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INFLUENCE OF TRANSIENT RESPONSE OF PLATINUM ELECTRODE ON NEURAL SIGNALS DURING STIMULATION OF ISOLATED SWINISH LEFT VAGUS NERVE

VPLIV PREHODNEGA ZNAČAJA PLATINASTE ELEKTRODE NA ŽIVČNI SIGNAL MED STIMULACIJO IZOLIRANEGA ŽIVCA VAGUSA SVINJE

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The main aim of the work was to measure transient response characteristics of interface between platinum stimulating electrodes and isolated swinish left cervical vagus nerve (segment), when electrical stimulating pulses are applied to preselected locations along the segment and elicited neural signals, also described as compound action potentials (CAPs), are recorded from particular compartments of the nerve.

The stimulating system was manufactured as a silicone self-coiling spiral cuff (cuff) with embedded matrix of ninety-nine rectangular electrodes (0.5 mm in width and 2mm in length), made of 45 µm thick annealed platinum ribbon (99.99 % purity), and a geometric surface of 1 mm^2.

For electrical stimulation, a current quasitrapezoidal, asymmetric and biphasic pulses with frequency of 1 Hz, were used. To test an influence of stimulating pulses having different parameters and waveforms on elicited CAPs, various degree of imbalance between an electric charge (charge) injected in cathodic phase as well as charge injected in anodic phase of a biphasic stimulating pulse, were deployed and compared.

To identify the differences in elicited CAPs however, an integral of the CAP cathodic phase as well as integral of the CAP anodic phase of stimulating pulse, were calculated and compared.

Results showed a strong component superimposed in the CAPs, considered as an ensemble artefact which greatly obscured the components of the CAPs, and various components did overlap.

Results also showed that stimulating pulses, having preset certain degree of imbalance between charge injected in cathodic and charge injected in anodic phase, elicited a slight change in a positive waveform deflection of CAP manifested under a cathodic phase as well as slight change in a negative waveform deflection of CAP manifested under an anodic phase. Furthermore, slight difference was observed in a CAP, expressed as integral cathodic positive deflection and as integral anodic negative deflection. However, it could be concluded that measured CAPs are not greatly influenced by the imbalance between a charge injected in cathodic and anodic phase of quasitrapezoidal, asymmetric and biphasic stimulating pulses.

Keywords: electrical stimulation, platinum electrodes, left vagus nerve, electrochemistry, electrical charge

Glavni namen dela je bil izmeriti prehodni značaj na prehodu med platinasto stimulacijsko elektrodo in delom izoliranega levega vratnega prašičjega živca vagusa (segment) med dovajanjem stimulacijskih impulzov na izbrana mesta vzdolž živca in hkratnim merjenjem živčnega signala, imenovanega sestavljeni akcijski potencial (CAP) na določenih predelih živca.

Stimulacijski sistem je bil izdelan v obliki silikonske spiralne objemke (cuff) z vdelano matriko devetindevetdestih pravokotnih elektrod (širina 0,5 mm in dolžina 2 mm), izdelanih iz žarjenega platinastega traku (čistost 99,99 %) in geometrijsko površino 1 mm^2.

Za električno stimulacijo so bili uporabljeni tokovni kvazitrapezni, asimetrični in izmenični impulzi frekvence 1 Hz.

Za preizkušanje vpliva stimulacijskih impulzov z različnimi parametri in oblikami na izzvani CAP je bila med katodno in anodno vneseni električni naboj uvedena določena stopnja neuravnoteženosti. Katodno in anodno vnesena naboja sta bila za izbrane impulze med seboj primerjana.

S ciljem ugotavljanja razlik v izzvanem CAP-u pa sta bila izračunana in med seboj primerjana integrala tako CAP-a, prisotnega pod katodno fazo, kot CAP-a, prisotnega pod anodno fazo zgoraj omenjenega stimulacijskega impulza.

Rezultati so pokazali močno komponento, položeno na CAP, ki je sestavljena motnja in ki znatno zamegli ter prekrije posamezne komponente CAP-a.

Rezultati so tudi pokazali, da zgoraj omenjeni stimulacijski impulzi s prednastavljeno določeno stopnjo neravnotežja med nabojem, vnesenim v katodni fazi, ter nabojem, vnesenim v anodni fazi, izzovejo majhne spremembe pri pozitivnem odklonu CAP-a, prisotnega pod katodno fazo, kakor tudi majhne spremembe v negativnem odklonu CAP-a, prisotnega pod anodno fazo.

Nadalje so bile opažene tudi majhne razlike v CAP-ih, izražene kot integral pod katodnim pozitivnim odklonom in kot integral pod anodnim negativnim odklonom.

Končno je mogoče skleniti, da neravnotežje med katodno in anodno vnesenim nabojem ni znatno vplivalo na izmerjene CAP-e.

Ključne besede: električna stimulacija, platinaste elektrode, levi živec vagus, elektrokemija, električni naboj

1 INTRODUCTION

In the past few decades, vagus nerve stimulation (VNS) has been the subject of considerable research with the goal to be used as method to treat a number of nervous system disorders, neuropsychiatric disorders, eating disorders, sleep disorders, cardiac disorders, endocrine disorders, and pain, among others.[1–3] In practically all studies in humans, VNS refers to non-selective stimulation of the cranial nerve X, known as the left vagus nerve, using specific electrode devices which development was based on different models provided by various research groups.The frequent result of non-selective stimulation however, is the occurrence of undesirable side effects.[4–6]

Peripheral nerve stimulation however, often requires the development of electrode systems that stimulate selectively a certain group of fibers in a nerve trunk without excitation of other nerve fibers.

However, the long-term use of such electrical stimulation requires that it is applied selectively and without tissue injury. Tissue injury and the corrosion of the stimulating electrode are both associated with high charge density stimulation.[7,8] For this reason, long-term stimulation of the nervous tissue requires the absence of irreversible electrochemical reactions such as electrolysis of water, evolution of chlorine gas or formation of metal oxides.

For a given electrode, there is a limit to the quantity of charge that can be injected in either anodic or cathodic direction with reversible surface processes. This limit depends upon the parameters of the stimulating waveform, the size of the electrode, and its geometry.[9–11] Namely, at the electrode-electrolyte interface there are capacitive mechanisms (charging and discharging of the electrode double layer, no electron transfer) and Faradaic mechanisms (chemical oxidation or reduction, reversible or irreversible).[12–14] If the voltage across the electrode-tissue interface is kept within certain limits, then chemical reactions can be avoided, and all charge transfer will occur by the charging and discharging of the double-layer capacitance. However, in many instances, the electrode capacitance is not sufficient to store the charge necessary for the desired excitation without the electrode voltage reaching levels where reactions could occur.[11,15]

The principal approach to control the interface voltage has been the use of charge-balanced biphasic stimuli that have two phases that contain equal and opposite charge. However, even with charge-balanced stimulating pulses it is possible that the interface voltage may reach levels where electrochemical reactions can occur.[16,17]

Platinum is among commonly used stimulating electrode materials that are capable of supplying high-density electrical charge to effectively activate neural tissue.[11] However, stimulation with a high charge density, pH shifts causing irreversible changes in tissue proteins, metallic dissolution products, gross hydrogen and oxygen gas bubbles, and oxidized organic and inorganic species, could occur[18]. Therefore, some platinum toxicity interactions in the body, actually not conclusively proven in human, could be expected. Namely, tests on laboratory mammals showed that soluble platinum compounds are much more toxic than insoluble ones while solid platinum wire or foil is considered to be biologically inert. Complexes with other dangerous metals and chemicals in the human body such as platinum salts, can cause several health effects, such as: DNA alterations, cancer, allergic reactions of the skin and the mucous membrane, damage to organs, such as intestines, kidneys and bone marrow and hearing damage.[19,20]

In the area of Functional Electrical Stimulation (FES) cuffs have been used in neuroprosthetic applications as stimulation electrodes as well as electrodes for the recording of the electroneurogram (ENG) for more than 35 years.[21] Twenty years later, this method was used for the first time in a chronic implantation in human subjects for the recording of the ENG for the use of feedback signal in a system for the correction of foot-drop.[22] While the time was passing, theoretical considerations and different models have stimulated and accompanied the development of cuffs[23,24] promoting them as the most successful biomedical electrodes for selective stimulation of different superficial regions of a peripheral nerve.[25,26] However, the long-term effectiveness and potential harmful effects of the cuffs on neural tissue in various applications is still not completely defined.

The present study addressed the mechanisms that could be involved in the modulation of recruitment properties of nerve fibres, and thus, in the modulation of CAP induced by both, the cathodic and anodic charge injected during selective stimulation of the isolated vagus nerve with developed cuffs and imbalanced quasitrapezoidal stimulating pulses having different parameters.

2 METHODS

The cuff was designed taking into consideration the results of histological examination of the swinish left vagus nerve, the model of selective electrical stimulation of particular superficial regions of the nerve and the model of selective stimulation of nerve fibers with different diameters.[27–29] The cuff and physical dimensions of the cuff were actually devised so to induce as low as possible radial pressure when installed on the nerve. Therefore, minimum mechanically induced nerve damage might be expected.

The cuff was manufactured by bonding two 0.05 mm thick silicone sheets together (Medical Grade Silicone Sheeting, Non-Reinforced, 6” × 8” × 0.002” Matt, SH-20001-002, BioPlexus Corporation, 1547 Los Angeles Avenue #107, Ventura, California 93004. USA.). At room temperature one sheet, stretched and fixed in that position, was covered with a layer of adhesive (RTV Adhesive, Acetoxy, Implant Grade, Part Number 40064, Applied Silicone Corporation, 270 Quail Court, Santa Paula, CA 93060, USA). A second un-stretched sheet was placed on top of the adhesive and the composite was compressed to a thickness of 0.15 mm until the whole curing process was completed. In normal laboratory conditions, the curing process was completed within 24 h. When released, the composite curled into a spiral tube as the stretched sheet contracted to its natural length.[26,27] As a result, the composite is soft self-sizing and flexible self-coiling spiral tube. When instaled on the nerve, the cuff wraps around the nerve and, because of its self-coiling property, adjusts automatically its inner diameter to the size of the nerve.

Ninety-nine rectangular electrodes with a width of 0.5 mm and length of 2 mm (geometric surface $g = 1$ mm^2, real surface area ≈ 1.4 mm^2[13], made of 45 µm thick annealed platinum ribbon (99.99 % purity), were then under microscope mechanically mounted on the third silicone sheet with a thickness of 0.05 mm. They were arranged in nine parallel groups each containing eleven electrodes, thus forming a matrix of ninety-nine electrodes.[28,29]

Afterwards, the electrodes were connected individually to the high frequency miniature and highly flexible isolated, multi-stranded and enameled finest copper wires (CU-lackdraht DIN 46 435, Φ 12 × 0.04 mm, Elektrisola, Reichshof-Eckenhagen, Germany). For experimental purpose, the junctions between platinum electrodes and multi-stranded wires were implemented using a special tin alloy. The multi-stranded wire was used, since it has the same average fatigue life as their individual constituent strands but the variance of that life is smaller. To maximize service life, it was concluded that wire strands should be manufactured at the smallest diameter possible (without introducing structural flaws). It was assumed that multi-stranded wires to the stimulating electrodes if routed carefully would play a minimal role on rotation of the cuff around the logitudinal axis and on translation in a longitudinal direction. Therefore, to ensure that the multi-stranded wires would not be the possible source of mechanical damage to the nerve, special care should be taken during instalation to route them so that enough slack would be left to avoid mechanical tensions being transmitted to the cuff. Afterwards, a self-coiling tube was mechanically opened and the silicone sheet with the matrix of electrodes was adhered onto an inner side of the tube.

In fabricted cuff, when the matrix was spirally rolled up, the longitudinal separation between nine parallel groups of electrodes was 2 mm and the circumferential separation between electrodes was 0.5 mm.

The dimensions of the nerve considered in cuff design were the:

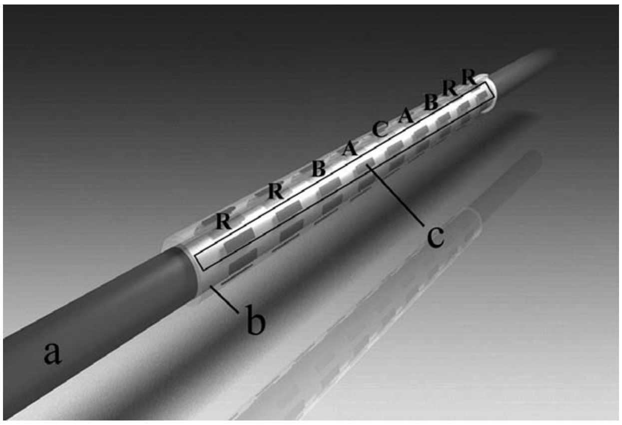

Figure 1: A perspective illustration of the the nerve segment (a) instaled into the 99-electrode cuff (b) and a position of specific electrodes within an arbitrary chosen longitudinal row of platinum electrodes (c). A-C-A represents triplets of electrodes within the stimulating section, B-represents blocking electrodes and R–R represents bipolar couples of electrodes for measurement of a CAP.
Slika 1: Prostorska risba segmenta živca (a) vstavljenega v 99-elektrodno objemko (b) in položaj posamezne platinaste elektrode v naključno izbrani vzdolžni vrsti elektrod (c). A-C-A je trojček elektrod v stimulacijski sekciji, B sta "bloking" elektrodi in R–R je bipolarni par elektrod v sekciji za merjenje CAP-a.

d – nominal diameter of the nerve: 2.5 mm
c – circumference of the nerve: 7.85 mm
l – total length of the cuff: 38 mm
w – approximate width of opened cuff: 12 mm

Figure 1 shows a finished ninety-nine-electrode cuff of 44 mm in total length and 2.5 mm in diameter (inner diameter of the first layer) and had 2.25–2.75 turns, snugly fitting the nerve in its resting position.

From the electrochemical point of view, a very important factor considered in the design of the cuff from different metals, was the stability of electrochemical potentials and the galvanometric behavior of stimulating electrodes in physiological media.

To supervise the electrode-electrolyte interface, the parameters of the stimulating waveform, injected via the stimulating electrode, were interchanged so to intentionally exceed the limits for reversible charge injection.[9,10,18]

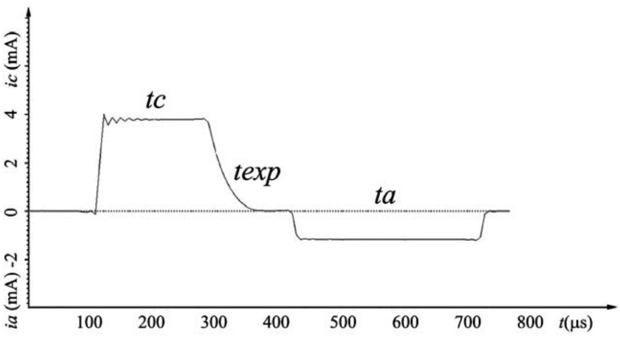

Figure 2: Parameters and waveform of the stimulating pulse
Slika 2: Parametri in oblika stimulacijskega impulza

Precisely, the absence or presence of both, reversible as well as irreversible electrochemical reactions, was controled exclusively via the imbalance between cathodically and anodically injected charge by the biphasic stimulating waveform.[7]

The stimulating pulse used in the study and shown in **Figure 2**, was current, biphasic, charge balanced and asymmetric pulse consisting of a precisely determined quasi-trapezoidal cathodic phase with a square leading edge with intensity i_c, a plateau with width t_c and exponentially decaying phase t_{exp}, followed by a wide rectangular anodic phase t_a/μs of a magnitude i_a.

To stimulate a determined group fibres within a particular compartment of a segment, stimulating pulses at frequency of 1 Hz were applied via stimulating cathode to preselected location.

An isolated, about 8 cm long segment of a swinish mid-cervical left vagus nerve, was installed within the cuff and mounted into the experimental chamber (**Figure 3**), according to the protocol approved by the ethics committee at the Veterinary Administration of the Republic of Slovenia, Ministry of Agriculture, Forestry and Food (VARS).

To maintain simulated physiological thermal conditions, the body of a measuring chamber machined out from Plexiglas, was heated to 37 °C using precision water circulator with range of control: ±0.003 °C .

To prevent extensive drying of the segment and maintain a natural "wet" surrounding of the nerve, the segment was occasionally flooded by the simulated cerebral perfusion fluid consisting of (in mM): MgCI2 2, CaCI2 2, KCI 2.5, NaCl 126, glucose 10, NaH2PO4·H2O 1.25, NaHCO3 26. At the same time, an interface between the stimulating electrode and neural tissue was maintained to closely mimic the physiological conditions.

The experiment consisted of four tests referred to as Test1-4, where given quasitrapezoidal stimulating pulses with preset parameters and waveform were delivered from a single channel precision custom designed stimulator to the triplet 5 in the stimulating section (ACA) of the cuff. A specific waveform and parameters of the individual pulses (1 Hz), were chosen by manual manipulation of the dials on the stimulator.

In the Test 1, the parameters and waveform were the following: i_c = 1.84 mA, t_c = 185 μs,

t_{exp} = 100 μs, τ_{exp} = 35 μs and i_a = 0.79 mA.

In the Test 2, the parameters and waveform were the following: i_c = 1.71 mA, t_c = 65 μs,

t_{exp} = 100 μs, τ_{exp} = 35 μs and i_a = 0.74 mA.

In the Test 3, the parameters and waveform were the following: i_c = 4.07 mA, t_c = 155 μs,

t_{exp} = 105 μs, τ_{exp} = 60 μs and i_a = 1.77 mA.

In the Test 4, the parameters and waveform were the following: i_c = 3.9 mA, t_c = 265 μs,

t_{exp} = 105 μs, τ_{exp} = 35 μs and i_a = 1.67 mA.

In the first three out of four stimulation tests, the CAP was measured simultaneously from the right end of the segment with with the couple of electrodes in the recording section (R–R) of the cuff, having the same longitudinal position as appointed triplet5 within the stimulating section (A-C-A).[30,31] In the Test 4 however, a selected recording couple was located at circumferentially opposite site according to an appointed triplet5.

In measurements of CAPs, signals recorded with an appointed couple of electrodes were delivered to a custom designed differential amplifier and amplified (A = 100).

The analogous signals of both, stimulating pulses and measured CAP signals, were digitized via an analogue-digital conversion board (DEWE-43, high performance data acquisition system designed and manufactured by the company DEWESOFT using data acquisition software DEWESoft 7.0.2 and stored on a Lenovo T420 portable computer).

To supervise the electrode-electrolyte interface established upon intentionally exceeded limits for reversible charge injection by different values of parameters and waveforms of selectively delivered stimulating pulses, a charge Q_c injected in cathodic phase as well as charge Q_a injected in anodic phase within precisely defined stimulus as shown above, were calculated and compared to each other. For this purpose, an integral of the i_c under a cathodic phase Q_c as well as the integral of the i_a under an anodic phase of the stimulus Q_c, were calculated. By doing this, the influence of the different stimuli on the offsets in the measured CAPs that might be elicited due to an imbalance between Q_c as well as Q_a, could be identified. Since all the stimuli were current pulses expressed in milliamperes, the corresponding charges Q_c *and* Q_a were expressed in nAs.

However, to identify the differences in CAPs, elicited by electrode-electrolyte interface established upon intentionally exceeded limits for reversible charge

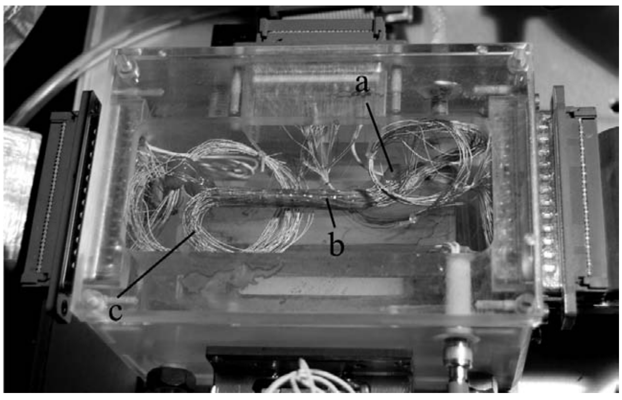

Figure 3: An isolated nerve segment (a), installed within the cuff (b) and multi-stranded copper wires (c), mounted into the experimental chamber

Slika 3: Izolirani segment živca (a), vstavljen v spiralno objemko (b), in bakrene pletenice (c), zmontirani v poskusno celico injection by different values of parameters and waveforms of selectively delivered stimulating pulses, an integral of the CAP in cathodic phase as well as integral of the CAP in anodic phase of stimuli were calculated and compared to each other. Since all the CAPs were voltage signals expressed in milivolts, the corresponding integrals were expressed in nV s.

All the offline signal analyses were performed on a Lenovo T420 portable computer using the Matlab R2007a programming tool.

3 RESULTS

Figure 4 shows measured CAPs while the segment was stimulated using quasitrapezoidal stimulus output waveforms and parameters, namely i_c, t_c, t_{exp}, *tau*, t_a and i_a, specifically preset in the abovementioned four tests. As could be seen in recorded CAPs, the **Figure 4** shown waveforms and values of the CAP were slightly obscured by stimulus artefacts and the transient response characteristics of an electrode/neural tissue interface and that of an inherent capacitance of the segment.

Table 1, however shows numerical values of calculated charge Q_c injected in cathodic phase as well as charge Q_a injected in anodic phase of precisely defined quasitrapezoidal stimuli selectively delivered to the triplet5 in Tests1–4. **Table 1** shows also values of calculated integral of corresponding CAPs in cathodic phase as well as in anodic phase of selectively delivered stimulating pulses.

A Test 4 however, was considered only in the sense of charge calculations while in a sense of integral calculation it was not considered. Namely, in the Test 4, corresponding CAP was measured using the couple of electrodes localed at circumferentially opposite site according to an triplet 5. Therefore, measured CAP could not contain action potentials of nerve fibres activated with an appointed triplet 5.

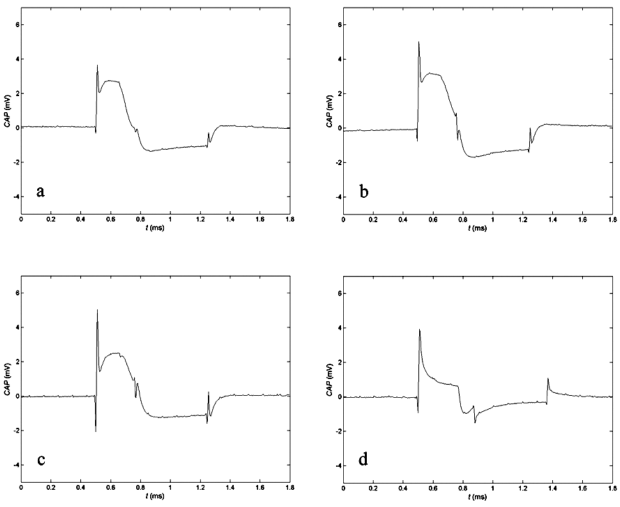

Figure 4: Specific waveform and values of CAPs measured in aforementioned four tests: a) CAP measured in the Test 1; b) CAP measured in the Test 2; c) CAP measured in the Test 3 and d) CAP measured in the Test 4

Slika 4: Oblika in vrednosti CAP-ov, izmerjenih v zgoraj omenjenih štirih preizkusih: a) CAP, izmerjen v testu 1; b) CAP, izmerjen v testu 2; c) CAP, izmerjen v testu 3 in d) CAP, izmerjen v testu 4

Table 1: Values and differences of cathodic Q_c and anodic Q_a charges and values and differences of Integrals1 of the CAP manifested under a cathodic phase and Integrals2 of the CAP manifested under an anodic phase of the stimulating pulses in Tests1-4

Tabela 1: Vrednosti in razlike katodnih Q_c in anodnih nabojev Q_a ter vrednosti in razlike Integrala 1 CAP-ov, zmontiranih pod katodno fazo in Integrala 2 CAP-ov, izraženih pod anodno fazo stimulusa pri testih 1–4

Variable	Test 1	Test 2	Test 3	Test 4
Q_c /(nA·s)	376.30	151.93	799.13	1107.82
Q_a /(nA·s)	376.42	352.63	832.27	803.42
ΔQ /(nA·s)	0.12	200.7	33.14	-304.4
Integral 1 /(nV·s)	250.57	113.01	665.22	300.38
Integral 2 /(nV·s)	59.85	61.50	76.15	44.95
ΔIntegral /(nV·s)	190.72	51.51	589.07	255.43

As result, **Table 1** shows the difference ΔQ of a cathodic Q_c and of an anodic charge Q_a as well as the difference of Integral1 of the CAP for cathodic phase and Integral2 of the CAP for anodic phase of the stimululating pulse for Tests1-4. Regardingly, in the Test2, for instance, a charge Q_c = 151.93 nAs was injected in cathodic phase and a charge Q_a = 352.63 nA s was injected in anodic phase, yielding a positive difference Q_{diff} = 200.7 nA s. This positive difference, being relatively high, could expectedly elicit some positive offset in the recorded CAP. In the Test4 however, a charge Q_c = 1107.82 nA s was injected in cathodic phase and a charge Q_a = 803.42 nA s was injected in anodic phase, yielding a negative difference Q_{diff} = –304.4 nA s. This negative difference, being also relatively high, could expectedly elicit some negative offset in the recorded CAP. Fortunately, it could be seen in **Figure 4**, that offset in all four measured CAPs, which could arise as a consequence of predefined imbalance in injected Q_c and Q_a, was not significant.

From the electrochemical point of view, it seems that all the reactions that occured at the cathode (A in **Figure 1**) due to a charge Q_c, injected via an i_c in the cathodic phase within a time t_c, were reversed, in part or in full, by the charge Q_a, injected via the anodic phase i_a within a time t_a. At each of the two triplet anodes in the same longitudinal row of electrodes (A–A in **Figure 1**), however, both the current and charge density would be equal to one fourth of the current and charge density occurring at the cathode. Namely, according to the model not presented in the paper, both of the triplet anodes and the two corresponding blocking electrodes (B–B in **Figure 1**), were electrically connected. Therefore, the electrochemical reactions that would occur at the single anode could not be of the irreversible type.

However, this pattern would inevitable worsen in case of stimulation in clinical practice where trains of repetitive stimulating pulses are applied. In this case, excursions of potential of electrodes within stimulating section due to predefined charge imbalance are additive in each stimulating pulse and the resulting excursion and consequently arised offset coud be significant.

4 DISCUSSION

An aim of the work was to contribute to the development of models and multi-electrode cuffs to be used for efficient and safe selective stimulation of autonomous peripheral nerves and for selective recording of CAPs at the same time.

The key developments in this technology were a cuff that can expand and contract to provide a snug yet non-compressing fit to the nerve, and a distributed matrix of platinum stimulating electrodes which made the performance of the cuff independent of it's positioning around the nerve. This design has strong potential for applications in neuro-prosthetic technology in future[9]. Namely, it would be very desirable to control different internal organs such as cardio-vascular system in patients with heart failure or atrial fibrillation by only one implanted system, e.g. on the lef cervical vagus nerve.

However, it is unavoidable to understand the response of peripheral nervous system elements to stresses that may occur in the complex interactions that take place between electrode and nerve secondary to VNS.

From CAP recording poinf of view, clinical use of implanted electrodes is hampered by a lack of reliability in chronic recordings, independent of the type of electrodes used. Namely, persistent presence of the electrode close to the neural tissue, causes a progressive local neurodegenerative disease-like state surrounding the electrode and is a potential cause for chronic recording failure.

However, from stimulation point of view, nerve fibers are located close to the stimulating electrode and also at a certain distance from it, the electrode should be able to inject enough charge to activate these fibers.[25,27] However, for multielectrode stimulating systems, containing miniature stimulating electrodes working at relatively high charge densities, it is very important that they are safe and electrochemically stable. Namely, to avoid harm to the vagus nerve in clinical use of the cuff, an inevitable requirement is the absence of irreversible electrochemical reactions such as electrolysis of water, evolution of chlorine gas or formation of metal oxides that could cause severe tissue injury associated with high charge density stimulation.[16,17]

Regarding both points of view, changes in the complex impedance of stimulating and recording electrodes in the cuff, chronically instaled onto a vagus nerve, should be characterized in a series of animal experiments.[32]

One weakness of a cuff manufacturing was a technically demanding and a time consuming process. Another weakness of a cuff was the use of a tin alloy at the junctions between platinum electrodes and multi-stranded wires. As this solution is not appropriate for a clinical practice, a mechanical connection as a more appropriate solution for further development of cuffs is in preparation. Beside, a perfect electrical isolation of all metals except electrode material is crucial for the life time of the system, othervise the mentioned reactions could occur.

Directions that our further work would be the following:

- Further development of the cuff for given clinical applications and accomplishment of electrochemical measurements under realistic conditions using the method of cyclic voltammetry, implementing the Ag/AgCl reference electrode.
- Development of the strategies to enhance a capability to obtainreliable long-term bipolar recordings of a CAP from a particular couple of platinum electrodes within the cuff.

It could be expected that mentioned directions, when performed, will lead to an additional enhancement of the cuff efficiency and to an overall more efficient and effective implantable devices.

5 CONCLUSIONS

The most important findings of the present study are the following:

- The strong component superimposed in the CAP was an ensemble artefact which came exclusively from the stimulating pulse via the transient response characteristics of an electrode/neural tissue interface and in part from an inherent capacitance of the segment.
- One could speculate that stimulus artefact traveled exclusively along the nerve surface via a capacitive nature of an interface, while recording electrodes measured an elicited voltage drop at the surface on the nerve.
- Single stimulating pulses, having preset certain degree of imbalance between a charge injected in cathodic phase Q_c and charge injected in anodic phase Q_a, elicited a slight change in a positive waveform deflection of CAP manifested under a cathodic phase as well as slight change in a negative waveform deflection of a CAP manifested under an anodic phase of the stimulating pulse.
- Measured CAPs are not greatly influenced by the imbalance between a charge injected in cathodic and anodic phase of quasitrapezoidal, asymmetric and biphasic stimulating pulses.
- The reactions that occured at the cathode due to an injected charge Q_c were reversed, in part or in full, by the charge Q_a.
- The electrochemical reactions that occured at the single anode could not be of irreversible type.

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