The effects of long chain polyunsaturated fatty acids on local activation properties in dogs vulnerable to atrial fibrillation

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***Abstract*— Marine derived long chain polyunsaturated fatty acids (PUFAs) were found to have benefits in reducing inducibility and maintenance of atrial fibrillation (AF) in a dog model. This study was conducted to evaluate the effect of PUFAs on local atrial electrical conduction properties acquired via a multi-electrode plaque sutured to the posterior wall of the left atrium of the heart in these dogs. Eleven dogs underwent simultaneous atrioventricular pacing (SAVP) for 2 weeks, and were organized into 2 groups: 5 dogs received no PUFAs (SAVP-PLACEBO), 6 dogs received Eicosapentaenoic or Docosahexaenoic acid derived from fish oils (SAVP-PUFA), where PUFAs were given for 21 days, starting 1 week prior to pacing and during the 2 week pacing period. Three features were extracted, which were the average conduction velocity, average intra atrial conduction time, and total activation time. The PUFA group had a faster average conduction velocity (0.82±0.19 m/s) than the PLACEBO group (0.47±0.21 m/s, P=0.02). Using the average conduction velocity feature, classification was performed with a linear classifier and leave-one-out method. In the SAVP-PLACEBO group, 60% of the dogs were correctly classified, and 66% of the dogs were correctly classified in SAVP-PUFA group, leading to an overall classification accuracy of 63.5%.**

I. INTRODUCTION & BACKGROUND

Atrial fibrillation (AF) is a common cardiac arrhythmia. Approximately 350,000 Canadians are affected by AF [1]. During AF, the regular electrical activity which leads to rhythmic contraction of the two upper chambers of the heart (the atria) is overwhelmed by chaotic electrical activity which causes uncoordinated contraction of the atria. As a result, blood is not pumped effectively out of the atria to the ventricles; this increases the possibility of blood clots and stroke [1]. Individuals with AF have a 3 to 5 times increased risk of stroke [1].

Previous studies in a dog model showed that marine derived long chain polyunsaturated fatty acids (PUFAs) have beneficial effects in the treatment of AF. In cases where AF vulnerability is induced by changes to atrial structure such as scarring and hypertrophy, PUFAs reduce incidence and maintenance of AF. A potential mechanism for this is a PUFA mediated decrease in conduction time across the atria [2], [3]. Increasing conduction velocity is known to decrease AF by improving the probability that chaotic electrical waves will collide and annihilate one another [4]. Previous attempts have been made to study "global" atrial conduction (from one atria to the other) and "local" atrial conduction (across a small area within one atrium) in this model [2], [3]. However, this study aimed to make a detailed analysis of the effect of PUFAs on "local" atrial electrical conduction properties in a small area of the posterior wall of the left atrium known to be important in the genesis of atrial fibrillation [5].

II. DATABASE AND PROTOCOL

The study was conducted on 11 dogs that were organized into 2 groups based on receiving PUFA (Eicosapentaenoic or Docosahexaenoic acid) or not. The 11 dogs underwent simultaneous atrioventricular pacing (SAVP) at 220 beats per minute for up to 2 weeks, in which both the atria and the ventricles were paced rapidly and simultaneously, resulting in substantial structural remodeling of the atria and vulnerability to AF. Experimentally induced episodes of AF were recorded from one of the following regions: right atrial appendage (RAA), left atrial appendage (LAA), superior vena cava (SVC), or inferior vena cava (IVC). The database of the study contains two groups:

1) SAVP-PLACEBO group: 5 dogs were paced for 14 days and received no PUFAs, and 5 episodes were recorded.
2) SAVP-PUFA group: 6 dogs were paced for 14 days and received PUFAs 7 days prior to pacing and also during the two weeks pacing duration (drug received for total of 21 days), and 9 episodes were recorded.

The Animal Use Protocol (AUP) was approved by St. Michael's Hospital, Toronto, ON, Canada and the investigation conforms to the Guide for the Care and Use of Laboratory Animals [6].

III. METHODS

The data acquired via the multi-electrode plaque attached to the posterior wall of the left atrium was loaded into a mapping software that displays one electrogram for each of the 23 electrodes in the plaque. A single beat was chosen for analysis and the instant of atrial activation was manually marked on each of the 23 electrograms by one of the authors (R.A.). Markings in 12 episodes out of a total of 14 episodes were verified by another author (A.R.). Atrial activation was selected at the point where the sharpest slope (the largest change in voltage with time, the highest dV/dt) occurred. The files were then exported and the marked atrial activations were used to construct two types of maps, an activation time map and a conduction velocity map. These maps were then used to extract distinguishable features in the 3 groups of dogs. Afterwards, the quantified features were fed into a

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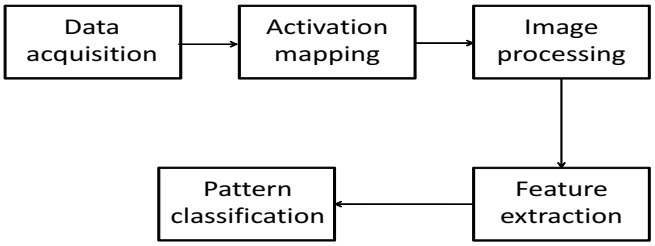


Fig. 1: Block diagram showing the methods followed to conduct the study

linear classifier to determine the significance of these features in separating the groups. Fig 1 presents a block diagram that includes the methods followed in this study.

A. DATA ACQUISITION

Data were recorded using a multi-electrode plaque that was sutured to the posterior wall of the left atrium. The plaque consists of 23 electrodes with an inter-electrode distance of 2.7mm, and an area of 192mm^2. Compared to the other recording systems used in previous studies, in addition to capturing the "local" atrial activations, this plaque also has more electrodes distributed with small inter-electrode distance in between, which in turn provides more precise information for the analysis and better resolution.

B. ACTIVATION MAPPING SOFTWARE

Data recorded by the plaque (electrograms taken from the 23 electrodes), were loaded into a mapping program which displays these electrograms and allows annotations. Local atrial activations were marked at the locations of the sharpest slope that is seen within a certain atrial beat. Atrial beats were identified to be between two consecutive ventricular beats. In some complex AF cases, the atrial beats couldn't be clearly determined, however, ventricular beats were clearly seen, and were therefore used as a reference to determine the atrial beats. For each episode in each dog, 3 atrial beats were analyzed. One when the recording started, one at the middle, and one at the end of the recording. After marking the local atrial activations in 3 beats in each episode, two parameters were exported: the geometry of the plaque along with the position of the 23 electrodes, and the corresponding activation time at each electrode. These two parameters were used to construct the activation time map and the average velocity map.

C. IMAGE PROCESSING

The position of the 23 electrodes (identified with black points in the activation time maps in Fig 2 and conduction velocity map in Fig 3) and their corresponding activation time were used to construct the activation time map and conduction velocity map in order to visualize the propagation of the electrical activity in the left atrium region and evaluate differences that may exist between the groups of dogs.

1) ACTIVATION TIME MAPS: The exported atrial activation time at each of the 23 electrodes were assigned into an array of <23x1> size. To construct the activation time map, another array of <67x59> size was interpolated from the original array where the points in the interpolated array that are positioned at location of the 23 electrodes carries the corresponding electrode activation time taken from the original array, and the activation time at all other points in the interpolated array are approximated by interpolating the activation time values in the closest electrodes around. This interpolated array was used only for visualizing purposes in order to view the propagation of the electrical activity and the pattern of the local atrial activations. Any other statistical information used in this study was taken from the original array that carries the actual information and not the interpolated values. The interpolated array was then mapped with a color scale that ranges from blue to red, with the blue colored regions being activated first, and red colored regions activated last. In order to compare the activation time maps for the different dogs, all the maps were generated with a maximum time of 60ms, which provides meaningful color variation that can be easily related to the propagation of electrical activity and the sequence of electrode activation. The black color in the activation time maps are in the regions where there is no data available and therefore is not included in the analysis. The color bar on the right side of the maps shows a range (blue to red) between 0 to maximum time (60 ms). In Figs 2a and 2d for example, it can be seen that the electrodes at the center of the plaque and the upper part of the plaque were activated earlier, followed by the activation of the electrodes in the lower part of the plaque.

However, other cases such as the one shown in Figs 2b and 2e didn't show enough information to understand the propagation direction of the electrical wave, this is because most of the electrodes were activated at an early time (below 30ms). For this case, the activation time map was exported with a maximum time of 20ms as shown in Figs 2c and 2f.

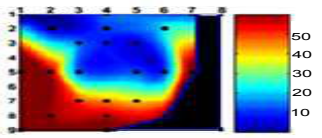
2) CONDUCTION VELOCITY MAPS: The conduction velocity was estimated by fitting a second order polynomial surface to the interpolated image data T(x,y), as given by equation 1 and described and given in [7]:

$$T(x,y) = ax^2 + by^2 + cxy + dx + ey + f \tag{1}$$

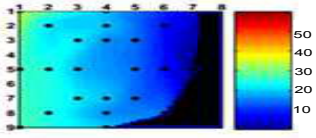
The speed of propagation was calculated by obtaining the partial derivatives of T(x,y) in both the x and y directions [7]. A window size of 5x5 was used to compute the gradient of the polynomial surface at any given point depending on the gradient of activation time in two points before, and two points after the points where the conduction velocity is computed as described in equation 2 and equation 3 [7].

$$V_e = \left| \begin{array}{c} \frac{dx}{dT} \\ \\ \frac{dy}{dT} \end{array} \right| = \left| \begin{array}{c} \frac{T_x}{T_x^2+T_y^2} \\ \\ \frac{T_y}{T_x^2+T_y^2} \end{array} \right| \tag{2}$$

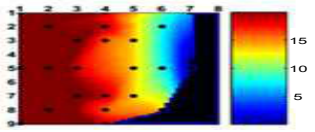
$$\text{where, } T_x = \frac{\partial T}{\partial x}, \text{ and } T_y = \frac{\partial T}{\partial y} \tag{3}$$



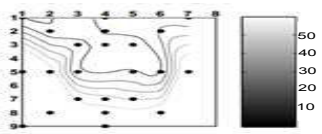
(a) Colored map (60ms maximum time)



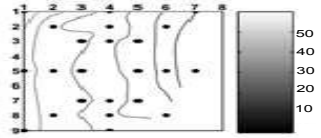
(b) Colored map (60ms maximum time)



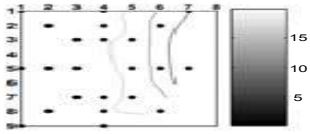
(c) Colored map (20ms maximum time)



(d) Gray scaled map (60ms maximum time)



(e) Gray scaled map (60ms maximum time)



(f) Gray scaled map (20ms maximum time)

Fig. 2: Activation time maps for sample episode 1 ((a), and (d)) and sample episode 2 ((b), (c), (e), and (f))

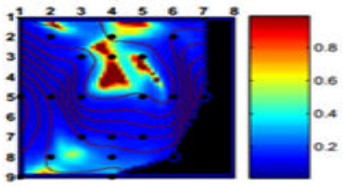


Fig. 3: Conduction velocity map (group: placebo)

The conduction velocity map is generated based on the location of the 23 electrodes (x and y direction) and their conduction velocity value, and then were interpolated for the other points. Average conduction velocity maps are a good representation of the direction and speed of propagation of the electrical wave. Fig 3 shows the conduction velocity map for the same beat analyzed in Figs 2b, 2c, 2e, and 2f. The black color in the conduction velocity maps are in the regions where there is no data available and therefore is not included in the analysis. The color bar indicates the range (blue to red) between 0 to maximum average conduction velocity (m/s).

D. FEATURE EXTRACTION

In order to quantify the effects of PUFAs, 3 features were selected to be analyzed in the studied dogs. The 3 features were first recorded for each single beat analyzed. As 3 beats were analyzed in every episode, the features were averaged over the 3 beats and every episode had a set of three features. To take into account the possible variations that might be present between different episodes, for example differences between RAA episode features and LAA episode features, the features of multiple episodes of a single dog were also averaged, ending with one set of 3 features for every dog. The selected features were previously used in literature to quantify the effect of PUFAs on AF inducibility [8]. The following is a list of the features extracted:

1) Average velocity (m/s): The average propagation speed of the electrical wave, which is the average conduction velocities over the 23 electrodes.
2) Average intra atrial conduction time (ms): The average atrial activation time, which is the average activation times over the 23 electrodes.
3) Total activation time (ms): The time needed for the electrical wave to move across the plaque (maximum activation time - minimum activation time).

E. FEATURE ANALYSIS AND CLASSIFICATION

After extracting the features, feature analysis was performed to identify any differences that might be present in placebo group features compared to PUFA group features. Feature analysis showed that the average conduction velocity was the most useful feature that significantly distinguished between placebo group and PUFA group.

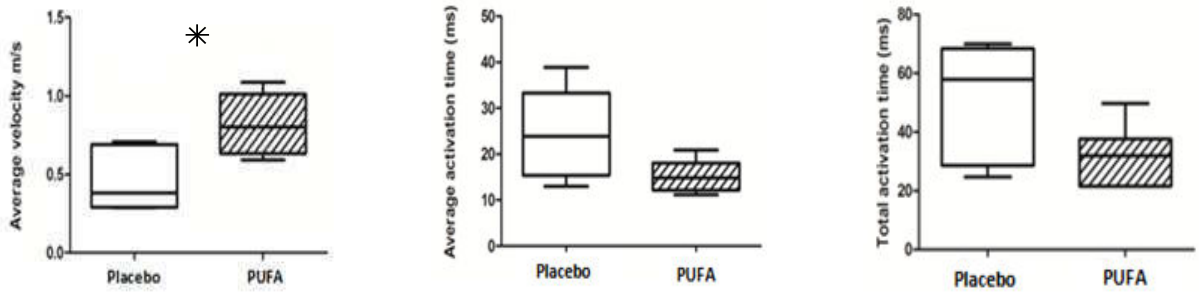
The extracted features were analyzed using linear discriminant analysis (LDA) based classifier. Cross validation was performed using leave-one-out (LOO) method, where a sample of the given data was taken out as a test sample and the classifier was trained with the left out samples, this process was repeated for all the samples [9]. The LOO method is better suited for analyzing small databases.

IV. RESULTS

To investigate the effects of PUFAs on the the average conduction velocity, average atrial conduction time, and total activation time, these features were examined using an unpaired t-test, using GraphPad Instat software [10], assuming equal variances in the two groups of dogs (placebo and PUFA). Tables I, II and III show the mean, standard deviation, the minimum and the maximum value of each of the three features in the two groups. The boxplots of the features for each group is provided in Fig 4. As shown in Fig 4a, the PUFA group has a faster average conduction velocity (0.819 m/s) compared to placebo group (0.468 m/s). Table IV is a confusion table showing the results of the supervised classification using the average conduction velocity feature. A Kruskal-Wallis test was also performed and statistical significance was observed for this feature in differentiating the groups.

V. DISCUSSION AND CONCLUSIONS

The 3 extracted features from this study, based on local atrial conduction properties, agree with previous literature showing a similar PUFA effect on global atrial conduction



(a) Average velocity (b) Average activation time (c) Total activation time

Fig. 4: Feature boxplots for placebo vs. PUFA

TABLE I: Results of the unpaired t-test performed on the average velocity feature

	Placebo	PUFA
Number of Dogs	5	6
Mean $\pm$ std [m/s]	0.47$\pm$0.21	0.82$\pm$0.19
Min - max [m/s]	0.29-0.71	0.0.59-1.08
P-value	0.0177 $<$ 0.05 (significant)	

TABLE II: Results of the unpaired t-test performed on the average conduction time feature

	Placebo	PUFA
Number of Dogs	5	6
Mean $\pm$ std [ms]	24$\pm$9.9	15$\pm$3.5
Min - max [ms]	13-39	11-21
P-value	0.065 $>$ 0.05 (not significant)	

properties [2], [3]. Both the mean, and the total activation time are longer in the placebo group when compared to the PUFA group, indicating that it is taking longer for the electrical wave to propagate across the plaque in the placebo group. This is corroborated by the velocity data, which shows conduction is significantly faster in the PUFA group.

Preventing conduction slowing caused by atrial structural remodeling (scarring, hypertrophy), can reduce AF incidence or duration [4]. A PUFA mediated increase in conduction velocity represents a potential explanation for the beneficial effects of PUFAs on AF vulnerability seen in these dogs [2], [3]. Previous findings relate faster conduction velocity to better Na^+ channel function, better cell-cell communication, and/or a reduction in fibrosis [4]. PUFAs may affect any of these factors in order to exert their beneficial effect.

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TABLE III: Results of the unpaired t-test performed on the total activation time feature

	Placebo	PUFA
Number of Dogs	5	6
Mean $\pm$ std [ms]	50$\pm$20.6	32$\pm$10.4
Min - max [ms]	25-70	21-50
P-value	0.082 $>$ 0.05 (not significant)	

TABLE IV: Cross validation using linear discriminate analysis with LOO method for the average velocity feature- % of classification

Method	Groups	Placebo	PUFA	Total
CV	Placebo	3	2	5
	PUFA	2	4	6
%	Placebo	60	40	100
	PUFA	33	67	100

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