Selective stimulation of the Vagus Nerve controls the blood pressure and simultaneously avoids significant side effects of Bradycardia and Bradypnea.

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Abstract
Recent hypertension therapy is foremost a pharmacological business with up to 30% of the patients not reaching normotension. Selective vagal nerve stimulation using the baroreflex to decrease the blood pressure could become the alternative treatment for such patients. We used multi-channel cuff electrodes wrapped around the vagal nerve to localize baroreceptive fibers prior to selectively stimulating them. The selectivity of the stimulation is essential as unselective stimulation can lead to cardiac, respiratory, or gastrointestinal side effects, which are unacceptable for chronic implants. For the localization of the fibers we recorded the ECG and blood pressure as reference signals. These signals were then used as triggers for the coherent averaging algorithm, which was necessary to raise the correlated field potentials above the noise level. Signals were recorded using a tripolar configuration along with a two-staged amplification. The former allowed cancelling out common mode noise, the latter compensated for impedance mismatch, a serious issue for all chronically implanted metal electrodes. Once baroreceptive fibers were localized, the electrode that was proximal to the fibers were used to selectively stimulate and trigger the baroreflex without triggering bradycardia or bradypnea.

Introduction
Increased blood pressure (hypertension) affects 25 % of the German population and is the worldwide number 1 risk to human health [1,2]. Even though most patients benefit from pharmaceutical treatments, blood pressure cannot be reliably lowered to physiological values in all of them [1]. Some patients are medically refractory and do not benefit from drug therapy at all. Electrical modulation of signals from afferent fibers originating in pressure sensors of the aortic arch and the carotid artery can trigger the so-called baroreflex and lower the blood pressure almost instantaneously [3,4]. The direct stimulation of those cervical pressure receptors is highly effective and almost free of side effects. However, surgical intervention and interfacing of the carotid artery are associated with high risk. Malfunction of an implant may lead to life threatening situations. Vagal nerve stimulation (VNS) instead is a common therapy for intractable epilepsy in clinical practice. Nerve surrounding electrodes are considered a safe implantation technology. Since the vagal nerve is one major communication pathway in the vegetative nervous system, global stimulation causes secondary effects such as nausea, vomiting, and even arrhythmia [5]. Since the afferent nerve fibers of the blood pressure sensors are located in the vagal nerve, selective stimulation of these fibers should lower the blood pressure without causing major side effects. Such selective stimulation could be addressed using intrafascicular electrodes. However, such electrodes are maximal invasive and sensitive to motion, which can damage the nerve and results in scars and functional impairment of the nerve [6]. Cuff electrodes are less invasive as they do not penetrate the perineurium, but they do not reach the same levels of selectivity at the same stimulation threshold as intrafascicular electrodes [6]. The application of cuff electrodes is however superior to intrafascicular electrodes, as there is no need for an exact alignment [7]. As the contacts of the cuff are placed extraneurally, the expected field potentials are in the range of a few µV. The recording of such low power signals against a noise floor that is about 10fold higher is challenging, especially if the method has to be compatible with the very limited computational power of an implantable embedded system. Therefore we did not consider rather complex operations like Fourier transformations or wavelet analysis as appropriate methods. Instead, we did rely on the coincidence of the baroreceptive fiber activity with the blood pressure and/or the ECG. The baroreceptors in the aortic arch respond to the pressure with a phasic volley of activity. This volley travels through the aortic depressor nerve (ADN), which merges with the vagus. The baroreceptive fibers of the ADN primarily consist of slow c-type fibers [8]. Due to the slow propagation a tripolar recording of this C-fiber mass activity will feature lower frequencies in the sum potential than field potentials from e.g.: fast A-fibers. In combination with a low pass filter (<300 Hz), tripolar recording and coherent averaging are sufficient to isolate the baroreceptive field potential and discriminate which electrode is proximal to the source of the signal [9]. In this work we describe what we learned from using the identified electrode to selectively stimulate the Vagus nerve.
Materials

Five Wistar rats were used for this experimental series. The experimental animals were initially anesthetized with Isoflurane (2 - 4 %) and analgesia was applied (Carprofen, 5 mg/kg bodyweight (BW)). The on-going anaesthesia was preserved by Isoflurane (1-2 % controlled by the respiration rate). During the experiments, the rats were placed on controlled heat mat and supplied with isotonic saline (1 ml/100 g BW). The left vagal nerve and the common carotid artery were exposed through a ventral neck incision. The carotid artery was blocked with arterial clamps. A tip catheter (ICP, Codman, 3F) was inserted and fixed in the proximal descending aorta. Then the cuff-electrode was wrapped around the vagal nerve without any pre-alignments of electrodes and nerve. Afterwards three ECG needle electrodes were inserted subcutaneously in the left and right hand as well as in the left foot. After an initial recording to locate the electrodes closest to the baroreceptive fibers, stimulation parameters were tested in arbitrary order to avoid adaptation processes. The stimulus parameters were tested primarily on the identified electrodes and for control purposes also across not identified electrodes. After the experiments the rats were sacrificed and the tissue was harvested for additional histological investigation.

The cuff electrode used in the experiments featured 24 contacts 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. Contact pads, interconnection lines and electrodes sites were sandwiched between two layers of polyimide (total thickness 11 µm). The thin-film metallization of 300 nm sputtered Platinum was coated with 1000 nm iridium oxide on the electrode sites. 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 an 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 electrodes and the 4 reference electrodes at a sample rate of 12207.03 Hz. The noise floor of the amplifiers was 0.9 μVRMS. A band-pass filter (Butterworth 2nd order, 20 to 200 Hz) was applied to the signals. The frequency bands below 20 Hz were rejected because they were present in all tripoles and they did not contain any baroreceptor information. Frequency components above 200 Hz were not synchronized or coupled with the blood pressure signal. The ECG electrodes and blood pressure sensor were also connected to the PZ3 pre-amplifier and recorded at the same sampling and filter settings as the electrode channels. The nerve signals were acquired as monopolar recordings. Succeeding signal processing on the PC included calculation of true tripoles of the electrodes, impedance balancing, filtering and coherent averaging to detect the baroreceptive activity. If any of the three electrodes belonging to a tripole showed an impedance mismatch, common mode artefacts that are eliminated by a tripole configuration became visible again. To balance this mismatch we used the following equation to alter the settings of the first stage of the amplification:

$$V_{bal} = (1+c)(V_i - V_j) + (1-c)(V_j - V_k)$$

With $V_{bal} =$ amplification of tripole x and c = adjustment variable between 1 and -1.

All details regarding our electrodes and the method of the coherent averaging can be found in [7].

Current controlled stimulation consists of biphasic rectangular pulses, which were generated and modified in the RZ2 module and fed into a custom-made 8-channel voltage-to-current-converter at a D/A conversion rate of 24414 Hz with 16 Bit resolutions. These pulses were adjusted to obtain charge-balance. The center electrode of the identified tripole was used as cathode against the two large peripheral ring electrodes. The standard proceeding was the following: We first located which tripole showed baroreceptive activity after filtering and coherent averaging. We then selected the center electrode of this recording tripole and proceeded each combination of stimulation parameter five times in an arbitrary order. All stimulation series consisted of 200 pulses. The following stimulation parameters were evaluated in our paradigm: repetition rate (30 Hz, 40 Hz and 50 Hz), stimulation amplitude (0.3 mA, 0.5 mA and 1 mA) and pulse width (0.1 ms, 0.3 ms and 0.5 ms). Individual stimulations were interrupted by an interval of at least 10 seconds. After finishing the 135 stimulation sequences for the baroreceptive electrode, the same parameters were tested with some of the remaining electrodes.

Results

After coherent averaging we found in all five experiments one or two tripoles that displayed baroreceptive activity. In four out of five cases these correlated activities featured a bipolar signal (see [7] for details). In one case the proximate electrode showed a damped oscillation as the correlated signal to the blood pressure. Stimulation across identified electrodes allowed blood pressure reduction while the remaining
electrodes only showed blood pressure decrease at very high stimulation levels (amplitude of 1 mA and stimulus duration of 0.5 ms) if at all. The stimulation over electrodes in close proximity to baroreceptive fibers vs. electrodes not being near the fibers unveils that typical side effects of unselective VNS seem to be located in separate areas of the vagus. The stimulation across an identified electrode (e.g. tripole 3 in Fig. 1) typically resulted in a long lasting reduction of the blood pressure, while the heart rate was only affected mildly and the respiration rate was not affected at all. The same stimulation (40 Hz stimulation frequency, 1 mA amplitude, 0.3 ms pulse duration) across another electrode (tripole 6), which did not show any baroreceptive signals during recording and which was not an adjacent neighbor of electrode 3, triggered no reduction of the blood pressure, but resulted in reduction of the heart rate (bradycardia) and a massive effect on the respiration (bradypnea) during stimulation.

![Figure 1](image1.png)

**Figure 1** A) Blood pressure (blue = systolic, red = MAP, green = diastolic) and heart rate during stimulation of channel 6 (from the non-baroreceptive tripole 6) Cuff-Electrode. B) The same for the baroreceptive channel 3. Note that the respiration can be identified by the oscillation of the blood pressure.

With the identified electrode it was possible to reduce the blood pressure way below safe values of 60% of pre-stimulus blood pressure. All three stimulation parameters seemed to influence the reduction of the blood pressure. From the three stimulation frequency chosen, 40 Hz clearly showed the strongest reduction.

![Figure 2](image2.png)

**Figure 2** Response of the mean arterial blood pressure (MAP) as a function of pulse duration (columns), stimulation frequency and stimulation amplitude for baroreceptive electrode 3 (CH3) and not baroreceptive electrode 6 (CH6). Each stimulus combination was tested five times in arbitrary order.

If however the stimulation amplitude was high (1 mA) and pulse width was large (0.3 ms or 0.5 ms), i.e.: the charge injection was high, the stimulation frequency became less important. In combination with all three parameters, we found the most suitable baroreflex results at 40 Hz, 0.3 ms pulse width and 1 mA stimulation.
Discussion
It was shown that the localization of baroreceptive fibers ensures both a selective stimulation of an almost side effect free baroreflex and that electrode dislocation can be easily compensated for by rerunning the localization procedure. The impedance balancing is useful to detect relative changes of electrode impedance, which typically occur in an implanted scenario. With the corrector factor (c) it is possible to level this for recordings. In all cases stimulations across the identified electrodes caused a drop of BP with hardly any bradycardia and no bradypnea. The combination of stimulation frequency, stimulation amplitude and pulse wi

Conclusion
We conclude that selective stimulation of the vagal nerve using multipolar cuff electrodes allows decreasing blood pressure while simultaneously only marginally co-triggering unwanted side effects like bradycardia and bradypnea. Fibers that carry blood pressure information have to be identified first, to allow a priori selection of adequate stimulation sites. In future experiments using more elaborate stimulation configuration (e.g. pentapolar stimulation or steering currents) the selectivity can be further improved.

References


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