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Selective Recording of Electroneurograms from the Left Vagus Nerve of a Dog during Stimulation of Cardiovascular or Respiratory Systems

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Abstract

Selective electroneurograms (ENGs) from superficial regions of the left vagus nerve of a dog were recorded with a 33-electrode spiral cuff (cuff) implanted on the nerve at the neck in an adult Beagle dog. The electrodes in the cuff were arranged in thirteen groups of three electrodes (GTE 1-13). To identify the relative positions of the particular nerve regions that innervated the heart and lungs, stimulating pulses (2 mA, 200 µs, 20 Hz) were individually delivered to all thirteen GTEs. It was shown that by delivering stimulating pulses to GTEs 4 and 9, heart rate, blood pressure and respiratory rate were modulated. Precisely, only when the stimuli were delivered to GTE 9, the heart rate began to fall and only when the stimuli were delivered to GTE 4 the rate of breathing decreased. To test the selectivity of recording the above-defined groups GTEs 4 and 9 and randomly chosen GTEs 1 and 7 were simultaneously used as recording GTEs while cardio-vascular or respiratory systems were stimulated by carotid artery compression, epinephrine injection and non-invasive, positive end-pressure ventilation. Results showed that stimulations elicited site-specific changes in ENG power spectra recorded from the superficial regions of the vagus nerve. Power spectrum of the ENG recorded with GTE 9, contained frequencies belonging to the neural activity elicited by compression of the carotid artery and injection of epinephrine. The power spectrum of the ENG recorded with GTE 4, contained frequencies belonging to the neural activity elicited by non-invasive, positive end-expiratory pressure ventilation. We concluded that the multi-electrode nerve cuff enables selective stimulation and recording of nerve activity from internal organs.

Key words: multi-electrode nerve cuff, electroneurogram, arterial pressure, heart rate, epinephrine, ventilation.

Introduction

There has been a big interest in the possible applications of functional electrical stimulation (FES) to the autonomic nervous system (21). However, only limited research has been conducted using superficial recordings of electrical activity from peripheral nerves of the autonomic nervous system (13, 24, 27).

Sensing of neural signals could be used to control functional electrical stimulation (FES) devices (18, 29, 30). Using the microneurography technique developed by Vallbo and Hagbarth (35) it has been possible to record afferent signals. The drawbacks of this technique are that the population of units which can be sampled is relatively small and that the recording electrode is easily dislodged if any significant

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movement of the surroundings occurs (9, 10, 23). The results published in Haugland and Sinkjar (14) and Nikolić *et al*. (22) demonstrate that these limitations can be circumvented by using tripolar nerve cuffs. They give a more global picture of neural activity, as nerve cuff signals enable considerable spatial and temporal averaging (25, 26).

When recording an ENG from sensory fibres, in the presence of signals other than neural activity, one must exclude electromyographic activity (EMG) as well as other noise sources that can be several orders of magnitude larger than the neural signals. It was shown by Triantis *et al*. (32) that, in ENG recording techniques, tripolar cuffs assist in the additional reduction of interference from sources outside the cuff such as EMG as well as other unwanted signals. The described arrangement where the three electrodes are mounted on the inside of a cylinder made from an insulating material is also referred to as a "quasi-tripolar" configuration (19). Exclusion of unwanted signals in the registration of the ENG is achieved when the outer electrodes of the tripolar nerve cuffs are short-circuited and a bipolar amplifier is used to measure the potential difference between the middle and outer electrodes.

The cuff length (*i.e.* electrode separation) is one of the important parameters that determines signal amplitude. Amplitudes of the recorded signals first increase, as the cuff length increases, and then reach saturation (1). Faster fibers require longer cuffs for saturation (31). Another important factor that determines signal amplitude is the volume of extracellular fluid surrounding the nerve segment inside the cuff because it shunts the electrical potentials to be recorded. A snugly fitting cuff gives considerably larger amplitudes compared with a cuff that has a relatively larger diameter. One advantage of the self-coiling cuff is that it squeezes the fluid out and increases the resistivity of the extraneural space within the cuff, thus increasing the signal amplitude. Therefore, the tripolar configuration has two advantages: [1] a superior resistance to electrical disturbances from the surrounding muscles and power lines, and [2] a high degree of mechanical stability. Nerve cuffs implanted on cutaneous nerves provided useful and reproducible whole-nerve recordings. The published results (13, 14, 22) also demonstrate the functional use of cutaneous mechanoreceptors recorded by an implantable whole-nerve cuff recording electrode. However, these whole-nerve recordings provide only one channel of information from the aggregate activity of many nerve fibres. Information recorded selectively with a multi-electrode spiral cuff could be effectively used for closed loop control of implantable stimulators (20, 24) that selectively activate neural pathway (*e.g.* stimulation of the nerves innervating the heart and thus for closed loop control of the cardio-vascular system). Attaining this ultimate goal, however, demands the development of a theoretical model and further animal experiments.

Accordingly, the first step in our study was to develop a model, methodology and complete set-up, including cuffs, for chronic and reliable selective recording of ENGs from superficial regions of the left vagus nerve of an adult Beagle dog.

Precisely, the first specific purpose of this study was to examine the feasibility of using the cuff for selective recordings of cardio-vascular output modulated by compression of the carotid artery at the carotid sinus and by neurally mediated syncope (NMS), induced by intravenous injection of epinephrine. The second specific purpose of this study was to examine the feasibility of using the cuff for recordings of respiratory output in anaesthetized and artificially ventilated dogs. The final focus of this study was to analyze the recorded ENGs in terms of frequency spectrum characteristics.

Materials and Methods

Model Predictions

Regulation of arterial blood pressure is accomplished by negative feedback systems incorporating baroreceptors located in the carotid sinus and in the aortic arch. The carotid sinus nerve, a branch of the glossopharyngeal nerve, innervates the carotid sinus baroreceptors while aortic arch baroreceptores are innervated by the aortic nerve, which then combines with the vagus nerve. Both nerves synapse in the brainstem (3, 11, 17). Baroreceptors of the carotid sinus are normally the dominant arterial baroreceptor (36). In dog, normal arterial blood pressure values are: systolic pressure 133.0 mmHg, diastolic pressure 75.5 mmHg, and mean arterial pressure (MAP) 98.6 mmHg (7).

Neurocardiogenic or vasovagal syncope (NMS) results from reflex-mediated changes in vascular tone or heart rate (5). NMS is characterized by peripheral vasodilation and a decrease in blood pressure, or hypotension, along with bradycardia, or by bradycardia alone (2, 37). These changes in heart rate and blood pressure are due to an increase in parasympathetic tone and a concomitant inhibition of sympathetic outflow (2, 37). We assumed in our experimental protocol, that NMS could be provoked with a single *i.v.* injection of epinephrine. Namely, it was shown (8) that epinephrine infusion into the carotid sinuses tunica externa increases discharges from the glossopharyngeal nerve potentially due to activation of the the baroreceptors. It was expected that the baroreceptors of both the carotid sinus as well as the aortic arch would respond to the injection of epinephrine with an increased firing rate. Consequently, increased superficial activity of both innervating nerves could be expected.

Increased pressure over the carotid artery at the carotid sinus provokes a reflex decrease in heart rate (bradycardia), which may provoke cardiac arrythmia. Namely, when the carotid sinus senses an increase in pressure, either blood pressure or external pressure, it may send a signal to slow the heart rate or decrease the blood pressure without slowing the heart rate (4, 6). Therefore, it was expected that the baroreceptors of the carotid sinus would respond to the aforementioned compressions with an increased firing rate while the aortic arch would remain relatively inactive since they are not directly stimulated (15, 28). It was proposed in the model that compression of the carotid artery at the neck with the fingers would have the same physiological effect as an *i.v.* injection of epinephrine. The result would be a decrease of the MAP, during the aortic pulse cycle. We suggest in the model that the activity of baroreceptors, innervated by the aortic nerve, travelling *via* a certain superficial region of the left vagus nerve to the brainstem, could be selectively recorded with the cuff implanted on the left vagus nerve.

The contribution of vagal afferents from the lung that are important in regulating the depth and frequency of breathing depends on the properties of the receptors in the tracheobronchial tree (2, 3). All types of receptor have their afferents in the vagal nerves. Therefore, we suggest in the model that afferent activity of the aforementioned receptors, travelling *via* a certain superficial region of the left vagus nerve to the brainstem, could be increased by non invasive positive end-expiratory pressure ventilation and selectively recorded *via* the cuff implanted on the left vagus nerve.

Finally, the present study addresses the hypothesis that a certain superficial region of the peripheral autonomic nerve is composed mainly of fibres innervating a single internal organ or gland (33).

Design of a Multi-Electrode Cuff

A cuff was made by bonding two 0.1 mm thick silicone sheets together. One sheet, stretched and fixed in that position, was covered by a layer of adhesive (MED-1511, NuSil, Carpinteria, CA, USA). A second unstretched sheet was placed on the adhesive and the composite was compressed to a thickness of 0.3 mm until the whole curing process was completed. When released, the composite curled into a spiral tube as the stretched sheet contracted to its natural length (20). The cuff, which was developed for selective electrical nerve stimulation, wraps around the nerve and, because of its self-coiling property, adjusts its diameter to the size of the nerve. Thirty-nine rectangular electrodes with a width of 0.6 mm and length of 1.5 mm were made of 50 μm thick platinum ribbon (99.99% purity) and mechanically connected to the insulated lead wires (AS 631, Cooner Wire, Chatsworth, CA, USA).

Afterwards, a matrix of thirty-nine 0.9 mm wide and 1.5 mm long openings arranged in three parallel groups each containing 13 positions at a distance of 0.5 mm were made in the third 0.1 mm silicone sheet. The distance between the three groups was 6 mm. Each of aforementioned thirty-nine electrode-lead wire compositions was then inserted into one of rectangular thirty-nine openings and fixed with silicone adhesive. At a front side of thus obtained sheet, the matrix of 33 electrodes was formed. At the back, side however, 33 lead wires were bent in the same direction to be connected to the common connector.

Back side of the silicone sheet with the matrix of electrodes was then bonded on the inner side of the mechanically opened cuff. The lead wires passed through to the outside of the cuff between silicone sheets. Each electrode marked with the same number within each of the three parallel spiral groups had the same position. Accordingly, thirteen groups of three electrodes (GTE) in the same line, in a longitudinal direction were formed.

The length of the cuff was optimized so that the surface of the nerve covered by the spiral cuff would be as small as possible to prevent damage associated with a reduced blood supply and excessive mechanical trauma of the nerve. To obtain maximum signal amplitudes, the length of a cuff should be close to the wavelength of neural action potentials (30-40 mm) or about 10 times greater than the inner diameter. Therefore, the cuff with an inner diameter of 2.5 mm was trimmed to a length of 18 mm as shown in the upper left corner of Fig. 1.

Cuff Implantation

The experiment was performed on an adult Beagle dog. The authors followed the guidelines contained in the Declaration of Helsinki and the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals in all experiments involving experimental animals. Under fully sterile conditions a gas sterilized (ethylene oxide) cuff was implanted according to the following protocol approved by the ethics committee of the Veterinary Administration of the Republic of Slovenia, Ministry of Agriculture, Forestry and Food. The animal was premedicated with medetomidine 40 μg/kg i.m. (Domitor, Orion Corp., Espoo, Finland) and methadone 0.2 mg/kg s.c. (Heptanon, Pliva, Zagreb, Croatia). The induction was performed with propofol 1.0 to 2.0 mg/kg *i.v.* (Diprivan, Zeneca Pharmaceuticals Ltd., Macclesfield,

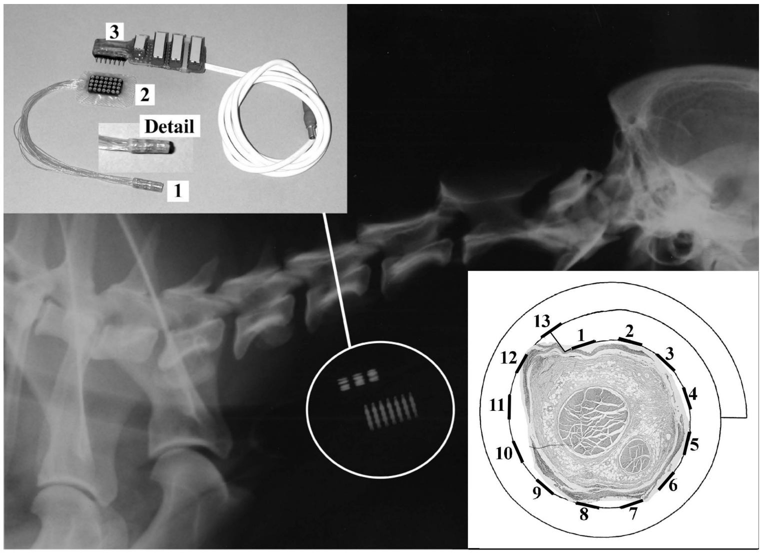

Fig. 1. X-ray of the 39-electrode spiral cuff implanted on the left vagus nerve of a dog. Upper left: (1) 39-electrode spiral cuff, (2) subcutaneous common connector, and (3) switch module. Lower right: a model of the cross-sectional geometry of the left vagus nerve within the cuff and relative position of GTEs 1 to 13; GTE 9 was adjacent to the superficial nerve regions innervating the heart and GTE 4 was close to the superficial nerve region innervating the respiratory musles.

Cheshire, England). General anaesthesia was maintained with isoflurane 0.8 to 1.5 vol. % (Forane, Abbott Laboratories Inc., Abbott Park, IL, USA) in 100 % O_2 (12, 16). When necessary, during surgery analgesia was sustained with ketamine 0.5 to 2.0 mg/kg *i.v.* (Ketamine, Veyx-Pharma GmbH, Schwarzenborn, Germany). Antibiotics (cefazolin 20 mg/kg *i.v.*; Cefamezin, Krka, Novo Mesto, Republic of Slovenia) were administered perioperatively. The room temperature was kept between 23.4° and 24.4°C and the temperature of the skin of the neck was also continuously monitored. The cuff was installed on the left vagus nerve at the neck as shown in Fig. 1. The leads of the implanted cuff were routed and fixated to the corresponding common connector under the skin. To ensure that the cuff's leads would not be a possible source of mechanical damage, special care was taken during implantation to route the leads so that enough slack would be left to avoid mechanical tension being transmitted to the cuff. Finally, the incision was closed and the animal awakened. Analgesia during the early recovery period was provided with methadone 0.3 to 0.5 mg/kg s.c. TID. Tramadol 8.0 mg/kg s.c. TID (Tramal, Grunenthal GmbH, Stolberg, Germany) was administered for a further two days.

After the last experiment, the animal was sacrificed using commercial euthanasia agent T61 administered *I.V.* (Hoechst, Frankfurt, Germany).

The whole study was performed within a time frame of three years. The first year was spent to develop the model, develop and fabricate the cuffs and to organize the materials, apparatus and facilities. The cuffs were actually implanted in two adult Beagle dogs. After the cuffs were implanted the next two months were spent to allow the animals to fully recover from anaesthesia and tissue healing. In both animals the cuff remained implanted for a period of two years, but during the last 6 months the nerves were not stimulated. Within the remaining period of 16 months, four recordings in each animal were performed. They were repeated about every five months. In this actually very complex study we tested mainly selectivity and reproducibility of the recordings and measured only basic parameters of physiological function in the tested organs. Due to the afore-mentioned complexity of the experimental work and the extent of the expected outcomes of the study a reliable statistical model could not be developed. Besides, in each animal unique conditions were established so only selectivity and intra-individual reproducibility could be tested. Accordingly, because of the afore-mentioned lack of a reliable statistical model, we present the results obtained in the last of four sessions conducted in the second implanted animal.

Connection of the Cuff's Electrodes to a Switching Module

All lead wires were connected to a special common connector implanted within the lateral subcutaneous tissue for the time between the experimental sessions. A special cable, which was shielded to minimize the pick-up of unwanted signals from the environment, was used to connect the common connector to the inputs

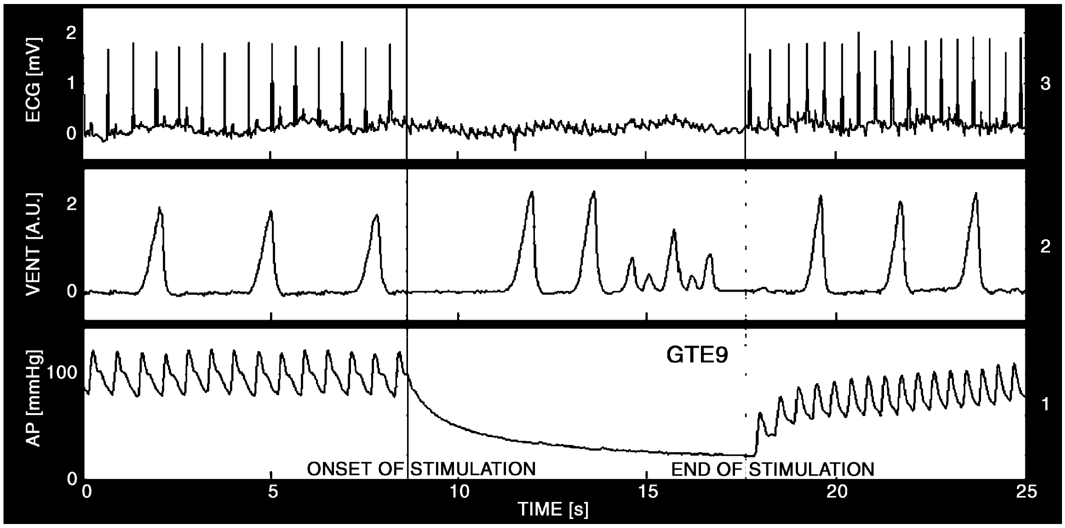

Fig. 2. The effect of selective stimulation of the left vagus nerve with GTE9 on ECG (trace 3), respiration (trace 2) and blood pressure (trace 1). Vertical lines indicate stimulation duration.

of the ENG amplifying system. At one end of the cable to be connected to the common connector was a switching module designed to fit its pins. The switching module permitted each GTE to be connected to the amplifier individually or in combination with other GTEs. In the switching module a "quasi-tripolar" recording configuration was achieved. Namely, the two outer electrodes of a particular GTE to be connected to one end of a stimulator/amplifier input were short-circuited while the corresponding central electrode was to be connected as to the other end. Furthermore, the common connector was designed to permit simple and reliable multiple use. This is very important because the common connector must be reimplanted several times between individual experiments without any damage.

Identification of Particular Nerve Regions and GTEs

The first recording session was performed two months after implantation to allow the animal to fully recover from anaesthesia and enable tissue healing. During the recording sessions, the dog was anaesthetized according to the afore-mentioned procedure. After taking the subcutaneously implanted common connector out of the body, it was thoroughly cleaned and dried. The common connector was then placed on the cleaned skin close to the wound. The common connector and the wound were covered with self-adhesive sterile surgical foil to isolate them from the ambient atmosphere during the experiment. The connection of the common connector to the inputs of the amplifier was made by perforating the self-adhesive sterile surgical foil with the pins of the switch module and inserting them in the common connector. It was crucial for the recordings that the connection permitted reliable mechanical and galvanical connections.

The switches of the switching module were alternately turned on so as to connect the electrodes of a particular GTE to the amplifier. Each of the thirteen GTEs was then denoted by a consecutive number as shown in the left lower corner of Fig. 1, where the geometric model of the cuff fitted on the left vagus nerve is presented. However, considering the dimensions of the nerve and the cuff, it was expected that certain GTEs remained out of contact with the superficial regions of the nerve.

The relative positions of GTEs closest to the superficial regions of the nerve innervating the heart and the respiratory muscles were determined experimentally. This was done by delivering stimulating pulses quasi-bipolarly to all 13 GTEs within the cuff. The superficial regions on the circumference of the nerve innervating the afore-mentioned organs that were in contact with the corresponding GTE were selectively stimulated using rectangular, biphasic, charge balanced, current pulses with an intensity of 1.3 to 2 mA and a frequency of 20 Hz.

The GTE 9 that elicited the largest measurable change in heart rate and blood pressure are shown in Fig. 2. Selective stimulation with GTE 9 elicited a sharp fall in blood pressure and heart rate with a simultaneous change in ventilation rate and amplitude. Both recovered after cessation of stimulation with GTE 9.

To validate changes in heart rate, the ECG from the first bipolar limb lead lead was recorded *via* stainless steel hypodermic needles. Simultaneously, arterial blood pressure was measured with a catheter inserted into the dorsal metatarsal artery. The catheter was attached to the disposable pressure transducer

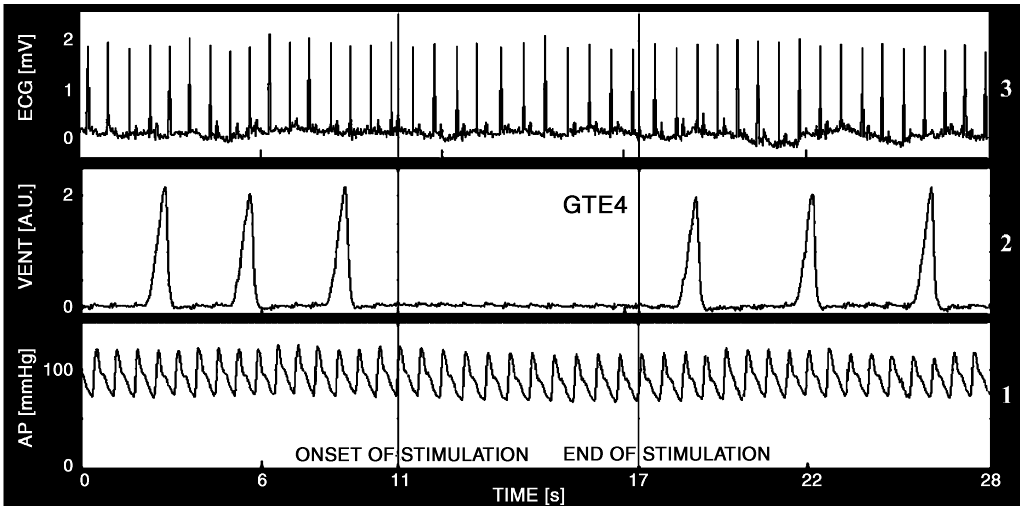

Fig. 3. The effect of selective stimulation of the left vagus nerve with GTE4 on respiration (trace 2), blood pressure (trace 1) and ECG (trace 3). Vertical lines indicate stimulation duration.

system (model: DPT-6000) for invasive blood pressure monitoring (Smiths Medical Deutschland GmbH, Kirchseeon, company section: pvb-Critical Care). The ECG and blood pressure signals were delivered to a custom designed differential amplifier and to a DigiPack 1200 high performance data acquisition system connected to a PC.

When stimuli were delivered to the GTE 4 that was in contact with the superficial region of the nerve innervating the respiratory muscles, the rate of respiration decreased as shown in Fig. 3. Stimulation with GTE 4 abolished ventilation during the duration of stimulation but did not affect the heart rate or blood pressure. Ventilation rate and amplitude were restored after cessation of stimulation with GTE 4. To validate the changes in the rate of respiration, produced by selective stimulation, variations of the circumference of the thorax were measured by a metal belt instrumented with a custom designed force transducer mounted around the chest. Then the signals obtained from the force transducer were delivered to a custom designed bridge amplifier and to a data acquisition system connected to the PC.

It was shown that by delivering stimulating pulses to the GTEs adjacent to the GTEs 4 and 9, heart rate, blood pressure and respiratory rate were significantly less effective modulated. In Figures 2 and 3, the rate of respiration is expressed in arbitrary units (A.U.). Namely, a force transducer attached tightly to the chest of a dog measured forces caused by variations of the thorax circumference.

Cuff Electrode Impedance

To obtain information crucial for reliable recording of the ENGs, we measured the impedances of electrodes within the cuff. This was done 2 months after implantation, just before the recording of ENG was performed. Impedances were measured with a Hewlett Packard vector impedance meter (Model 4800A) at frequencies ranging from 1 to 10 kHz. Since the surface of the cuff electrodes was relatively large, their impedance (Z) was low. Impedances of the connected and short-circuited outer electrodes and of the connected central electrodes, both measured at a frequency of 1 kHz, were approximately 0.25 kΩ and 0.4 kΩ, respectively. An external ground electrode was tightly placed around the thigh, resulting in low impedance.

ENG Recording and Analysis

The short-circuited outer electrodes in each of the two indicated GTEs 4 and 9, and randomly chosen GTEs 1 and 7, were then simultaneously connected through an explanted connector and a switch module to one end of the custom designed ENG preamplifier, and the corresponding central electrode was connected to the other end. A hypodermic needle, inserted into the subcutaneous tissue in the area above the spine, was used as the ground electrode and connected to the ENG preamplifier common input.

All system preamplifiers for ENG recordings were designed with OPA-27 and OPA-37 (Burr-Brown) ultra-low noise precision operational amplifiers (34). Each preamplifier consisted of three amplifying stages. The first (input) and the second stage were designed using OPA-27 operational amplifiers. Input differential resistance was set to 1M (and the gain of both stages to A = 25. The output of the second stage was fed to a passive band-pass

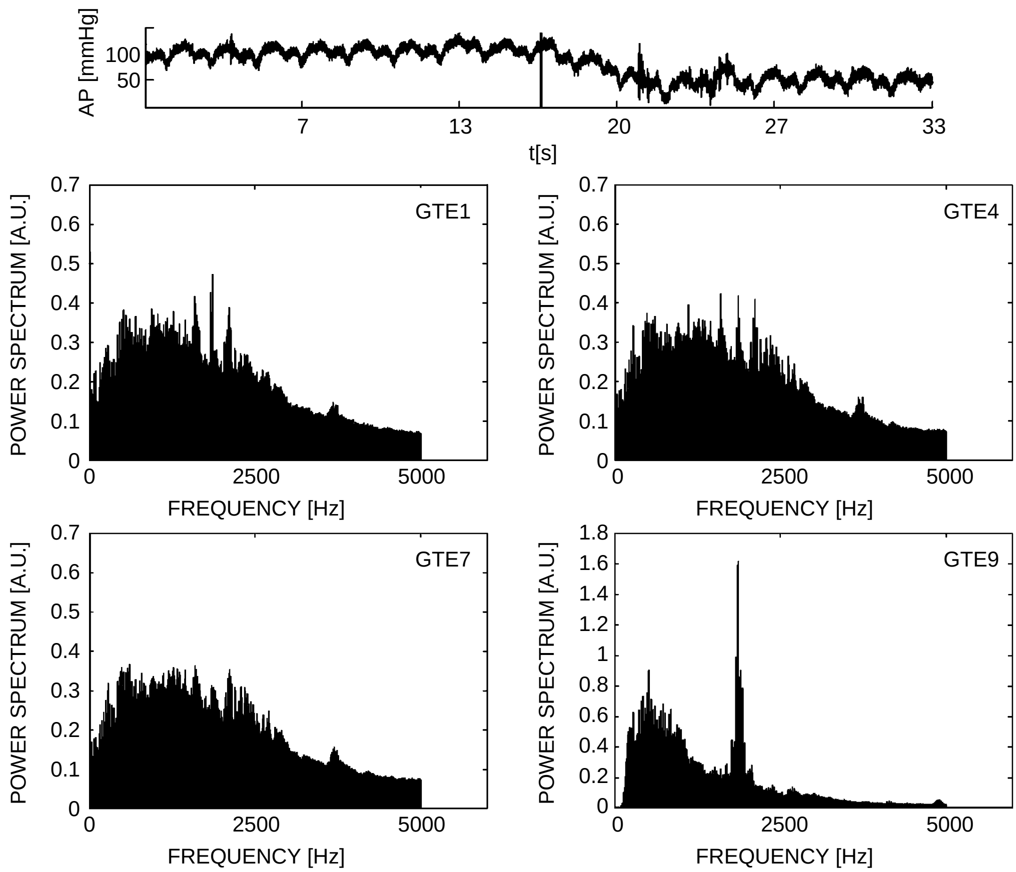

Fig. 4. The effect of unilateral compression of the left carotid artery on blood pressure and ENG power spectra of GTEs 1, 4, 7 and 9. The top section of Fig. 2 shows a blood pressure trace before and after compression and the bottom section the ENG power spectra of GTE 1, 4, 7 and 9 during carotid artery compression. (time frame 17 s to 33 s). Vertical line indicates the start of carotid artery compression.

filter (700 Hz to 10 kHz). The third (end) stage with an OPA-37 operational amplifier provided an adjustable gain to compensate for attenuation by the passive filter and to set the total gain of the preamplifier to A = 10,000.

In the main amplifier, set to a gain of A = 10, the ENG was further bandpass filtered using a high-pass filter (5^{th} Order Chebyshev) with a lower cut-off frequency of 500 Hz (-3dB) to remove EMG contamination, and a low-pass filter (3^{rd} Order Bessel) with a higher cut-off of 10 kHz (-3dB). The complete ENG amplification system contained an independent preamplifier and the main amplifier. Both units were powered by two 12 V rechargeable sealed lead-acid batteries. The total amplification of the system was A = 100,000.

The recorded ENGs were fed to a DigiPack 1200 high performance data acquisition system and sampled at 20 kHz. The system includes a Digidata 1200A analogue to digital (A/D) adapter, designed and manufactured by Axon Instruments, and high speed data acquisition software (AxoScope 8.2).

Heart rate and blood pressure were modulated by two separate interventions: [1] manual compresion of the carotid artery at the neck for 15 s and [2] a single *i.v.* bolus of epinephrine (0.1 ml/kg, 1:200). Non-invasive, positive end-expiratory pressure ventilation (10 cm of H_2O) was used to modulate respiration. Mechanical respiration was delivered to the lungs through a mouthpiece that was connected to a respirator.

Changes in ENG activity, recorded under each GTE within the defined superficial region of the nerve, that were elicited by compression of the carotid artery, injection of epinephrine and non-invasive intermittent positive-pressure ventilation were evaluated by calculating the power spectra of corresponding ENGs. For this purpose a fast Fouriere transformation (fFt) using Matlab 6.5 within a band of frequencies ranging from 1 kHz to 5 kHz was performed.

Results

Unilateral compression of the left carotid artery decreased blood pressure and had a significant effect

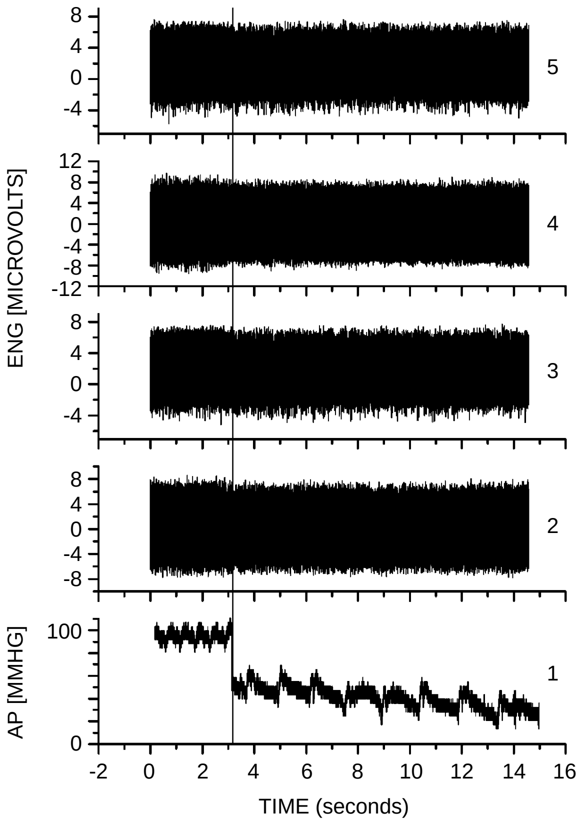

Fig. 5. ENGs recorded by 4 of 13 GTEs before epinephrine was applied (left to the vertical line) and while and after epinephrine was applied (right to the vertical line). Bottom trace: the trajectory of corresponding blood pressure and heart rate.

only on the ENG power spectrum recorded by GTE 9 (Fig. 4). MAP (mathematical mean value) decreased from 100 mmHg (systolic pressure 120 mmHg, diastolic pressure 80 mmHg) to 50 mmHg (systolic pressure 70 mmHg, diastolic pressure 30 mmHg). Carotid artery compression did not influence the heart rate - it was 103 bpm before and after compression. Only the GTE 9 ENG power spectrum showed frequencies which could be attributed to intensified superficial nerve activity due to compression of the carotid artery. The spectrum was bimodal with the higher mode (1.75-1. 96 kHz) with a power peak of 1.6 A.U. at 1.82 kHz) and the lower frequency components (70 Hz-1.27 kHz) which have much lower amplitudes except at a power peak of 0.9 A.U. at 506 Hz.

Fig. 5 shows four raw ENGs recorded before (left to the vertical line) and after (right to the vertical line) epinephrine was injected. Accordingly, trace (b) shows the ENG recorded by GTE1, trace (c) shows the ENG recorded by GTE4, trace (d) shows the ENG recorded by GTE7, and trace (e) shows the ENG recorded by GTE9. The bottom-most trace (a) of the figure, representing arterial blood pressure, shows a significant decrease in mean blood pressure and bradycardia after epinephrine was applied.

The effect of a single *i.v.* dose of epinephrine on the heart rate and blood pressure is presented in Fig. 6. Epinephrine injection caused a significant decrease in mean blood pressure and bradycardia. MAP decreased from 100 mmHg (systolic pressure 120 mmHg, diastolic pressure 80 mmHg) to 25 mmHg (systolic pressure 45 mmHg, diastolic pressure 5 mmHg), and heart rate decreased from 117 bpm to 40 bpm. Only the ENG power spectrum recorded with GTE 9 showed frequencies which could be attributed to intensified superficial nerve activity due to the injection of epinephrine. The spectrum was bimodal; a higher mode (1.62-2.1 kHz) with a power peak of 1.55 arbitrary unit (A.U.) at 1.86 kHz and lower frequency components (100 Hz-1.62 kHz) which have much lower amplitudes, except at a power peak of 0.75 arbitrary units (A.U.) at 506 Hz.

Non-invasive positive end expiratory pressure ventilation elicited a significant change in the ENG power spectrum recorded with GTE 4, situated close to the superficial nerve region innervating the lungs (Fig. 7). Namely, only the ENG power spectrum recorded with GTE 4 showed frequencies which could be attributed to intensified superficial nerve activity elicited by non-invasive, positive end-pressure ventilation. The spectrum was bimodal; a higher mode (1.98-2.20 kHz) with a power peak of 1.4 A.U. at 2.117 kHz and lower frequency components (100 Hz-1.98 kHz) which have much lower amplitudes, except at a power peak of 1 A.U. at 302 Hz.

In Figures 6 and 7, showing power spectra, the intensity of frequencies is validated in arbitrary units (A.U.).

Discussion

We have demonstrated for the first time that multi-electrode spiral cuffs are an effective method for long-term, selective recording of ENGs from superficial regions of autonomic peripheral nerves innervating internal organs. Specifically, it was shown that ENGs selectively recorded from superficial regions of the left vagus nerve, innervating the heart and the lung, containing both efferent and afferent activity. We present in this study that natural sensory activity in superficial regions of the left vagus nerve of a dog innervating above-mentioned organs, arising most probably from their receptors such as barroreceptors and recorded selectively with a chronically implanted cuff, potentially contains useful information about the visceral function.

It was shown in our study that the heart and the lung ENGs contained frequencies that can be attributed to communicating activity between the afore-

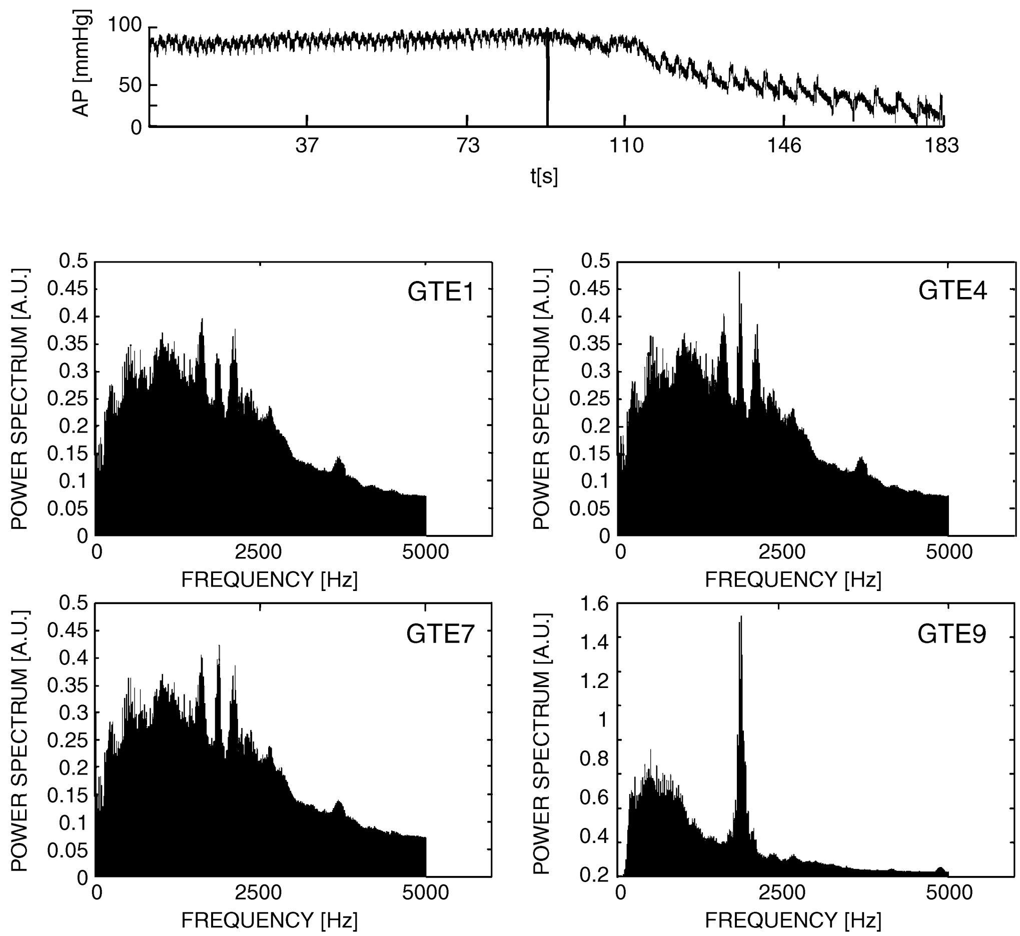

Fig. 6. The effect of a single *i.v.* dose of epinephrine on the heart rate, blood pressure and ENG power spectra of GTE 1, 4, 7 and 9. The power spectra were calculated within the period from the injection of epinephrine at the 92. second to the end of recording at the 183. second. Vertical line indicates the time of epinephrine injection.

mentioned organs and the central nervous system. In the presented study the activity of the parasympathetic left vagus nerve rose immediately after three separate interventions: carotid artery compression, non invasive positive end-expiratory pressure ventilation and *i.v.* epinephrine administration. However, afferent and efferent activities could not be distinguished.

Our study contributed to the development of multi-electrode spiral cuffs for ENG recording and at the same time to the development of multi-electrode spiral cuffs for stimulation of peripheral nerves of an autonomic nervous system, innervating internal organs and glands. The cuff had yielded reliable and reproducible recordings for 16 months. The signal-to-noise ratios of the left vagus nerve recordings obtained with the cuff electrode were in an acceptable range and could detect modulations of superficial nerve activity by changes in cardio-vascular and respiratory drive.

A major advantage of the cuff electrodes over any other type of electrodes was their mechanical stability, which significantly improved the reproducibility of the recordings both in terms of signal amplitude and frequency content. However, it was shown by Triantis *et al*. (32) that cuff imbalance related to cuff asymmetry, cuff end-effects and interference source proximity could degrade the performance of ENG amplifier configurations, such as the quasi-tripole and the true-tripole. The performance of an ENG amplifier configuration can be improved by using the adaptive-tripole that automatically compensates for cuff imbalance (1, 19).

One weakness of a multi-electrode cuff manufacture is a technically demanding and a time consuming process. Namely, production time per cuff is more than 14 workdays. Furthermore, most of the cuff production stages, which are actually artistic work, have to be performed under a ×30 magnifying lens. Nevertheless, even for an experienced person it is not possible to achieve a success rate greater than 10-30%. The rejection rate is more than 60% because of uneven spacing among the cuff electrodes. The technical solutions described in our study could be

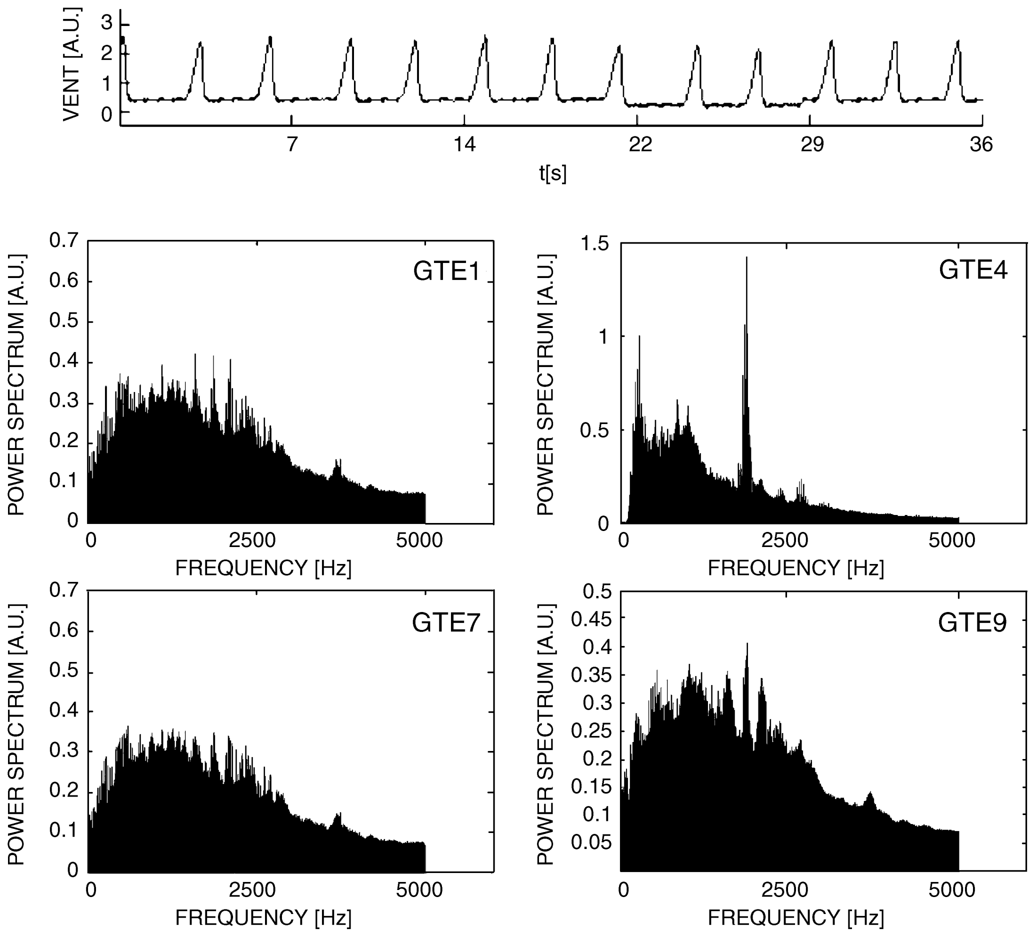

Fig. 7. The effect of non-invaisive, positive end-expiratory pressure ventilation (duration 36 s) on ENGs power spectra recorded by GTEs. Top section: ventilation during non-invasive, positive end-expiratory pressure ventilation. Bottom section: ENG power spectra of GTEs 1, 4, 7 and 9.

used in various animal and human basic studies concerning neurophysiology of internal organs, and their relation to bodily changes and diseases. Multi-electrode spiral cuffs on peripheral autonomic nerves could be used for simultaneous stimulation and recording of nerve activity. ENGs recorded selectively with a multi-electrode cuff could be useful as a potential feedback signal for control of visceral function of different internal organs and glands. Our future studies will focus on the analysis of selectively recorded ENGs to extract information which could be potentially useful for neurological evaluation of diseases in internal organs. Therefore, the long-range goal of our research will be to understand how the various branches of the autonomic nervous system regulate visceral function.

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