ($P < 0.03$, ANOVA) from a mean value of 123 ± 23 ms at PCL 400 ms to 85 ± 11 ms at PCL 150 ms. Importantly, both DR and AR stages displayed significantly shorter ARI at long PCLs compared to BR (−14 ± 14% and −10 ± 14% at PCL 400 ms, respectively, $P < 0.04$), that blunted the ARI rate adaptation ($P = 0.77$ and $P = 0.86$, respectively, ANOVA). Figure S2 of the supplemental material shows similar findings regarding the kinetics of ARI expressed as a function of DIs.

In contrast, CT showed rate dependence in remodeling. In the BR stage, 2:1 atrial capture occurred and prevented rapid rates from causing CT prolongation. After remodeling, CT increased with PCL (AR, $P < 0.03$, ANOVA) as capture failure occurred at higher pacing rates (i.e., PCL 130 ms). CT was significantly ($P < 0.01$) longer during (DR) and after (AR) compared to before (BR) remodeling at most PCLs. CV values, however, were similar between DR and AR at any PCL (BR vs AR, P = NS), and exhibited rate dependence only after remodeling (AR) from a mean value of 74 ± 15 cm/s at PCL 400 ms to 67 ± 8 cm/s at PCL 130 ms ($P < 0.01$, ANOVA). In summary, weeks of intermittent AT decreased right atrial ARI and ERP values, blunted ARI rate dependence but did not affect CV. Conduction slowing occurred after remodeling only because of facilitation of atrial capture at rapid rates.

Remodeling Promotes Atrial Capture During Rapid Pacing

Intermittent 2:1 capture intermingled with 1:1 atrial capture in early stages of pacing, but became less pronounced as remodeling shortened ARI and ERP. Panel A of Figure 3 shows a sequence of 76 stimuli from a 400-beat EP protocol at PCL 150 ms before (BR, top) and after (AR, bottom) 2

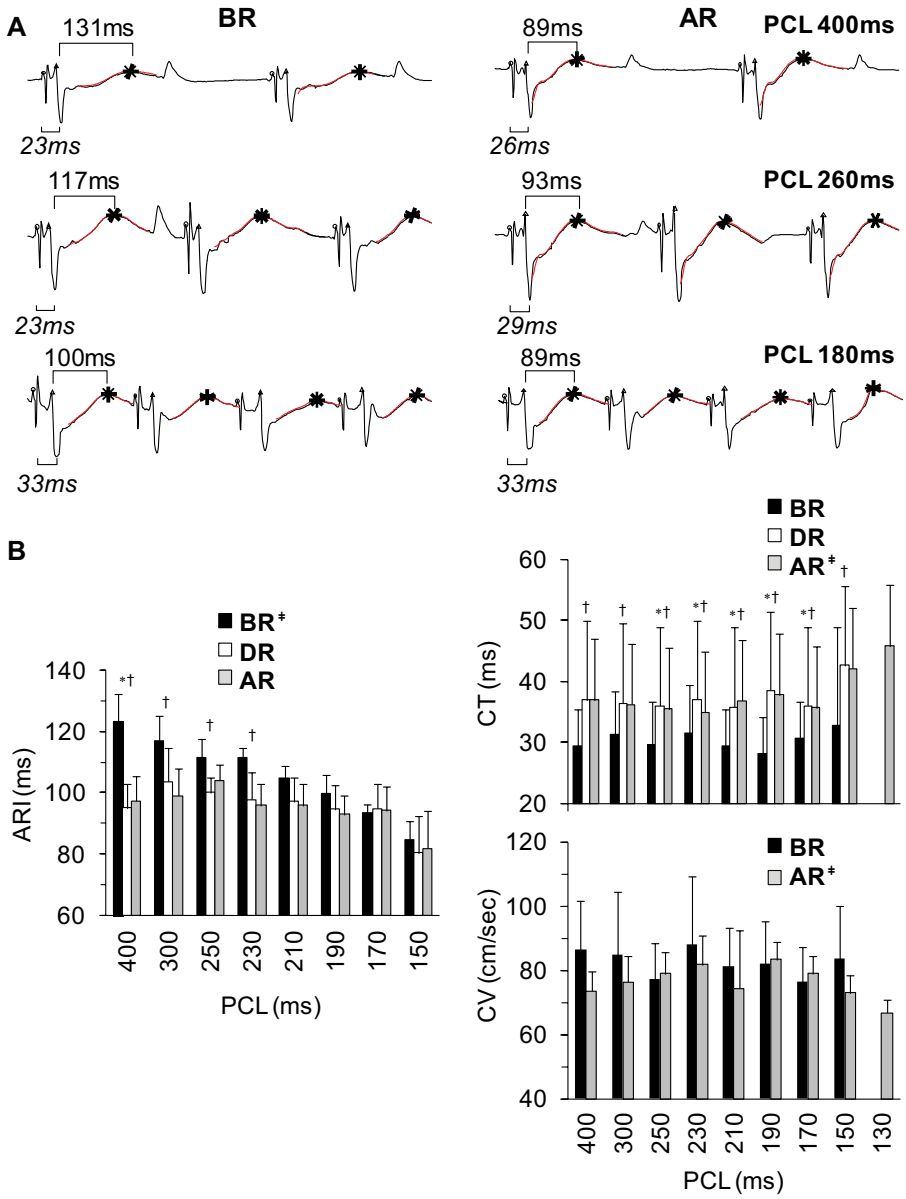

Figure 2. *Intermittent AT blunts ARI rate dependence and prolongs CT. Panel A: representative RA EGMs at decreasing PCLs before (BR, left) and after (AR, right) remodeling. Numbers on the top pertain to ARI and on the bottom to CT. Panel B: summary data (n = 8 sheep, mean ± SE) of ARI (left), and of CT and CV (right) kinetics as a function of PCL before (BR), during (DR), and after (AR) remodeling. *P < 0.05 BR versus DR; †P < 0.05 BR versus AR; ‡P < 0.03 for trend.*

weeks of intermittent AT in a representative sheep. The inset shows a magnified sequence of 2:1 capture. The proportion of 1:1 capture increased from 2% before to 61% after remodeling. Panel B shows the cumulative percentage of 2:1 capture (n = 8, mean ± SE) as a function of PCL during the 400-beat EP protocols before (BR), during (DR) and after (AR) remodeling. All 3 remodeling stages showed a gradual increase in intermittent capture with shortening PCL ($P < 0.001$, ANOVA). No significant difference in kinetics was observed between groups (P = ns, MANOVA). However, a 10 ms shift in the PCL threshold at which the proportion of 2:1 capture became significantly greater from that at longer PCL was noticed after (AR, 130 ms, grey arrow) compared to before (BR, 140 ms, black arrow) remodeling. The PCL range (maximum minus minimum) at which 2:1 capture occurred narrowed from before (BR, 110 ms) to during (DR, 80 ms) to after (AR, 60 ms) remodeling. In summary, weeks of intermittent AT facilitate atrial capture at shorter cycle length tachycardias (modeled as shorter PCL).

Remodeling Stages and Rate Dependence of Re-ALT

Atrial Re-ALT was detected in all sheep, and its amplitude was inversely related to PCL. Panel A of Figure 4 shows from top to bottom representative unipolar RA EGMs, and time series of CT, ARI, diastolic interval (DI), T_a, and Re-ALT (ΔT_a) at PCL 260 ms (left) and 180 ms (right) before remodeling. Beat-to-beat alternation of RA EGM T-wave is emphasized by alternating black and white arrows. At PCL 260 ms, subtle beat-to-beat T_a alternans is visible on the atrial EGM that was not accompanied by alternans of EGM depolarization wave nor of CT as shown in the time series. Decreasing the PCL from 260 ms to 180 ms increased the mean Re-ALT magnitude (ΔT_a) from 140 ± 68 μV to 291 ± 71 μV, prolonged the mean CT from 22 ± 1 ms to 35 ± 4 ms, decreased the mean ARI from 115 ± 4 ms to 102 ± 2 ms and the mean DI from 145 ± 4 ms to 80 ± 6 ms. Note that at PCL 260 ms both ARIs and DIs displayed some beat-to-beat alternans as well (mean ΔARI 7 ± 2 ms and mean ΔDI 12 ± 3 ms) that was concordant, with long ARI preceded by long

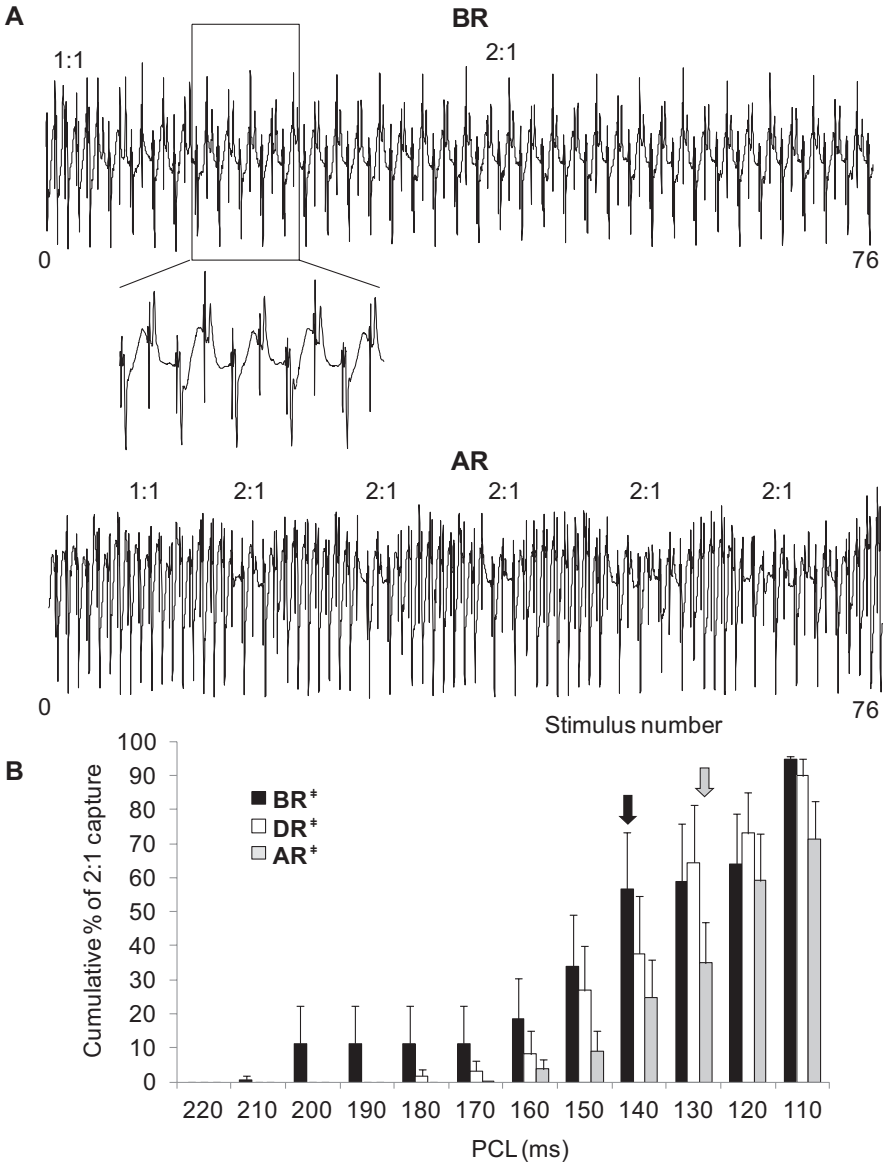

Figure 3. *Remodeling promotes atrial capture during rapid pacing. Panel A: illustrative RA unipolar EGMs at PCL 150 ms with intermittent 1:1 and 2:1 atrial capture of variable duration. The inset shows a magnified sequence of 2:1 capture before remodeling (BR). Panel B: cumulative percentage of 2:1 capture (mean ± SE) as a function of PCL during the 400-beat pacing protocols before (BR), during (DR), and after (AR) remodeling. Arrows indicate the PCL thresholds for 2:1 capture before (BR, black) and after (AR, grey) remodeling. ‡ $P < 0.001$ for trend.*

DI and short ARI preceded by short DI. At PCL 180 ms, however, ARI alternans became less visible and DI alternans disappeared, while magnitude of ΔTa alternans increased. Panel B shows summary data (n = 7, mean ± SE) of mean Re-ALT as a function of PCL for the 3 remodeling stages. Re-ALT could not be measured in one sheep as the T_a could not be reliably identified. Note the significant ($P < 0.001$, ANOVA) increase in mean Re-ALT magnitude with shorter PCL of similar magnitude across all remodeling stages (P = ns between stages, MANOVA). The PCL threshold at which Re-ALT significantly differed from background noise was found to be at 300 ms before (BR), and 260 ms during (DR) and after (AR) remodeling.

Broadening of AF Susceptibility with Remodeling

Overall, 18 nonsustained AF episodes were observed in 4 out of 8 sheep during the 400-beat EP protocols. The number of AF episodes increased from before (BR, n = 3) to during (DR, n = 6) to after (AR, n = 9) as shown in Table 1. Panel A of Figure 5 shows RA EGMs at PCL 150 ms before (BR, top) and after (AR, bottom) remodeling in a representative sheep. Before remodeling, 1:1 atrial capture was present over the 400-beat protocol. After remodeling, after 4 weeks of intermittent AT, an episode of nonsustained AF was observed, preceded by 1:1 capture and both Re-ALT (alternating red and blue arrows) and depolarization wave alternans (thin red arrows) as well. Panel B shows summary data (n = 8 sheep) of cumulative percentage of AF (mean ± SE) over the 400-beat protocols as a function of PCL for the 3 remodeling stages. Note that apart from an outlier at PCL 200 ms before remodeling (BR, black columns), the PCL range during which AF surged was broader after (AR, min-max PCL 60 ms) compared to before (BR, min-max PCL 40 ms) and during (DR, min-max PCL 30 ms) remodeling. Note the high AF burden (∼100%) for the 2 sheep that were not in 2:1 capture at PCL 100 ms after remodeling (AR, grey column). In summary, weeks of intermittent AT broadened the PCL range that triggered AF and increased the number of AF episodes.

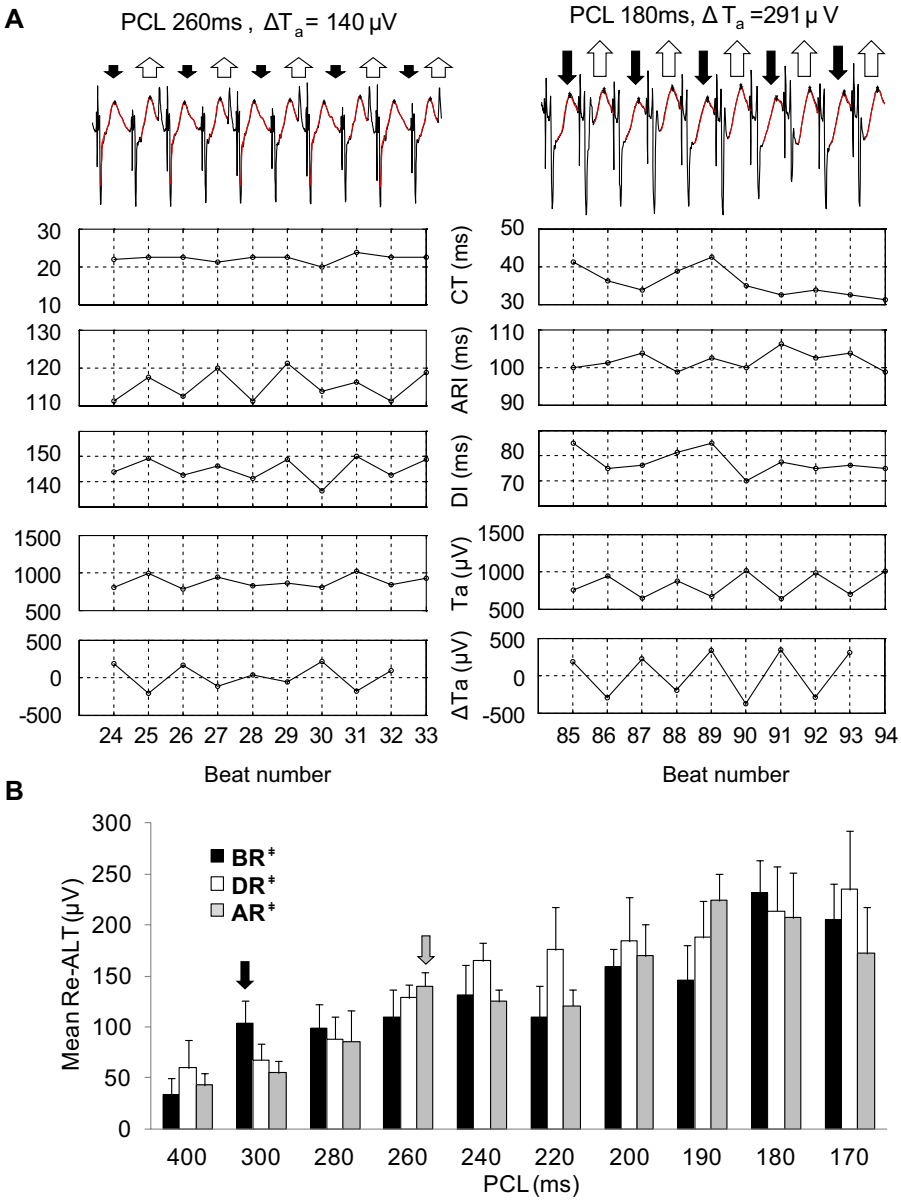

Figure 4. *Rate dependence of Re-ALT. Panel A: from top to bottom RA unipolar EGMs at PCL 260 ms (left) and 180 ms (right) and time series of CT, ARI, DI, T_a, and ΔT_a in a representative sheep before remodeling. T_a alternans is highlighted by alternating black and white arrows. Panel B: summary data ($n = 7$ sheep, mean ± SE) of Re-ALT (ΔT_a) as a function of PCL before (BR), during (DR), and after (AR) remodeling. Arrows indicate the PCL thresholds for Re-ALT before (BR, black) and after (AR, grey) remodeling. ‡$P < 0.001$ for trend.*

Kinetics of Re-ALT and CT Before AF

Table 1 reports the distribution of patterns for the 17 AF episodes that were analyzable during rapid pacing. One episode was excluded because of poor EGM quality. Among the 17 remaining episodes, AF was preceded by Re-ALT in 76% of cases, by complex T_a oscillations in 12%, by a gradual CT prolongation (CT+) in 53%, and occurred when 1:1 capture resumed in another 12% for which analysis of repolarization could not be performed. Panel C of Figure 5 shows Re-ALT (mean value 231 ± 118 μV) and a gradual CT prolongation (from 49 ms to 90 ms) at PCL 200 ms but no depolarization wave alternans before AF surged. Panel D of Figure 5 reports the summary data (n = 4 sheep, mean ± SE) of mean Re-ALT (left) and CT (right) values measured over the last 9 beats before AF. The magnitude of Re-ALT before AF was higher after (AR, 565 ± 52 μV, 8 episodes) compared to before (BR, 202 ± 41 μV, 2 episodes) and during (DR, 245 ± 33 μV, 5 episodes) remodeling, and 3 times higher than the mean value at the shortest PCL shown in Figure 4B (172 μV at PCL 170 ms). Likewise, the mean CT value after remodeling (AR, 91 ± 6 ms) was twice that before (BR, 43 ± 6 μV) and during (DR, 37 ± 2 μV) remodeling, and that at the shortest PCL shown in Figure 2C (42 ms at PCL 150 ms). CV estimated from interelectrode distance averaged 64 ± 28 cm/sec before (BR) and further dropped to 46 ± 24 cm/s after (AR) remodeling. On top of each column are reported the PCLs [mean ± SD] at which AF occurred. Note the shorter value after (AR, 121 ± 19 ms) compared to before (BR, 165 ± 49 ms) and during (DR, 146 ± 5 ms). The limited numbers of episodes precluded a full statistical analysis.

Discussion

This study reports in an ovine model that intermittent RA AT mimicking focal triggers for AF decreased ERP and

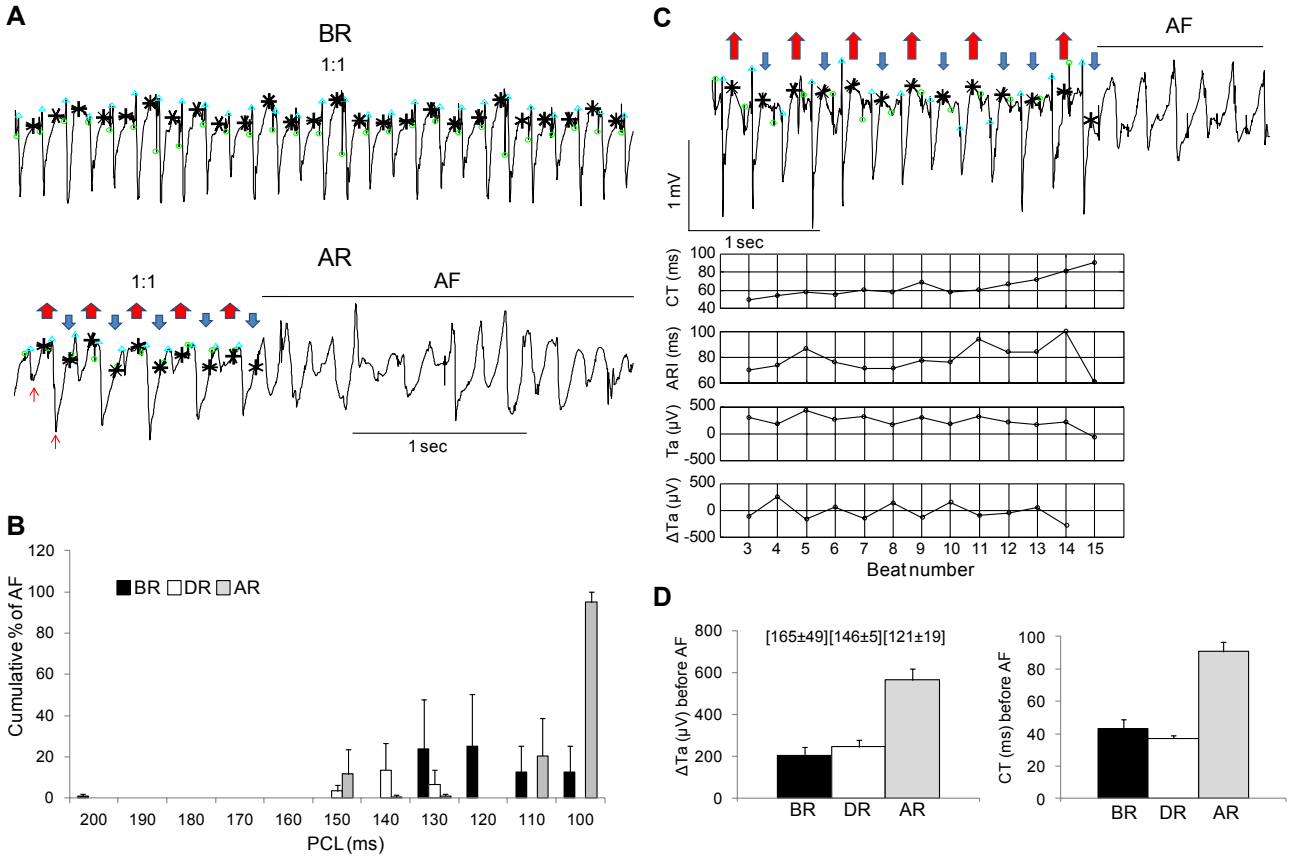

Figure 5. *Broadening of AF susceptibility with remodeling. Panel A: RA unipolar EGMs at PCL 150 ms before (BR, top) and after (AR, bottom) in a representative sheep. Before remodeling, rapid pacing resulted in 1:1 atrial capture with some complex fluctuations of T_a. After remodeling, rapid pacing triggered an episode of nonsustained AF preceded by alternans of depolarization (thin red arrows) and repolarization (black dots, thick red and blue arrows) waves. Panel B: summary data (n = 8 sheep, mean ± SE) of the cumulative percentage of pacing time in AF during the 400-beat pacing protocol as a function of PCL before (BR), during (DR), and after (AR) remodeling. Panel C: from top to bottom RA unipolar EGM and corresponding CT, ARI, T_a, and ΔT_a time series before an episode of pacing-induced AF (black line) at PCL 200 ms. Panel D: summary data (n = 4 sheep, mean ± SE) of ΔT_a (left) and CT (right) values for the last 9 beats preceding AF before (BR, black columns, n = 2 episodes), during (DR, white columns, 5 episodes) and after (AR, grey columns, 8 episodes) remodeling. The PCLs [mean ± SD] at which AF episodes occurred are reported on top of each column.*

ARI, and blunted the ARI rate dependence, which, in turn, facilitated atrial capture at high rates and increased the susceptibility to AF. Despite blunted ARI rate adaptation, RA Re-ALT was maintained during remodeling, and further increased in amplitude just before rapid-pacing induced AF. These data support the hypothesis that intermittent *in vivo* AT causes a pro-fibrillatory preconditioning via Re-ALT and conduction slowing at rapid rates, which creates a milieu for the emergence of wavebreaks, reentry and AF.

Intermittent Right Atrial Tachycardia Shortens ARI and Promotes Atrial Capture at Rapid Rates

These data provide a mechanistic explanation for the widespread clinical observations that repetitive paroxysms of AF have a cumulative effect on electrical remodeling that facilitate transitions to persistent AF. On one hand, cumulative tachycardia may be expected to cause remodeling while, on the other, periods of sinus rhythm may be expected to facilitate the opposing effects of reverse remodeling.

Our study uses ARI as validated surrogates for APD to clarify how intermittent clinical AT may facilitate AF.[14] ARIs correlate with local APD or ERP, and can track APD changes.[3,14,21-23] Weeks of intermittent AT in our model shortened ARI across a wide range of PCLs and blunted its rate adaptation. The "dose-response" relationship of intermittent AT on remodeling showed that ARI alterations were similar between 3 and 7 weeks of intermittent AT, which agrees with the rapid time course of electrical remodeling from burst pacing.[1-4,6]

Notably, shortening of ARI and ERP by intermittent AT facilitated atrial capture at high rates (Fig. 3) that may accelerate remodeling. Although this finding is commonly observed in early electrical remodeling, some clinical and experimental conditions show varying effects of pro-fibrillatory interventions on atrial ERP. For instance, lone AF,[24] hypercapnia,[25] and aging[26] in humans are associated with prolonged ERP, AF models based on the heart failure phenotype show preserved ERP,[9] while obesity shortened ERP.[27] The interplay of each of these clinical variables could affect vulnerability to and sustainability of AF. Recently, Echebarria *et al.* explored the role played by traveling discordant alternans in the onset of 2:1 conduction block in numerical simulations.[28] Whether traveling discordant alternans is mechanistically involved in the transition from 1:1 to 2:1 capture in our sheep model remains undetermined because of our fixed recording site. However, the lack of far field potentials and similar observations of 2:1 capture in humans

TABLE 1
Repolarization and CT Dynamics Preceding Pacing-Induced AF

	Complex N (%)	Re-ALT N (%)	CT+ N (%)	CT- N (%)	2:1 to 1:1 to AF N (%)
BR	1	1	2	0	1
DR	1	4	3	2	1
AR	0	8	4	4	0
Total	2 (12)	13 (76)	9 (53)	6 (35)	2 (12)

AR = after remodeling; BR = before remodeling; Complex = complex fluctuations of T_a magnitude; CT+ = prolongation of conduction time; CT- = lack of change in conduction time; DR = during remodeling; N = number of episodes; Re-ALT = repolarization alternans.

using monophasic APD recordings[18] suggest capture failure rather than conduction block as the underlying mechanism.

Intermittent Atrial Tachycardia Promotes Conduction Slowing at Rapid Rates

Clinically, multiple conditions affect electrophysiology beyond atrial repolarization, and likely interact in patients with AF in a complex manner. Aging, obesity, and sleep apnea all prolong RA and LA CT,[25-27] and prolongation of P-wave duration (PWD) as a surrogate for bi-atrial CT is associated with an elevated risk of AF.[29] In lone AF patients, Stiles *et al*. showed dilation of RA and LA, prolongation of PWD and of bi-atrial CT, and fractionation of bi-atrial electrograms compared to controls.[24] In chronic AF patients, Yu *et al*. observed that while ERP normalized within 4 days following restoration of SR, there was a less marked normalization in PWD, RA and LA CT at long and short PCLs.[30]

Using a chronic and *in vivo* model of RA AT that models intermittent tachycardia "triggers" of AF, our study shows that weeks of intermittent AT prolonged apparent CT at long and short PCLs due to structural remodeling (atrial dilation) but did not slow atrial CV. These findings are concordant with observations in the goat model on an absence of atrial fibrosis and CV slowing, and unaltered connexin expression up to 16 weeks of burst pacing,[2,3] although Interspecies differences likely exist. Lin *et al*. recently reported increased expression of extracellular matrix proteins in a porcine model of burst pacing.[12] Moreover, AF models based on a heart failure phenotype consistently show CV slowing and atrial fibrosis, maintained ERP and ERP rate adaptation[8,10] and increased expression of extracellular matrix genes and proteins.[5,8,9] In our study, preserved CV after several weeks of intermittent AT may be explained by the modest and intermittent increase in heart rate during AT and by the lack of heart failure as illustrated by the preserved LVEF until sustained AF occurred.

Conduction also displays rate dependence[18,31,32] that changes with remodeling.[31] Persistent AF patients show broader CT restitution, such that less rate acceleration is required to induce conduction slowing than paroxysmal AF patients who show steeper CT restitution.[31,32] In contrast, control patients without clinical AF lacked CT restitution and AF susceptibility.

A strength of our study is that each sheep served as its own control, allowing us to define the effect of a high burden of AT on conduction dynamics. Before remodeling took place, CT restitution was flat and AF inducibility low, as in control subjects.[31] As atrial rates rose, CT prolonged slightly before intermittent capture prevented further conduction slowing. Weeks of intermittent AT converted flat CT and CV restitutions into rate-dependent kinetics. In our model, this nonlinear restitution after remodeling was enabled by shortening of ARI and ERP, both enabling high pacing rates that promoted conduction slowing, especially just prior to AF initiation. In summary, a high burden of AT caused electrical remodeling that promotes atrial conduction slowing at high rates.

Electrical Remodeling Promotes Re-ALT at Rapid Rates

Re-ALT reflects beat-to-beat alternation of APD, a dispersion of repolarization that promotes wavebreak, conduction block and re-entry.[16,33] Re-ALT has been involved in transitions from atrial flutter[17,33] and rapid pacing[14,18] (Fig. 5) to AF, and from SR to VT.[20] In a chronic ovine model, we recently reported that Re-ALT is measurable *in vivo* using the standard pacemaker technology employed in the present study, is rate dependent and independent of prolongation and alternans of CT[14] (Fig. 5). In the present study, atrial Re-ALT continued despite flattening of ARI kinetics by weeks of intermittent AT. This is in line with recent works showing a poor relationship between APD restitution and Re-ALT.[18,34,35] A unique finding of our study is that atrial remodeling postponed capture failure toward higher pacing rates (mean PCL 121 ms, i.e., 496 bpm) that allowed Re-ALT to reach very high magnitudes (565 μV) before AF (twice that measured at PCL 170 ms in Fig. 4). Based on our data, the CL of PVT reported in humans (175 $\pm$ 30 ms)[36] falls within a window of high Re-ALT.

Mechanisms of Increased Susceptibility to AF

We found 2 modes of AF induction during remodeling that caused sustained AF. First, in 88% of episodes, Re-ALT or complex oscillations of atrial repolarization occurred in tandem with slowed conduction that immediately preceded AF. Importantly, a progressive increase in the number of AF episodes was observed during remodeling that paralleled decreases in ARI/ERP and the presence of capture failure only at higher rates. Rapid atrial capture, in turn, further amplified Re-ALT and slowed conduction in the last beats preceding AF (Fig. 5C). Hence, the higher Re-ALT magnitude and CV slowing preceding AF after remodeling were an extension of rate-dependent properties. The second mechanism, in 12% of cases, was resumption of 1:1 capture during rapid pacing that may have led to conduction block and reentry, but that could not be explored further due to our single site recording. This rate dependence is similar to experimental and clinical AF triggered by premature beats.[1,32] These observed mechanisms may explain clinical and experimental induction of AF via premature beats[1,4,32] or rapid pacing.[37] In prior work, rapid pacing causes slowed conduction that enables unidirectional block of conduction,[19] abrupt changes in propagation vector[31] and reentry,[37,38] paralleled by a gradual increase in Re-ALT before AF,[14,18,19] suggesting interaction of Re-ALT and slow conduction to facilitate wavebreak. In a modeling study, premature beats triggered AF by creating transient discordant Re-ALT leading to conduction block and reentry.[33] The interaction of Re-ALT and conduction slowing in patients at PCLs similar to that of PVT (175 $\pm$ 30 ms)[36] may create a dynamic milieu for wavebreaks, in line with modeling[33] and experimental data from ventricular[16,39] and atrial[19] mapping studies. Recently, interventions restoring conduction within the atria[40] or dampening Re-ALT[35,41]

proved to be of potential benefit. Overexpression of Cx43 and Cx40 within the atria shortened PWD, restored conduction and decreased AF susceptibility in a rapid pacing AF model,[40] while overexpression of SERCA2A[41] and modulation of DIs by pacing reduced the magnitude of Re-ALT[35] and exerted antifibrillatory properties.[41]

Study Limitations

Our study has some limitations that deserve comment. First, uncertainties remain as to whether our observations apply to the LA, as we inserted pacemaker leads only into the RA. However, similar APD and Re-ALT dynamics have been reported between RA and LA in patients with paroxysmal and persistent AF.[18,31,32] Second, our study analyzes a single atrial site, that limits conclusions on spatial dispersion of repolarization, conduction block and spatially discordant (i.e., arrhythmogenic) Re-ALT. Third, the analysis of unipolar EGMs does not provide direct mechanistic insights into cellular and subcellular mechanisms of increased susceptibility of AF, and future studies should analyze the relationship between T_a alternans and conduction slowing with local depolarization and repolarization properties using optical mapping. Fourth, it is possible that the trend toward shorter ERP after (AR) compared to before (BR) remodeling only takes place at the stimulation site. Stimulation and recording electrodes could not be switched as a limitation of pacemaker design. However, ARIs at the recording electrode were shorter at multiple PCLs before (BR) versus after (AR) remodeling, suggesting that the trend toward shorter ERP extended beyond the stimulation site.

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Supporting Information

Additional Supporting information may be found in the online version of this article at the publisher's website:

Figure S2. Summary data (n = 8 sheep, mean ± SE) of activation recovery interval (ARI) as a function of diastolic interval (DI) before (BR) and after (AR) remodeling. A natural logarithm function is used to fit the data.