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Detection of baroreceptor activity in rat vagal nerve recording using a multi-channel cuff-electrode and real-time coherent averaging

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Abstract— **Electrical stimulation of afferent nerve fibers originating from pressure sensors can trigger the baroreflex to reduce blood pressure and might be an alternative to treat patients with hypertension. In this study, baroreceptor compound activity was detected using multi-channel cuff-electrode recordings on rat vagal nerve. In order to isolate the vagal nerve signals from external potentials (such as ECG- and EMG-coupling), a tripolar measuring technique was applied. To eliminate noise and neural signals corresponding to other organs, coherent averaging was used. The baroreceptor-correlated signals appear predominantly in one of the electrode channels, presumably close to the corresponding neural substrate. This localization was done in real-time.**

I. INTRODUCTION

HYPERTENSION is a typical disease of civilization, a worldwide threat to patient's health and a burden to health care systems. While most patients can rely on pharmaceutical treatments, this option is ineffective for some due to compliance or ineffectiveness of drugs [1]. Overriding the afferent signals from pressure receptors in the aortic arch and the carotid artery can trigger the so-called baroreflex regulation. This negative feedback loop regulates down the blood pressure almost instantaneously. Technical solutions dealing with the stimulation of the pressure receptors or with the afferents of these receptors in the vagal nerve have been subject to research for many years, however, most of the approaches lack the option of feedback control and/or selective stimulation [2,3]. Stimulation of the receptors in the carotid artery (carotid sinus) is highly selective in terms of addressing the correct target for the stimulation and thus almost free from side effects [4]. Still, this approach deals with high risks as the surgical intervention addresses the carotid artery and minute errors or malfunctions of the implant can result in life threatening situations. Stimulations targeting the vagus nerve (VNS) are common practice for severe epilepsy therapy and are considered a safe implantation technology. Downside is, the vagal nerve is the major communication path for many organs. Stimulation of the complete cross section of the vagal nerve results in secondary effects such as chest and throat pain, cough, voice alteration, nausea, vomiting and even arrhythmia [5]. The localization of blood pressure relevant fibers is therefore the first step to embark on selective stimulation of such fibers. For therapeutic use, fully implantable devices are mandatory that have to work with robust and fast localization algorithms using embedded systems with limited (computing) power, e.g based on MSP430 (Texas Instruments) microcontrollers.

Highly selective recording and stimulation can be achieved by maximum invasiveness. Intrafascicular multi-channel electrode [6] deliver access to small groups of fibers but penetrate the perineurium and increase the risk of larger tissue response and nerve damage. Cuff-electrodes do not reach the level of selectivity [6] at the same stimulation thresholds but allow electronic switching of channels without the need of exact surgical prealignment of the electrode sites with respect to the nerve. Using a hand manufactured multi-channel cuff electrode Rozman et al. described recording of baro-receptive activity from 39 tripolar electrode channels [7]. In that study, dog vagal nerve responses to two different blood-pressure modulation mechanisms were measured: manual carotid artery compression and epinephrine application. As a result, the Fourier transform of one triple electrode showed a single peak at 1.86 kHz. In this study, we developed a multipolar polyimide thin-film cuff-electrode based on an established process technology [8]. The recording of action potentials or action potential like responses (with Fourier transforms generally featuring broad peaks in the range of 1.2 to 2.5 kHz) is rather unlikely in cuff-electrode scenarios. We intend to record nerve compound activity and therefore focused on the low frequency range below 300 Hz. To be as close as possible to the real scenario of an implant, we aimed to isolate the baroreceptive signals from 8 tripolar cuff recording channels without artificial stimulation of the blood pressure of the test animal. We correlated the recordings from the tripolar electrodes with pressure signals from a tip catheter to perform coherent averaging.

II. MATERIALS AND METHODS

A. Animal Preparation

The data for this study were recorded from three Wistar rats. The rats were initially anesthetized 2-4 % Isoflurane and 5 mg/kg bodyweight Carprofen was administered s.c. for analgesia. For ongoing anesthesia 1-2 % Isoflurane was permanently administered. The dose was regulated based on the respiration rate. The rats were placed under a heat lamp and administered isotone ringer solution (3 ml/h, s.c.). An

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incision in the neck was made to expose the left vagus nerve and the common carotid artery. First the carotid artery was blocked using arterial clamps. In the descending carotid the tip catheter (ICP, Codman, 3F) was inserted and fixed in the proximal descending aorta. After fixation of the tip catheter the cuff-electrode was wrapped around the vagal nerve without any pre-alignments of electrodes and nerve. Finally three needle electrodes were inserted subcutaneously in the chest for ECG recordings. Recordings in every experiment were always without blood-pressure variations. After the experiments the rats were sacrificed to allow extraction of the nervous tissue for additional histological investigation. All proceedings were in full accordance with and approved by the Ethics Committee for Animals Experiments in Baden-Württemberg, Germany.

B. Cuff electrode

The cuff electrode used in the experiments (Figure 1) featured 24 electrodes arranged in eight tripoles around the cuff perimeter (45° spacing). All ring electrodes were used to record the vagal nerve signals. Four large ring electrodes facing inside the cuff adjacent to the recording sites and two electrodes facing outside were used as reference or ground, respectively. The cuff total length was 10 mm, the inner diameter was 0.6 mm and distance between cross sectional electrodes was 2 mm.

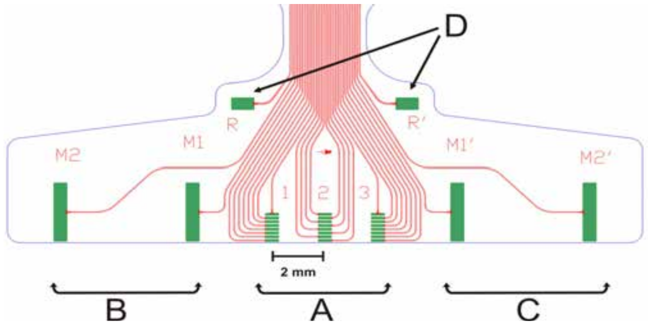

Fig. 1: Schematic of Cuff electrode used. The array comprises 8 tripoles (A), 4 counter electrodes (B,C) facing inside the cuff and two reference electrodes facing outside (D).

Contact pads, interconnection lines and electrodes sites were sandwiched between two layers of polyimide (total thickness 11 µm, [7]. The thin film metallization of 300 nm sputtered Platinum was coated with 1000 nm Iridium Oxide on the electrode sites.

C. Recording Setup

For the experiments we used a PZ3 system (Tucker Davis Technology, Florida, USA), which contains low noise pre-amplifiers for the signal conditioning, attached to a RZ2-module, which holds two digital signal processors (DSPs) and allows some digital/analog inputs/outputs to preprocess the signals. The RZ2 was connected to a PC via a PCIe interface card. The PZ3 pre-amplifier was set for recording monopolar signals from each of the 24 electrode and the 4 reference electrodes at a sample rate of 12207.03 Hz. The noise floor of the amplifiers was 0.9 μV_{RMS}.

The nerve signals were acquired monopolar and were processed in the PC to detect the baroreceptor activity. This processing included calculation of true-tripoles of the electrodes, filtering and coherent average. In addition, ECG and blood pressure were recorded with the same settings on separate input channels of the setup.

D. Coherent Averaging

The baroreceptor signals are masked by the recording noise and other neural activities not correlated to the blood-pressure. These potential decrease the signal-to-noise ratio (SNR). Despite the masking baroreceptive signals can be uncovered using the coherent analysis since their activity is correlated to the blood-pressure. The principle consists on taking the average of N snippets from a recorded channel (in our case: recorded tripole). Each snippet can start from e.g. the QRS complex of the ECG recording or from the artifacts of the monopolar recording, acting as a trigger signal. Assuming $x_p(t)$ to be the baroreceptor information synchronized with ECG and blood pressure signals and $n_k(t)$ the noise and other neural potentials, the recording of one channel $y_k(t)$ can be expressed as:

$$y_k(t) = x_p(t) + n_k(t) \qquad (1)$$

Not changing the blood pressure the baroreceptor activity is supposed to be stable for each snippet and the average of N snippets becomes:

$$\bar{y}(t) = x_p(t) + \frac{1}{N}\sum_{k=1}^{N} n_k(t) \qquad (2)$$

Since the noise is not correlated with the ECG the right half of the term tends to 0 with N increasing to infinity. Thus:

$$\bar{y}(t) \approx x_p(t) \qquad (3)$$

The snippet triggering acuity is essential for the coherent average method as each jitter smoothens the baroreceptive signal as well. Even tough the local minimum or local maximum of the recorded blood pressure did qualify for a trigger signal, we got best results using the QRS complex.

To save computation time during the data analysis, the coherent average method was optimized for a real-time application. The proposed algorithm consists on the following elements:

- Calculate the time reference for each snippet based on two consecutive QRS complexes (triggering for a given threshold).
- Use a recursive IIR average structure instead of allocating all snippets and averaging all at the end.

The recursive IIR filter only needs allocating the current snippet and last snippet for each tripole. The optimization is proposed as follows:

Let $x_k(n)$ be the current snippet for tripole and $y_k(n)$ the coherent average filter output, where k is the snippet number and n is the discrete time variable. Let N be the total number of snippets. The standard averaging technique would perform the operation:

$$y_N(n) = \frac{1}{N}\sum_{i=1}^{N} x_k(n) \qquad (4)$$

Assuming a hypothetical buffer structure with snippet locations, the output of the averaging filter would be:

$$y_k(n) = \frac{N-1}{N} y_{k-1}(n) + \frac{x_k(n)}{N} \tag{5}$$

Additional filtering was applied using a band pass filter (20 to 200 Hz) prior to the coherent implementation. This filter was primarily used to reject mechanical and thermal noise generated by surgical activities. The filter properties were determined using spectrograms of the ongoing recording.

III. RESULTS

A. Tripolar vs. Monopolar

The true-tripole configuration allows the elimination of potentials from the recording, which are not located inside the nerve. Continuous 0.8 and 1 kHz bands found in the recording are frequency components intrinsic to the cuff electrode. The electrode dimensions and the spacing between the electrodes determine these frequencies [9]. Such frequency bands were irrelevant for the baroreceptor activity detection and rejected with the band-pass filter applied before the coherent average implementation. Similar to the monopolar configuration, unbalanced tripoles contain ECG artifact. If the tripole unbalancing is a result of broken electrodes, the ECG artifact is increasing along with the respiration EMG artifact: (Figure 2)

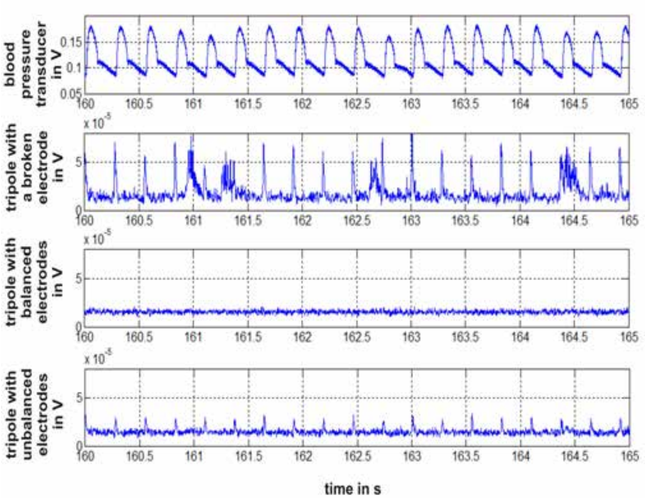

Fig. 2: Comparison of balanced and unbalanced tripoles and tripoles with a broken electrode.

In the monopolar recording, the electroneurogram (ENG) is dominated by ECG artifacts (below 500 Hz) and respiratory EMG artifacts (flames with approx. 1 second gap, when the blood pressure decreases locally).

B. Filtering

Using the data from the spectrograms, a 20 to 200 Hz band-pass filter (Butterworth 2nd order) was applied to the signals. The frequency bands below the 20 Hz were rejected because they were present in all tripoles and they do not content any baroreceptor information. Frequency components above 200 Hz were not synchronized or coupled with the blood-pressure signal. After the filtering, the tripoles were calculated. ENG of unfiltered, filtered, balanced and unbalanced without and with coupling exhibited different activity (Figure 3).

C. Coherent Averaging

The coherent average implementation was successful in all three experiments. The advantages of the real-time coherent average implementation are:

- Reduction of 93 to 97% in the computation time (i. e. 0.5 seconds are needed instead of 7 minutes)
- Data amount reduction: 10 to 50 snippets (instead of 400) were enough for the pattern convergence.
- Identical patterns obtained from both methods (conventional and real-time implementation)

Baroreceptive signals were visible after coherent averaging (Figure 4, channels 4 and 5) even in an unbalanced tripole, i.e. bipolar recording without full artifact subtraction of the common mode signal.

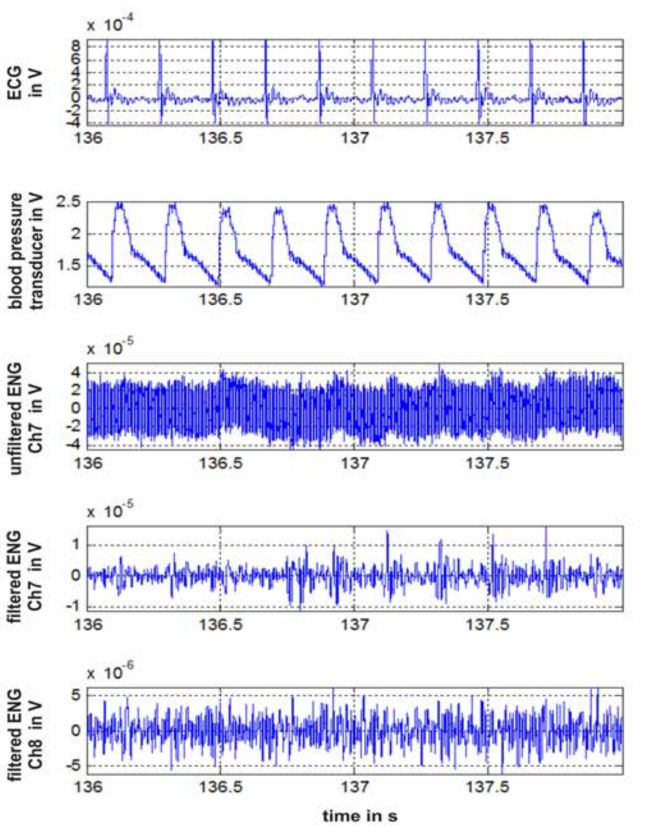

Fig. 3: Top down: Traces of ECG, blood pressure, unfiltered and balanced tripole 7, filtered and balanced tripole 7 (with coupling), filtered and balanced tripole 8 (no coupling).

IV. DISCUSSION

It was shown that coherent averaging in realtime is a suitable approach for detection of baroreceptive activity with true tripole cuff electrodes, a configuration superior to mono- and bipolar configurations with respect to artifact suppression. We suggest that signals were detected on the channels in vicinity to the signal carrying nerve fibers.

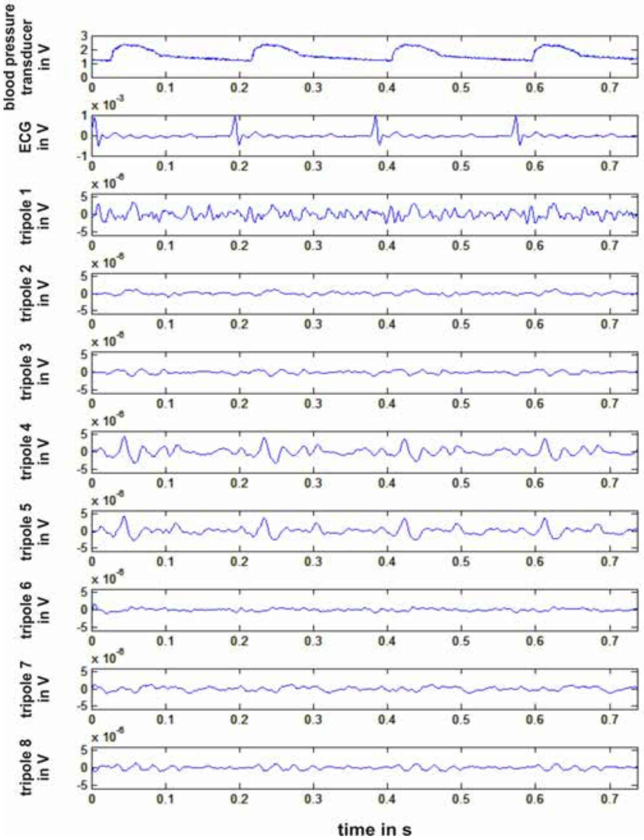

Fig. 4: Convergence of coherent average with unbalanced electrode on channel 1. In this example the baroreceptive signal is found on two adjacent channels (4 and 5).

Low frequency ENG recordings below 300 Hz limits the computational demands for implantable devices (like digital signal processors) and accelerates the processing in a tremendous way as it only takes a few seconds to calculate a coherent average. Coherent averaging improves signal to noise ratio and allows even in broken tripoles artifact reduction of common mode signals and extraction of baroreceptor information when additional ECG information is available. If the electrodes of a cuff are in close vicinity to bursting neurons it might be possible to record potentials that feature peaks in the characteristic frequency band between 1.2 and 2.5 kHz (for APs). However, to find baroreceptive fibers always on the outmost surface of a large nerve like the vagus nerve is rather unlikely. Field potentials have less temporal information than APs but can be recorded further away from their source. The compound nerve activity might contain more information not yet addressed by our algorithms comparable to local field potentials: distance from the source and systolic blood pressure. Future work will address these tasks. Eventually, ECG and blood pressure recordings can be omitted but serve as calibration and control signals in the current study.

V. CONCLUSION

In this work, an algorithm for the real time analysis of baroreceptor activity inside the vagus nerve in rats has been presented. Using a multi-channel cuff-electrode and a software-based true-tripolar measurement, exhibits synchronized activity with the blood pressure recordings. Similar activity has already been reported [10] but was recorded with needle electrodes on a dissected nerve. Extracting the signal with the cuff-electrode presented the challenge of separating the desired signal from the rest of the signals travelling inside the nerve. For that purpose, the coherent average resulted in a valuable tool, which eliminated signals that are not correlated to the heart activity. Additionally this approach requires only low calculation power and time, which is appropriate for fully implantable devices. In the future, these results will be used to select the areas for selective vagus nerve stimulation to trigger the baroreflex and reduce blood pressure.

REFERENCES

[1] H.R. Black, W.J. Elliott, G. Grandits, P. Grambsch, T. Lucente, W.B. White, J.D. Neaton, R.H. Grimm, Jr.L. Hansson, Y. Lacourciere, J. Muller, P. Sleight, M.A. Weber, G. Williams, J. Wittes, A. Zanchetti, R.J. Anders, "Principal results of the Controlled Onset Verapamil Investigation of Cardiovascular End Points (CONVINCE) trial." *Jama*, 289(16): p. 2073-82, 2003.

[2] W. Fan, M.C. Andresen, "Differential frequency-dependent reflex integration of myelinated and nonmyelinated rat aortic baroreceptors." *Am J Physiol* 275: 632-640, 1998.

[3] P.M. De Paula, J.A. Castania, L.G. Bonagamba, H.C. Salgado, B.H. Machado, "Hemodynamic responses to electrical stimulation of the aortic depressor nerve in awake rats." *Am J Physiol* 277: 31, 1999.

[4] K.A. Illig, M. Levy, L. Sanchez, G.D. Trachiotis, C. Shanley, E. Irwin, et al., "An implantable carotid sinus stimulator for drugresistant hypertension: surgical technique and short-term outcome from the multicenter phase II Rheos feasibility trial." *J Vasc Surg* 44: 1213-1218, 2006.

[5] H.A. Sackeim, A.J. Rush, L.B. Marangell, Z. Nahas, M.M. HM, S. Geroge, "Vagus Nerve Stimulation: A new Tool for Brain Research and Therapy." *Society of Biological Psychiatry*, vol. 47, pp. 287-295, 2000.

[6] J. Badia, T. Boretius, D. Andreu, C. Azevedo-Coste, T. Stieglitz, X. Navarro, "Comparative analysis transverse intrafascicular multichannel, longitudinal intrafascicular and multipolar cuff electrodes for the selective stimulation of nerve fascicles." *J. Neural Engineering* 8, 036023, 13 pages, 2011.

[7] J. Rozman. R. Ribaric, "Selective recording of electroneurograms from the left vagus nerve of a dog during stimulation of cardiovascular or respiration system", *Chinese J of Physiology*, 50(5),240-250, 2007.

[8] T. Stieglitz, H. Beutel, M. Schuttler, J.U. Meyer, "Micromachined, Polyimide-based Devices for Flexible Neural Interfaces.“ *Biomedical Microdevices*, 2 (4), pp. 283-294, 2000.

[9] G.S. Dhillon, K.W. Horch, "Neuroprosthetics, Theory and Practice.“ Utah, USA : *Scientific World*, 2004.

[10] N.H. West, B.N. Van Vliet, "Responses to Circulatory Pressure, and Conduction Velocity, of Pulmocutaneous Baroreceptors in Bufo Marinus.“ *Journal of Physiology*, vol.388, p. 41-53, 1987.