Electrical Impedance Properties of Deep Brain Stimulation Electrodes during Long-Term In-Vivo Stimulation in the Parkinson Model of the Rat

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Abstract. Deep brain stimulation (DBS) is an invasive therapeutic option for patients with Parkinson's disease (PD) but the mechanisms behind it are not yet fully understood. Animal models are essential for basic DBS research, because cell based *in-vitro* techniques are not complex enough. However, the geometry difference between rodents and humans implicates transfer problems of the stimulation conditions. For rodents, the development of miniaturized mobile stimulators and adapted electrodes are desirable. We implanted uni- and bipolar platinum/iridium electrodes in rats and were able to establish chronical instrumentation of freely moving rats (3 weeks). We measured the impedance of unipolar electrodes *in-vivo* to characterize the influence of electrochemical processes at the electrode-tissue interface. During the encapsulation process, the real part of the electrode impedance at 10 kHz doubled after 12 days and increased almost 10 times after 22 days. An outlook is given on the quantification of the DBS effect by sensorimotor behavioral tests.

Keywords: EIS, Intracerebral electrodes, Basal ganglia, Subthalamic nucleus, Rat brain, Chronic instrumentation, 6-OHDA, Parkinson's disease.

1 Introduction

Parkinson's disease (PD) is a widespread degenerative disorder of the central nervous system that affects motor function, speech, cognition and vegetative functions. The cardinal symptoms such as tremor, rigidity, bradykinesia and postural instability result mainly from the death of dopaminergic cells in the substantia nigra pars compacta

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J. Gabriel et al. (Eds.): BIOSTEC 2012, CCIS 357, pp. 287–297, 2013.

and the subsequent lack of dopaminergic inputs into the striatum. This causes an alteration of the activity pattern in the basal ganglia [2]. Deep brain stimulation (DBS) is a novel therapeutic option for PD as well as an increasing number of neuropsychiatric disorders. Before DBS became a therapeutic intervention, electric stimulation of basal ganglia had been used to guide neurosurgeons to the precise position for a surgical lesion, the ultimate therapy of a late-stage PD. The main advantage of DBS over surgical lesions is the reversibility and possibility to modulate stimulation parameters [3]. The small volume of the target region for DBS in the human brain requires a highly specific adaption of the electrodes which need to be thoroughly tested in animal models, including different materials and geometries. So far, DBS-data of animal models of PD are scarce. During *in-vivo* stimulation, the properties of the DBS electrodes are changing as a function of time caused by electrochemical processes at the surface of the implant and the subsequent tissue response [5]. The tissue response is a foreign substance reaction. Its intensity depends on the material [Grill and Mortimer, 1994] and is correlated with the thickness of the adventitia finally encapsulating the implant [18]. Adventitia formation causes a steady change in the impedance of the electrodes leading to changes in the attenuation of the stimulating signal. As a result, the efficiency of the surrounding tissue stimulation is changing [12]; [13]; [7]. One opportunity to minimize this problem is to choose an appropriate electrode material. Previous investigations of our group [6]; [8] have shown that the use of stainless steel electrodes is not appropriate because of the corrosion and erosion processes intensified by electrolytic electrode processes. Electrochemically induced alterations are negligible for inert platinum electrodes, even though electrode processes may still influence the surrounding tissue [5]. For an optimal adjustment of the DBS signal, the kinetics of the electrode-impedance alterations caused by the adventitia formation must be taken into account [12]; [13].

2 Materials and Methods

2.1 Animal Treatment

Forty, adult, male Wistar Han rats (240-260 g) were obtained from Charles River Laboratory, Sulzfeld, Germany) and housed under temperature-controlled conditions in a 12 h light-dark cycle with conventional rodent chow and water provided ad libitum. The rats were subject to the following treatments:

- anesthesia (40 rats)
- 6-OHDA-lesioning (40 rats, 2 rats died while surgery)
- electrode implantation (38 rats (2 rats died while surgery): 15 unipolar electrodes, 21 bipolar electrodes)
- chronical instrumentation (26 rats: 21 rats with bipolar electrodes, 5 rats with unipolar electrodes)
- impedance measurement without chronical instrumentation (10 rats with unipolar electrodes)

The study was carried out in accordance with European Community Council directive 86/609/EEC for the care of laboratory animals and was approved by Rostock's Animal Care Committee (LALLF M-V/TSD/7221.3-1.2-043/06).

Anesthesia. The rats were anesthetized by ketamine-hydrochloride (10 mg per 100 g body weight, i.p., Ketanest S®, Pfizer, Karlsruhe, Germany) and xylazine (0,5 mg per 100 g body weight, i.p., Rompun®, Pfizer). Before surgery, the eyes of the rats were medicated with Vidisic (Bausch and Lomb, Berlin Germany). After surgery, the wound was sutured and the rats received 0.1 ml novaminsulfone (Ratiopharm, Ulm, Germany) and 4 ml saline subcutaneously. Rats were exposed to red light (Petra, Burgau, Germany) until normalization of vital functions.

6-Hydroxydopamine (6-OHDA) Lesioning. The 6-OHDA (Sigma, Deisenhofen, Germany) lesioning was performed by stereotactic surgery in adaption to Strauss et al. [17]. The lesions of the right medial forebrain bundle of rats were induced by injection of 26 µg 6-OHDA in 4 µl saline with 1 g/l ascorbic acid delivered over 4 min via a 5 µl hamilton microsyringe (Postnova Analytics, Landsberg/Lech, Germany). The coordinates relative to bregma were: anterior-posterior (AP) = -2.3 mm, medial-lateral (ML) = 1.5 mm and dorsal-ventral (DV) = -8.5 mm [16].

Electrode Implantation. Electrodes were implanted into the subthalamic nucleus (STN), which is the most common target region for treatment of PD patients. The surgical procedure was performed using a stereotactic frame (Stoelting, Wood Dale, IL, USA) modified according to Harnack et al. [9]. To support the surgical procedure, a cold light source (KL 1500 LCD, Schott, Mainz, Germany) was used in combination with a stereo-microscope (Leica, Wetzlar, Germany). The skull was opened by a dental rose-head bur (Kaniedenta, Germany). The coordinates relative to bregma were: anterior-posterior (AP) = -3.5 mm, medial-lateral (ML) = 2.4 mm and dorsal-ventral (DV) = -7.6 mm [16]. A dental drill was used to bore an additional hole in the skull for an anchor screw. The electrode was fixed to the skull by an adhesive-glue bridge (Technovit 5071, Heraeus, Germany) to the anchor screw. After all, a subcutaneous wire was implanted. The suture exit hole was located in the middle of the back of the rat.

The counter-electrodes (dental wires and suture clips in combination with the unipolar electrodes) were implanted into the neck of the rats.

Chronical Instrumentation. Commercial rat jackets (Lomir Biomedical, Quebec, Canada) with a backpack were used to fix all electronic components of the miniaturized custom-made stimulator system (Rückmann und Arndt, Berlin, Germany) to the rat. The DBS stimulator and the battery were located in the backpack of the rat jacket. The DBS electrode and the battery were connected to the DBS stimulator via plug connectors (RS Components GmbH, Mörfelden-Walldorf, Germany). Both were soldered with a lead-free solder tin (RS Components GmbH) and insulated with a biocompatible shrink tubing (RS Components GmbH).

Impedance Measurement. Ten rats were used to measure the kinetics of the electrode impedance alterations caused by the adventitia formation at the surface of unipolar electrodes. During the measurement, the rats were anesthetized. The measuring procedure was performed every day over a measuring period of 12 days (in experiment 1) and over 22 days (in experiment 2). In the first experiment we used a dental wire as counter-electrode and in the second step an array of suture clips (Allgaier Instrumente GmbH, Frittlingen/Tuttlingen, Germany) to evaluate the influence of the counter-electrode (shape and position) on the measurement results of the impedance.

2.2 Stimulation- and Counter-Electrodes

For impedance measurements, we designed unipolar platinum-iridium (Pt/Ir) microelectrodes which were covered with polyesterimide insulation and custom-made by Polyfil (Zug, Switzerland; Fig. 1). In a first step, dental wires made of biocompatible, nickel-free steel alloy (18% Cr, 18% Mn, 2% Mo, 1% N, remander iron) of 1 mm diameter and 10 mm length were used as counter-electrodes (see: Fig. 4a) in combination with the unipolar DBS electrodes. In a second step, an array of counter-electrodes was pierced into the necks of a number of rats, to evaluate the influence of the counter-electrode on the impedance measurement. For this, suture clips (see: Fig. 4b) were used. Electrochemical electrode effects were negligible at the counter-electrode due to the low current density at its large surface.

We also designed and implanted bipolar Pt/Ir electrodes (Fig. 2) with two stacked tips to test the effects of nonaxial symmetric field distributions. Further, this electrode type does not require the implantation of counter-electrodes.

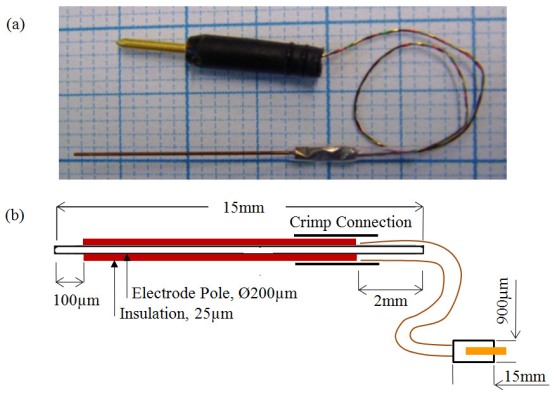

Fig. 1. Photograph (a) and scheme (b) of the unipolar Pt/Ir electrode (Polyfil, Zug, Switzerland). The electrode pole was a round wire made from Pt90Ir10 with a diameter of 200 µm. The length of the non-insulated tip of the electrode pole was 100 µm. The insulation consists of polyesterimide 180 with a thickness of 25 µm [15].

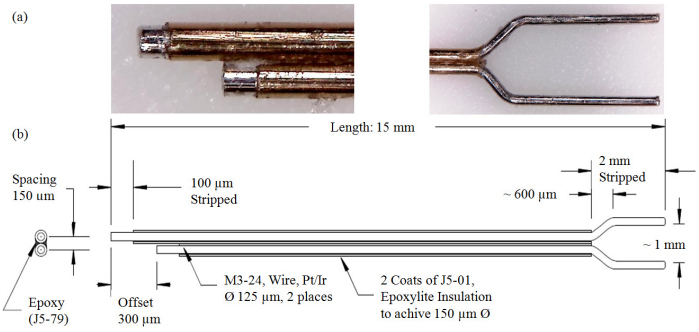

Fig. 2. Photograph (a) and scheme of the bipolar Pt/Ir electrode (FHC, Bowdoin, ME, USA). The two electrode poles were round wires made from Pt90Ir10 with a diameter of 125 µm. The lengths of the non-insulated tips were 100 µm. The thickness of the epoxylite insulation was 25 µm.

2.3 Electric Impedance Spectroscopy (EIS)

Electric impedance spectroscopy (EIS) is a common measuring technique for determining the electrical properties of tissues [4]. It is used in a wide range of applications, such as breast cancer detection [10], the monitoring of the lung volume [1] and in material sciences. EIS is nondestructive and therefore suitable for the characterization of the DBS electrodes during the encapsulation process.

Equipment. The EIS measurements were conducted with an impedance spectrometer Sciospec ISX3 (Sciospec Scientific Instruments, Pausitz, Germany) and a test fixture HP16047D (Hewlett-Packard, Japan) connected to a personal computer with Sciospec-measuring software. The two connectors of the test fixture were connected to the DBS and the counter-electrodes.

Measurement. To characterize the electrode properties during encapsulation, the impedance was recorded in the frequency range from 100 Hz to 10 MHz over a period of two weeks after implantation. Frequency range, amplitude, number of points and the averaging of the impedance spectrometer were programmed by the measuring-software (Sciospec). 401 frequency points were logged which were distributed equidistantly over a logarithmic frequency scale. The measuring voltage (peak to peak) was 12.5 mV_{PP}. The measuring-software logged the measuring data of the impedance spectrometer (real and imaginary parts of the impedance vs. frequency) by saving them as a data file.

Before each measurement, the impedance spectrometer was calibrated by open, short and load measurements. Each measurement was repeated three times to improve the statistical significance. The measurements were repeated every day for one week and every second day during the second week.

The stimulation pulse usually applied in DBS has a frequency of 130 Hz and a pulse width of 60 µs. Because of the steep slopes of the needle-shaped pulse, the signal is rich in high harmonic frequencies [5]. For this reason, we measured the impedance within the wide frequency range from 100 Hz to 10 MHz, which is beyond the range of up to 10 kHz reported by Lempka et al. [12].

Impedance Theory. The electrical impedance describes the magnitude ratio between the applied AC voltage and the resulting current flowing with a certain phase shift. Mathematically speaking, the impedance Z* is a complex number with the unit [Ω], which is composed of a real (Z´) and an orthogonal imaginary part (Z´´) marked by the complex unit $j = \sqrt{-1}$:

$$Z^* = \mathrm{Re}(Z^*) + j \cdot \mathrm{Im}(Z^*) = Z' + j\,Z'' \tag{1}$$

For interpretation of the measuring data, an equivalent circuit model is required to be fitted to the measuring data. The aim was to model electrochemical processes and adherent cell growths by combinations of resistors, capacitors and constant phase elements [12].

Data Analysis. The logged data were transferred to Matlab (The MathWorks™, Version 7.9.0.529) to calculate means and standard deviations. For their graphic representation, they were finally copied to Sigma Plot 11.0 (Systat Software, 11.0, Build 11.2.0.5).

2.4 Electron Microscopy Study of Electrode Encapsulation by Tissue

Concentric bipolar microelectrodes with an inner pole diameter of 75 µm and an outer pole diameter of 250 µm (CB CSG75; FHC, Bowdoinham, ME) were placed into the STN of two anesthetized rats. The rats were stimulated for 3 h with biphasic constant-current pulses with a repetition frequency of 130 Hz and pulse duration of 60 µs at 250 µA with a stimulus generator (Multichannel Systems, Reutlingen, Germany). The electrodes were removed and one electrode was incubated in trypsin solution (Trypsin-EDTA (1x) in HBSS W/O CA&MG W/EDTA.4NA, Gibco, UK) at 37°C for 1 h. The other electrode was postfixed overnight in 4% glutaraldehyde (Merck, Germany) in PBS.

Electrodes were washed, fixed in 4% glutaraldehyde in PBS, washed again, post-fixed in 1% osmium tetroxide, dehydrated in acetone, and subjected to critical-point drying (EMITECH K850, Ashford, Kent, UK). The samples were sputtered with colloidal gold using a Sputter Coater (BAL-TEC SCD 004, Schalksmühle, Germany) before examination with a scanning electron microscope (DSM 960 A, Zeiss, Oberkochen, Germany).

3 Results

3.1 Electrode Implantation

We implanted 36 Pt/Ir electrodes (15 unipolar electrodes, 21 bipolar electrodes) in Parkinsonian rats and combined the bipolar electrodes with chronical instrumentation. This allowed for stimulation under spontaneous movement conditions without fixing the rats to an apparatus. For an optimal adjustment of the DBS signal in future experiments; we measured in pilot tests (without chronical instrumentation) the kinetics of the electrode impedance alterations caused by adherent cell growth at the surface of the implanted unipolar electrodes.

Verification by Ink Injection. As a first test, the localization of the electrode tip in the target region was verified by ink injection via a 5 µl Hamilton micro syringe of approximately the same size as the implanted stimulation electrode (Fig. 3).

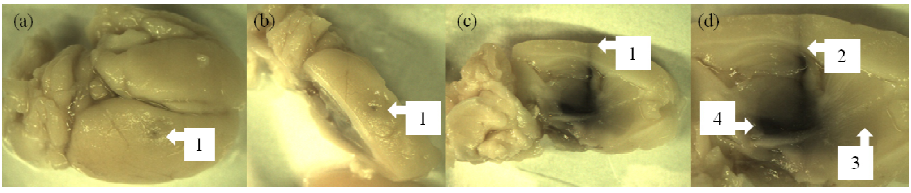

Fig. 3. Rat brain fixed in formalin, (a): top view of a rat brain with a puncture resembling an electrode canal (1), (b): sagittal section of one hemisphere with the puncture (1), (c): hemisphere where tissue was removed to display the injection canal, (d): enlarged photo of (c) with the ink injection canal (2), striatum (3), and other parts of the basal ganglia (4)

Counter-electrode Implantation. For the EIS measurements, counter-electrodes were pierced through the intact scalp close behind the cut for the implantation of the stimulation electrode. In a first step, a dental wire of biocompatible steel alloy used as

a counter-electrode was implanted into the neck of two rats, shown in Fig. 4a. In a second step, an array of suture clips was implanted into the neck of 8 rats, shown in the scheme in Fig. 4b.

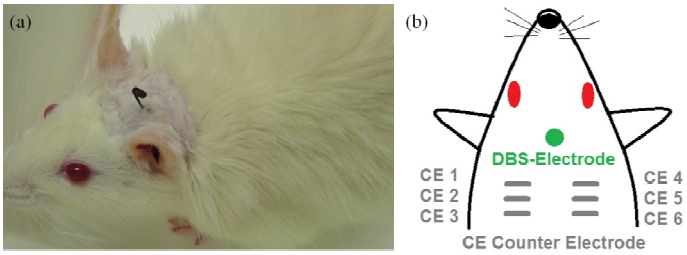

Fig. 4. Photograph of an implanted dental wire of biocompatible steel alloy (a) and scheme of an array of suture clips (b) used as counter-electrodes for the EIS measurement

3.2 Chronic Instrumentation

Chronic instrumentation (in combination with the bipolar DBS electrodes) was established and applied to freely moving Parkinsonian rats (Fig. 5a). For handling, the rats had to be trained every day over a period of 5 weeks. Following electrode implantation, a biocompatible subcutaneous wire was implanted (Fig. 5b). After each stereotactic surgery (6-OHDA lesioning and electrode implantation), the rats were left alone for one week to allow for wound healing. Then, the rat jackets were put on. All electronic components (Fig. 6c) were located in the backpack of the rat jacket. This allowed for a stimulation of up to 3 weeks when the batteries were changed every second day and the jacket once a week. The DBS electrode and the battery were connected to the DBS stimulator via plug connectors. The weight of all components of the chronic instrumentation (including battery) was 5 g.

Previous investigations of our own group [6]; [8] allowed for a stimulation of only 3 h when the rats were fixed to an apparatus.

The custom-made miniaturized DBS stimulator system was manufactured in cooperation with the company Rückmann und Arndt, Berlin, Germany.

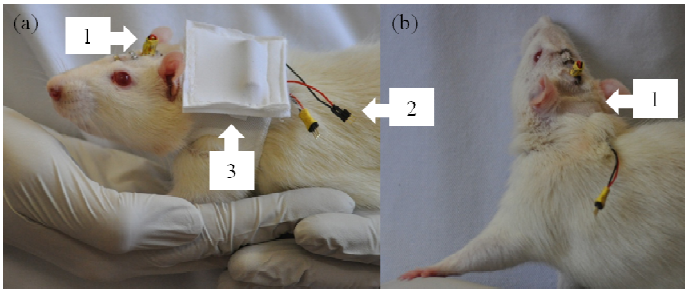

Fig. 5. Photograph of a freely moving rat one week after implantation with an implanted bipolar DBS electrode and chronic instrumentation consisting of (1) bipolar Pt/Ir electrode with subcutaneous cable and plug connector, (2) plug-connector of the DBS stimulator and (3) DBS stimulator and battery in the carrying bag of the rat jacket

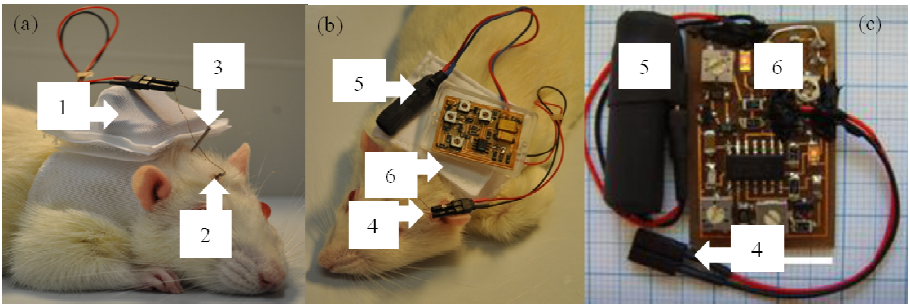

Fig. 6. Photograph of a rat with a unipolar electrode that was anesthetized for implantation. The chronic instrumentation consisted of: (1) rat jacket with carrying bag, (2) unipolar Pt/Ir electrode, (3) counter-electrode made from dental wire, (4) plug connector electrodes, (5) battery, (6) DBS stimulator.

3.3 Impedance Measurement

Our data showed the general tendency of an impedance increase during the encapsulation process. The measuring results are shown in Fig. 8 and 9. The value of Z´ has almost doubled after 12 days (Fig. 8a) and increased almost 10 times (Fig. 9a) after 22 days compared to its initial value at 10 kHz. In parallel, also the absolute value of Z´´ (Fig. 8b and 9b) decreased. The results match with findings of Lempka et al. [12] for the monkey brain who described the formation of the adventitia as a foreign substance reaction as the main reason for the impedance increase. Our own electron-microcopy study showed that within 3 h post implantation, a considerable number of cells firmly adhered to the stimulation electrodes (Fig. 7).

The findings we obtained with the implantation of an array of suture clips indicated that the influence of the position, shape and material of the counter-electrode on the impedance measurement results was negligible.

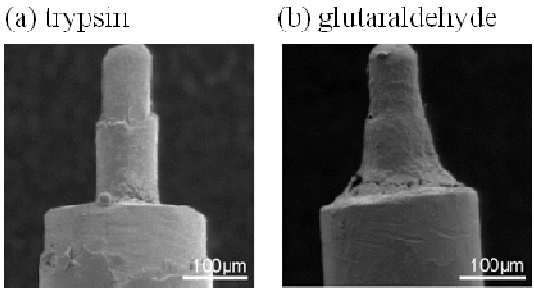

Fig. 7. Scanning electron-microscopical images of electrodes that had been implanted and used for 3 h of stimulation. (a): Electrode cleaned by trypsination prior to electron microscopy. (b): Electrode with fixed adhering tissue.

Our data showed an impedance decrease at the second day after implantation followed by a significant increase from the third day on. Interestingly, the same impedance behavior has already been reported by Lempka et al. [12, p. 6, Fig. 4]. However, no explanation has been given for this phenomenon.

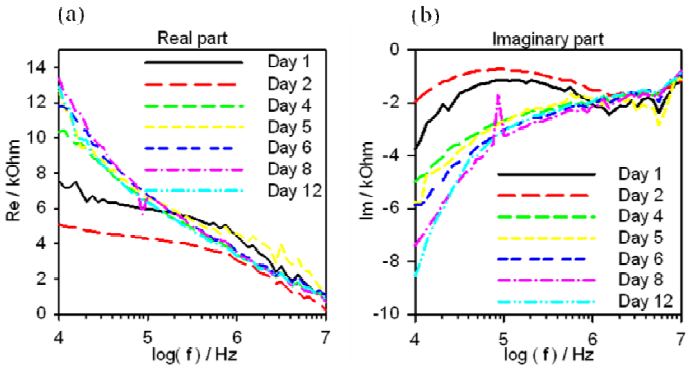

Fig. 8. In *vivo* impedance change of a unipolar Pt/Ir electrode measured against a dental-wire counter-electrode over 12 days ((a) Re = Z´; (b) Im = Z´´)

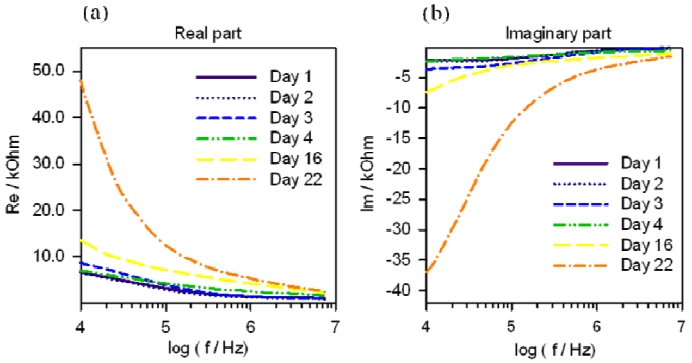

Fig. 9. In *vivo* impedance change of a unipolar Pt/Ir electrode measured against a suture-clip counter-electrode over 22 days ((a) Re = Z´; (b) Im = Z´´)

4 Conclusions and Perspectives

Pilot experiments in the rat model have shown that the impedance of unipolar DBS electrodes is significantly increasing with time after implantation. The main reason is the formation of adhering tissue that encapsulates the implant. This tissue response is a foreign body reaction. Its intensity depends on the material. The stimulation efficiency can be improved with an appropriate electrode material. Previous investigations of our own group have shown that stainless steel electrodes are not appropriate. The use of platinum/iridium could improve the stimulation efficiency.

The characterization of the encapsulation process enabled us to readjust our miniaturized prototype stimulator system to adapt the stimulation parameters according to the stimulation duration. To the best of our knowledge, we established for the first time chronic instrumentation of completely freely moving rats for long-term experiments (of up to 3 weeks). Also for the first time, iron-free electrodes have been applied to Parkinsonian rats for performing DBS experiments over the same observation time.

In future experiments, we aim for a comparative study of uni- and bipolar electrodes with a conventional and a modified stimulation program. The quantification of the DBS effect on locomotion, exploration and anxiety will be analyzed by drug- and non-drug induced behavioral tests. For these experiments, the operating life of the battery of the miniaturized DBS stimulator has to be prolonged.

An equivalent circuit model will be developed for a better understanding of the measuring data in order to extract encapsulation parameters. These investigations aim at clarifying the phenomenon of the impedance drop at the second day after implantation.

Potential effects at the electrode-tissue interface will be analyzed by histological, immunochemical and electron-microscopical methods.

Acknowledgements. K.B. is grateful for a stipend of the German Research Foundation (DFG, Research Training Group 1505/1 "welisa"). T.K. acknowledges financing by a project of the Federal Ministery of Economics and Technology (BMWi, V230-630-08-TVMV-S-031). Part of the work was conducted within a project financed by the Federal Ministry of Education and Research (BMBF, FKZ 01EZ0911). The authors are grateful to Dr. R. Arndt for a fruitful cooperation on the stimulator development and to Dr. J. Henning for help with the electron microscopy study. We would like to thank the staff of the electron microscopy center at the University of Rostock's Medical Faculty for outstanding technical support.

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