SUPPLEMENTAL MATERIAL
SUPPLEMENTAL METHODS

This research was performed in accordance with the Unit of Laboratory Animal Medicine Policies, Guidelines and Standard Operating Procedures at the University of Michigan.

Langendorff perfused heart preparations

Five healthy male sheep weighing 30–40 kg were used in the study. The animal protocol was approved by the University Committee on Use and Care of Animals of the University of Michigan and conforms to the Guide for Care and Use of Laboratory Animals by the United States National Institutes of Health. Sheep hearts were isolated and Langendorff-perfused as described previously. Briefly, following anesthesia induced by forelimb Propofol (4–6 mg/kg) IV administration, animals were heparinized (50 mg), intubated and ventilated. Thereafter, a midline sternotomy exposed the hearts which were quickly removed to result in a rapid death by exsanguination. Removed hearts were cannulated through the aorta and connected to a Langendorff-perfusion system with recirculating oxygenated Tyrode’s solution at a flow rate of 200–240 mL/min (pH 7.4, 35.5–37.5°C). The composition of the Tyrode’s solution was (mM): NaCl 130, KCl 4, MgCl₂ 1, CaCl₂ 1.8, NaHCO₃ 24, NaH₂PO₄ 1.2, glucose 5.6 and albumin 0.04 g/L. After atrial transseptal puncture to equilibrate left and right atrial pressures, we ligated all orifices except the inferior vena cava which we cannulated with an outflow tube connected to a pressure transducer. Intra-atrial pressure was calibrated and controlled by adjusting the height of the outflow tube. Once connected, the ventricles were crated to keep the heart in a horizontal position with anterior aspects pointing upward and intra-atrial pressure maintained at 5 cm H₂O for the duration of the experiment to mimic diastolic left atrial pressure. Electrodes continuously collected bipolar recordings from the left and right atrial epicardium for rhythm monitoring. We ensured that the heart was in healthy sinus rhythm at the beginning of each experiment and then administered a bolus of blebbistatin (10–20 μM, Enzo Life Science, NY, USA) and induced ventricular fibrillation to abolish motion artifacts in the atria during optical mapping.

AF inductions

AF onset, including initiation and early stabilization periods, were studied following the addition of 0.25 μM carbachol to the circulating perfusate. AF was induced with an S1-S2 programmed pacing protocol as follows: 10 S1 stimuli at twice the capturing amplitude were administered at 300 msec intervals and an S2 extra-stimulus was administered from the LA free wall at progressively shorter coupling interval until AF was induced or tissue became refractory. For this study, induced AF episodes were classified as self-sustained if lasted at least 40 sec. Longer AF episodes were cardioverted by bi-atrial electric shock and hearts permitted to stabilize in sinus rhythm before repeating the AF induction pacing protocol. Up to about 30 trials of AF inductions were conducted in each heart. The S1-S2 induction protocol guarantees no external stimulus is present during the Initiation and Early
Stabilization periods. Sixteen randomly selected non-sustained episodes and all 20 sustained AF episodes were included in the analysis. Analysis is focused on two periods of the AF onset: The first 7 sec period post S2 is termed here the Initiation period and the 10 sec period 30 sec post S2 is termed here the Early Stabilization period.

**Wide-view endoscopic optical mapping (Figure S1)**

The novel wide-view endoscopic mapping approach used in this study is illustrated in Figure S1. The approach builds on a new objective lens unit comprising of negative and correcting lenses assembly designed to extend the original borescope field of view when immersed in the translucent Tyrode’s solution filled atrial cavities. Panel A shows the basic optical properties of the developed wide-view objective lens unit demonstrated by imaging the inside of a 75 mm diameter sphere with multiple uniform size holes of 11 mm in diameter each using the same system as in the experiments. The center of the right image (green circle) is the sphere point farthest from the objective lens and the periphery of the image corresponds to the sphere area nearest to the lens. The lens projection of the spherical surface onto the image is accomplished by local scale transformation whereby the radial axis is contracted and the angular axis is stretched from the center to the periphery of the image. At the periphery, about 37 pixels from the center of the image, the 11 mm circular hole (yellow ellipse) is seen to be radially contracted by 40% (orange lines) and angularly stretched by 35% (cyan lines).

For imaging the endocardial surfaces of the atria lesions were cut at left ventricular apex and right ventricular free wall to enable insertion of two dual-channel solid borescopes (Everest VIT, GE Inspection Technologies, Huerth, Germany) toward the left and right atria (panel B). The distal ends of the borescopes were equipped with new wide-view objective units and advanced through the ventricular lesions to position the objective unit at the plane levels of the left and right atrioventricular valves, thus enabling viewing the entire endocardial atrial surfaces. The geometrical shape of the atria chambers is complex and variable, but its general concave shape relative to the objective lens renders the endocardial wide-view optics with less geometrical distortions as compared with a wide-view image of flat or convex surfaces. Color images at ambient light taken via the borescopes in its mapping position are showing panoramic internal views of the atria, including their septa, roofs, vena cava, pulmonary veins and the free walls (panel C). The system is imaging the proximal aspects of the appendages (which are shallower in sheep than in humans).

Following the positioning of the borescopes and their objectives, the lesions were sealed to maintain a stable intra-atrial pressure and the proximal ends of the borescopes were coupled to two synchronized 14 bits CCD cameras (SciMeasure, Decatur, GA) on the acquisition channels, and to two light guides delivering 532 nm excitation light on the illumination channel. The two cameras and borescopes were then used to record voltage-sensitive di-4-ANEPPS fluorescence from the endocardial surfaces of the two atria in 10 sec long movies of 80×80 pixels each at 600 frames/sec. Movies of the right and left atria were filtered in time and space and their background intensity subtracted to yield the dynamic time series
of the fluorescence as previously performed\textsuperscript{7-9}. Panel D of Figure S1 shows background intensity images from a representative heart marked with locations of 4 sample fluorescence traces during AF shown in panel E. As can be appreciated from the traces, the amplitude of the signals varies between recording sites; however, at a minimum of about 100 arbitrary digital units levels of fluorescence the cycles of the AF activation waves are clearly detectable\textsuperscript{17}. Sample videos are provided as Videos S1-S6.

**Optical data analysis**

Analyses included spatial and temporal quantifications of phase-, frequency- and time-domain parameters\textsuperscript{18,46} as follows:

**Phase-domain analysis:** The action potentials phase in each pixel was computed using the Hilbert transform. In phase maps, propagation is from high to low phases modulo $2\pi$. Phase singularity points (SPs) in maps, defined as a location towards which all phases converged, were identified manually and their time and coordinates marked blind to subsequent analyses. All marked SPs were included in analyses. Rotors were defined as waves pivoting around an SP for $>1$ cycle. SPs of rotors were tracked to determine their trajectory and lifespan. SPs lasting less than one rotation were considered wavebreaks.

**Frequency-domain analysis:** Fluorescence movies were divided into 3-sec long segments for analysis of the time-course of the dominant frequency (DF) defined as the frequency with the maximum power at each pixel in each segment. We defined maximal DF (DF\textsubscript{max}) in DF maps as the highest DF present in $>20$ contiguous pixels.

**Time-domain analysis:** For fluorescence time-series of each pixel, up- and down-strokes were automatically detected between peaks and the cycle length (CL) was calculated as the time interval between 50\% of peak-to-peak amplitudes in sequential upstrokes\textsuperscript{46}. CLs of all pixels formed a new movie whereby each frame presented the atria-wide CLs for each pixel during the duration of each cycle.

**Cycle length analysis:** CLs in two locations and settings were collected from frames of the CL movies: (i) The CLs at the rotors sites before and during the rotor SP appearance were used to determine the rotor-induced CL changes at the rotors sites themselves. Signals at rotors’ cores may have low amplitude hence a CL at a coordinate of interest was determined by averaging values from a $3\times3$ pixels array centered at that coordinate in the CL maps. (ii) The minimal CL (CL\textsubscript{min}) $>5$ percentile anywhere in the atria was used to determine the atria-wide fastest activation rate at a given moment.

**Acceleration and deceleration:** Reduced or increased values of CL in time at rotor-site and CL\textsubscript{min} across the atria are considered acceleration or deceleration, respectively, at their corresponding locations. CL at rotor sites was evaluated before and during the rotor presence to study the effect of rotor formation on local acceleration or deceleration. Atria-wide CL\textsubscript{min} values at movie frames immediately before and after rotor SPs presence, and
100 msec after a non-rotor SPs presence, were used to study the acceleration or deceleration effects of rotors and non-rotors SPs on the fastest activation rate in any location across the entire atria.

Action potential duration: For fluorescence time-series of each pixel during pacing activity, up- and down-strokes were automatically detected. Action potential duration at 70% repolarization (APD_{70}) was calculated for each pixel between 50% peak-to-peak upstroke level and subsequent down-stroke at 70% peak-to-peak repolarization level.

Rotor drift distance and velocity: The start and finish pixel sites of the SPs trajectory of a rotor were used to determine its begin-to-end drift vector. For each rotor, the drift vector was decomposed into its radial and angular components which were multiplied by a factor of 1+0.4R/37.3 and 1-0.35R/37.3 respectively to account for the panoramic optical projection distortion (R being the distance between the center of the vector and the center of image in pixels; see Figure 1A). The magnitude of the drift vector following a multiplication by a conversion factor of 1.859 was considered the drift distance expressed in millimeters. The rotor drift velocity was then calculated by the ratio of the drift distance to the rotor lifespan. As the rotor drift distance and velocity were calculated from the begin and end flanking points of the SP drift trajectory, they refer to the effective whole rotor drift and not to the meandering of its instantaneous SP location.

SUPPLEMENTAL RESULTS

Atrial activation during pacing (Figure S2)

The distribution of atrial action potential duration at 70% repolarization (APD_{70}) across the RA and LA was analyzed during the S1 pacing stimuli at 300 msec intervals prior to the premature stimulus (see Supplemental Methods and Figure 1) and is presented in Figure S2. Figure S2A shows the endocardial RA and LA panoramic activation time (ACT) map of the last of the 10 S1 waves paced at 300 msec intervals in the same heart shown in Figure S1 (see also Video S1). Pacing was from the LA free wall (asterisk) and the ACT map shows an uninterrupted propagation toward the RA with a complete bi-atrial conduction time of approximately 75 msec^{47}. The APD_{70} for averaged S1 waves in the same representative heart was 109.7±7.4 msec for the RA and 96.2±13.3 msec for the LA. We further analyzed the regional and whole atria APD_{70} distribution measured in 19 movies of S1 waves paced at 300 msec intervals and preceding inductions of sustained AF in 5 hearts (Figure S2B). We found that APD_{70} varied across the various bi-atrial anatomical regions (Two-way ANOVA test p<0.0001) and averaged APD_{70} for all RA regions was longer than the for all LA regions (112.6±19.3 msec vs. 93.9±9.9 msec respectively, p<0.0001)^{48}.

Initial breakthroughs and rotor activation patterns (Figures S3 and S4)

Breakthrough (BT) of an initiating wave and repeat pivoting rotor patterns of activity are of particular interest as possible drivers and targets in ablation to terminate AF^{19,49}. Both patterns were observed in our experiments (Figure S3A) here and we first characterize them...
during the period immediately following the S2 extra stimulus. Panel A of Figure S2 shows sample phase snapshots of BT and rotor patterns in an AF induction; immediately subsequent to the S2 wave a radial wave propagation from a BT activation is demonstrated in the area between the ridge and free wall of the LA; immediately thereafter, a single counterclockwise rotational activity is seen around an SP at the SVC area; about 30 sec later, multiple SPs indicating either wavebreaks or rotor activity are seen throughout both atria (see Figure S3).

Analysis of 20 sustained AF inductions showed that the first activation patterns following the S2 wave were BTs in 16 (80%) and rotors in 4 (20%) cases (Figure S3B). The first BTs were 13/16 in the LA and 3/16 in the RA. In all the inductions that resulted in sustained AF, rotors followed the BT activity with the first ones appearing mostly in the RA; 13 times in the RA, 4 in the LA and in 3 cases they appeared simultaneously in the RA and the LA (Fisher's exact test two-tailed p=0.0081).

The sequence of BTs appeared sometimes at an alternating atrium and could not be linked to each other in space. We however characterize the time intervals between sequential BTs of new waves to better understand their possible role in the AF onset. Figure S3C is a scatter plot of the BT intervals vs. the cycle number in the sequence (first interval is between the S2 and the first BT waves) for inductions leading to sustained (red) or non-sustained (blue) AF. The plot reveals large scattering of BT intervals, however a linear regression analysis demonstrates a separation between the non-sustained AF and sustained AF; inductions whose activity resulted in non-sustained AF (n=16) showed no significant trend in BT intervals over time (blue, y=0.9x+123.9 msec, R²=0.0119, p=0.368) and inductions whose activity resulted in sustained AF (n=20) showed decreasing BT intervals over time (red, y=-4.3x+113.6 msec, R²=0.0309, p=2.63e-4). The analysis in Figure S3 and the sample sequence of phase maps in Figure S4 demonstrates that our mapping of the activation patterns during the initial stage of the sustained AF reveals BTs activity that accelerates and transforms into rotational activity.

**Inductions not resulting in sustained AF (Figure S5)**

A sample of phase movie snapshots during an induction that failed to result in a sustained AF is shown in Figure S5. One hundred sixty-four msec after the S2 stimulus in the LA, a first non-paced activation wave is seen to originate in a BT in the RA and to propagate uninterrupted across the RA and the LA. A 2nd non-paced similar activation pattern originating in a BT in the RA is seen at 287 msec after S2 (123 msec BTs interval). Following additional 2 BT activations (not shown), a 5th and 6th BT activation patterns are visible at 680 and 829 msec post S2, respectively (159 msec BTs interval). Overall, the 4 patterns of activations during the 6 non-paced BT waves presented in Figure S5 do not show SPs at any time and are followed by a period of quiescence, shown at a sample 977 msec snapshot. Subsequently to the non-activation period, 936 msec after the 6th BT activity, sinus rhythm was resumed as seen by the first sinus wave originating at a BT in the RA illustrated at 1765 msec post-S2.
Time-course of regional distribution of rotor presence (Figure S6)

The regional distribution of rotor appearance during the Initiation and Early Stabilization stages is presented in Figure S6 following these steps: First, all SPs belonging to rotors were annotated for the region where they appear in each movie frame. Second, for each region the number of SPs in 10,000 frames (16.7 sec) were counted separately for the Initiation and Early Stabilization stages. Third, the percentage of rotor presence in each region was calculated as the ratio of the counted number of SPs for that region to the total number of SPs counted for all the regions. And finally, the regional percentages for the 20 AF inductions were averaged and presented as stacked bar columns.

Thus, Figure S6 presents a quantification of the time with rotational activity lasting more than one cycle across all RA and LA regions, expressed as the percentage of the total time with rotational activity anywhere and anytime, separately during Initiation (left column) and during Early Stabilization (right column). The SPs of a total of 572 rotors were tracked in 30,695 frames (about 51.2 sec) during Initiation and 45,211 frames (about 75.3 sec) during Early Stabilization. During Initiation, there was a trend for SPs of rotors to be more abundant in the RA (55.8±18.9%) than in the LA (44.2±18.9%; p=0.0621). As AF progresses to Early Stabilization, we observed a significant shift in the amount of rotors presence only in two areas: The LA roof rotors presence increased from 5.7±6.1% to 10.7±10.5% (p=0.0187) and the RA free wall decreased from 35.5±18.7% to 25.6±16% (p=0.0121). Overall, during the transition to Early Stabilization the RA rotors presence tended to decrease from 55.8±18.9% to 44.3±23.9% and LA rotors presence tended to increase from 44.2±18.9% to 55.7±23.9% (p=0.0670), practically reversing the rotors presence distribution during Initiation to have less RA rotors than LA rotors during Early Stabilization, but not to a significant level (p=0.1364). Please note that RA and LA percentage values are complementary to 100% for each induction and therefore their STD values, as well as the t-test p values characterizing the transition from Initiation to Early Stabilization, are identical. In addition, the value of Alpha may be adjusted to 0.025 to determine the significance of the between RA and LA differences and transitions from Initiation to Early Stabilization observed in this analysis.

Time-domain acceleration of activity (Figure S7)

For greater insight into the dynamic process of acceleration we generated CL movies of beat-by-beat activation intervals for all pixels. For each movie frame (instantaneous CL map) we selected the minimal CL (CLmin) and analyzed the time course of CLmin in binned periods of 166 msec (100 frames). Figure S7A shows the time course of CLmin during Initiation (left) and Early Stabilization (right) stages of a sample induced AF. Linear best fit shows an acceleration trend (reduction of CLmin) during Initiation (p<0.0001) contrasted by a constant CLmin during Early Stabilization (p=0.8435). Composite analysis of 20 inductions of sustained AF is presented in Figure S7B. The linear regression intercept (left) indicates the CLmin at the beginning of the two stages decreased from 71±25 to 57±16 msec (p<0.0001); the linear regression slope (right) indicates the CLmin trends during the two
stages is increasing from $-1.65\pm2.2$ to $0.04\pm0.02\times10^{-3}$, $p=0.0019)$. Overall, the gradual reduction in CLmin during Initiation and its eventual steadiness later during Early Stabilization parallels the time course of DFmax shown in Figure 2B.

**Time-course of regional DFmax distribution (Figure S8)**

Figure 2 of the manuscript describes the time course of the DFmax across the entire atria during the AF onset. Here we describe in Figure S7 in more details the time-course of the DFmax specific to each of the atria regions as outlined in the anatomical diagrams in Figures 2, S2 and S3. DFmax data for 20 AF inductions presented in Figure S8 show a variability across the different regions and times. On average the PLA (red symbols) is hosting the DFmax during all 4 periods of time analyzed, albeit not always significantly higher than the other regions. The corresponding average values of the DFmax at the PLA are $11.11\pm0.58\text{ Hz (0-3 sec, }p=0.0586)$, $13.03\pm0.58\text{ Hz (4-7 sec, }p=0.5764)$, $15.13\pm0.58\text{ Hz (30-33 sec, }p=5.2e-05)$ and $14.67\pm0.58\text{ Hz (37-40 sec, }p=0.0675)$. Interestingly, the average DFmax at the SVC (green symbols) region is second highest and not statistically different from the highest DFmax at the PLA at the 0-3 and 37-40 periods ($11.08\pm0.67\text{ Hz; }p=0.9279$ and $14.22\pm0.71\text{ Hz; }p=0.6012$, respectively). It is also interesting to note that despite the PLA presenting the highest average DFmax in each period, on an individual induction basis, the other LA regions of the ridge, the free wall and the roof (red arrows) presented similar or higher DFmax values. Finally, the higher average DFmax values in the LA free wall as compared with the RA free wall (blue and dark green symbols, respectively) over the 4 time periods analyzed in Figure S8 is consistent with previous epicardial optical mapping studies of cholinergic AF in isolated sheep heart showing also that the LA free wall was activated at a faster DF than the RA free wall\textsuperscript{14}.

**Time-course of rotors appearance and lifespan (Figure S9)**

The CLs localized to rotor sites, at cycles prior and during the rotors presence and identified in 20 inductions of AF, were collected using the tracking method shown in Panel A of Figure 3. A total of 217 and 357 rotors detected during Initialization and Early Stabilization stages, respectively were analyzed in Figure S9 for appearance rate and time lifespan in each consecutive 1 sec long bins of time. ANOVA tests revealed that there were no significant differences between inter-bin rates of appearance at the two stages (Panel A; left, $p=0.4743$ and right, $p=0.1253$, respectively) and rotors appeared in the 20 inductions in series at 5 sec average rates of $2.0\pm1.37$ vs. $3.29\pm2.09$ rotors/sec during Initiation and Early Stabilization, respectively ($p<0.0001$). The number of rotors detected decreased with their number of lifespan cycles before disappearance, as in previous studies\textsuperscript{14}, and they completed up to 6 cycles during Initiation and up to 10 cycles during Early Stabilization before dissipating. Although the 5 sec average number of their cycles lifespan in the 20 inductions was similar ($1.75\pm1.03$ vs. $1.76\pm1.2$ rotations per rotor; $p=0.9048$; not shown), their 5 sec average time lifespan decreased from $224.1\pm118.8$ to $196.6\pm110.4$ msec, respectively, ($p=0.0074$. Panel B). ANOVA tests on inter-bin time lifespan of rotors in Panel B revealed significant variability during Initiation (left, $p=0.0062$) and non-significant variability during Early Stabilization.
(right, p=0.5423), providing another parametrization for the dynamic activation process of AF onset in our model.

**Reduction and increase of atrial CLmin during rotors and non-rotors SPs (Figures S10-S12)**

Both accelerations and decelerations of the activity across the atrial were observed during the presence of rotors and non-rotors SPs. Figure 4A shows a sample case in which the LA-wide minimal CL is reduced following a rotor appearance and disappearance (acceleration). In Figure S10 we show an opposite sample case in which the LA-wide CLs are prolonged (deceleration) following the presence of a rotor. The sample pixel signal in Figure S10 is showing that the 2 CLs before the rotor appearance are 75 and 72 msec and following the rotor disappearance the CLs shown prolong to 84 and 92 msec. The corresponding LA-wide CL maps show a general prolongation of CLs with increasing areas of CLs >140 msec (red-yellow areas). We analyzed the cumulative contribution of rotors and non-rotors SPs presence on the CLs across the entire two atria to provide insight on the transition of the AF from accelerating during Initiation to stable activation rate during Early Stabilization, despite the constant acceleration of activity at the rotor site during the two AF stages as shown in Figure 3B.

The CLmin values across the entire atria varied greatly between different inductions and stages of AF and are analyzed in Figures S11 and S12. In Figure S11, CLmin prior to rotors were compared with CLmin subsequent to the same rotors. We analyzed changes in atria-wide CLmin values for 210 rotors during Initiation, and 362 rotors during Early Stabilization. The post-rotor CLmin decreased compared to pre-rotor CLmin in 113 (53.8%) of cases during Initiation and 156 (43.1%) of cases during Early Stabilization. During Initiation (left, n=210), the 113 pairs demonstrating acceleration showed CLmin reduction of 20±18.9 msec and the 97 pairs demonstrating deceleration showed CLmin increase of 16.4±15.5 msec (Wilcoxon test p<0.0001). Overall, the effect of all rotors analyzed during Initiation demonstrated a cumulative CLmin shortening from 90.2±32.3 msec to 87.3±33 msec (paired t-test p=0.0439). During Early Stabilization (right, n=362), the 156 pairs demonstrating acceleration showed CLmin decrease of 15.2±13.7 msec and the other 206 pairs demonstrating deceleration showed CLmin increase of 14±13.9 msec (Wilcoxon test p<0.0001). Overall, the effect of all rotors analyzed during Early Stabilization demonstrated a non-significant cumulative CLmin alteration from 77.6±26 msec to 78.7±26.9 msec (paired t-test p=0.1671). Aggregate analysis of the pairs of CLmin values during Initiation and Early Stabilization showed there was a significant average atria-wide reduction by 3.5±25.2 msec (p=0.0439) during each rotor at Initiation, and a non-significant average atria-wide increase by 1.2±20 msec (p=0.1671). Thus, the rotors presence is associated with atria-wide acceleration of AF at Initiation versus stability of AF rate at Early Stabilization (p=0.0112).

In contrast to the effect of rotors, the non-rotor SPs was not associated with reduction of the atria-wide CLmin. In Figure S12 pairs of CLmin values immediately prior and subsequent to 313 non-rotor SPs during Initiation and 696 non-rotor SPs during Early Stabilization were
compared after normalization to the earliest CLmin in each movie to account for the large variability across pairs from different inductions. Differences between normalized mean values of pre-SP and post-SP CLmin were less than 1% during both Initiation (p=0.583) and Early Stabilization (p=0.235). The normalized CLmin differences also did not differ between Initiation and Early Stabilization (p=0.4497). Thus, only the cumulative activity of accelerating rotors, and not non-rotor SP presence in general, is associated with acceleration of AF during onset.
**Figure S1. Experimental setup and panoramic data.**

**A)** Basic properties of the new wide-view lens unit demonstrated in viewing the inside of a training baseball ball of about 75 mm in diameter. Left: The developed wide-view objective lens unit is attached to a borescope and a CCD camera as described below and is placed inside a spherical 75 mm diameter training baseball ball with circular holes (11 mm diameter) for scaling of the inside view. Top ruler scale is in mm. Right: Circular holes of the sphere are visible through the wide-view lens and the camera system. The central 11 mm diameter hole (green circle) is the farthest from the lens and the peripheral holes (yellow ellipse) are the closest to the lens. The projection of the sphere on the image is accomplished by a radial contraction and angular stretch of the periphery relative to the center of the image. At the periphery, 37.3 pixels from the center of the image, the 11 mm circular hole (yellow ellipse) is seen to be radially contracted by 40% (orange lines) and angularly stretched by 35% (cyan lines).

**B)** View of the experimental setup showing the two cameras (bottom of image) attached to two borescopes equipped with wide-view objective lenses inserted through the ventricles’ apexes (VA) to map the RA and LA in the...
Langendorff perfused isolated sheep heart (top of image). C) Visual color image of the endocardial surfaces of the RA (left) and LA (right) obtained via the borescopes and the wide-view lenses. D) Sample background fluorescence intensity images of the two atria obtained with the cameras in B. E) Sample traces of dynamic fluorescence obtained in pixels marked in D. Amplitude of signals exceeded 100 arbitrary digital units.
Figure S2. Atrial action potential duration during S1 pacing.

A) Top: Endocardial RA and LA panoramic activation time (ACT) map during the last of 10 S1 paced waves (at 300 msec intervals) in the same heart shown in Figure S1. Pacing is from the LA free wall (asterisk). Middle: Representative single pacing recordings during the last 3 S1 waves are shown. Bottom: APD$_{70}$ map for the averaged S1 waves in the same heart. RA APD$_{70}$ is 109.7±7.4 msec and LA APD$_{70}$ is 96.2±13.3 msec.

B) Top: A representative endocardial image and outline of 8 anatomical RA and LA regions. Please note that the inferior vena cava as well as the distinction between the left and right pulmonary veins (PVs) in the sheep atria are not visible in some cases and we therefore combine their data into unifying superior vena cava (SVC) and PVs regions, respectively. Bottom: The individual, mean and STD APD$_{70}$ for the anatomical regions outlined measured during S1 pacing preceding inductions of sustained AF (N=5, n=19). APD$_{70}$ varies across regions.
(Two-way ANOVA test $p<0.0001$) and $\text{APD}_{70}$ for all RA regions is longer than the for all LA regions ($112.6\pm19.3$ msec vs. $93.9\pm9.9$ msec respectively, $p<0.0001$).

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Figure S3. Initial activation patterns and their progress.

A) Anatomy image and activation patterns in a sample AF induction panoramic mapping of the RA and LA endocardial surfaces. From top to bottom panels: (i) The anatomy image of the panoramically mapped RA and LA (SVC, superior vena cava; PVs, pulmonary veins). (ii) A breakthrough (BT, asterisk) pattern at the edge of the LA free wall region during initiation of AF. (iii) A rotor pattern is followed in the RA. (iv) During early stabilization, a complex activation includes multiple reentries and BTs across both atria. B) Patterns of activity in 20 self-sustained AF inductions in N=5 hearts. Left: Incidences of BTs and rotors as first activity after S2. Center: Location of first BTs after S2. Right: Location of first rotor, either immediately after S2 or after BTs. Two-tailed Fisher's exact test: p=0.0081. C) Linear regression analysis of cycle length (CL) between sequential BTs following S2 vs. the BTs cycle number in non-sustained AF inductions (blue, N=5, n=15) and sustained AF inductions (red, N=5, n=20). F-test for linear comparisons: p=0.006.
Figure S4. A sequence of phase movie snapshots during a representative AF induction.

One-hundred thirty-five msec after the S2 pacing in the LA (not shown), a new wave breakthrough endocardial (BT, asterisk) appears in the LA septal area with a delayed BT in the RA. Following, at 187 msec, another BT appears at the RA with a delayed BT in the LA. Thereafter an SP is formed in the RA and becomes a rotor that lasts for more than one rotation (snapshots at 275 to 545 msec). The concomitant activity in the LA is regular with BTs at all snapshots. Arrowhead circles: location and chirality of a rotor.
Figure S5. A sequence of phase movie snapshots during an induction that failed to result in a sustained AF.

One hundred sixty-four msec after the S2 stimulus in the LA, a first non-paced activation wave is seen to originate in an RA breakthrough (BT, asterisk) and to propagate uninterrupted across the RA and the LA. A 2nd non-paced similar activation pattern originating in a BT in the RA is seen at 287 msec after S2. Following additional 2 BT activations (not shown), a 5th and 6th BT activation patterns are visible at 680 and 829 msec post S2, respectively. Subsequently, a period of quiescence pause in activity across the RA and LA is presented at 977 msec post S2, which is followed by a sinus rhythm wave originating at a BT in the RA presented at 1765 msec post S2.
Figure S6. Rotor distribution across atrial regions.

Stacked bar representation of percentage of time that the rotors existed in each of the atrial regions at Initiation (left) and Early Stabilization (right) of AF. Numbers for each region represent the mean±STD percentage time rotors were present in that region relative to all rotors (in all regions and all times) in the 20 inductions (N=5, n=20; total number of rotors at Initiation was 216, total number of rotors at Early Stabilization was 356). Percentage values for all regions sum up to 100% for each induction. Anatomical regions are according to the anatomy diagram in panel A of Figure S2. Comparisons were made by t-test for normal distributions (Shapiro-Wilk test) and by Wilcoxon signed-rank test for non-normal distributions. Statistical significance level is Alpha=0.025 for dual comparisons. See Supplemental Results section for more details.
Figure S7. Maximal dominant frequency (DFmax) in different atrial regions calculated at 4 different time periods following the S2 stimulus.

Colored symbols are mean±STD values for 20 AF inductions in 5 hearts for each region listed in the legend on top (20 dot symbols in each column correspond to 20 DFmax values at each particular location). Anatomical regions are according to the anatomy diagram in panel A of Figure S2. DFmax values at the PLA were compared with the DFmax values of regions with the nearest lower mean value by t-test.
Figure S8. Time-course of cycle lengths (CLs).

A) Representative induction example of minimal CL (CLmin) averaged over the mapped atria every consecutive 200 msec bin during Initiation (left) and Early Stabilization (right) stages of a sample AF induction. Linear best fit with 95% confidence lines are superimposed on graphs (red) and presented as formulas of with intercepts and slopes. B) Comparisons between Initiation and Early Stabilization stages for intercepts (left) and slopes (right) of CLmin time curves in AF inductions (N=5, n=20). Red markers indicate mean (horizontal bar), 95% confidence of the mean (box) and STD (error bars) values. Wilcoxon signed rank test was performed for all comparisons.
Figure S9. Appearance rate and time lifespan of rotors in each consecutive 1 sec long bins of time during Initiation and Early Stabilization of 20 AF inductions in 5 hearts.

A total of 217 and 357 rotors during Initialization and Early Stabilization stages, respectively, were analyzed. A) Left: Numbers of rotors that appeared in each sequential 1 sec long bin during Initiation. Right: Numbers of rotors that appeared in each sequential 1 sec long bin during Early Stabilization. There were no significant differences between inter-bin rates of appearance at the two stages (ANOVA tests. Initiation: p=0.4743, Early Stabilization: p=0.1253). Rotors appeared in the 20 AF inductions in series at 5 sec average rates of 2.0±1.37 vs. 3.29±2.09 rotors/sec during Initiation and Early Stabilization, respectively (p<0.0001). B) Left: Rotor durations for 217 rotors during Initiation. Right: Rotor durations for 357 rotors during Early Stabilization. ANOVA tests on inter-bin time lifespan of rotors revealed significant variability during Initiation (left, p=0.0062) and non-significant variability during Early Stabilization (right, p=0.5423). The 5 sec average time lifespan of the rotors decreased from 224.1±118.8 to 196.6±110.4 msec, respectively, (p=0.0074). Red symbols
are mean±STD values for 20 AF inductions (A), and for rotors (B), for each time bin in 5 hearts.
Figure S10. A sample case of decelerating LA CLs from before to after a rotor presence during the AF Initiation.

Fluorescence signal from a site near a rotor with corresponding phase (top row) and CL (bottom row) maps. Numbers superimposed on the fluorescence signal and in the CL maps indicate corresponding CLs in msec. Asterisks in maps indicate location of the fluorescence signal near the rotor. A stable rotor with undetected drift is present between 0 and 270 msec. The CLs in the sample fluorescence signal (top) prolong from tor values of 75 and 72 msec prior to the rotor formation to values of 84 and 92 msec after its disappearance. CL map after rotor disappearance (273 and 370 msec) is seen to have larger red-yellow areas and less blue areas compared with the CL map before rotor formation (0 and -100 msec) indicating a deceleration during the rotor presence. Pre-rotor and Post-rotor black arrow markers in the fluorescence trace indicate times of determining atria-wide CLmin in the analyses presented in Figures 4, S10 and S11. Arrowhead circles, sample rotor location and chirality. Arrows, general propagation directions. Dashed line circles, sample non-rotor SP locations.
Figure S11. Analysis of rotors effect on atria-wide CLmin.

The CLmin values were determined from instantaneous panoramic CL maps pre- and post-rotors (regardless of their location) during Initiation (left) and Early Stabilization (right) of 20 induced AF episodes in 5 hearts. In each graph the CLmin pre- and post-rotors are paired and colored blue if post-rotor CLmin is larger than pre-rotor CLmin (deceleration), and in red if post-rotor CLmin is smaller than pre-rotor CLmin (acceleration). Red markers indicate mean (horizontal bar), 95% confidence of the mean (box) and STD (error bars) values. During Initiation (left, n=210), 113 (53.8%) pair cases demonstrated CLmin acceleration of (mean±STD) -20±18.9 msec and 97 pair cases demonstrated CLmin deceleration of 16.4±15.5 msec (Wilcoxon test p=3.81e-13). Cumulative analysis of all post- vs. pre-rotor CLmin during Initiation demonstrated a CLmin shortening from 90.2±32.3 msec to 87.3±33 msec (paired t-test p=0.0439). During Early Stabilization (right, n=362), 156 (43.1%) pair cases demonstrated CLmin acceleration of -15.2±13.7 msec and 206 pair cases
demonstrated CLmin deceleration of 14±13.9 msec (Wilcoxon test p=1.57e-22). A cumulative analysis of all post- vs. pre-rotor CLmin during Early Stabilization demonstrated a non-significant cumulative CLmin alteration from 77.6±26 msec to 78.7±26.9 msec (paired t-test p=0.1671).
Figure S12. Analysis of non-rotors (SPs with lifespan <1 rotation) effect on atria-wide CLmin.

CLmin values were determined from instantaneous panoramic CL maps pre- and post-SP (regardless of their location) during Initiation (left, n=313) and Early Stabilization (right, n=696) of 20 induced AF episodes. In each graph the CLmin pre- and post-SP are normalized to the first pre-SP CLmin in the corresponding Initiation or Early Stabilization movies, paired, and colored blue if post-SP CLmin is larger than pre-SP CLmin (deceleration), and in red if post-SP CLmin is smaller than pre-SP CLmin (acceleration). Among the 313 pairs Initiation pairs (left), 145 (46.3%) showed acceleration and 168 showed deceleration. Among the 696 Early Stabilization pairs (right), 278 (39.9%) showed acceleration and 418 showed deceleration. Red markers indicate mean (horizontal bar), 95% confidence of the mean (box) and STD (error bars) values. Cumulative analysis of post-vs. pre-SP CLmin in Initiation pairs (left) demonstrates non-significant alteration of normalized CLmin from 105.8±32.0% to 105.5±32.3% (mean±STD, paired t-test p=0.583). Similar analysis of Early Stabilization pairs (right) also demonstrates a non-significant normalized CLmin alteration from 102.8±32.0% to 102.9±32.4% (mean±STD, paired t-test p=0.235).
Supplemental Video Legends:

Video S1. Fluorescence movie of panoramic mapping of the endocardial RA and LA surfaces on the left and right sides of the frames, respectively. The movie shows waves crossing the LA and RA following the S1 pacing stimuli at the lateral aspect of the LA (right side of the movie). Movie is shown at a slowed frame rate. Best viewed with Windows Media Player.

Video S2. Fluorescence movie of panoramic mapping of the endocardial RA and LA surfaces on the left and right sides of the frames, respectively. The movie shows initially the tail of the last S1 wave in the RA, followed by a S2 wave and thereafter the initiation stage of AF. Movie is shown at a slowed frame rate. Best viewed with Windows Media Player.

Video S3. Fluorescence movie of panoramic mapping of the endocardial RA and LA surfaces on the left and right sides of the frames, respectively. Movie was recorded during the early stabilization stage of AF (about 30 sec following the S2 stimulus). Movie is shown at a slowed frame rate. Best viewed with Windows Media Player.

Video S4. Phase movie of panoramic mapping of AF initiation. The endocardial RA and LA surfaces are on the left and right sides of the frames, respectively. The movie shows initially the tail of the last S1 wave in the RA, followed by a S2 wave and thereafter the initiation stage of AF. Movie is shown at a slowed frame rate. Best viewed with Windows Media Player.
Video S5. Phase movie during the early stabilization stage of AF (about 30 sec following the S2 stimulus). The endocardial RA and LA surfaces are on the left and right sides of the frames, respectively. Movie is shown at a slowed frame rate. Best viewed with Windows Media Player.

Video S6. Phase movie of the LA at the Initiation stage showing a rotor drift as described in Figure 5. Movie is shown at a slowed frame rate. Best viewed with Windows Media Player.