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Reconsidering the Multiple Wavelet Hypothesis of Atrial Fibrillation

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Abstract

Background: Moe/Abildskov proposed the multiple wavelet hypothesis of atrial fibrillation (AF) based on observations in the canine vagal nerve stimulation (VNS) AF model. Data from mapping studies in *in vitro* canine AF model by Allesie et al. evaluated the Moe/Abildskov hypothesis, concluding that a critical number of wavelets sustained AF.

Objective: To reassess VNS mapping data using the same methods used by Allesie to evaluate Moe's multiple wavelet hypothesis.

Methods: Using the canine VNS AF model in 6 dogs, 510 unipolar AEGs were recorded simultaneously from both atria. Activation sequence maps were produced from sustained AF during VNS in each dog. Per Allesie, consecutive 10ms activation windows were analyzed during 300ms. Repetitive activation analysis was applied to Moe's canine VNS AF model.

Results: The number of wavefronts in each AF episode was 0–8 in Allesie's studies measured by sequential atrial mapping; 0–10 in our bi-atrial simultaneous mapping studies. In both studies, an electrically silent period was observed in each atrium, and was reactivated by wavefronts emanating from focal sources. Allesie postulated that an electrically silent atrium was reactivated by a wavefront propagating from the other atrium. However, in our bi-atrial simultaneous mapping studies, each electrically silent atrium was reactivated by a distinct focal source.

Conclusions: Data from both studies showed a similar number of wavefronts, similar AF activation patterns, and periods of an electrical atrial silence reactivated by focal sources. Also, in our studies, independent focal sources initiated wavefronts reactivating the atria, thereby explaining the mechanism maintaining AF.

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Keywords

mechanism of atrial fibrillation; mapping; focal source; reentry; vagal nerve stimulation

The Journal Subject Codes

Arrhythmia and Electrophysiology (Atrial Fibrillation)

Introduction

For many decades, the multiple wavelet hypothesis (multiple wandering wavelets) has been a major conceptual mechanistic model of AF. Moe and Abildskov proposed the multiple wavelet hypothesis of AF based on observations during induced AF in the canine vagal nerve stimulation (VNS) model.^{1, 2} But no mapping was done. According to the multiple wavelet hypothesis, AF is maintained by multiple wavelets randomly propagating throughout both atria along varying routes, primarily determined by refractory periods of the tissue. Moe et al. developed a computational model that indicated that 23 – 40 such random wandering wavelets were necessary to sustain AF.³ Later, data from mapping studies of induced AF in a Langendorff perfused, acetylcholine infused, in vitro canine atrial model by Allesie et al. evaluated the Moe hypothesis, and concluded that only 4 – 6 simultaneously circulating, random, wandering wavelets were necessary to sustain AF.⁴ Based on their mapping studies, they assumed that periods of an electrically silent atrium most likely were reactivated by a wavefront propagating from the other atrium, even though the latter was not mapped. However, our recent mapping studies of the original canine Moe/Abildskov model showed that in contrast to their prediction, focal sources drove the atria, thereby maintaining AF.⁵ All wavefronts emanated from focal sources, largely either colliding or merging with each other at variable sites, or encountering refractory tissue. Although Allesie et al. studied a different canine model than our studies, both studies observed that all wavefront propagation consisted of collision or merging with another wavefront or encountering refractory tissue. The objective of our study was to evaluate the Moe/Abildskov multiple wavelet hypothesis by applying the same methods and criteria used by Allesie et al. to our mapping data of the Moe/Abildskov model. When considering Moe's multiple wandering wavelet hypothesis, we further investigated repetitive activation patterns during AF in the canine Moe/Abildskov model.

Methods

Animal experimental protocols were approved by the Case Western Reserve University Institutional Animal Care and Use Committee. All studies were performed in accordance with the guidelines specified by our Institutional Animal Care and Use Committee, Department of Agriculture Animal Welfare Act, Public Health Service Policy on Humane Care and Use of Laboratory Animals, and Association for Assessment and Accreditation of Laboratory Animal Care International.

Six adult mongrel canines weighing 18–23 kg were studied and anesthetized with pentobarbital (20– 30 mg/kg intravenously), maintained on isoflurane 2% gas anesthesia,

and mechanically ventilated using a Boyle anesthesia machine. Using standard techniques previously described,⁵ a sternotomy was performed to expose the normal heart in a pericardial cradle. A pair of plunge wire electrodes were placed in the epicardial surface of the right atrial appendage (RAA), posteroinferior left atrium (PLA), and the mid-portion of Bachmann's bundle (BB) for monitoring heart rhythms, and for cardiac pacing as needed. The right cervical vagus nerve was prepared for electrical stimulation. As a physiological test, the vagus nerve was stimulated to produce sinus cycle length (CL) prolongation of at least 50% using a custom-made, tripolar, flat interface nerve electrode (frequency: 20 Hz, pulse width: 2 ms, amplitude: 2–7.5 mA). During VNS resulting to achieve at least 50% sinus CL prolongation, AF was induced by rapid atrial pacing for 3–7 beats at a CL of 75 milliseconds from one of the atrial epicardial electrodes (RAA, BB, or PLA), and maintained by constant VNS (> 5 minutes). With the cessation of VNS, sustained AF spontaneously terminated within 1 min. The entire protocol was recorded using a Bard LabSystem PRO (Bard Electrophysiology, MA, USA) to record ECG lead II with atrial electrograms (AEGs) from the RAA, BB, and PLA sites. Four of six canines (#1–4) in this study were the same animals as in the recent study.⁶ An additional two animals (#5–6) were unique to this study only.

Data Acquisition:

During AF, AEGs were recorded from arrays of 510 electrodes placed on the epicardial surface of both atria along with one ECG channel and one marker channel (total: 512 electrodes). An electrode array containing 438 unipolar electrodes arranged in pairs was placed on the right atrium (276) and on left atrium (162), and secured in place with a hook-and-loop belt (Velcro straps). Another array of 48 electrodes arranged in pairs was placed separately on BB, and one of 24 electrodes was placed separately between the pulmonary veins. The interelectrode distance of each bipolar pair was 0.7 mm, and the distance between each bipolar electrode pair and its neighbor site ranged from 2 to 6 mm. Using our custom-made, simultaneous, bi-atrial, high density, epicardial mapping system, all AEGs were individually amplified at 1000 gain, band-pass filtered at 0.05–500 Hz, sampled at 1 kHz, and digitized at 12 bits. Data were transferred in real-time and stored on a personal computer for offline analysis (CEPAS, Cuoretech Pty Ltd, Sydney, Australia).

Activation sequence analysis:

Analysis was based on sequential time windows determined by the shortest CL during AF. For each episode, data from 12 consecutive time windows from one or more selected portions of the recorded episode were analyzed, and the activation sequences maps were drawn with 10 ms isochrones. Once the activation sequence maps were constructed, focal activation sites were identified, and the morphology of the unipolar AEGs was characterized at those sites. Per Allessie et al.,⁴ 30 consecutive 10 ms activation windows were analyzed over a period of 300 ms. One critically important difference between our study and Allessie was that we recorded simultaneously from a total of 510 epicardial electrodes covering the epicardium of both atria, whereas Allessie et al. recorded from 192 endocardial electrodes sequentially from each atrium. Also, we further examined the reactivation of the electrically silent periods during AF. All bipolar AEGs were subjected to CL variability and dominant frequency (DF) analyses to detect mean CL, standard deviation, and DF.⁷ Each analysis of

bipolar AEGs was performed on 4-sec segments of data. DF maps were generated. Data are presented as the mean CL $\pm$ standard deviation, since 1,000/DF is equivalent to the mean CL.

Repetitive activation pattern analysis:

We applied our repetitive activation analysis to the Moe/Abildskov canine VNS AF model. Local activation times were selected using the previously described algorithm.⁷ Each bipolar site was assigned a 2 $\times$ 2 grid of surrounding neighbor's sites. Activation times of each bipolar site, and four surrounding bipolar sites were calculated in the determination of focal QS or RS activation, a rotor activation, and a wavefront entering from the edge of mapping array (See supplementary). Repetitive activation pattern analysis was performed using customized software (CEPAS, Madry Technologies, Sydney, Australia). To identify repetitive activation patterns and their sources, i.e., focal QS or RS activation, rotors, or a wavefront entering from the edge of mapping array, we analyzed 4 seconds of data during sustained AF in each dog. A repetitive activation pattern was defined as three or more consecutive beats producing consistent, recurrent activation wavefront propagation patterns.

Statistical analysis:

Data are presented as the mean $\pm$ standard deviation using Minitab (Minitab Inc, State College, PA).

Results

Analysis of AF Activation Patterns using the same methods and criteria as Allesie et al.

Six episodes of sustained AF during VNS were analyzed using the same methods and criteria used by Allesie et al. Table 1 shows summary data for the six studies. The number of wavefronts identified in the atria of each episode of AF was 0 – 8 in the Allesie et al. studies, and 0 – 10 (mean 4.2 ± 1.2) in our studies. Also, Allesie et al. were only able to map one atrium at the time, and found electrically silent periods in each atrium during AF. In our bi-atrial simultaneous mapping studies, electrically silent periods occurring in both atria simultaneously were observed in 2 episodes for 20 ms. Additionally, electrically silent periods were observed in an atrium (1 – 5 per episode, mean duration 26 ± 16 ms, range 10 – 50 ms). Figure 1 shows a 30 consecutive 10 ms activation window from only the RA by Allesie et al. (panel A), and from both atria from our mapping data (panel B). In panel A, during windows 18 and 19, electrically silent periods were observed in the RA, and were reactivated by wavefront emanating from focal activation (window 20). During windows 13 and 14 in panel B, electrically silent periods were observed in both atria, and were reactivated by wavefronts emanating from focal sources in the posterior LA and RA. In both aforementioned studies, electrically silent periods were observed in each atrium, and were reactivated by wavefronts emanating from focal sources (foci or breakthrough sites). Allesie et al. postulated (assumed) that an electrically silent atrium was reactivated by a wavefront propagating from the other atrium due to the inability to map both atria simultaneously. However, in our bi-atrial simultaneous mapping studies, each electrically silent atrium was reactivated by a distinct focal source (QS morphology, fig 2C), and was not reactivated by wavefronts propagating from the other atrium. Neither atrium was

reactivated by epicardial wavefronts propagating from the other atrium. In all episodes, the LA was reactivated by wavefronts emanating from focal sources in the posterior LA, left BB, left inferior pulmonary vein (PV) area, and left superior PV area. The right atrium (RA) was reactivated by wavefronts emanating from focal sources at the high RA, sulcus terminalis, and right BB.

Analysis of AF Activation Patterns and Dominant Frequency Analysis

Figure 2 is a representative example of the same episode (figure 1B) of sustained AF due to focal sources of different CL. Panel A shows the activation sequence map of four consecutive beats during AF on both atria. Panel B shows the bipolar AEGs from selected sites *a* through *f* recorded simultaneously during AF from a focal QS activation (site *b*) and 5 nearby sites (*a*, *c*, *d*, *e*, and *f*). Selected AEGs are shown along with burst symbols and propagation arrows illustrating activation in panel A. Panel C shows selected bipolar AEGs along with each unipolar component (QS morphology) of the bipolar AEG from the focal QS activation (site *b*). Focal QS activation originating near the posterior LA produced a wavefront that propagated toward the left atrial appendage, where it collides with another wavefront from a focal RS activation in the middle of BB. It also propagates toward the RA, where it collides with a wavefront from a focal QS activation in the RA. The repetitive activation patterns generated from focal QS activation produced collisions of wavefronts at variable sites or encountered refractory tissue. DF analysis of 4 seconds of data during the same episode (fig 1B and fig 2) is shown in Figure 3. DF analysis demonstrated that a group of adjacent sites with a single DF peak at 8 Hz was present in a portion of the LA, confirming the presence of an area of repetitive activation which corresponded to 1:1 atrial activation during AF. In short, DF analysis supports the presence of a regular activation source sustaining AF.

With the cessation of VNS, focal sources disappeared, and AF terminated in all episodes. Figure 4 shows the spontaneous termination of the episode from fig. 2 after stopping VNS. Panel A shows the last five consecutive 80 ms windows of activation sequence. Panel B shows the bipolar AEG from the same focal QS site *b* in fig. 2, along with each unipolar component. Following termination of VNS, panel A shows that the two sites of focal QS activation were still present (the posterior LA free wall and the RA). When all wavefronts emanating from focal sources extinguished, and there was no further focal activation, there was an electrically silent period of 477 ms in both atria. This pause may be due to overdrive sinus node suppression prior to resuming sinus rhythm.

Repetitive Activation Pattern Analysis of AF

Table 2 shows a summary of the repetitive activation pattern analysis during AF. In all AF episodes, repetitive focal activations were observed in both atria from focal QS and RS AEGs (mean 2.2 ± 1.2 , range 1 – 4; and mean 2.8 ± 1.9 , range 1 – 6, respectively; total mean 5 ± 2.6 , range 2 – 7). Wavefronts entering from the edge of mapping array that produced a repetitive activation pattern were observed in 5 episodes (mean 3.2 ± 2.2 , range 0 – 6). The durations of repetitive activations patterns from each source were 1) mean 1.6 ± 1 sec., range 0.7 – 3.3 secs. from focal QS AEGs; 2) 1 ± 0.5 sec., range 0.4 – 2.3 secs. from focal RS AEGs; and 3) 1.2 ± 0.6 sec., range 0.4 – 2.4 secs. from a wavefront entering from the edge

of mapping array. During AF, at least 3 regions of a repetitive activation pattern were observed at all times. No rotors were detected. Repetitive activation patterns were observed during AF in the Moe/Abildskov model. However, no multiple wandering wavelets perpetuating AF were seen.

Discussion

In our simultaneous, bi-atrial, high density mapping studies in the canine Moe/Abildskov model, the major findings of our study were 1) using method and criteria by Allesie et al. in the canine Moe/Abildskov model, independent focal sources initiated wavefronts reactivating the atria, thereby explaining the mechanism maintaining AF in the Moe/Abildskov model of AF; 2) a minimum of 3 repetitive activation patterns were observed at all times; 3) No multiple wandering wavelets were found. Therefore, the repetitive activation patterns emanating from focal activation sources played a critical role in the maintenance of AF.

Repetitive activation patterns during AF in the animal model and in patients

Over the past 60 years, the multiple wavelet hypothesis has been a dominant conceptual model of AF. However, with improved mapping technologies applied to electrophysiological studies of AF in animal models and patients, a repetitive activation pattern (driver mechanism) of AF has been demonstrated. In *in vitro* animal models of AF using cholinergic stimulation, many mapping studies have been performed for several decades. In a right atrial preparation, with increasing acetylcholine concentrations, activation patterns converted from multiple wandering wavelets to a repetitive reentrant circuit.⁸ Also, optical mapping in a sheep atrial model perfused with acetylcholine demonstrated repetitive rotating activation (stable rotor) as a predominant mechanism that sustains AF.^{9, 10} Also, a recent study using optically mapped canine cholinergic AF preparations demonstrated that the predominant mechanism for maintaining AF was due to a repetitive focal activation.¹¹ A study in *in vitro* explanted human right atrial cholinergic AF preparations has recently shown that epicardial focal activation is due to repetitive transmural reentrant activation around atrial fibrosis that drives the atria, thereby sustaining AF.¹² Also, we are aware that the same group, using simultaneous optical and clinical contact mapping with cardiac magnetic resonance imaging in *in vitro* explanted human hearts, demonstrated that clinical contact mapping with intramural 3D fibrosis distribution may help distinguish between true-positive localized repetitive activation sources and false-positives, which could improve the success of targeted ablation.¹³ In an *in vivo* canine model of AF, either congestive heart failure or canine sterile pericarditis, using high density contact mapping, a repetitive reentrant circuit or focal sources was demonstrated as a predominant mechanism sustaining AF.^{14, 15} In patients with persistent and long-standing persistent AF, there is enough evidence that AF is driven by localized repetitive activation sources in the atria, such as focal sources or different types of reentry, including rotors.^{16–25} Using different types of mapping technologies and analysis algorithms, targeted ablation of localized repetitive activation patterns during AF have been successfully performed to achieve acute termination of AF.^{16, 17, 21, 23–25} Although there is a controversy regarding reported predominant activation

patterns (rotational vs. focal), neither repetitive activation patterns supports the multiple wavelets hypothesis.

Clinical Implication

Although AF in the Moe/Abildskov model is not spontaneous and is an acute model in a normal heart, it has several clinical implications. Our data from this study may be the mechanism of vagally mediated AF. The data we have described demonstrate that repetitive focal sources at different CL can perpetuate AF. This, in turn, suggests a potential target for AF ablation.

Limitation

Moe and Abildskov used pentobarbital (a vagolytic agent) anesthesia, but we only used pentobarbital for induction of anesthesia. However, we used the same method to calibrate vagal stimulation strength, so that this different methodology is not an issue. AEGs from the atrial septum and endocardium were not recorded in our study, precluding the identification of the source of the repetitive focal RS activation or repetitive wavefront entering the edge of the array. Also, the measurement of electrically silence periods was performed in the epicardium of both atria. However, the Moe/Abildskov model we studied is an acute model in a normal heart. Therefore, it is unclear if there were epi-endo dissociation activations or epi-endo reentry. So, we cannot rule out the possibility that repetitive focal RS activation was the result of epi-endo reentry or endocardial reentry. Finally, ablation of repetitive focal activation to terminate AF could not be performed, as all activation sequence analyses during AF were performed offline.

Conclusion

Data from both studies showed a similar number of wavefronts and similar activation patterns during AF. The data also showed periods of electrical atrial silence reactivated by focal sources. In our simultaneous, biatrial, high density mapping studies, independent repetitive focal sources initiated wavefronts reactivating the atria, thereby explaining the mechanism maintaining the Moe/Abildskov model of AF. No multiple wandering wavelets perpetuating AF were seen. No multiple wandering wavelets or rotors were found. Additionally, due to the fact that repetitive activation patterns (3) were observed during AF, the multiple wavelet hypothesis of AF, considered for many years to be the mechanism that sustains AF, is not supported by our data from the Moe/Abildskov model.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A. 30 Consecutive 10 ms activation windows from ONLY the right atrium by Allesie et al.

B. 30 Consecutive 10 ms activation windows from both atria from our mapping data (dog #1)

Figure 1.

30 consecutive 10 ms activation window from only the RA by Allesie et al. (panel A), and both atria from our mapping data (panel B, Dog #1). **Panel A:** During windows 18 and 19, electrically silent periods were observed in the RA and were reactivated by wavefront emanating from focal activation (window 20). **Panel B:** During windows 13 and 14, electrically silent periods were observed in both atria, and were reactivated by wavefronts emanating from focal sources in the posterior LA and RA. **LA**, left atrium; **RA**, right atrium.

A. AF activation sequence map of 4 consecutive 80 ms windows

B. Bipolar AEGs from selected sites *a* through *f*

C. Selected bipolar AEG along with each unipolar component (QS morphology) of the bipolar AEG from the sustained focus (*site b*)

Figure 2.

A representative example of the same episode (fig. 1B) of sustained AF due to focal sources of different CL. **Panel A:** Activation sequence map of four consecutive beats during AF in both atria. **Panel B:** Bipolar AEGs from selected sites *a* - *f* recorded simultaneously during AF from a focal QS activation (*site b*) and five nearby sites (*a*, *c*, *d*, *e*, and *f*). Selected AEGs are shown along with burst symbols and propagation arrows illustrating activation in panel A. **Panel C:** Selected bipolar AEGs along with each unipolar component (QS morphology) of the bipolar AEG from focal QS activation (*site b*). See text for discussion.

Dominant Frequency analysis

Figure 3.

DF analysis of 4 seconds of data from the same episode (fig. 1B and fig. 2). DF analysis demonstrated that a group of adjacent sites with a single DF peak at 8 Hz was present in a portion of the LA, confirming the presence of an area of repetitive activation patterns, which corresponded to 1:1 atrial activation. **DF**, dominant frequency; **LA**, left atrium

A. Termination of sustained AF after cessation of VNS.

B. Selected bipolar AEG along with each unipolar component of the bipolar AEG from the same focus (site b in fig 2)

Figure 4.

Spontaneous termination of the episode from fig. 2 after stopping VNS. **Panel A:** The last five consecutive 80 ms windows of activation sequence during AF followed by sinus rhythm.

Panel B: Bipolar AEG from the same focal QS site b in fig. 2, along with each unipolar component. When all wavefronts emanating from focal sources became extinguished and there was no further focal activation, there was an electrically silent period of 477 ms in both atria, followed by sinus rhythm. **AEG**, atrial electrograms; **VNS**, vagal nerve stimulation

Table 1.
Summary of analysis data during AF using the same methods and criteria used by Allesie et al.

Dog	# foci/window (range)	Mean of # wave front (range)	Electrically silent period in each atrium (ms)
1	1.5 (0 – 3)	3 ± 1.6 (0 – 5)	10 (RA)
			30* (RA)
			20* (LA)
			50 (RA)
2	1.4 (1 – 2)	4 ± 1.5 (2 – 7)	20 (RA)
3	1.0 (0 – 2)	5 ± 1.3 (2 – 7)	10 (LA)
4	2.4 (1 – 4)	4 ± 1.9 (2 – 9)	10 (RA)
			20 (RA)
			10 (LA)
5	2.7 (0 – 3)	6 ± 1.9 (4 – 10)	10 (LA)
6	1.8 (1 – 2)	2.8 ± 1.4 (0 – 6)	20 (RA)
			50* (RA)
			40* (LA)
			40 (LA)
			50 (RA)
Mean	1.8 ± 0.6	4.2 ± 1.2	26 ± 16

LA, left atrium; RA, right atrium;

* , 20 ms bi-atrial silent periods

Table 2.

Summary of the repetitive activation pattern analysis during AF

Dog	Focal QS activation		Focal RS activation		Rotor activation		Wavefront entering the edge of the array	
	#	Duration (location)	#	Duration (location)	#	Duration (location)	#	Duration (location)
1	4	3.3 sec (LA)	3	1 sec (RA)	-	-	4	2.4 sec (RAA)
		2.2 sec (LA)		1.7 sec (RA)				2.3 sec (RA)
		2 sec (LA)		1.3 sec (LA)				1.9 sec (LAA)
		1.4 sec (RA)						1.4 sec (BB)
2	1	4 sec (RA)	2	2.3 sec (RA)	-	-	6	2.3 sec (LA)
				0.4 sec (LA)				1.3 sec (RA)
								1.2 sec (LA)
								0.7 sec (LA)
3	2	1.1 sec (LA)	6	1 sec (LA)	-	-	5	1.3 sec (BB)
		1 sec (RA)		1 sec (LA)				1.1 sec (RA)
				0.8 sec (RA)				1 sec (LA)
				0.7 sec (RA)				0.7 sec (LA)
4	3	1.4 sec (LA)	4	0.9 sec (RA)	-	-	2	0.9 sec (RA)
		1.1 sec (RA)		0.8 sec (RA)				0.7 sec (LA)
		0.7 sec (RA)		0.6 sec (RA)				
5	2	1.1 sec (LA)	1	1.3 sec (LA)	-	-	-	
		0.7 sec (RA)						
6	1	1.2 sec (RA)	1	1.2 sec (RA)	-	-	2	1 sec (RA)
								1 sec (RA)
Mean	2.2 ± 1.2	1.6 ± 1 sec	2.8 ± 1.9	1 ± 0.5 sec	-	-	3.2 ± 2.2	1.2 ± 0.6 sec