Kinetics of Atrial Repolarization Alternans in a Free-Behaving Ovine Model

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

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

Kinetics of Atrial Repolarization Alternans. ***Introduction:*** **Repolarization alternans (Re-ALT), a beat-to-beat alternation in action potential repolarization, promotes dispersion of repolarization, wavebreaks, and reentry. Recently, Re-ALT has been shown to play an important role in the transition from rapid pacing to atrial fibrillation (AF) in humans. The detailed kinetics of atrial Re-ALT, however, has not been reported so far. We developed a chronic free-behaving ovine pacing model to study the kinetics of atrial Re-ALT as a function of pacing rate.**

Methods: **Thirteen sheep were chronically implanted with 2 pacemakers for the recording of broadband right atrial unipolar electrograms and delivery of rapid pacing protocols. Beat-to-beat differences in the atrial T-wave apex amplitude as a measure of Re-ALT and activation time were analyzed at incremental pacing rates until the effective refractory period (ERP) defined as stable 2:1 capture.**

Results: **Atrial Re-ALT appeared intermittently but without periodicity, and increased in amplitude as a function of pacing rate until ERP. Intermittent 2:1 atrial capture was observed at pacing cycle lengths 40 ms above ERP, and increased in duration as a function of pacing rate. Episodes of rapid pacing-induced AF were rare, and were preceded by Re-ALT or complex oscillations of atrial repolarization, but without intermittent capture.**

Conclusion: **We show in vivo that atrial Re-ALT developed and increased in magnitude with rate until stable 2:1 capture. In rare instances where capture failure did not occur, Re-ALT and complex oscillations of repolarization surged and preceded AF initiation.** *(J Cardiovasc Electrophysiol, Vol. 23, pp. 1003-1012, September 2012)*

Atrial fibrillation, excitability, reentry, repolarization alternans

Introduction

Atrial fibrillation (AF) initiates when triggers such as pulmonary vein (PV) tachycardias interact with substrates.[1,2] The exact nature of the electrophysiological substrates favoring transition from PV tachycardia to persistent AF remains unclear. Repolarization alternans (Re-ALT), a beat-to-beat alternation in action potential duration (APD) or amplitude, has been identified as a mechanism by which dispersion of repolarization is promoted, leading to wavebreaks and reentry,[3–6] and has been implicated in transitions to AF from pacing,[7–9] following atrial flutter[5] or myocardial infarction.[7] Recently, Re-ALT and complex APD oscillations have been shown to play an important role in the transition from rapid pacing to AF.[10] Atrial Re-ALT required progressively faster rates for patients with persistent AF, patients with paroxysmal AF, and controls. However, the detailed kinetics of atrial Re-ALT has not been reported so far. We developed a chronic free-behaving ovine pacing model to study in vivo the kinetics of atrial Re-ALT as a function of pacing rate.

Methods

Animal Experimental Setup and Data Acquisition

Two pacemakers, each with a single lead screwed into the right atrium (RA), were implanted in 13 male sheep (74 ± 18 kg) under general anesthesia (2 mg/kg i.v. propofol, 1–5% isoflurane):[11] a Vitatron™ T70 model was used for the recording of atrial unipolar electrogram (EGM) because of its unique broadband signal characteristics (sampling frequency

This work was supported by the Swiss National Fund for Scientific Research (grant numbers 325200-112668, 320030-127229) to Dr. Pruvot, the CardioMet Pôle, Medtronic™ Switzerland and Vitatron™. Dr. Narayan was supported by a grant from the NIH (grant number HL83359).

Dr. Pruvot reports honoraria from Medtronic. Dr. Narayan reports Fellow support from Medtronic, Boston Scientific, St. Jude Medical, and Biotronik, and honoraria from Medtronic, St. Jude Medical, and Biotronik. Dr. Narayan is co-inventor of intellectual property owned by the University of California Regents and licensed to Topera Inc. However, Topera does not fund any research. Dr. Fromer reports honoraria from Medtronic and St. Jude Medical. Drs. Ruchat and Tenkorang report research support in the form of outdated pacing leads and pacemakers from Medtronic and Vitatron. Other authors: No disclosures.

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

Manuscript received 30 December 2010; Revised manuscript received 7 March 2012; Accepted for publication 12 March 2012.

doi: 10.1111/j.1540-8167.2012.02336.x

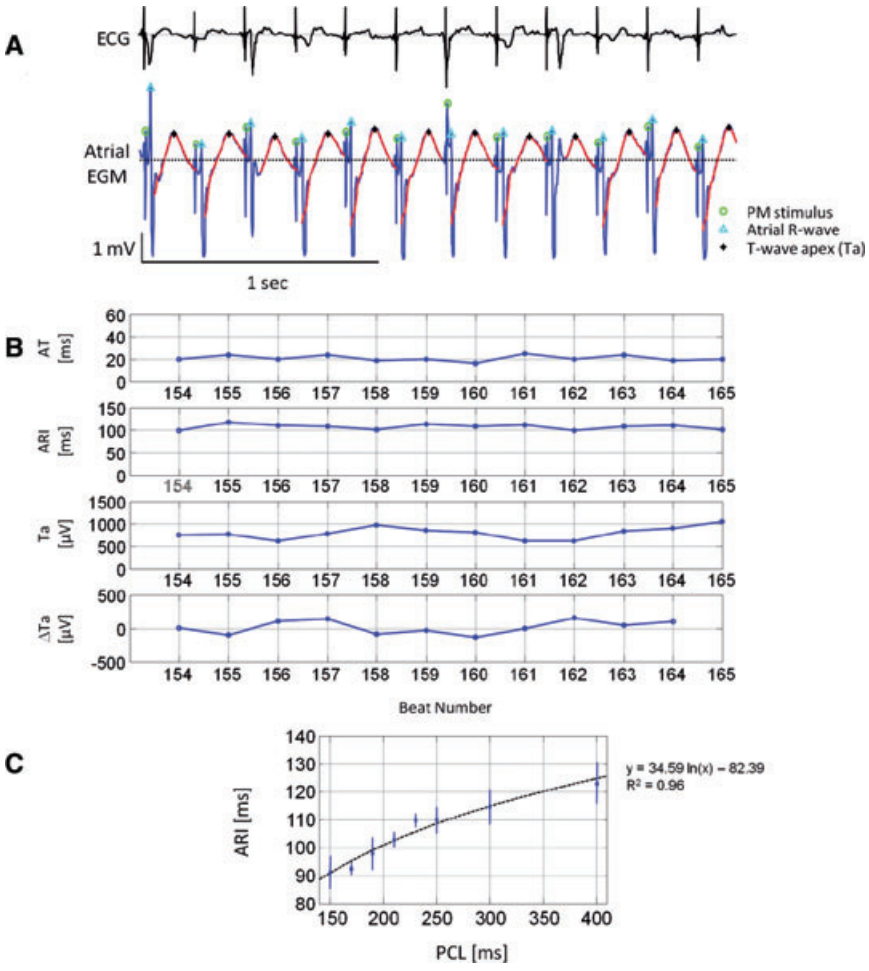

Figure 1. *Ovine atrial unipolar EGM and time series. A representative example of subcutaneous bipolar ECG (top) and corresponding unipolar electrograms (EGM, bottom) at a pacing cycle length (PCL) of 210 ms is shown in panel A. Green circles denote pacemaker (PM) stimuli, cyan triangles the first atrial depolarization wave (R-wave), and black stars atrial T-wave apexes (T_a). Repolarization waves are highlighted by red bold curves. Panel B shows from top to bottom time series of AT, activation recovery interval (ARI), T_a and beat-to-beat difference in T_a (ΔT_a) from the EGM shown above. Panel C illustrates the nonlinear (i.e., logarithmic fit) relationship between ARI (mean ± SD) and PCL determined in a subset of 8 sheep.*

800 Hz, 0.4 Hz high pass filter), and a Vitatron™ Prevent AF pacemaker to deliver customized electrophysiological pacing protocols because of its reprogramming capacity. Atrial EGM and subcutaneous ECG were recorded with a wireless Holter device (TMSi™, B_Holter). At many pacing cycle lengths (PCLs), 2:1 AV conduction prevented the reliable measurement of Re-ALT as the far-field ventricular depolarization impinged on the preceding atrial repolarization. In order to prevent 2:1 AV ratios or other far-field ventricular interferences, the AV junction was ablated and a right ventricular lead was implanted to maintain AV synchrony in a subset of sheep (n = 4, 63 ± 6 kg).[11] Recordings were acquired at least 1 month following device and leads implantation after light sedation (xylazine 0.2 mg/kg). Experiments were carried out in accordance with the European convention for the protection of vertebrate animals used for scientific purposes.

Pacing Protocols

Atrial Re-ALT thresholds, kinetics of the Re-ALT amplitude and activation time were determined as a function of PCL. The pacing protocols consisted of atrial pacing for 400 beats starting at PCL 400 ms with 10 ms decrement until stable 2:1 capture (i.e., approximating the effective refractory period, ERP). The amplitude of stimulation (2.3 ± 0.6 V, 0.5 ms) was at least twice diastolic threshold and remained constant over the pacing protocols.

Signal Analysis

ECG baseline wandering was suppressed with a Butterworth high-pass filter with a 1.5 Hz cut-off frequency. A wavelet denoising method was applied on both EGM and ECG signals and ventricular activity was cancelled in sheep with AV block to improve Re-ALT quantification. Pacemaker stimuli, atrial depolarization and repolarization waves, and T-wave apex (T_a) were then identified on the EGM (Fig. 1, panel A). The filtering, ventricular cancellation, identification and T_a error in time, and amplitude are detailed in the Supporting Information. Re-ALT sequences were determined from time series of beat-to-beat differences of atrial T_a, similarly to the method developed by Swerdlow *et al*. for unipolar EGMs provided by ICD.[12] Re-ALT amplitude, when present, was also measured and averaged for the last 9 beats preceding episodes of rapid pacing-induced AF. Because complex oscillations of atrial repolarization have also been reported preceding arrhythmia onset,[10] we measured the mean beat-to-beat difference in T_a for the last 9 beats before induced AF; oscillations of atrial T_a were considered complex when beat-to-beat T_a varied in a nonalternating pattern by >50 μV, which has been measured as the background level of T_a variations in our model (see Fig. 2, panel C). Activation time (AT, panel B of Fig. 1) of unipolar EGM was defined as the time interval between the pacemaker stimulus and the maximum of the atrial depolarization wave (R_a), and alternans of AT (AT-ALT) as beat-to-beat variation in AT $>$ 1.25 ms (i.e., time interval between 2 samples). Diastolic intervals (DIs) were measured as

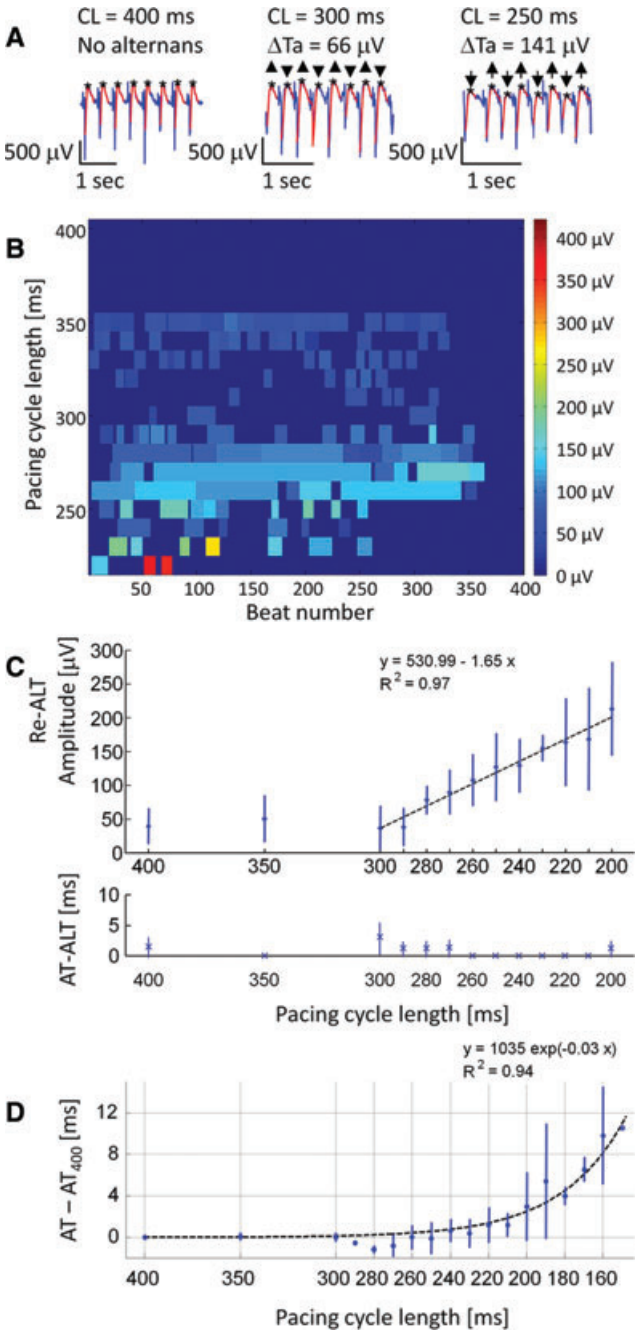

Figure 2. *Rate dependence of Re-ALT. Panel A shows examples of atrial EGM at decreasing PCLs. Panel B displays the Re-ALT magnitude over a 400-beat pacing protocol. Significant Re-ALT sequences are displayed as rectangles of variable duration (number of beats, x-axis) and amplitude (0–400 μV, colorscale) at decreasing PCLs (400 to 220 ms, y-axis). Panel C reports (n = 4 sheep with AV block) mean ± SD of the Re-ALT amplitude (top) and activation time alternans (AT-ALT, bottom), and panel D mean ± SD of AT prolongation (defined as the difference from baseline value [AT-AT 400 ms]) as a function of PCL.*

the time interval elapsed from the preceding T_a to the following R_a. Activation recovery intervals (ARIs) were measured from R_a to the following T_a because at fast pacing rates the end of the atrial repolarization wave could not be reliably determined as it was impinged by the following depolarization. Although ARIs are moderately correlated with local APD or refractory periods,[13-15] they are able to track adequately APD changes.[15] To support the relationship of ARIs to APDs in our model, the rate dependence of ARI was evaluated in a subset of 8 sheep. Panel C of Figure 1 shows that ARI decreased significantly ($P < 0.05$) as a function of PCL from 123 ± 7 ms at 400 ms PCL to 91 ± 16 ms at 150 ms PCL. Panel B of Figure 1 also illustrates representative examples of time series of AT, ARI, T_a amplitude, and its beat-to-beat differences (ΔT_a) at PCL 210 ms from the EGM shown in panel A. Although subtle variations in ΔT_a were observed, they did not fulfill criteria for Re-ALT (see the Statistical Analysis section).

Statistical Analysis

Statistical tests were performed using Matlab version 7.9 (Mathworks Inc., Natick, MA, USA). Time series of T_a were used to determine Re-ALT sequences, which were considered significant when the 2 following conditions were fulfilled: (1) T_a was alternating for ≥ 5 consecutive beats; (2) even and odd T_a distributions were statistically different based on a Student *t*-test ($P < 0.01$, 2 sided). The same criteria were applied to AT alternans (AT-ALT), which was considered significant if >1.25 ms. Multiple one-way ANOVA was used to test differences in ARI, Re-ALT amplitude, AT and AT-ALT as a function of PCL. Fisher's g-statistic was used to assess whether Re-ALT sequences appeared in a periodic manner.[16] Fisher's exact test was used to analyze the 2×2 contingency table. A $P < 0.05$ was considered significant.

Results

Kinetics of Atrial Repolarization Alternans

Atrial Re-ALT was detected in all sheep (with and without AV block), and its amplitude increased as a function of pacing rate. Panel A of Figure 2 illustrates representative examples of atrial unipolar EGMs at decreasing PCLs. Smoothed repolarization waves are shown in red and T_a by black stars. Re-ALT was absent at 400 ms PCL, but appeared and increased in magnitude at shorter PCLs. The decrease of PCL to 300 ms and 250 ms was associated with progressive increase in Re-ALT amplitude from a mean peak-to-peak difference (ΔT_a) of 66 μV at 300 ms to 141 μV at 250 ms PCL. Panel B shows a color map of amplitude and beat locations (*x*-axis, from 1 to 400 beats) of Re-ALT for an entire stimulation protocol (*y*-axis, range 400–220 ms PCL) in an AV block sheep with ventricular pacing at 40 bpm (1,500 ms) to dissociate far-field ventricular activity from atrial EGMs. Subtle Re-ALT was observed at long PCLs (400–300 ms, dark blue). For PCLs ≤ 290 ms, a progressive increase in duration and amplitude (light blue) of Re-ALT was noticed. Importantly, the Re-ALT amplitude was much higher (red) at short PCL (220 ms) before the first instance of 2:1 capture (dark blue following the red marks at PCL 220 ms). Panels C and D show summary data of the kinetics (mean ± SD) of Re-ALT, AT-ALT, and AT prolongation at decremental PCLs (*x*-axis) in sheep with AV block (n = 4) during right ventricular pacing at 40 bpm. Notably, the Re-ALT amplitude increased linearly ($R^2 = 0.971$, $P < 0.001$) from PCL 290 ms and became significant from the background level (40 ± 25 μV at PCL 400 ms) at PCLs ≤ 230 ms ($P < 0.05$), which was defined as the atrial Re-ALT threshold. We further evaluated the presence and amplitude of Re-ALT induced by realistic noise on simulated EGMs. Noise-induced Re-ALT (5.2 ±

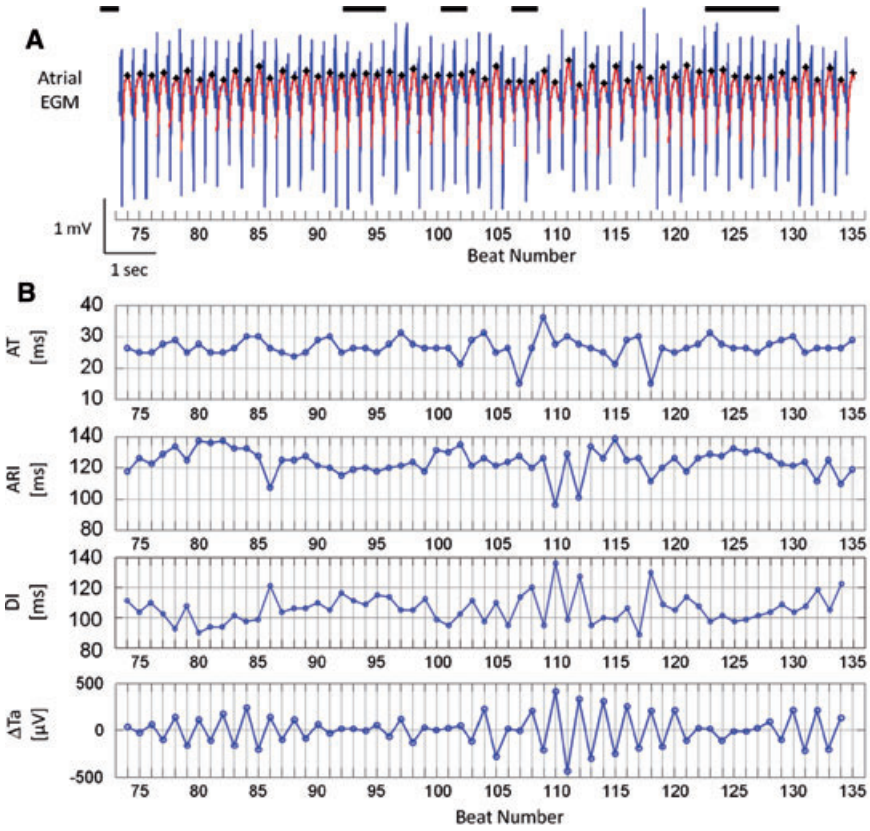

Figure 3. *Re-ALT is intermittent but not periodic. Panel A shows an EGM with intermittent Re-ALT at PCL 230 ms. T_a are marked by black stars and nonalternating periods by black lines. Panel B depicts the corresponding time series of AT, ARI, diastolic intervals (DI), and beat-to-beat differences in T_a (ΔT_a).*

0.5 μV) was present but appeared much smaller than the amplitude of experimentally measured Re-ALT (i.e., 155 $\pm$ 19.5 μV at PCL 230 ms) as detailed in the Supporting Information. Panel D of Figure 2 shows the kinetics of AT minus its mean value measured at PCL 400 ms (AT_{400}) as a function of PCL. AT remained stable until PCL 210 ms increased exponentially, and became significantly different from the baseline value at PCLs $\leq$190 ms ($P < 0.05$). Then, the potential contribution of alternans of AT to Re-ALT was evaluated. Panel C shows that AT-ALT was absent over the entire pacing protocol. In summary, both the occurrence and amplitude of atrial Re-ALT, and the kinetics of AT were rate dependent, but alternans of AT was not observed.

Atrial Re-Alt Was Intermittent Despite Continued Pacing

The color map (panel B) of Figure 2 shows that once atrial Re-ALT had developed, it was not steady during the time course of the pacing protocol. The atrial Re-ALT amplitude fluctuated over time but without periodic pattern (Fisher's g-statistic, P = ns). Panel A of Figure 3 shows a representative example of atrial unipolar EGM taken between beats 74 and 135 of a 400-beat protocol at PCL 230 ms. T_a are emphasized by black stars. Sequences without significant Re-ALT are highlighted by black lines of variable duration at the top of the recording. Re-ALT appeared intermittently and in a nonperiodic pattern with rises and declines of the magnitude separated by periods of no Re-ALT of variable duration as well. Panel B of Figure 3 shows the corresponding time series of AT, ARI, DI, and Re-ALT (ΔT_a). Notably, depolarization wave amplitudes in panel A and AT in panel B did not show any beat-to-beat alternation, suggesting that the mechanisms underlying atrial Re-ALT at intermediate PCLs primarily involve repolarization rather than depolarization. In contrast, ΔT_a clearly exhibited periods of Re-ALT of the variable magnitude and duration. At the maximum amplitude of ΔT_a alternans (beat number 103 to 119 and 131 to 135), ARI and DI also showed significant beat-to-beat alternation, but of shorter length compared to ΔT_a. ARIs alternating at maximal ΔT_a alternans showed a concordant relationship with DIs: short ARIs (e.g., beats 110 and 112) were preceded by short DIs (e.g., beats 109 and 111), and long ARIs (e.g., beats 111 and 113) by long DIs (e.g., beats 110 and 112). The dependency of APD (and its surrogate: ARI) on the preceding DI,[4,6,10,17,18] termed the ARI-DI restitution, has been thought to be mechanistically involved in Re-ALT when the slope of the relationship is >1.[19] Figure 3, panel B, shows that the ARI-DI restitution does not drive ΔT_a alternans as ARIs and DIs alternated less frequently than ΔT_a did. To further examine whether the ARI-DI restitution may play a limited role in driving ARI alternans at maximal ΔT_a alternans, an ARI-DI restitution limited to beats 103–119 showing both ARI and DI alternans was drawn (data not shown). Although ARIs decreased as a function of DIs, the maximum restitution slope determined from a linear fit[10,18] for the shortest 40 ms DI segment (range: 89–111 ms) was <1 (0.6). Altogether, these observations suggest that atrial Re-ALT is not driven by the ARI-DI restitution.

Intermittent Atrial Capture

The pacing protocol was pursued until stable 2:1 atrial capture, which occurred at mean PCL 128 $\pm$ 19 ms (i.e., steady state ERP). Interestingly, we observed periods of intermittent 2:1 atrial capture intermingling with 1:1 capture as shown in Figure 4. The mean PCL at which the first periods of intermittent 2:1 atrial capture appeared was 154 $\pm$ 24 ms. Panel A of Figure 4 illustrates an example of intermittent

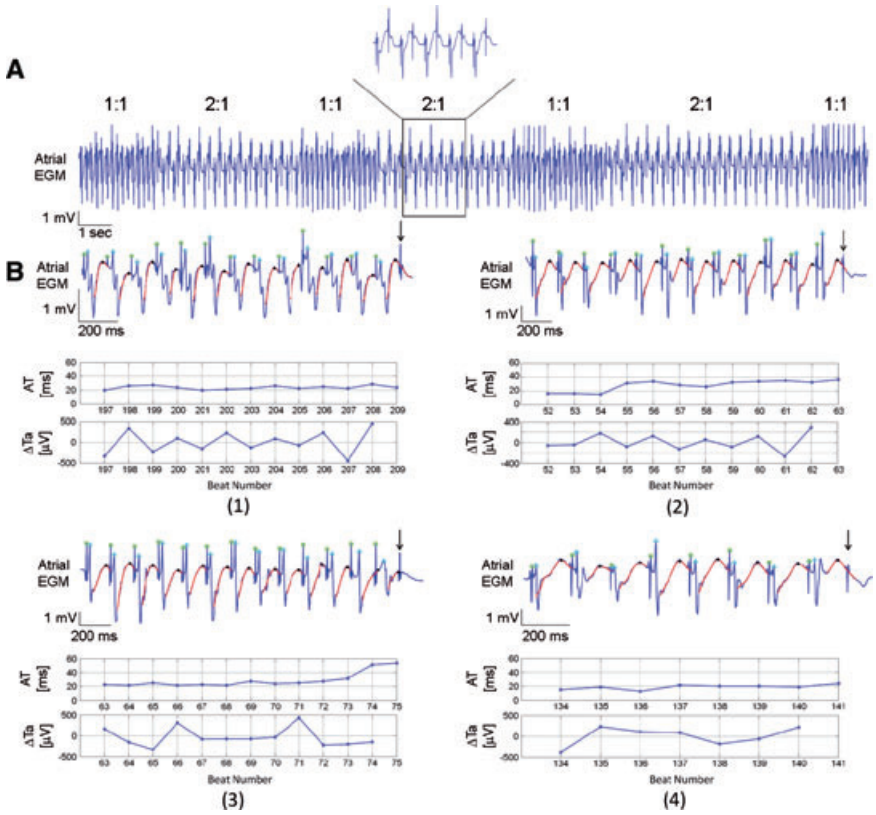

Figure 4. *Intermittency of atrial capture. An illustrative unipolar EGM with intermittent 1:1 and 2:1 atrial capture of variable duration at PCL 160 ms is shown in panel A. Panel B illustrates the 4 different patterns of EGM and corresponding AT, ARI, and ΔT_a time series until the first beat of 2:1 capture (arrow).*

TABLE 1

Re-ALT and AT Prolongation Before Capture Failure—Prevalence of the 4 Patterns Illustrated in Figure 4

	Presence of Re-ALT	Absence of Re-ALT	
Stable AT	40% (B1)	11% (B4)	51%
Increased AT	33% (B2)	16% (B3)	49%
	73%	37%	

AT = activation time; Re-ALT = repolarization alternans.

1:1 and 2:1 atrial capture. No clear periodicity was observed as periods of 2:1 capture varied in duration. Panel B shows representative examples of atrial unipolar EGMs and their corresponding AT and ΔT_a time series prior to 2:1 atrial capture. Four different patterns of AT and Re-ALT dynamics were observed among a total of 186 transitions from 1:1 to 2:1 capture. Atrial EGMs display 1:1 atrial capture until the first noncaptured beat (arrow). Panel B1 shows Re-ALT with a progressive increase in ΔT_a magnitude (maximum ΔT_a 450 μV) and no AT change preceding 2:1 capture. Panel B2 shows the second pattern characterized by a similar increase in Re-ALT (maximum ΔT_a 280 μV) but with a gradual increase in AT (from 26 ms to 36 ms). Panel B3 shows the third pattern characterized by the lack of any significant Re-ALT. AT, however, markedly increased 4 beats prior to capture failure (from 28 ms to 54 ms). Panel B4 shows the last pattern characterized by the absence of any Re-ALT and AT changes preceding 2:1 capture. Table 1 displays the prevalence of each of the 4 patterns of AT and ΔT_a changes among the 186 episodes. 40% of the episodes were of the type as shown in panel B1 and 33% in B2. Importantly, Re-ALT was observed in 73% and AT prolongation in 49% of episodes. Only 11% of the overall episodes exhibited neither changes in AT nor Re-ALT (panel B4). Using Fisher's exact test, no significant difference in Re-ALT prevalence ($P = 0.14$) was observed between sequences with and without AT prolongation, supporting the lack of relationship between Re-ALT occurrence and AT prolongation before capture failure. In the 49% of episodes showing AT prolongation before capture failure, the mean number of beats over which AT prolonged was 4.9 ± 2.5 (95% CI, 2–8).

Also, the faster the pacing rate, the longer the periods of intermittent 2:1 capture until steady state ERP. Panel A of Figure 5 shows (top tracing) the first episodes of 2:1 capture at PCL 200 ms. The middle tracing shows the same sheep at a shorter PCL (170 ms), with more frequent and longer periods of 2:1 capture. The bottom tracing shows stable 2:1 atrial capture at steady state ERP (PCL 160 ms). For all pacing protocols between the first beats of capture failure and steady state ERP, durations of 2:1 capture sequences were totaled, divided by the duration of the pacing protocol and expressed as the percentage of cumulative 2:1 atrial capture. Panel B of Figure 5 shows summary data based on 35 recordings in 7 sheep starting at PCL that was 40 ms above steady state ERP and decremented by steps of 10 ms. Importantly, the cumulative percentage of intermittent atrial capture increased as the PCL shortened. At ERP, cumulative 2:1 capture was 95% as 1:1 capture of a few beats' duration was observed at the initiation of the pacing protocol. In a subset of 4 sheep (20 recordings) whose ARIs were reliable enough, the last beat of 2:1 capture and the first beat of 1:1 capture were compared (panel C of Fig. 5). AT remained similar (from 34 ± 14 to 31 ± 12 ms, P = NS) but ARI decreased significantly (from 97 ± 18 to 89 ± 19 ms, $P < 0.05$) at resumption of 1:1 capture, which is consistent with a decrease in APD promoting the recovery of 1:1 capture. Also, note the transient Re-ALT at resumption of 1:1 capture.

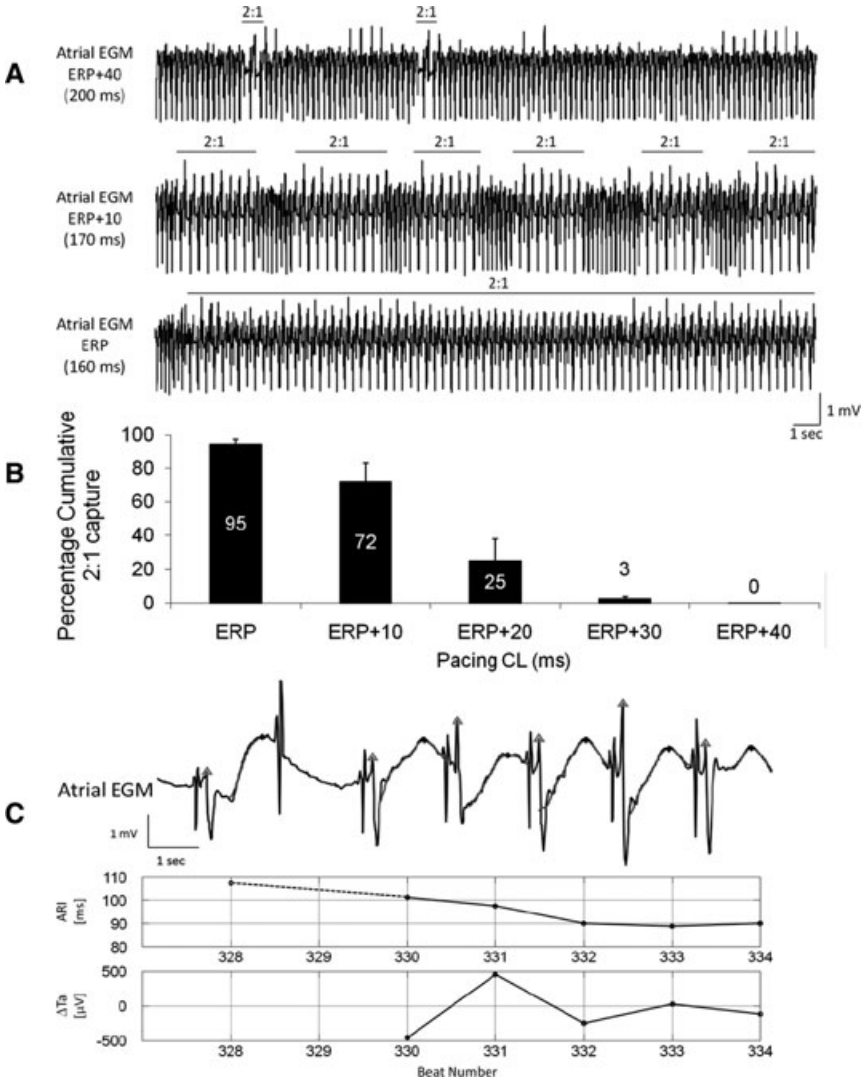

Figure 5. *Duration of 2:1 atrial capture is rate-dependent. Panel A displays representative examples of unipolar EGM at decreasing PCLs. Rare 2:1 atrial captures of short duration are seen at PCL 40 ms above effective refractory period (ERP, top). Middle tracing illustrates the increase in 2:1 capture duration 10 ms above ERP. Bottom tracing shows 1:1 atrial capture of short duration followed by stable 2:1 capture. Panel B shows mean ± SD of cumulative percentage of 2:1 atrial capture as a function of PCL in a subset of 7 sheep. In panel C are shown EGM (top), ARI (middle), and Re-ALT (ΔT_a, bottom) time series at resumption of 1:1 capture. ARI decreased before, and Re-ALT emerged at resumption of 1:1 capture.*

Atrial Fibrillation Episodes

Forty-four protocols totaling 758 bursts of 400 beats were performed starting at PCL 400 ms until steady state ERP, of which 12 (1.6%) triggered 20 episodes of nonsustained AF (nsAF) at mean PCL 150 ± 36 ms. Seventeen episodes occurred during 1:1 capture, and 3 following the transition from 2:1 to 1:1 capture. Eighty percent (16/20) of the AF episodes were preceded by Re-ALT (mean magnitude of the 8 last ΔT_a: 366 ± 285 μV) and 15% (3/20) by complex T_a oscillations (mean magnitude of the 8 last ΔT_a: 180 ± 5 μV). One episode was triggered by a single S1 at resumption of 1:1 capture at PCL 120 ms. Prolongation of AT was observed in 55% (11/20), absent in 40% (8/20) and not analyzable in one episode (5%). Note that the amplitude of Re-ALT (366 ± 285 μV) preceding AF was well above the maximal value (210 ± 70 μV) measured at PCL 200 ms (shown in Fig. 2C). Panel A of Figure 6 shows an illustrative example where Re-ALT (ΔT_a) emerged and AT prolonged at resumption of 1:1 capture, leading directly to AF. Note that the similar dynamics of Re-ALT and AT prolongation with episodes of 2:1 capture (see Fig. 4). Panel B of Figure 6 shows an example of complex T_a oscillations preceding the initiation of AF by rapid pacing. To further illustrate the observed atrial repolarization dynamics preceding nsAF, Poincaré plots of the beat-to-beat T_a difference (ΔTa_{n+1}) as a function of its preceding value (ΔTa_n) were built. ΔT_a alternans (Fig. 6A) tended to aggregate at 2 distinct locations on either side of the "zero" alternans line, while complex ΔT_a oscillations spanned the graph (Fig. 6B) before nsAF. In summary, both alternans and complex oscillations of atrial repolarization were commonly observed before rapid pacing-induced AF.

Discussion

This study reports the detailed kinetics of atrial Re-ALT during rapid atrial pacing. We show in vivo that Re-ALT developed and increased in magnitude with rate until stable 2:1 capture. In rare instances where 1:1 capture was maintained during rapid pacing, nsAF surged. Our work also shows the feasibility of recording atrial Re-ALT using available pacemaker technology to provide a surrogate of APD alternans.

Rate Dependence of Atrial Repolarization Alternans

Atrial Re-ALT has been observed using unipolar recordings,[20] MAP,[5,8,10,17,18] and optical mapping.[7,21] This study is the first to our knowledge to report the detailed kinetics of atrial Re-ALT and its interplay with capture failure based on unipolar EGMs using a method similar to the one

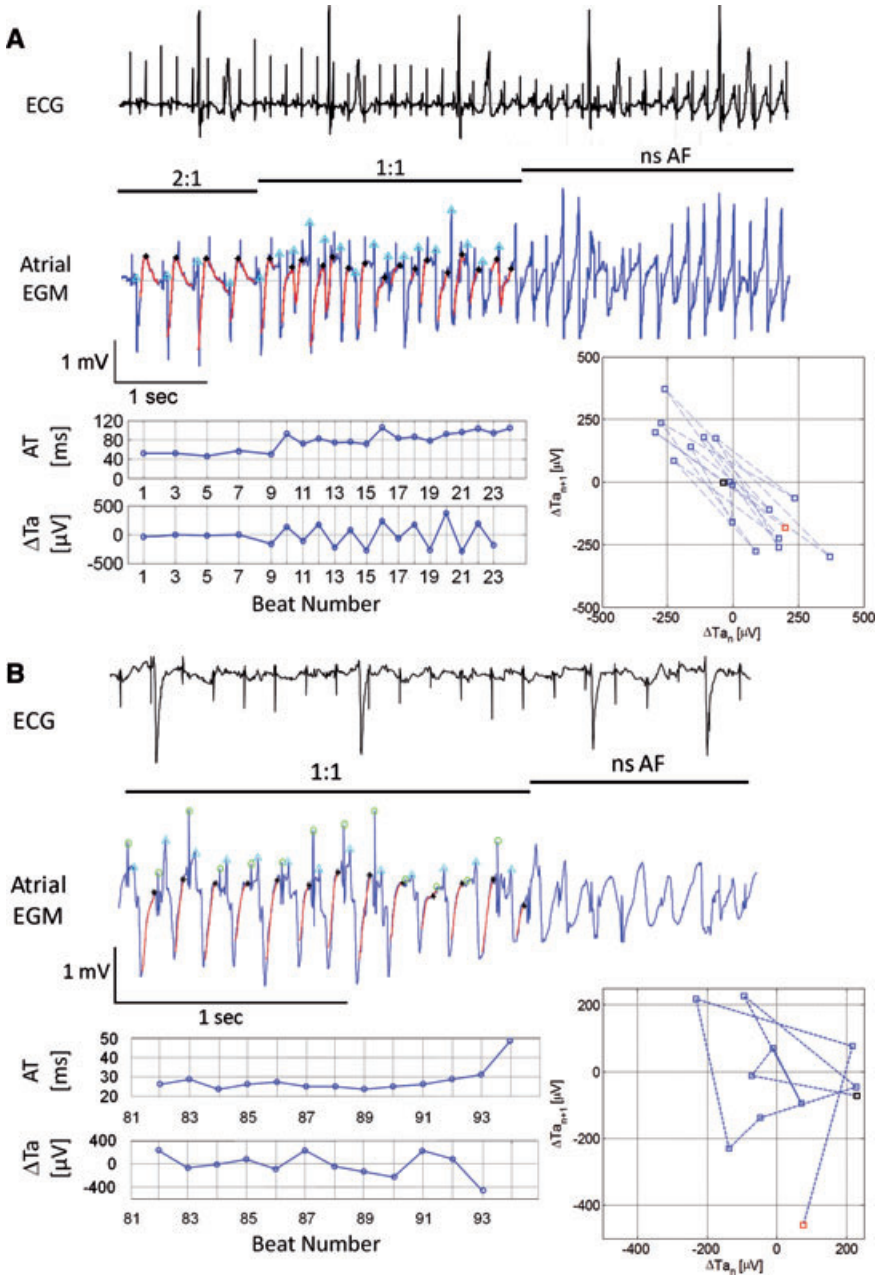

Figure 6. *Rapid pacing-induced AF. From top to bottom are shown ECG, atrial EGM, corresponding AT and ΔT_a time series, and Poincaré plots of ΔT_a during rapid pacing. Panel A shows an episode of nonsustained AF (nsAF) at resumption of 1:1 capture at PCL 180 ms. Note the gradual increase in atrial Re-ALT (ΔT_a) and AT prolongation, but the lack of capture failure before nsAF. Panel B shows an example of pacing-induced nsAF at PCL 130 ms preceded by complex T_a fluctuations. Note the large beat-to-beat difference in T_a (range ~200 μV) and AT prolongation without capture failure. The Poincaré plots show that ΔT_a alternans (upper graph) tends to aggregate at 2 distinct locations on either side of the "zero" alternans line, while complex ΔT_a oscillations span the graph (lower plot) before nsAF.*

developed by Swerdlow *et al*. in patients with ICD.[12] Atrial Re-ALT was rarely observed at long PCLs, but progressively increased in duration and linearly in amplitude starting at PCL 280 ms. The PCL at which Re-ALT amplitude became significantly different from the background level (230 ms) was similar to the value measured in control subjects (i.e., 218 ± 30 ms) referred for catheter ablation of supraventricular tachycardia and in cultures of atrial myocyte monolayer (250 ms),[21] supporting the adequacy of our model to study the kinetics of Re-ALT in vivo.[10] Importantly, right atrial Re-ALT arose at PCLs where AT was neither prolonged nor alternating, indicating that atrial Re-ALT, as for the ventricles,[3,10,22] primarily involves beat-to-beat alternation in the repolarization time course. This is further supported by Tsai *et al*., who recently reported Re-ALT in cultures of atrial myocytes during rapid field pacing.[21] Atrial Re-ALT arose from alternans of intracellular calcium, and was further promoted by atrial stretch-induced defective intracellular calcium reuptake. Also, no consistent relationship was found between ΔT_a alternans and ARI and DI alternans or the kinetics of the ARI-DI restitution. ΔT_a alternans was observed at times of no ARI and DI alternans. This is in line with recent clinical[10] and experimental studies[6,21] that found no relationship between APD-DI restitution kinetics and alternans, but a role of intracellular calcium cycling alternans in driving repolarization alternans.

Intermittency of Atrial Repolarization Alternans

Selvaraj *et al*. reported in heart failure patients spatially out-of-phase (i.e., discordant) intermittent ventricular Re-ALT using unipolar signals.[23] Mirinov *et al*. showed that intermittent Re-ALT is related to unstable nodal lines separating out-of-phase regions spanning the heart surface, which are mechanistically linked to slow APD accommodation following a change in PCL.[24] Based on atrial unipolar signals, we observed sequences of intermittent Re-ALT appearing in

a nonperiodic pattern. The rise and decline in the Re-ALT amplitude preceding nonalternating periods is suggestive of nodal lines spanning the RA, but cannot be proven as recordings were performed at a single site. Interestingly, atrial ARIs displayed alternation in duration only at the maximum amplitude of T_a alternans. Experimentally, atrial ARIs are able to track APD changes,[15] hence alternation of ARIs indicates APD alternans. The repolarization wave of bipolar and unipolar signals is the result of repolarization gradient of surrounding excitable tissue.[13] The peak of the T-wave is timed with the maximal voltage gradient between neighboring APs and the height of the T-wave with the magnitude of that maximal voltage gradient.[13] Alternation in T_a amplitude without ARI alternans suggests alternation in the steepness of the phases 2 and 3 of neighboring APs but not necessarily of the AP duration. This hypothesis is supported by the recent clinical observation that alternans of phase 2 of MAPs better correlates with T-wave alternans than alternans of the duration of phase 3 of the AP.[10] However, in general, both phases alternate together (see Fig. 1 in Ref. 25). Alternatively, some inaccuracy in the detection of the timing, but not of the amplitude of T_a, may have postponed the detection of ARI alternans. In summary, ovine right atrial repolarization kinetics share common features with human and rodent ventricles including the rate dependence and the intermittency of Re-ALT.

Potential Mechanisms of Intermittent Atrial Capture

Brooks *et al*. observed 40 years ago that atrial Re-ALT and intermittent capture occurred at similar rapid pacing rates.[20] Their observation that an increase in stimulus strength converted 2:1 into 1:1 atrial capture argued for decreased excitability as the underlying mechanism of intermittent capture. More recently, Watanabe *et al*. showed in canine RA using MAPs that the ERP measured during Re-ALT after a long APD is longer than that after a short APD.[8] Hence, atrial Re-ALT combined with AT prolongation may have facilitated the first noncaptured beat by delaying the ERP to a point where the next stimulus is refractory. This hypothesis is further supported by MAP recordings performed in humans suffering from AF as shown in Figure S1 of the Supporting Information. Panel A shows left atrial (LA) Re-ALT at slow pacing rate, and panels B (LA) and C (right atrial, RA) Re-ALT and capture failure preceded by AT prolongation that progressively postponed the repolarization until the stimulus fell within the ERP. This, in addition to the observation that intermittent capture in the sheep was seen in a range (i.e., 40 ms) of PCLs just above ERP, strongly suggests reduced excitability. However, other mechanisms must be involved in the maintenance of intermittent capture. The rate dependence of the duration of intermittent capture and the decrease in ARI preceding resumption of 1:1 capture are both suggestive of a time-dependent process. Further studies beyond the scope of our research are required to elucidate these mechanisms. Our results may also hint at a potential mechanism by which rapid atrial rhythms may fail to cause AF in certain patients by means of capture failure as recently reported during rapid pacing.[10]

Repolarization Alternans and Mechanism of AF

In the seminal work of Haissaguerre *et al*.,[1] PV tachycardias triggering AF had a mean CL of 175 ± 30 ms that fell within human (<218 ± 30 ms),[10] ovine (i.e., 230 ms to ERP) and atrial myocyte monolayer culture (250 ms to ERP)[21] PCL range during which atrial Re-ALT occurred. In this study, during the rare instances where 2:1 capture did not occur with rapid pacing, nsAF episodes were observed and preceded either by overt Re-ALT or by complex T_a oscillations, whereas AT prolonged or remained stable. Interestingly, complex APD oscillations have recently been reported to herald rapid pacing-induced AF in humans as well.[10] Taken together, these observations suggest that alternans and complex oscillations of atrial repolarization, but not AT prolongation, may be one of the mechanisms facilitating transition from focal tachycardia to AF by promoting dispersion of repolarization and wavebreaks as reported recently in humans with rapid pacing.[10] Whether digital pacemaker technology with broadband signal characteristics could be used to predict the susceptibility to and imminence of AF in humans and animal models of AF need to be further investigated. Our study may also hint at the limited clinical success of overdrive pacing in suppressing AF burden[26] as increasing the pacing rate may promote Re-ALT and dispersion of repolarization. Patients suffering from AF and models of rapid-pacing display multiple atrial alterations including a decrease in ERP[27] that may potentially shift Re-ALT to lower rates[10] and 2:1 capture to higher rates, both reducing the effectiveness of this potentially protective mechanism.

Study Limitations

Our study bears limitations that deserve some comments. First, our experimental model of rapid pacing does not provide the typical electroanatomical substrate reported in animal models and humans suffering from AF.[28] Second, some uncertainties remain as to whether our observations apply to the stimulation site as the recording electrode was remote by 2 cm on average, and to the LA as the pacemaker leads were implanted into the RA. Similar APD and Re-ALT dynamics between the RA and LA, however, were recently reported by our group in humans suffering from paroxysmal and persistent AF.[10] Figure S1 of Supporting Information shows in humans RA and LA MAP recordings with atrial Re-ALT and delayed AT preceding capture failure at the pacing site that shared all the characteristics of the transitions to 2:1 capture observed in our ovine model. Third, our model is limited to a single RA site, which prevents us from drawing any conclusion on rapid pacing-induced dispersion of repolarization and discordant (i.e., arrhythmogenic) alternans. Fourth, the analysis of unipolar EGM repolarization presumably provides information about atrial repolarization properties during rapid pacing. It does not provide, however, direct mechanistic insights into cellular and subcellular mechanisms and future studies should analyze the relationship between T_a alternans and local repolarization properties using optical mapping.[24]

Acknowledgments: The authors would like to thank P. van Dam and H. de Bruyn for the development and embedding of the customized pacemaker protocols.

References

1. Haissaguerre M, Jais P, Shah DC, Takahashi A, Hocini M, Quiniou G, Garrigue S, Le Mouroux A, Le Metayer P, Clementy J: Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med 1998;339:659-666.

2. Ausma J, Wijffels M, Thone F, Wouters L, Allessie M, Borgers M: Structural changes of atrial myocardium due to sustained atrial fibrillation in the goat. Circulation 1997;96:3157-3163.
3. Pastore JM, Girouard SD, Laurita KR, Akar FG, Rosenbaum DS: Mechanism linking T-wave alternans to the genesis of cardiac fibrillation. Circulation 1999;99:1385-1394.
4. Weiss JN, Karma A, Shiferaw Y, Chen P-S, Garfinkel A, Qu Z: From pulsus to pulseless: The saga of cardiac alternans. Circ Res 2006;98:1244-1253.
5. Narayan SM, Bode F, L. Karasik P, Franz MR: Alternans of atrial action potentials during atrial flutter as a precursor to atrial fibrillation. Circulation 2002;106:1968-1973.
6. Pruvot EJ, Katra RP, Rosenbaum DS, Laurita KR: Role of calcium cycling versus restitution in the mechanism of repolarization alternans. Circ Res 2004;94:1083-1090.
7. Miyauchi Y, Zhou S, Okuyama Y, Miyauchi M, Hayashi H, Hamabe A, Fishbein MC, Mandel WJ, Chen LS, Chen PS, Karagueuzian HS: Altered atrial electrical restitution and heterogeneous sympathetic hyperinnervation in hearts with chronic left ventricular myocardial infarction: Implications for atrial fibrillation. Circulation 2003;108: 360-366.
8. Watanabe I, Masaki R, Nuo M, Oshikawa N, Okubo K, Okumura Y: Effect of 60 minutes of rapid atrial pacing on atrial action potential duration in the in-situ canine heart. J Interv Card Electrophysiol 2003;8:165-171.
9. Hiromoto K, Shimizu H, Furukawa Y, Kanemori T, Mine T, Masuyama T, Ohyanagi M: Discordant repolarization alternans-induced atrial fibrillation is suppressed by verapamil. Circ J 2005;69:1368-1373.
10. Narayan SM, Franz MR, Clopton P, Pruvot EJ, Krummen DE: Repolarization alternans reveals vulnerability to human atrial fibrillation. Circulation 2011;123:2922-2930.
11. Pruvot E, Jousset F, Ruchat P, Vesin J-M, Prudat Y, Zerm T, Fromer M: Propagation velocity kinetics and repolarization alternans in a free-behaving sheep model of pacing-induced atrial fibrillation. Europace 2007;9:vi83-vi88.
12. Swerdlow C, Chow T, Das M, Gillis AM, Zhou X, Abeyratne A, Ghanem RN: Intracardiac electrogram T-wave alternans/variability increases before spontaneous ventricular tachyarrhythmias in implantable cardioverter-defibrillator patients: A prospective, multi-center study. Circulation 2011;123:1052-1060.
13. Janse MJ, Sosunov EA, Coronel R, Opthof T, Anyukhovsky EP, de Bakker JMT, Plotnikov AN, Shlapakova IN, Danilo P Jr, Tijssen JGP, Rosen MR: Repolarization gradients in the canine left ventricle before and after induction of short-term cardiac memory. Circulation 2005;112:1711-1718.
14. Anyukhovsky EP, Sosunov EA, Feinmark SJ, Rosen MR: Effects of quinidine on repolarization in canine epicardium, midmyocardium, and endocardium: II. In vivo study. Circulation 1997;96:4019-4026.
15. Vigmond EJ, Tsoi V, Yalin Y, Page P, Vinet A: Estimating atrial action potential duration from electrograms. IEEE Trans Biomed Eng 2009;56:1546-1555.
16. Wichert S, Fokianos K, Strimmer K: Identifying periodically expressed transcripts in microarray time series data. Bioinformatics 2004;20: 5-20.
17. Kim B-S, Kim Y-H, Hwang G-S, Pak H-N, Lee SC, Shim WJ, Oh DJ, Ro YM: Action potential duration restitution kinetics in human atrial fibrillation. J Am Coll Cardiol 2002;39:1329-1336.
18. Narayan SM, Kazi D, Krummen DE, Rappel W-J: Repolarization and activation restitution near human pulmonary veins and atrial fibrillation initiation: A mechanism for the initiation of atrial fibrillation by premature beats. J Am Coll Cardiol 2008;52:1222-1230.
19. Qu Z, Garfinkel A, Chen P-S, Weiss JN: Mechanisms of discordant alternans and induction of reentry in simulated cardiac tissue. Circulation 2000;102:1664-1670.
20. Brooks CM, Gilbert JL, Janse MJ: Failure of integrated cardiac action at supernormal heart rates. Proc Soc Exp Biol Med 1964;117: 630-634.
21. Tsai CT, Chiang FT, Tseng CD, Yu CC, Wang YC, Lai LP, Hwang JJ, Lin JL: Mechanical stretch of atrial myocyte monolayer decreases sarcoplasmic reticulum calcium adenosine triphosphatase expression and increases susceptibility to repolarization alternans. J Am Coll Cardiol 2011;58:2106-2115.
22. Narayan SM, Bayer JD, Lalani G, Trayanova NA: Action potential dynamics explain arrhythmic vulnerability in human heart failure: A clinical and modeling study implicating abnormal calcium handling. J Am Coll Cardiol 2008;52:1782-1792.
23. Selvaraj RJ, Picton P, Nanthakumar K, Mak S, Chauhan VS: Endocardial and epicardial repolarization alternans in human cardiomyopathy: Evidence for spatiotemporal heterogeneity and correlation with body surface T-wave alternans. J Am Coll Cardiol 2007;49: 338-347.
24. Mironov S, Jalife J, Tolkacheva EG: Role of conduction velocity restitution and short-term memory in the development of action potential duration alternans in isolated rabbit hearts. Circulation 2008;118: 17-25.
25. Wan X, Laurita KR, Pruvot EJ, Rosenbaum DS: Molecular correlates of repolarization alternans in cardiac myocytes. J Mol Cell Cardiol 2005;39:419-428.
26. Camm AJ, Sulke N, Edvardsson N, Ritter P, Albers BA, Ruiter JH, Lewalter T, Capucci PA, Hoffmann E: Conventional and dedicated atrial overdrive pacing for the prevention of paroxysmal atrial fibrillation: The AFTherapy study. Europace 2007;9:1110-1118.
27. Anné W, Willems R, Holemans P, Beckers F, Roskams T, Lenaerts I, Ector H, Heidbüchel H: Self-terminating AF depends on electrical remodeling while persistent AF depends on additional structural changes in a rapid atrially paced sheep model. J Mol Cell Cardiol 2007;43:148-158.
28. Platonov PG, Mitrofanova LB, Orshanskaya V, Ho SY: Structural abnormalities in atrial walls are associated with presence and persistency of atrial fibrillation but not with age. J Am Coll Cardiol 2011;58:2225-2232.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Clinical observations

Figure S1. Human observations. ECG (top), MAP (middle) and coronary sinus EGM (CS, bottom) recordings during decremental PCLs. Panel A shows MAPD alternans at PCL 400 ms during 3:1 and 2:1 AV conduction. Panels B and C show left (LA, PCL 180 ms) and right (RA, PCL 170 ms) atrial MAPD alternans in 2 patients. Both MAPD alternans (alternating numbers) and prolongation of activation time (AT_{MAP}, red numbers) suggestive of a transient reduction in excitability gradually increased until the first beat of capture failure (arrow). Also note the presence of MAPD alternans phase reversal as shown by 2 consecutive long (panels A and B) and short (panels B and C) beats.

Preprocessing

Ventricular activity cancellation

Figure S2. Ventricular activity cancellation using median template subtraction. Panel A shows an atrial EGM from a sheep with AV block containing far-field ventricular activity. Panel B shows the ventricular template obtained by computing the median of consecutive segments. Panel C shows the improved atrial repolarization wave after subtraction of the median template.

Error estimation in the determination of atrial T wave apexes from EGMs

Estimation of noise-induced Re-ALT

Figure S3. Fit of the T wave. Panel A shows a simulated EGM where the exact location of the T_a is marked by a black star. Panel B shows the EGM with additive white Gaussian noise (SNR = 10 dB) and the corresponding fits in red. Panel C shows the same EGM after wavelet denoising and polynomial fit in red.

Table S1. Errors in the identification of T_a timing and amplitude on simulated EGMs with decreasing noise levels. The upper rows report the mean error ± standard deviation in

the temporal location of T_a [ms] using the Savitzky-Golay fit before (B) and after wavelet denoising (C). The lower rows report the mean error ± standard deviation in the amplitude of T_a relatively to the amplitude of the T wave in [%] using the Savitzky-Golay fit before (B) and after wavelet denoising (C).

References