Recording and Identification of Cardiac Neuron Activity in the Right Atrium Ganglionated Plexus

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Abstract

Recent multichannel electrode array technology has enabled the simultaneous recording of multiple cardiac neurons located in ganglia on a beating heart. These new bioelectric signals are contaminated by the electrical activity of the atrial muscle just underneath. These atrial waveforms may mask relevant neuronal activity. In this paper, we evaluate the application of a principal component analysis technique to suppress atrial activity (AA) and reveal hidden neuronal activity. Neuronal signals were recorded in situ using a 16-channel electrode in an open-chest, anesthetized dog in sinus rhythm. Validation of AA cancellation was performed by comparing neuron spike waveforms extracted from within AA with those found in AA-free time intervals. Results showed that consistent neuronal waveforms can be identified within AA in order to improve the detection of neuron firings.

1. Introduction

The heart receives sympathetic and parasympathetic innervation through the intrinsic cardiac nervous system [1]. Cardiac neurons notably contribute to the control and the regulation of heart rate and contraction. Afferent and efferent terminations as well as sympathetic efferent post-ganglionic neurons are known to be located in patches of fatty tissue on the atrial surface [2]. These ganglionated plexi have been hypothesized to contain local circuit neurons acting as local processor of information (the so-called "little brain in the heart" [3]) to coordinate regional cardiac function. There are growing evidences that an imbalance in the electrical activity of cardiac neurons is involved in the initiation and maintenance of atrial arrhythmias [4,5]. Advances in neurocardiology raised the need for reliable in situ monitoring of the electrical activity of the cardiac neurons in the ganglionated plexi.

Electrophysiological recordings in ganglionated plexi are performed by inserting an electrode in the nervous tissue, enabling the measurement of extracellular potentials (spikes) generated by neuronal action potential [1]. In the atria, this task is complicated by two problems. First, the electrode is placed directly on a beating heart and moves with it. Second, the signals generated by cardiac neurons may be masked by the superimposed atrial activity (AA). The first issue is typically addressed by means of a probe tethered by a flexible lead. Proposed solutions to the second problem have been so far limited to blanking the signals during AA [1,6], thus ignoring possible neuronal activity in these intervals. This paper presents a first attempt to extract information about cardiac neurons during local atrial depolarization, which will be crucial for future studies during atrial fibrillation.

This AA cancellation problem is similar to the subtraction of ventricular activity in the ECG during an atrial arrhythmia [7,8]. By analogy, considerable gain in AA removal performance is expected from the use of multiple simultaneous signals. While multichannel electrodes are commonly used in the brain, recordings in intrinsic cardiac ganglia have been so far essentially limited to one or a few electrodes [1]. We are using linear multielectrode arrays, in which electrodes are far enough from each other to record different neurons, but sufficiently close so that AA manifestation (atrial waveforms) should remain similar in all of them. Principal component analysis (PCA) appears to be a natural tool in this situation. In this paper, we follow this approach and evaluate its applicability to multichannel cardiac neuron recordings in dogs.

2. Methods

2.1. Experimental recordings

Mongrel dogs underwent bilateral open chest surgery. The activity generated by neurons located in the right atrium ganglionated plexus (RAGP) was recorded for 25 minutes by means of a multichannel microelectrode array

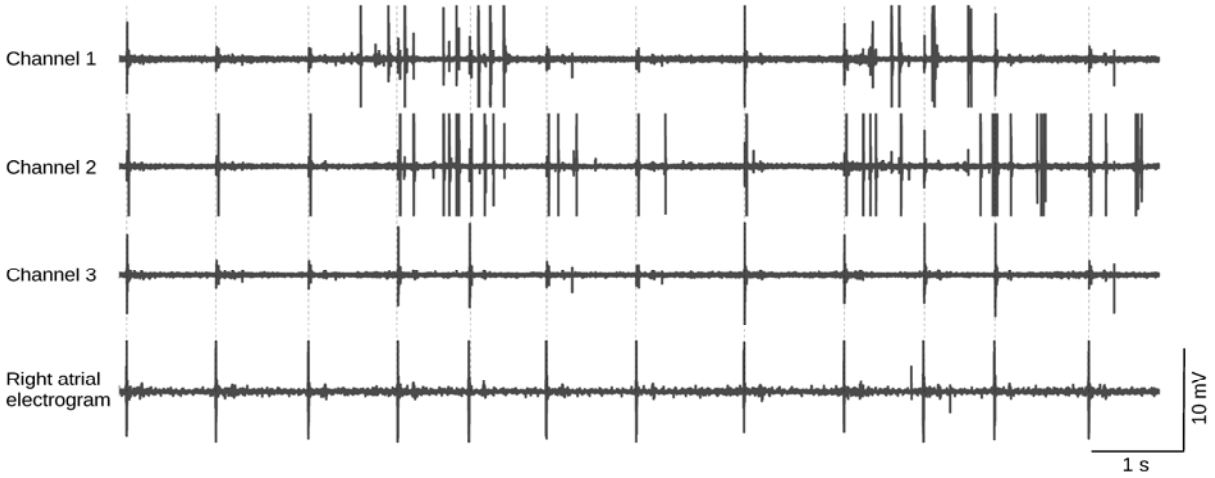

Figure 1. Electrical activity recorded in the right atrial ganglionated plexus (only 3 channels are displayed) and right atrial electrogram representing myocardial activity. Note that some events (AA) are aligned in all channels.

(Linear Microelectrode Array, MicroProbes Inc., Guithersberg, MD) *in situ* in baseline conditions under anesthesia and controlled respiration. This microelectrode array, consisting of 16 platinum/iridium electrodes (25 µm diameter electrode with an exposed tip of 2 mm; impedance 0.3-0.5 MΩ at 1 kHz), was embedded in the right atrial fat that contained the RAGP such that its tip was placed adjacent to right atrial myocardium. In addition, an electrode was sewn to the atrial myocardium close to the RAGP to provide a reference atrial electrogram and assist AA identification. When low amplitude neuronal activity was observed by visual inspection in a channel during the setup phase of the experiment, the gain of that channel was manually adjusted. The gain therefore varied across the channels.

The signals were digitized at a sampling frequency of 5.6 kHz via a Cambridge Electronics Design (model 1401) data acquisition system.

2.2. AA waveform detection

The 17 signals (16 neuronal channels + electrogram) were analyzed offline using the Spike2 software (Cambridge Electronics Design). The signals of the two distal microelectrodes of the array were discarded because of low signal-to-noise ratio, leaving 14 neuronal channels. Figure 1 shows three different channels as well as the right atrium electrogram. Neuronal activity is clearly visible on the first two channels, while it remains very low on the third one. When the local cardiac tissue depolarizes, it generates a waveform not only on the electrogram, but also simultaneously on all other data channels (see Fig. 1).

Events (both neuronal responses and AA since they have similar amplitude) were detected using a threshold-based method provided in Spike2. Because of the inter-electrode distance, the activity of a single neuron can usually not be seen from more than two adjacent channels. To discriminate between neuronal response and AA, we therefore assumed that any event present simultaneously in three or more channels was AA. To facilitate the procedure, AA detection was performed on a subset of four channels (called four-trode) with low neuronal activity (selected by visual inspection). The right atrial electrogram served to ensure that AA detected in neuronal signals was indeed caused by an atrial activation. Note that atrial electrogram waveforms were approximately but not exactly aligned with the AA in neuronal channels since the myocardial electrode and the RAGP electrode were distant by a few millimeters.

2.3. Spike sorting outside AA

Spike sorting consists in grouping neuronal waveforms into clusters based on their shape [9]. Each waveform of a group presumably corresponds to the firing of the same neuron. As a first step, a 26-ms window was blanked in all channels around each detected AA waveform. Automatic spike sorting techniques available in Spike2 were applied to extract neuron spike trains. The standard approach for neuronal activity identification stops here. The next two paragraphs describe an attempt at finding neuronal spikes within the blanked intervals.

2.4. AA cancellation

Signals were then exported from Spike2 to Matlab for further analysis. Each AA window was processed separately. An example of signal waveforms in an AA window is shown on the left panel of Fig. 2. After mean subtraction, the signals were normalized by their standard deviation to compensate for channel-specific gain (see e.g. signals 4, 5, 7 and 9 in Fig. 2).

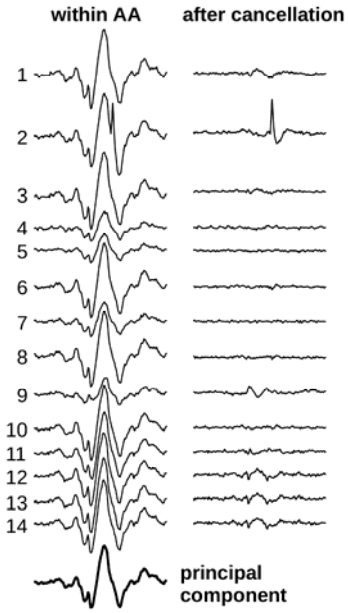

Figure 2. First column: signals (14 channels) containing an atrial activity (AA) waveform. Second column: the same signals after AA cancellation. The bottom trace shows the principal component.

For each atrial beat, the 14 signals in the corresponding AA window were represented as a 146-by-14 data matrix (windows were 146 samples long). AA cancellation was performed based on principal component analysis (PCA) of that matrix [10]. In all but rare cases, the first principal component (an example is shown at the bottom of Fig. 2) captured at least 70-90% of the variance. This confirmed that AA shape was very similar in all channels. The signals were then reconstructed after suppression of the first principal component. Removing the second principal component did not further improve the identification of neuronal activity in the residual, presumably due to its orthogonality constraint. The right panel of Fig. 2 shows examples of residuals after AA cancellation. One of them (channel 2) seems to contain a neuronal activity.

2.5. Spike identification within AA

To establish that spikes observed within AA after cancellation (and detected by thresholding) were real neuronal responses, two criteria were used. First, when simultaneous spikes occurred in more than two channels, they were considered as cancellation artifact. Otherwise, the spike waveform was compared to the templates of neuronal waveforms identified outside AA using Spike2. The maximum cross-correlation served as a quantitative measure to select the best candidate and associate the spike with a previously identified neuron.

3. Results

In the 25 min studied, 1600 AA waveforms were detected (64 per min). These corresponded to the segments that were blanked in the standard approach. Some AA corresponding to atrial activations were not detected. They had significantly lower amplitude (e.g. premature beats) than neuronal response and were not blanked in the standard approach because they did not prevent the identification of neuronal response. Decreasing the threshold to detect them would increase false positive detection of neuronal response.

Outside AA, a total of 18 neuronal waveforms were identified in the 14 channels, presumably corresponding to the activity of 18 different neurons. Their firing rates ranged from 0.06 to 2.09 Hz in baseline conditions, in agreement with previous recordings in the RAPG [1]. Table 1 lists the number of neuronal spikes outside AA for the 10 neurons having the highest firing rates.

Table 1. Number of neuron spikes inside and outside atrial activity (AA).

neuron	#spikes outside AA	#spikes within AA	fraction inside
1	3129	35	1.1%
2	1888	23	1.2%
3	1641	24	1.4%
4	1333	17	1.3%
5	1251	11	0.9%
6	1023	14	1.4%
7	897	7	0.8%
8	687	20	2.8%
9	584	25	4.1%
10	549	7	1.3%
others	1850	38	2.0%
total	14832	221	1.5%

AA cancellation provides a tool for identifying additional neuronal spikes. The channel 2 of Fig. 2 has a spike in the residual after cancellation. Its amplitude is in the range of the amplitudes of spikes identified outside AA. To further confirm that this spike is a neuronal response, its waveform was compared to similar spike waveforms outside AA. Figure 3 gives a few examples of spikes with similar shapes found inside and outside AA. For a variety of morphologies, waveforms were consistent inside and outside AA. As a results, it was possible to associate each spike inside AA with a cluster of waveforms (i.e., with a neuron) outside AA. Table 1 summarizes for the neurons with highest firing rates the number of spikes inside and outside AA. Spikes inside AA contributed to about 1 to 2% of the total identified spikes, while the cumulated length of all AA represented 2.8% of total signal duration.

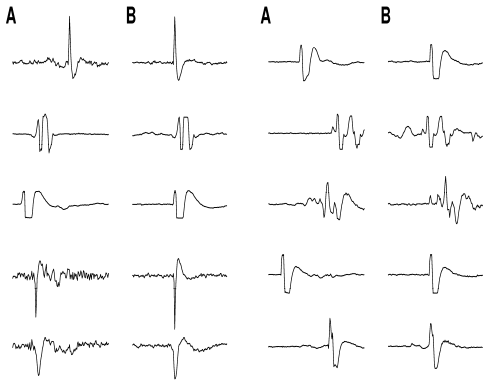

Figure 3. **(**A) Examples of neuronal responses within an AA waveform after cancellation. (B) Best match with a neuronal response found outside AA.

4. Discussion

This paper demonstrates the value of multichannel neuronal recording for investigating the intrinsic cardiac nervous system. First, the detection of simultaneous spikes in multiple channels facilitates the identification of AA. This is of particular importance since amplitude and shape alone are sometimes not sufficient to reliably classify waveforms as AA. Second, a simple PCA algorithm enabled us to reveal neuronal activity masked by AA. Combined with powerful spike sorting techniques in Spike2, this type of multichannel analysis opens new perspectives in neurocardiology by permitting to study neuron population dynamics and network interactions in atrial ganglionated plexi in relation to cardiovascular, chemical, mechanical or neuronal inputs/outputs [11].

When AA waveforms are blanked, neuron firing rates are underestimated. Our results suggest that the systematic error in firing rate is of the order of 1.5% during sinus rhythm, which is small as compared to changes in firing rates resulting from external input (e.g. increased blood pressure). When the activity of a neuron is cardiovascular-related (i.e., when it fires at specific phases within the cardiac cycle), there may be a physiological reason for the presence or absence of firing during the AA. This may explain why the average firing rate in the AA is slightly lower than expected (1.5% of the spikes in 2.8% of signal duration). Another reason could be misdetection of lower amplitude waveforms due to cancellation artifacts.

The problem of removing AA becomes more critical during episodes of atrial arrhythmias. In these very relevant conditions, atrial rate increases markedly so that the cumulated duration of AA may represent up to 15-20% of signal duration, resulting in a more severe underestimation of firing rates. Future work will evaluate the applicability of our approach to these signals.

5. Conclusion

The application of multichannel microelectrode arrays to neurocardiology created new signal processing challenges. A combination of PCA and template matching enabled us to get more insight into RAGP neuronal activity hidden in AA. These tools and their future developments will form the basis for deeper investigations of neuronal activity in relation to the occurrence of atrial arrhythmias.

Acknowledgements

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