Substrate size as a determinant of fibrillatory activity maintenance in a mathematical model of canine atrium

Renqiang Zou, James Kneller, L. Joshua Leon, and Stanley Nattel

1Department of Medicine and Research Center, University of Montreal and Montreal Heart Institute, and 2Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec; and 3Department of Electrical Engineering, University of Calgary, Calgary, Alberta, Canada

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ATRIAL FIBRILLATION (AF) is the most common sustained arrhythmia in humans, is an important contributor to human morbidity and possibly mortality, and presents a variety of therapeutic challenges (29). The mechanisms underlying AF have been a subject of long-standing debate and recent intense investigation (31). Some of the basic properties of fibrillation induced by faradic stimulation were described by MacWilliam (23) in the late 1800s, along with the discoordinating effects of vagal nerve activation on atrial fluttering caused by faradic currents. MacWilliam also showed that ventricular fibrillation could persist in isolated sections of ventricular tissue. Garrey (8) extended MacWilliam’s approach and noted that the ability of isolated tissue to fibrillate depends on its mass, introducing the notion of a “critical mass” for fibrillation related to conditions required for multiple circuit reentry. This idea was later incorporated in Moe’s “multiple wavelet hypothesis” (26). An alternative conceptual framework championed by Lewis (20) was the idea of a primary reentrant circuit (a “mother wave”), with fibrillatory activity due to an irregular response of the atrial substrate.

Experimental support has been presented for both of these paradigms. AF persistence across species varies with their cardiac size, with AF being much longer lasting in larger hearts (28). On the other hand, the ability of the mouse heart to sustain fibrillation has been presented as a challenge to the critical mass hypothesis (41), and an analysis of dominant frequencies and phase singularity (PS) properties during AF in isolated sheep hearts has led to the suggestion that one or a small number of reentrant generators rather than multiple wavelet reentry underlies AF (4).

Testing the “critical mass” notion in biological tissues is difficult because paradigms that allow for the examination of a range of tissue sizes (e.g., different species, models producing atrial dilatation, developmental changes, etc.) are also associated with discrepancies in atrial electrophysiological properties. Mathematical models of atrial activity do not share this limitation: the size of the computational substrate can be varied while the underlying electrophysiological properties are kept constant.

We (18) have previously analyzed the properties of AF in a mathematical model of cholinergic AF with a two-dimensional substrate having realistic cellular and tissue electrical properties for the dog. In a 5 × 10-cm substrate (taken to represent normal canine atrial dimensions), AF is generally maintained by a single dominant spiral wave with wavebreak in a heterogeneous substrate (18). The role of substrate dimensions was not addressed, and we are unaware of other studies that assessed the role of substrate dimensions specifically. In the present investigation, we analyzed the relationship between atrial substrate size and AF persistence, along with associated mechanisms, in the model. We evaluated activity under 12 discrete conditions of acetylcholine (ACh) concentration ([ACh]) and distribution in each of five rectangular atrial substrates over an 11.1-fold range of surface areas. Induced activity was followed for as long as it was maintained, to a maximum of 20 s.

METHODS

Model description and implementation. We used the Ramirez-Nattel-Courtemanche (RNC) canine atrial action potential (AP)
model, as previously described (17, 34). The cell membrane was modeled as an electrical capacitor-resistor system with ionic current (I_{ion}) given by

\[ I_{ion} = I_{Na} + I_{K} + I_{Ca} + I_{Kr} + I_{cl} + I_{Ca^{2+}} + I_{K,ca} + I_{Cl} + I_{Na},K + I_{Na,K} + I_{Cl} \]

where \( I_{ion} \) includes contributions of Na\(^+\) current (\( I_{Na} \)); inward rectifier K\(^+\) current (\( I_{K} \)); transient outward current (\( I_{Kr} \)); ultrarapid (\( I_{Kr,ad} \)), rapid (\( I_{Kr} \)), and slow (\( I_{Ks} \)) delayed rectifier K\(^+\) currents; L-type Ca\(^{2+}\) current (\( I_{Ca} \)); Ca\(^{2+}\)-activated Cl\(^-\) current (\( I_{Ca,cl} \)); ACh-dependent K\(^+\) current (\( I_{K,ca} \)); sarcolemmal Ca\(^{2+}\) pump current (\( I_{Ca,p} \)); Na\(^+\)/Ca\(^{2+}\) exchanger current (\( I_{Na,ca} \)); Na\(^+\)-K\(^+\)-ATPase current (\( I_{Na,ATP} \)); and background Na\(^+\) (\( I_{Na,nu} \)), Ca\(^{2+}\) (\( I_{Ca,nu} \)), and Cl\(^-\) currents (\( I_{Cl} \)). APs were calculated by numerical integration of the relation

\[ \frac{dV}{dt} = \frac{I_{ion} + I_{stim}}{C_m} \]

where \( V \) is voltage, \( t \) is time, \( I_{stim} \) is the stimulus current, and \( C_m \) is membrane capacitance.

A two-dimensional rectangular atrial substrate was modeled as previously described (16, 18). The substrate consists of discrete cables representing muscle fibers, with each composed of a chain of myocytes that form a longitudinal syncytium. Fixed transverse resistors (10 MΩ) represent lateral coupling. Fiber resistivity and interfiber resistances were chosen to match experimental propagation characteristics. The discretization factor (segment length per length constant) was kept <0.1 to prevent numerical distortions (15, 36). Voltage-dependent expressions involving exponentials were precomputed with 50-μV resolution and stored in a table to increase computational efficiency (42). Activity was analyzed in substrates of 3 \( \times \) 6, 4 \( \times \) 8, 5 \( \times \) 10, 7.5 \( \times \) 15, and 10 \( \times \) 20 cm, including two smaller and two larger substrates than in our previous studies (16, 18). To change tissue size, we varied both the number of cables (180, 240, 300, 450, and 600 cables, respectively) and the number of cells per cable (360, 480, 600, 900, and 1,200, correspondingly). The atrial conduction velocity (CV) anisotropy ratio was 2.4:1, and longitudinal CV was 80 cm/s, compatible with experimental measurements of CV in the canine atrium (21).

**RESULTS**

**Substrate dimensions and AF maintenance.** We ran simulations for 60 size/[ACh]/[ACh] distribution conditions. For each condition, we first defined a S1S2 interval that induced at least five cycles of spontaneous activity and then analyzed the activity induced. This was possible for all but the smallest substrate (3 \( \times \) 6 cm), in which only one [ACh]/[ACh] distribution condition allowed for the induction of repetitive activity. Substrate size was clearly a strong determinant of AF duration (Fig. 1A). The roles of [ACh] and [ACh] distribution are illustrated in Fig. 1, B and C, respectively. The results for each mean [ACh] (Fig. 1B) are the average for all [ACh] distributions at that concentration, whereas the results for each [ACh] distribution (Fig. 1C) are the average for all [ACh]s at that distribution. For any mean [ACh] or [ACh] distribution, AF duration increased progressively as substrate dimension increased. Whereas mean [ACh] significantly determined AF duration, the spatial distribution of [ACh] was not a systematic determinant of AF duration. To verify that arrhythmic behavior was independent of the initial S1-S2 conditions, we varied the S1-S2 interval in the average-sized substrate (3 \( \times \) 10 cm) by 5-ms increments and observed up to 5 s of activity initiated by three different S1-S2 intervals for each [ACh] concentration/distribution condition. The results (Table 1) indicate that AF duration was relatively consistent for each [ACh] concentration/distribution condition and showed no systematic dependence on initial conditions.

**Mechanisms of perpetuation and termination of fibrillatory activity.** Figure 1 indicates that substrate size is indeed a determinant of the maintenance of fibrillatory activity. To evaluate potential underlying mechanisms, we studied the activation pattern during each simulation.

In our previous analysis of simulated AF in a 5 \( \times \) 10-cm substrate (18), most cases of longer-lasting AF were maintained by single dominant rotors. In the present study, activity triggered by S2 was always initially maintained by a single
clockwise rotor with its core tip located in a zone of low [ACh]. The fate of this rotor depended largely on [ACh]. For the highest mean [ACh] (corresponding to the largest [ACh] differences between peak and trough [ACh]), the initial rotor tended to remain strongly anchored in the same low-[ACh] zone. Figure 2 shows an example in a 4 × 8-cm grid. Figure 2A is an atrial TMP map at a single time point during sustained fibrillatory activity. A star indicates the position of the dominant rotor core tip, the dashed arrow shows the direction of rotation, and solid arrows show the directions of wavefront propagation. An animation of TMP and PS maps is shown in on-line movie 1, with an evolving TMP map at the top and corresponding PS trajectories at the bottom (see http://ajpheart.physiology.org/cgi/content/full/00252.2005/DC1). PS trajectories are color coded, with PSs lasting >1 s in green, PSs lasting >1 cycle but <1 s in blue, and PSs lasting <1 cycle in red. Instantaneous PS points are shown as bright color-coded dots, with the preceding 50 ms of each trajectory as a thick line and the complete previous PS trajectory history as thin lines. Running simulation time is shown in digital format. For the example illustrated, PS meander occurred but was never sufficient for the primary rotor core tip to escape its initial low-[ACh] zone location. Wavebreak occurred when the emanating impulse impinged on other low-[ACh] zones, generally producing two short-lived counterrotating daughter wavelets that mutually annihilated or drifted off the substrate surface. Mutual annihilation of counterrotating secondary waves resulting from wavebreak was strongly favored because of the sharp rotation of such waves, which restricted them to the same low-[ACh] zone.

Figure 2B shows all PS trajectories superimposed on an [ACh] map. The location of the primary rotor tip in a single low-[ACh] zone is evident, as are daughter wavelets, which self-annihilated by colliding with each other. The PS lifespan histogram (Fig. 2C) shows a large number of short-lived PSs
and one PS at the primary rotor wave tip that remained stable throughout the simulation. APs in the low-[ACh] core region (Fig. 2D) show great amplitude variability and a lower dominant frequency (Fig. 2E) than in the adjacent high-[ACh] zone (Fig. 2, F and G). Both display the irregular periodicity of fibrillatory activity.

Single rotor-based AF, as shown in Fig. 2, occurred typically at the highest mean [ACh] (7.5 nM). Of 17 simulations at 7.5 nM mean [ACh], AF was sustained by single rotors for the full simulation in 14 simulations (82%; Table 2). In 10 of these, a single stable rotor maintained AF throughout the simulation (SG-type activity, as in Fig. 2), whereas in 4 the initial rotor eventually terminated but was immediately replaced by another long-lasting rotor generated by a daughter wave. Fibrillatory activity was then maintained by SSG. Figure 3 and on-line movie 2 (see http://ajpheart.physiology.org/cgi/content/full/00252.2005/DC1) show an example in the 10-cm substrate. Figure 3A shows APs in the low-[ACh] core of the predominant rotor. Activation is clearly irregular. The PS trajectory map (Fig. 3B) shows the principal generator rotor in the upper center as well as two transient rotor zones lasting >1 s (green trajectories) in other low-[ACh] regions. Figure 3C shows TMP maps at four time points corresponding to the extinction and replacement of a primary rotor. The clockwise rotor is shown at 8,401 ms, with its core tip PS at the star. At 8,891 ms, two counterrotating daughter wavelets have formed with core tip PSs (small stars) in the same low-[ACh] zone. The primary rotor collided with one of these wavelets (dashed red line), causing mutual annihilation. However, the remaining daughter wave formed a stable rotor that took over primary generator function, as shown at 9,076 and 9,401 ms. The sequence of events can be visualized in corresponding running time in on-line movie 2. SSG activity tended to occur with larger [ACh] distributions, which allowed for the transient formation of multiple wavefronts with core tips within individual (relatively large) low-[ACh] zones.

In three cases, daughter waves succeeded in creating independent secondary rotors. For one of these, single rotors eventually resumed domination. In another, MGZ activity developed and remained stable for the entire simulation in a 5 × 10-cm substrate (for an example of MGZ activity, see Fig. 5 and the associated discussion below). In one case, MGZ activity terminated rapidly in the 4 × 8-cm substrate.

A consistent feature of the highest mean [ACh] simulations was strong anchoring in low-[ACh] regions, which tended to prevent drift of the primary rotor away from its initial location, as well as sharp curvature of daughter waves that result from wavebreak against other low-[ACh] zones, which favored mutual annihilation and prevented the production of long-lasting secondary rotors. Primary rotors were unlikely to ter-

Fig. 2. Atrial activity in a substrate of size 4 × 8 cm with mean [ACh] of 7.5 nM and [ACh] distribution of 1.67 cm. A: atrial transmembrane potential (TMP) map at 2,551 ms of the simulation (voltage scale at right). The large white star indicates the primary generator core tip phase singularity (PS) location, with the dashed arrow indicating the direction of rotation. Solid arrows indicate other wavefront propagation directions. B: PS trajectory map superimposed on the [ACh] distribution grid ([ACh] scale to right). C: PS lifespan histogram. D: action potentials (APs) from the core of the primary spiral (position shown by * in B). E: fast Fourier transform (FFT) of activity in D. F: APs from the periphery of the primary rotor (position shown by # in B). G: FFT of activity in F. (See also on-line movie 1.)
### Table 2. AF mechanism and duration in relation to substrate size, [ACh], and [ACh] distribution

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NI, noninducible, i.e. despite systematic S1S2 variation by 1 ms, no S1S2 could be identified that induced repetitive arrhythmic activity; SG, single dominant generator rotors; SSG, series of single dominant generator rotors; MGZ, multiple generator zones; SG-MGZ-SSG, single generator followed by multiple generator zones followed by reorganization to a series of single generators; MGZ-SG, multiple generator zones followed by a single generator rotor. For 1.875 and 3.75 nM mean [ACh] conditions, no AF could be induced in the 3 × 6-cm substrate.

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**Fig. 3.** Atrial activity in a substrate of size 10 × 20 cm with mean [ACh] of 7.5 nM and [ACh] distribution of 3.3 cm. A: atrial APs from the core region of the primary generator rotor. B: PS trajectory map superimposed on the [ACh] distribution grid ([ACh] scale to right). C: TMP maps at times indicated during the simulation. The large white star indicates PS at the core tip of the initial primary rotor. The dashed red line at 8,891 ms indicates annihilation between initial primary rotor and a daughter wave. The white star with the internal red asterisk indicates a generator rotor arising from a daughter wave at 8,891 ms. Dashed arrows show the direction of primary rotor rotation. Other arrows indicate directions of other wavefronts. For a discussion, see text (see also on-line movie 2).
minate by collision with boundaries or with secondary waves, because of strong anchoring. Consequently, inducible fibrillatory activity was long lasting at the highest mean [ACh] for the majority of simulations at all substrate sizes, and substrate size was not a major determinant of AF persistence under this condition.

In contrast to the well-anchored primary rotors observed at the highest mean [ACh], at lower mean [ACh]s (with smaller spatial [ACh] gradients), primary rotors tended to drift away from their initial locations. Primary rotor drift favored annihilation against a boundary or a daughter wave. In smaller sheets, primary rotor extinction due to such drift usually terminated AF. Figure 4 and on-line movie 3 (see http://ajheart.physiology.org/cgi/content/full/00252.2005/DC1) show an example of this type of activity in a $4 \times 8$-cm substrate with the lowest mean [ACh] (1.875 nM) and a 2-cm [ACh] distribution distance. APs from a low-[ACh] zone are shown in Fig. 4A, and the PS trajectory map is shown in Fig. 4B. TMP maps showing the progression of activity are provided in Fig. 4C. $S_2$ initiated a single clockwise-rotating rotor toward the middle of the substrate (Fig. 4C, top left). The PS at the core tip of this spiral showed much more meander (green trajectory in Fig. 4B) than for the example shown in Fig. 2, moving from the initial low-[ACh] area toward a low-[ACh] area to the upper left of the substrate at $\sim 880$ ms, returning toward the middle of the substrate (Fig. 4C, top right and bottom left), and then moving to another low-[ACh] area to the lower right part of the substrate at $\sim 2,300$ ms (Fig. 4C, bottom right). The latter area is adjacent to a border of the grid, and AF terminated at $\sim 2,400$ ms when the primary rotor drifted to the lower right corner and extinguished. In this example, several intermediate-lasting rotors established themselves temporarily in the lower left and far left portions of the grid, as reflected by the blue trajectories in Fig. 4B, but were annihilated by collisions with other waves or with borders. Because no longer-lasting secondary rotors were available to assume generator function at the time of primary generator rotor extinction, AF terminated.

The most important role of substrate size in determining AF maintenance was noted for the lowest mean [ACh]s. Figure 5 and on-line movie 4 (see http://ajheart.physiology.org/cgi/content/full/00252.2005/DC1) illustrate a simulation with the same [ACh] and spatial distribution as in Fig. 4 but in the largest computational substrate (10 x 20 cm). Because pairs of counterrotating secondary wavelets were not restricted in location by strong functional anchoring, they could avoid annihilation by separating from each other, leading to longer-lasting daughter waves that took over generator function upon initial primary rotor extinction. Reentry began with a single rotor in a central low-[ACh] area (see on-line movie 4). About 1 s after the initiation of reentry, a pair of counterrotating daughter waves was generated in an adjacent low-[ACh] zone and failed to annihilate against each other. One of them drifted to an adjacent, third low-[ACh] zone. Similar events continued to occur over time, with intermediate-lasting daughter waves avoiding annihilation by detaching from the low-[ACh] areas in which they were formed and drifting to adjacent areas. The number of intermediate- and long-lasting (blue and green PS) rotors was much larger than in the smaller substrate shown in Fig. 4. In addition, more low-[ACh] zones were located at a distance from boundaries, making extinction of spiral wave generators at boundaries less likely. Consequently, AF was sustained by multiple coexisting spiral wave generators that

![Fig. 4. A: atrial activity recorded from a low-[ACh] zone in a substrate of size $4 \times 8$ cm with mean [ACh] of 1.875 nM and [ACh] distribution of 2.0 cm. B: PS trajectory map superimposed on the [ACh] distribution grid ([ACh] scale to right). C: snapshots of TMP at times indicated. For a discussion, see text (see also on-line movie 3).](http://ajheart.physiology.org/)

Substrate Size and Atrial Fibrillation Maintenance

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formed, lasted for varying periods of time and then annihilated against invading wavefronts, only to be replaced by new generators.

TMP maps during this MGZ type of activity, as illustrated in Fig. 5A, resembled classical multiple circuit reentry. PS trajectory maps (Fig. 5B) showed numerous zones of long-lasting (green) and intermediate-lasting (blue) generator activity. The PS histogram in this case (Fig. 5C) showed 39 PSs lasting >1 s, reflecting a large number of generator rotors over the course of 20 s of activity. However, the longest-lasting PS was present for 1.79 s (in contrast to 20 s for examples of sustained SG activity), indicating the tendency of even long-lasting generators to be annihilated and the need for multiple generators to ensure continued activity. APs showed highly irregular activity (Fig. 5D), and the power spectrum was much more broad based (Fig. 5E) than for activity maintained by one or successive single generators (e.g., compare with Fig. 2, E and G). Sustained MGZ activity clearly did not depend on the persistence of a single primary generator but rather on the presence of multiple shorter-lasting generators able to maintain activity at all times.

In contrast to the highest mean [ACh] substrate, for which MGZ activity occurred in only three simulations, MGZ was observed for seven simulations at the lowest mean [ACh] and nine simulations for the intermediate mean [ACh] (Table 2). The duration of activity maintained exclusively by MGZ reentry showed a strong dependency on substrate size at the two lower mean [ACh] values, with such activity lasting under 1 s in the 4 × 8-cm substrate, <4 s in the 5 × 10-cm substrate, up to 10 s in the 7.5 × 15-cm substrate, and between 15 and 20 s in six of seven cases in the 10 × 20-cm substrate.

Properties associated with different mechanisms of AF maintenance. Two important contributors to perpetuation of activity were noted. The occurrence of stable SG activity was closely related to mean [ACh], with longer-lasting SG and SSG activity being very likely at high [ACh] irrespective of substrate size. Low mean [ACh] was tended to be associated with unstable, meandering primary generators, with AF persistence relying on MGZ mechanisms linked to substrate size.

Given the critical importance of mean [ACh] in governing the mechanisms underlying persistent activity in our model, we assessed the effects of [ACh] and [ACh] distribution on APD values and heterogeneity. Figure 6 shows the relationships between mean [ACh] and spatial [ACh] distribution on one hand and mean APD at and APD gradient (difference between largest and smallest APD at a frequency of 6 Hz). The primary determinant of mean APD was clearly mean [ACh] (Fig. 6A). Larger spatial [ACh] gradients were associated with slightly larger mean APD values, likely because of reduced electrotonic influences of short APD at high [ACh] regions on the longer APD zones. The APD gradient was predominantly determined by [ACh] but then, presumably because larger peak to trough [ACh] distances resulted in reduced APD smoothing by electrotonic interaction. Mean [ACh] was a
smaller determinant, tending to produce larger APD gradients as mean concentration increased. Mean [ACh] was also a significant determinant of activation rate during AF. The dominant frequency in the FFT histogram in the low-[ACh] core zone and in the high-[ACh] periphery of spiral wave generators averaged $8.7 \pm 0.1$ and $10.4 \pm 0.1$ Hz, respectively, at $1.75$ nM mean [ACh]; $9.3 \pm 0.1$ and $11.4 \pm 0.1$ Hz at $3.5$ nM; and $9.6 \pm 0.1$ and $12.1 \pm 0.1$ Hz at $7.5$ nM. The larger dominant frequencies at higher [ACh] reflect the shorter APD values, resulting in sharper rotation of reentrant wavefronts and smaller rotor cores.

The number of PSs reflects the number of wavebreaks that can potentially form daughter wave generators to sustain MGZ reentry. Figure 7 shows an analysis of the relationships among mean [ACh], [ACh] distribution, and substrate size on one hand and the number of PSs normalized to observation time (in ms). Larger mean [ACh] tended to decrease PS numbers (Fig. 7A), presumably by limiting drift, and smaller [ACh] distribu

tion distances increased PS numbers modestly by increasing the number of low-[ACh] zones per substrate (Fig. 7B). However, substrate size was clearly the strongest determinant of the number of PSs.

**DISCUSSION**

We used a mathematical model of canine atrial tissue to evaluate the role of substrate dimensions in determining the persistence of fibrillatory activity. Continuous activity could be maintained either under conditions that produced very stable primary generator rotors or conditions that resulted in substantial rotor meander and a high probability of eventual extinction of individual generator sources. The role of substrate dimension was most important for conditions associated with unstable meandering rotors, because the maintenance of continuous activity required the existence of a large enough number of longer-lasting rotors that extinction of all simultaneously was unlikely. For stable generator rotor conditions, fibrillatory activity, once successfully induced, tended to remain stable irrespective of substrate size.

**Relationship to previous conceptual models of AF.** There are essentially three conceptual schemas of AF, all rooted in notions developed in the early 20th century: 1) multiple circuit reentry; 2) single circuit reentry; and 3) sustained rapid ectopic activity (31). Garrey (8) and Mines (25) were the first proponents of multiple circuit reentry as the mechanism underlying AF. Moe (27) refined the multiple reentry circuit schema with his "multiple wavelet hypothesis" and investigated the conditions promoting fibrillatory activity in a computer model. Lewis (20) held that AF is due to a single primary rapidly rotating wave, with wavefront break-up against variably refractory tissue producing fibrillatory activity. For many years, the multiple circuit model was preeminent, with substantial support from clinical and experimental evidence (reviewed in Refs. 30 and 31). Lewis’ mother wave concept has recently received support from a range of experimental observations that point to single dominant rotors underlying AF in a variety of experimental paradigms (4, 5, 14, 24, 45).

When we initially developed the model described in the present study, we noted that AF was generally maintained by a single primary generator rotor (18). In the present study, we extended this work to a range of atrial substrate dimensions and to a longer simulation time, applying a recently developed method for automated PS identification and tracking (51) that permits the analysis of the complex activity that occurs in larger atrial substrates. This allowed us to refine the ideas that emerged from our previous analysis, by indicating that either dominant rotors (similar to Lewis’ mother wave) or multiple unstable reentrant generators (essentially multiple wavelet reentry) can underlie fibrillatory activity in the model, depending on the functional properties and dimensions of the atrial substrate. To our knowledge, the present analysis is the first to identify in a mathematical tissue model a spiral wave counterpart to Moe’s multiple wavelet hypothesis of AF.

We found that the MGZ mechanism, corresponding to multiple circuit reentry, was less likely than single rotor mechanisms (SG and SSG) to maintain fibrillatory activity for all conditions but the largest substrate. This finding supports the evolving notion that single rotor reentry with fibrillatory conduction is an important, and possibly predominant mechanism,
for the maintenance of AF (4, 14, 24, 40). However, in larger, diseased atria, mechanisms corresponding to MGZ activity may become more important and may account for greater difficulty in AF suppression and prevention.

Relationship to experimental evidence regarding AF mechanisms. Detailed experimental evidence for multiple wavelet reentry in cholinergic AF was first presented in 1985 (1). Subsequent studies provided epicardial mapping evidence supporting multiple circuit reentry in vagal (47) and tachycardia remodeling AF (9). The role of atrial wavelength as a determinant of AF provided further support for multiple wavelet reentry (35). However, other evidence has called into question the role of multiple circuit reentry. Rapid left atrial activity, often with an apparent dominant generator zone, underlies cholinergic AF in isolated sheep hearts (4, 14, 24, 40). In these studies, most wavelets were generated by passive wave break (4). In the present analysis, either single dominant rotors (Figs. 2 and 3 and on-line movies 1 and 2) or continuous unstable generator activity (Fig. 5 and on-line movie 4) sustained fibrillatory activity. Our observation of SG AF in very small substrates supports observations of fibrillation in mouse hearts (41) and the notion that such fibrillation may not require multiple circuit reentry.

Several of our observations parallel findings in experimental studies of cholinergic AF. ACh alters cardiac electrophysiology primarily by activating $I_{K_{ACH}}$ (18). $I_{K_{ACH}}$ is particularly important in atrial tissue and, when activated, can produce atrial hyperpolarization (removing voltage-dependent Na$^+$ current inactivation) and cause striking AP shortening. These effects provide greater source current for impulse conduction and allow for more rapid spiral wave rotation, accelerating and stabilizing atrial reentry (18). The importance of ACh actions are compounded by the spatial heterogeneity of effects associated with parasympathetic stimulation of the heart (22), which produces zones of lower [ACh] that serve as functional barriers, and therefore anchoring points, in close proximity to areas of higher [ACh] that serve as functional barriers, and therefore anchoring points, in close proximity to areas of higher [ACh]/action. These properties are analogous to the changes induced by altered amplitude of K$^+$ current, $I_{K_{1}}$, which appears to be a significant determinant of the stability of reentry and fibrillation in both atrial (33) and ventricular (37) tissues. We noted in the present study that lower mean [ACh]s were associated with meandering unstable rotors, whereas at higher concentrations single rotors stabilized and dominated activity. Schuessler et al. (39) observed multiple reentry circuits at moderately high [ACh] but single, dominant rotors that controlled the rhythm at the highest [ACh]s in canine atrial preparations. Ikeda et al. (12) noted meandering unstable wavefronts in the canine right atrium exposed to ACh. They subsequently described rapid nonstationary wavefronts during cholinergic AF in the canine right atrium, but stationary wavefronts associated with the shortest APDs, <30 ms (48).
Role of tissue size in AF maintenance. Garrey (8) first pointed to tissue size as a determinant of the likelihood of fibrillation, a notion supported by the persistence of AF in animals with different cardiac sizes (28). Atrial size is a significant determinant of clinical AF (10). Surgical procedures that reduce left atrial size may prevent recurrent AF (7, 38), and some of the benefit from linear ablation in AF may be due to decreased left atrial size (2). Smaller left atria are associated with higher conversion rates from AF (6). Our study supports the role of atrial size as a determinant of AF maintenance and provides potential mechanistic insights.

Consideration of the model. Compared with Moe’s original computer model of AF (27), the model we used is much more realistic. Moe’s model used a 992-unit array, with each unit having 5 discrete excitability states governed by an algebraic expression. The relative refractory period was much longer than in intact tissue, and there were no elecetrotonic interactions between functional units. Our model is based on cells of physiological size with APs controlled by realistic mathematical representations of canine ionic currents, with APs responding to various conditions similarly to experimental observations (34). Coupling, conduction, and anisotropic properties are appropriate for the dog (18).

On the other hand, the cellular substrate geometry is limited to a two-dimensional rectangular array. Three-dimensional complexities of in vivo atria, such as holes (venae cavae, pulmonary veins, and mitral and tricuspid valves), structural complexity (pectinate muscles and crista terminalis), and tissues with specific properties (septum and pulmonary veins) are not included. A more geometrically realistic three-dimensional model of the atria has been described (43) and provides insights into the potential involvement of various geometric structures in AF. However, other recent modeling studies of AF have similarly used two-dimensional planar arrays (32, 50), which remain instructive for asking specific questions such as the ones we posed in the present study. It will be interesting to determine in future work whether the principles defined in the present analysis continue to hold in more geometrically realistic models. Despite the simplifications inherent in our model, it has been shown in previous studies to agree closely with a range of experimental observations (16, 18) and with the results of more complex three-dimensional models (44). Of interest, the frequency peaks in FFT spectra in the present study are comparable to previous observations during vagal AF in the anesthetized dog (22, 46, 47).

We have previously observed that high, homogeneous [ACh]s stabilize reentry and greatly reduce meander of the spiral wave core tip (18). In the present study, we note that this anchoring effect of high [ACh]s results in very stable dominant reentrant circuit mechanisms by suggesting that either may support a sufficient number of meandering rotors that the probability of all being extinguished simultaneously is very low, a mechanism that cannot occur in smaller substrates. These observations support the notion that larger substrates favor AF persistence but indicate that the importance of this complicating feature of cardiac disease, geometric complexities, autonomic factors, etc. In addition, our model deals only with fibrillatory activity of the reentrant type and is not relevant to forms of AF that may be due predominantly to rapid ectopic activity as believed to arise from the pulmonary veins in some patient populations (31).

In conclusion, we found that atrial substrate size is a potentially important determinant of the maintenance of fibrillatory activity in our model. Unlike our previous study, which found a predominance of single rotor mechanisms in a substrate of fixed, 5 × 10-cm dimensions, the present analysis shows that either single dominant rotors or multiple unstable rotor-based reentry can underlie persistent fibrillatory activity in an atrial model. Although AF can be maintained in relatively small substrates by single dominant rotor mechanisms, larger substrates allow for persistent AF more easily because they can support a sufficient number of meandering rotors that the probability of all being extinguished simultaneously is very low, a mechanism that cannot occur in smaller substrates. These observations support the notion that larger substrates favor AF persistence but indicate that the importance of this phenomenon depends on the mechanisms underlying AF. Furthermore, these findings speak to recent debates regarding the relative importance of single driver rotors versus multiple reentrant circuit mechanisms by suggesting that either may maintain AF depending on atrial size and electrophysiological properties.

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REFERENCES


40. Wu TJ, Kim YH, Yoshima M, Athill CA, Ting CT, Karagueuzian HS, and Chen PS. Promotion of atrial fibrillation by 10.220.32.247 on August 28, 2017 http://ajpheart.physiology.org/ Downloaded from