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CHRONIC STIMULATION OF AN AUTONOMOUS NERVE WITH PLATINUM ELECTRODES

KRONIČNA STIMULACIJA AVTONOMNEGA ŽIVCA S PLATINASTIMI ELEKTRODAMI

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A spiral nerve cuff (cuff), including thirty-nine platinum electrodes, arranged in thirteen spiral sets of three electrodes (triplet), was installed on the mid-cervical left vagus nerve (vagus) of a Beagle dog. The relevant position of the particular compartment and the optimal stimulation intensities were identified by delivering stimulating pulses to each triplet. The largest mean change in the components of both the ECG and hemodynamic changes was elicited with a stimulating intensity i_c = 2.5 mA applied to triplet9. Every two weeks the aforementioned compartment was stimulated for 20 min with rectangular, charge-balanced, biphasic, current pulses with the parameters: width 100 µs, intensity i_c = 2.0 mA, frequency 20 Hz, and a time delay between the biphasic phases of 100 µs for the time period of 18 months. It was calculated that to induce neural damage a total injected charge of approximately 172.8 mA s per phase at a charge density of 0.1 µA s/mm^2 was injected. At the end of the trial, the neural and encapsulation tissue subjacent to the central group of triplet 9 and the neural and encapsulation tissue subjacent to the silicone insulation were dissected free and analyzed. The extent of the pathology of the neural tissue caused by the presence of the cuff and/or by the electrochemical reactions and the surface of the stimulating cathode in triplet 9 were investigated using light microscopy. The results showed that the usage of a cuff was associated with a build up of connective tissue around the cuff as well as within the cuff. The results also showed that some tissue injury occurred beneath the stimulating cathode of triplet 9. Finally, the results showed an absence of any anomaly at the flat geometric surface of 2 mm^2 in stimulating the cathode of the triplet 9, which could be attributable to irreversible electrochemical reactions.

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

Spiralna objemka, ki je vsebovala devetintrideset platinastih elektrod, razporejenih v trinajst skupin po tri elektrode (trojček), je bila kirurško vstavljena na osrednji del levega vratnega živca vagusa psa pasme Beagle. Položaj določenega področja živca in optimalna jakost stimulacije sta bila določena z dovajanjem stimulacijskih impulzov na vsakega od trojčkov. Največjo povprečno spremembo komponent elektrokardiograma in hemodinamskih sprememb je izzvala jakost stimulacije i_c = 2.5 mA, dovedena na trojček 9. Omenjeni predel je bil v obdobju 18 mesecev vsaka dva tedna stimuliran 20 min s pravokotnimi, nabojsko uravnoteženimi izmeničnimi tokovnimi impulzi z naslednjimi parametri: širina 100 µs, jakost i_c = 2.0 mA, frekvenca 20 Hz in fazni zamik 100 µs. Izračunano je bilo, da je bil za povzročitev poškodbe živca skupni vnesen naboj približno 172.8 mA s. Živčni in ovojnični tkivi ob osrednji skupini elektrod v trojčku in tkivi ob silikonski izolaciji so bili na koncu poskusa izrezani in analizirani. Razsežnosti patologije živčnega tkiva zaradi elektrokemijskih reakcij in prisotnosti objemke ter površina stimulacijske katode trojčka 9 sta bili preiskani s svetlobno mikroskopijo. Rezultati so pokazali, da je uporaba objemke povezana z gradnjo veznega tkiva okoli objemke in v njej. Rezultati so tudi pokazali, da je pod stimulacijsko katodo trojčka 9 nastala poškodba živca. Končno so rezultati pokazali, da na ravni geometrijski površini stimulacijske katode trojčka 9 ni bilo poškodb, ki bi jih lahko pripisali nepovratnim elektrokemijskim reakcijam.

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

1 INTRODUCTION

The electrical activation of the nervous system provides a means to exert external control over body systems that are normally under the control of the nervous system.[1,2,3] Peripheral nerve stimulation often requires the development of electrode systems that stimulate the selectively a certain group of fibers in a nerve.[4] However, the long-term use of such electrical stimulation requires that it is applied selectively and without causing any tissue injury.[5,6,7] Tissue injury and the corrosion of the stimulating electrode are both associated with high-charge-density stimulation.[8,9] For this reason, the long-term stimulation of the nervous tissue requires the absence of irreversible electrochemical reactions, such as the electrolysis of water, the evolution of chlorine gas or the formation of metal oxides.[10,11] For a given electrode there is a limit to the quantity of charge that can be injected in either the anodic or cathodic direction with reversible surface processes.[12] This limit depends upon the parameters of the stimulating waveform,[13] the size of the electrode, and its geometry. The charge required for stimulation with miniature electrodes, however, often exceeds the limits for reversible charge injection. Therefore, an important component in the design of stimulating-electrode systems is the stimulating electrode itself; its properties determine the nature and kinetics of the charge transfer between the electrons moving in the external circuit and the ions moving through the electrolytes within the tissue.

Nerve cuffs are among the most successful of stimulating electrode systems.[3,14,15] Acute studies from our laboratory have demonstrated that cuffs could be used to selectively activate the different superficial regions of a peripheral nerve.[15] However, the long-term effectiveness and potentially harmful effects of these types of electrodes on neural tissue in various applications is still not completely defined.

The present study addressed the mechanisms involved in the production of neural damage induced by both the chronical physical presence of the implanted cuff and by the prolonged selective stimulation of the mid-cervical left vagus of a dog. The study reported here also seeks to investigate the changes on the surfaces of particular stimulating electrodes that could result the long-term selective stimulation of the vagus of a dog, using the method of light microscopy.

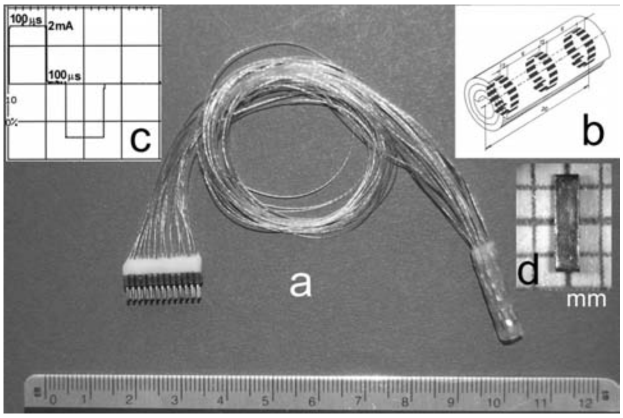

Figure 1: (a) 39-electrode cuff, (b) Simplified perspective illustration of the cuff, (c) Conceptual illustration of a current stimulating pulse pair, (d) Magnified view of one electrode within the triplet.

Slika 1: (a) 39-elektrodna objemka, (b) enostavna prostorska risba objemke, (c) shematska risba tokovnega stimulacijskega impulza, (d) povečana slika ene elektrode v trojčku

2 METHODS

The cuff design was devised to induce as low as possible radial pressure when installed on the vagus so that any mechanically induced vagus damage might be minimized. Accordingly, 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 and by a second unstretched sheet. Afterwards, the composite was compressed to a thickness of 0.3 mm. When released, after the curing process was completed, the composite curled into a spiral tube as the stretched sheet contracted to its natural length.[4,15] Thirty-nine rectangular electrodes (1.5 × 0.6 mm) were made of cold-rolled and annealed 0.05-mm-thick platinum ribbon (99.99 % purity), yielding a geometric area of 2 mm^2 in each electrode. However, according to Brummer et al. (1977),[5,8] one geometric square milimeter of a smooth platinum electrode corresponds to about 1.4 ("real") mm^2. Therefore, the real surface of these electrodes may have been an area of at least 2.8 mm^2.

Furthermore, the faradaic processes available on Pt have been classified as reversible or irreversible.[12] For some platinum electrodes, reversible charge injection limits for cathodic pulses range from 0.25×10^{-6} A s/mm^2 to 35×10^{-6} A s/mm^2 for Ir oxide electrodes. The relatively large contact area of the single platinum electrode shown in Fig. 1d, when implanted on the vagus, resulted in low impedances /*Z*/ (about 1.73 kΩ at 1 kHz, and about 1.36 kΩ at 10 kHz). The impedance of the single electrode was measured "in vivo" 20 d after implantation. The electrodes were mounted on a third silicone sheet with a thickness of 0.1 mm where they were arranged in three parallel groups, each containing 13 electrodes at a distance of 0.5 mm. The distance between the groups was 6 mm. As a result, thirteen triplets in a longitudinal direction were formed (**Figure 1b**).

To avoid as much as possible an occurrence of electrochemical potentials between the parts made of different metallic materials and consequently unwanted irreversible electrochemical reactions that could occur in physiological media, the electrodes were connected to the lead wires using the technology of a simple mechanical connection (Pressing). The lead wires were made of fine-stranded and Teflon-insulated stainless-steel wires (Cooner Wire, Chatsworth, USA, Type AS631). The sheet with the arranged electrodes was then bonded on the inner side of the mechanically opened cuff. Finally, the cuff with the inner diameter of 2.5 mm was trimmed to a length of 20 mm, as shown in **Figure 1a**.

To stimulate mostly B-fibers, the model proposed a stimulating pulse train composed of charge-balanced biphasic stimuli with a frequency of 20 Hz containing a combination of current cathodic and anodic phases.[13] The cathode and anode current steps of the stimuli used were 2 mA, while the cathode and anode halves were 100 ms in width. The time delay between the biphasic phases was settled at 100 ms. Finally, the maximum charge required for the selective stimulation of B-fibers within the particular region of the vagus was proposed.

$$Q/\text{mm}^2 \text{ (geometric)/phase} = \\ = 2 \cdot 10^{-3}\text{A} \times 100 \cdot 10^{-6}\text{s}/2\ \text{mm}^2 = 0.1 \cdot 10^{-6}\ \text{A s/mm}^2 \quad (1)$$

The relationship between the charge density per phase, or Q/mm^2 (geometric)/phase (expressed in units of microcoulombs per square milimeter per phase of the charge balanced waveform) expressed in the equation (1), and the total injected charge to tissue injury was investigated by light microscopy after 18 months of selective stimulation.

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. Under fully sterile conditions a gas sterilized (ethylene oxide) cuff was implanted on the mid-cervical left vagus (**Figure 2a**), 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), (Telephone: +386 1 300 1300, URL: http://www.vurs.gov.si.

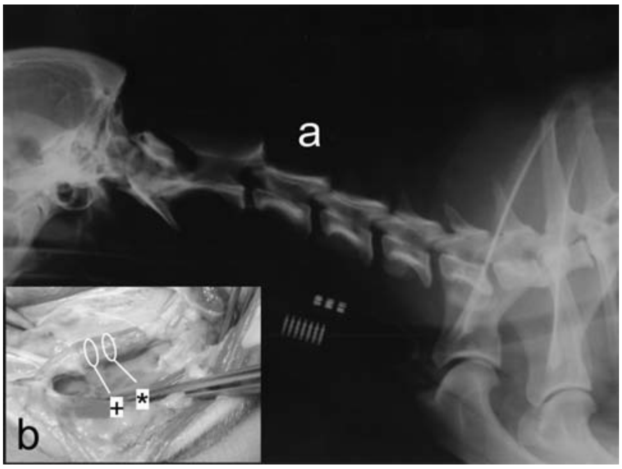

Figure 2: (a) X-ray of the cuff implanted on the vagus of a dog, (b) Insulated vagus within the cuff and sites where the segments (*) and (+) for histo-pathological analysis were cut out

Slika 2: (a) Rentgenska slika objemke, implantirane na pasji vagus, (b) izprepariran vagus znotraj objemke in mesta odvzema segmentov (*) in (+) za histopatološko analizo

Four weeks after the implantation the first stimulating session was performed. To determine in which position a triplet would be in contact with a particular compartment, innervating mainly the atrioventricular and to some extent the sinoatrial node, a stimulating pulse train of the aforementioned stimuli with an intensity of i_c = 2.5 mA was delivered for about 10 s to all 13 triplets. The triplet that elicited the largest change in the heart rate and the blood pressure, i.e., triplet 9, was considered as being relevant for the selective stimulation. More precisely, when the stimuli were delivered to triplet 9, the heart rate and the blood pressure began to decrease.

Afterwards, in each session the compartment was continuously stimulated for 20 min, using the aforementioned stimuli, while the intensity i_c was settled at 2.5 mA, as shown in **Figure 1c**. The session was repeated every two weeks for a time period of 18 months. It was calculated that to induce neural damage a total injected charge of approximately 172.8 mA s per phase was injected.

After the stimulation sessions were completed, the animal was euthanized by an overdose of an inhalant anesthetics isoflurane introduced from a vaporizer. The authors followed the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia from June 2007.

Finally, for the histo-pathological analysis the vagus together with the cuff represented in **Figure 2b**, was dissected free and cut at both sides of the cuff, thus providing a 20-mm-long composite segment.[6,9,17] Afterwards, two cylindrical specimens were cut out from a composite segment: 1) a 6-mm-long specimen,was cut from the composite subjacent to the silicone insulation between one of the outer and the central group of triplet electrodes (stimulating cathode) (**Figure 2b**+) and 2) a 1.5-mm-long specimen was cut from the composite subjacent to the central group of triplet electrodes (stimulating cathodes) (**Figure 2b***).

For the histo-pathological analysis, the specimens were fixed in 4 % formaldehyde solution phosphate buffered at pH 7.4, processed according to standard histological procedures and embedded in paraffin. Transverse, 4-μm tissue sections obtained by an ultramicrotome were stained with hematoxylin and eosin or by Giemsa stain.

For the metallurgical observations, however, a central electrode (stimulating cathode) of triplet 9 in the second 1.5-mm-long specimen was gently, without any mechanical deformation, removed from the silicone, cleaned and rinsed with acetone. In an evaluation of the galvanometric behaviour of this electrode using the electrochemical technique of cyclic voltammetry, an operational potential window between hydrogen and oxygen evolution in a protein-containing solution (Eliott's buffered solution, pH 7.3) was delineated. In other words, keeping the electrode potential within this window during the stimulation guaranteed that no water-electrolysis reaction occurred. This electrode, a stimulating cathode, was then introduced into the optical microscope.

3 RESULTS

Compared with the region of the vagus remote from the cuff, the vagus subjacent to the cuff showed a granuloma formation and a thickening of the connective

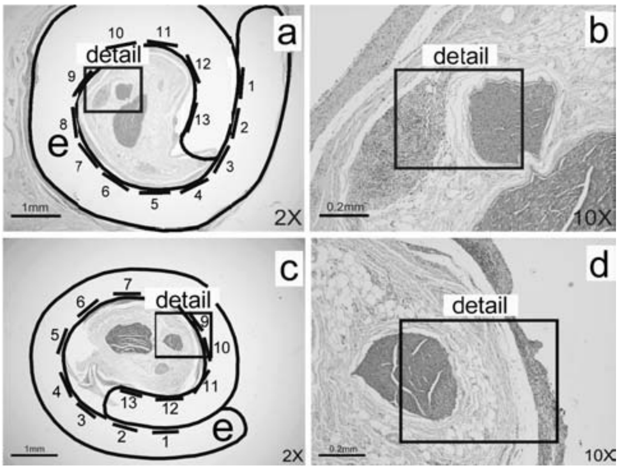

Figure 3: (a) Low-power view of a transverse section through the specimen (+), cut beneath the silicone insulation, (b) Magnified view of detail in (a), representing a granuloma-like tissue and a branch of vagus tissue, (c) Low-power view of a transverse section through the specimen (*), cut beneath the central group of triplet electrodes (stimulating cathodes), (d) Magnified view of detail in (c), representing a granuloma-like tissue and a branch of vagus tissue, (e) Silicone insulation.

Slika 3: (a) Malo povečana slika prereza skozi vzorec (+), odrezan ob silikonski izolaciji, (b) povečan detajl v sliki (a), ki je slika tkiva granuloma in veje tkiva vagusa, (c) malo povečana slika prereza skozi vzorec (*), odrezan ob osrednji skupini elektrod v trojčku (stimulacijske katode), (d) povečan detajl v sliki (c), ki je slika tkiva granuloma in veje tkiva vagusa, (e) silikonska izolacija tissue (the radial pressure measured in the cuff was about 245 Pa). Furthermore, the histological changes seen in the region of the vagus tissue that was stimulated with the adjacent triplet9 were obviously different from the region of the vagus tissue that was surrounded by the cuff, but was not in contact with the triplets.

Figure 3 (a) and (b) contains a transverse section at magnifications of 2 and 10 through the middle of the specimen (+), cut from the vagus subjacent to the silicone insulation. Frame (a) is a low-power view (2X) of the section through the vagus specimen and silicone insulation (e). A precise reconstruction of an "in vivo" situation showed that the vagus was well accommodated within the cuff. There was a build up of connective-tissue encapsulation around and within the cuff.

Frame (b) shows a magnification of detail 1 at 10X, including granuloma-like tissue on the left, and a branch of neural tissue on the right. This tissue included a mix of fibroblasts, collagen, and mostly foreign body cells. Most of the fibres appeared histologically normal. However, proliferation of subperineural connective tissue and a decrease in the density of axons could be observed.

Figure 3 (b) and (c), shows an analogous transverse section at magnifications of 2 and 10 through the middle of a specimen subjacent to the central group of triplet electrodes (stimulating cathodes). Frame (c) is a low-power view (2X) of the section, showing that the vagus was easily accommodated within the cuff. Frame (d) is a magnification of detail 1 at 10X, showing a vagus tissue and a build up of connective tissue. The vagus tissue is seen on the left, and the connective tissue encapsulation is seen on the right. Through most of the vagus tissue, the density of fibres in this stimulated region appeared normal, except at the right side, where a decrease in the density of axons could be observed.

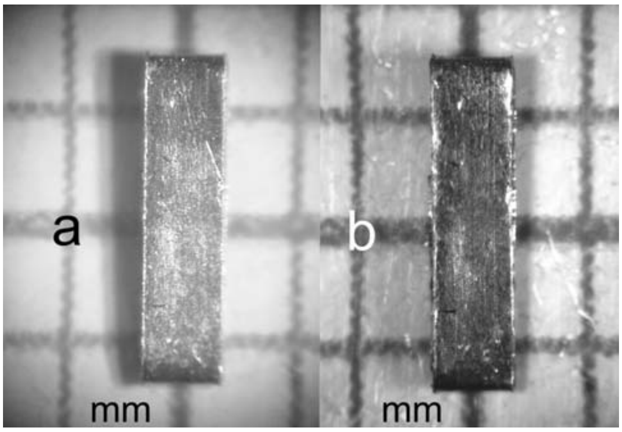

Figure 4: (a) Low-power microscopic image of the triplet9 central electrode (stimulating cathode) before implantation, (b) Low-power microscopic image of the triplet9 central electrode (stimulating cathode), pulsed with the described stimulating pulses, after 18 months of stimulation.

Slika 4: (a) Malo povečana mikroskopska slika osrednje elektrode (stimulacijske katode) trojčka 9 pred implantacijo, (b) malo povečana mikroskopska slika osrednje elektrode (stimulacijske katode) trojčka 9 po 18-mesečni stimulaciji.

The cyclic voltammogram showed that hydrogen evolution began at about –0.8 V, while the evolution of oxygen began at about 1.0 V. As expected, a train of the biphasic, rectangular, current pulses applied during the stimulation resulted in a series of potential steps between –0.8 V and 1.0 V, with respect to the Saturated Calomel Electrode.

Finally, **Figure 4a** shows a low-power microscopic image of a triplet 9 central electrode (stimulating cathode) before implantation, while **Figure 4b** shows a low-power microscopic image of the aforementioned electrode, pulsed with described stimulating pulses, after 18 months of stimulation.

4 DISCUSSION

The majority of fibres comprising the vagus of a dog are small-diameter non-myelinated C-fibers; the rest are intermediate-diameter myelinated B-fibers and large-diameter myelinated A-fibers are organized longitudinally along the length of the nerve.[16] However, the acute responses of a cardio-vascular system on vagus stimulation generally requires mostly B-fibre stimulation, while stimulation of the A- and C-fibres is to be avoided. Since in reality, B-fibres are located close to the stimulating electrode and also at a certain distance from the electrode, the electrode should be able to inject enough charge to depolarize these fibres.[1,2] For multi-electrode stimulating systems containing miniature stimulating electrodes working at relatively high charge densities, it is very important that they are electrochemically stable. The technology of such systems usually includes the use of numerous parts made of different metals. To ensure an appropriate electrochemical stability all the connections and parts except the surface of the stimulating electrode must be covered by a material with as high as possible electrical resistivity.

The characteristics of the morphological abnormalities observed after 18 months of stimulation were consistent with those observed in previous studies of cuff electrodes.[9] In fact, it was recently shown that the mere presence of an electrode invariably resulted in a thickened epineurium and in some cases increased peripheral endoneurial connective tissue beneath the electrodes.18 It could be explained by the radial pressure within the cuff, which could induce an interference with intraneural blood flow. Nevertheless, the stimulus parameters used in the study somewhat exceeded those that would be required in most clinical applications of electrical stimulation. Therefore, an increased thickness of the connective tissue could be expected. In the study, a vagus was exposed to a relatively high rate of movement between the cuff and the nerve trunk. A direct mechanical interaction between the cuff and the vagus was an obvious means by which such damage might been inflected. However, there are other mechanisms that may also contribute: pressure caused by the formation or excessive fibrous encapsulation around the cuff, the transmission of forces from adjacent muscles to the cuff and hence to the nerve, and some undue tension in the cuff's leads, even if they are carefully routed during the implantation.

5 CONCLUSIONS

- The use of a cuff was associated with the build up of connective tissue around the cuff as well as within the cuff.
- A moderate tissue injury occurred beneath the stimulating electrode of the triplet 9.
- No changes were observed on the surfaces of the stimulating triplet9 electrode investigated after 18 months of stimulation using optical microscopy.
- It can be concluded that irreversible electrochemical reactions potentially induced by relatively high charge biphasic stimulating pulses were not elicited.
- The technical solutions described may find potential applications in neuroprosthetic technology for neurologically impaired patients, especially for the selective control of internal organs and glands, such as the cardiovascular system, and their relation to bodily changes and diseases.
- One weakness of the cuff manufacture was the technically demanding and a time consuming process.
- The latter technology of the mechanical connection could be the solution for further development of multi-electrode systems for the electrical stimulation of the nerve tissue.
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6 REFERENCES

[1] J. T. Mortimer: Motor Prostheses. Pages 155–187 in *Handbook of Physiology,* Section 1. J. B. Brookhart, V. B. Mountcastle, (section eds.), V. B. Brooks (volume ed.), S. R. Geiger (executive ed.), American Physiological Society, Bethesda, MD., 1981

[2] W. F. Agnew, D. B. McCreery (Editors): Neural Prostheses, Fundamental Studies, Prentice-Hall, Inc., A Division of Simon & Shuster, Englewood Cliffs, New Jersey, 1990

[3] G. G. Naples, J. T. Mortimer: A nerve cuff electrode for peripheral nerve stimulation. *IEEE Trans. Biomed. Eng.,* BME-35 (**1988**), 905–916

[4] J. D. Sweeney, D. A. Ksienski, J. T. Mortimer: A nerve cuff technique for selective excitation of peripheral nerve trunk regions. *IEEE Trans. Biomed. Eng.,* BME-37 (**1990**), 706–715

[5] S. B. Brummer, M. J. Turner: Electrochemical considerations for safe electrical stimulation of the nervous system with platinum electrodes. *IEEE Trans. Biomed. Eng.,* 24 (**1977**), 59–63

[6] D. B. McCreery, W. F. Agnew, T. G. H. Yuen, L. Bullara: Charge density and charge per phase as cofactors in neural injury induced by electrical stimulation. *IEEE Trans. Biomed. Eng.*, 37 (**1990**), 996–1001

[7] W. F. Agnew, D. B. McCreery: Considerations for safety with chronically-implanted nerve electrodes. Epilepsia (Supplement 2) 31 (**1990**), S27–S32

[8] S. B. Brummer, M. J. Turner: Electrical stimulation with Pt electrodes. I. A method for determination of "real" electrode areas. *IEEE Trans. Biomed. Eng.* 24 (**1977**), 436–439

[9] W. F. Agnew, D. B. McCreery, T. G. H. Yuen, L. A. Bullara: Evolution and resolution of stimulation induced axonal injury in peripheral nerve. Muscle Nerve, 10 (**1999**), 1393–402

[10] L. S. Robblee, T. L. Rose: The electrochemistry of electrical stimulation. *Proc. 12th Ann. Conf. IEEE Eng. in Med. & Biol. Soc.,* Philadelphia, (**1990**), 1479–1480

[11] M. D. Bonner, M. Daroux, T. Crish, J. T. Mortimer: The pulse-clamp method for analyzing the electrochemistry of neural stimulating electrode. *J. Electrochem. Soc.,* 140 (**1993**), 2740–2744

[12] L. S. Robblee, T. L. Rose: Electrochemical guidelines for selection of protocols and electrode materials for neural stimulation. In *Neural Prostheses:* Fundamental Studies. W. F. Agnew, D. B. McCreery, Eds. Englewood Cliffs, Nj: Prentice-Hall, 25–66, 1990

[13] J. Rozman, B. Pihlar, P. Strojnik: Surface examination of electrodes of removed implants. *Scand. J. Rehab. Med. Suppl.,* 17 (**1988**), 99–103

[14] J. T. Mortimer, W. F. Agnew, K. Horch, G. Creasey, C. Kantor: Perspectives on new electrode technology for stimulating peripheral nerves with implantable motor prostheses. IEEE T. Rehabil. Eng., 3 (**1995**), 145–154

[15] J. Rozman, B. Sovinec, B. Zorko: Multielectrode spiral cuff for ordered and reversed activation of nerve fibres. *J. Biomed.* Eng., 15 (**1993**), 113–120

[16] S. Sunderland: Nerves and nerve injuries. E.& S., Livingstone Ltd., Edinburgh and London, 1968

[17] D. B. McCreery, W. F. Agnew, T. G. H. Yuen, L. A. Bullara: Relationship between stimulus amplitude, stimulus frequency and neural damage during electrical stimulation of sciatic nerve of cat. *Med. Biol. Eng. Comput.,* 33 (3 Spec No) (**1995**), 426–9

[18] T. G. H. Yuen, W. F. Agnew, L. A. Bullara, S. Jacques, D. B. McCreery: Histological evaluation of neural damage from electrical stimulation: considerations for the selection of parameters for clinical application. *Neurosurgery,* 9 (**1981**) 3, 292–9