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Development of an implantable microstimulation system for chronic DBS in rodents

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Abstract—**High frequency deep brain stimulation (DBS) of certain basal ganglia nuclei (e.g. subthalamic nucleus, STN) has emerged as a powerful neuromodulatory approach in the treatment of late stage Parkinson's disease patients. However, the underlying mechanisms of action are not fully understood. We have therefore established an implantable DBS device for small laboratory animals (e.g. rats) that allows the reliable and safe application of continuous DBS for at least 3 weeks. We could further show that miniaturized monopolar electrodes comprising activated iridium are suitable for continuous stimulation of small brain structures like the STN without inducing severe insertion or stimulation related injuries.**

I. Introduction

THE use of high frequency deep brain stimulation (DBS) for the treatment of movement disorders and other neuro-psychiatric conditions has become a widely accepted modulatory therapeutic approach in the field of functional neurosurgery. The target selection and the stimulating parameters of DBS depend on the condition to be treated [1]. Most experience exists with DBS derived from its application in Parkinson's disease (PD). In PD patients DBS of the subthalamic nucleus (STN) is regarded as the state-of-the-art treatment for motor complications in advanced disease stages. Despite the growing number of patients treated (world-wide more than 75.000 PD patients) and the ongoing extension of the indication of DBS to other (neuro-psychiatric) disorders, the mechanisms of action of DBS are not completely understood.

For this reason the application of DBS in animal models with the overwhelming majority of preclinical research utilizing rats as experimental subjects is a common strategy to investigate the basic mechanisms of DBS *in-vivo* [2]. Although numerous preclinical studies in rats provided important insights in the mechanisms of action of DBS, the interpretation of these findings is often limited by the application of short-term stimulation periods (i.e. minutes to hours). Thus, it may be concluded that the quality of experimental DBS studies will improve as experimental DBS setting more mimics the clinical situation with continuous DBS over long periods (i.e. weeks to months). For this paradigm an appropriate stimulation hardware system for small laboratory animals (i.e. rodents) is an important pre-requisite. Recently, numerous preclinical studies in rats have been published in which long-term DBS (i.e. days to weeks) was applied via an externalized stimulation system to overcome the temporal issue in the transfer of a clinically used therapy into the experimental field [2, 3]. However, an implantable stimulation hardware system, which restrains the biological, physiological and behavioral integrity of the research subject to a minimum, would better reflect clinical practice. Such an implantable device would allow the small laboratory animal to be housed in their regular environment, without being restricted in their hygiene and mobility, and would reduce the risk of damage of the externalized device and its sub-assemblies as well as infection [4].

We have therefore developed a miniaturized, completely implantable, and programmable DBS system for rats (including accessory equipment and stimulating electrodes) which allows the application of continuous and chronic STN-DBS that can be safely applied without inducing tissue damage when using monopolar electrodes comprising activated iridium [5].

II. The Implantable DBS Device for Rats-Technical Aspects

The microstimulation system for rats comprises three sub-assemblies: (i) a miniaturized impulse generator (IPG, length: 35mm, width: 17mm), (ii) a programmer connected to an inductive coil allowing the transfer of stimulation protocols, and (iii) an electrode – lead – system.

(i) The IPG basically consists of a microcontroller (PIC16C54) attached to a reed switch, a DC-to-DC converter, several constant current sources and a crystal oscillator which serves as the internal system clock for all software programmed processes of the PIC16C54. Its frequency (77.5 kHz) is used to create pulsed signals for several ports switching the different current sources and the transmission signal. The microcontroller processes electromagnetically transmitted data of the stimulation protocol following transformation into temporally

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incremental (digitized) signals. These signals drive the DC-to-DC converter and the constant current sources which are also interconnected with the microcontroller. The DC-to-DC converter transforms the battery power (~ 3V) to a higher amplitude (~ 18V) using a voltage booster (MAX630) to power up the constant current sources when a pulse is processed by the microcontroller. An electromagnetic emitter connected to the DC-to-DC converter allows a feedback sending of the stimulus protocol to the programmer. The constant current sources, which produce a stable output current (I_{out}) consist of a current generator, a capacitor (C1) and several resistors (R_1 and R_2). At rest (no current flow), the maximal voltage drop at the capacitor is $U_{Cmax} = I \cdot R_2$. When the circuit is closed (current flow), this voltage decreases to $U_{Cmin} = I \cdot (R_1 \cdot R_2 / R_1 + R_2)$. The opening and closing of this circuit is enabled by a microcontroller regulated switch comparable to digital-to-analog converter allowing to verify the ratio of its "on-" and "off-mode" by a pulse-width-modulation (PWM). Due to the PWM, the mean voltage drop at the capacitor is modulated between the limits $U_{Cmin} \leq U_C \leq U_{Cmax}$. The mean voltage U_C drives the constant current sources whose output current I_{out} (proportional to U_C) can be pulsed through two transistor circuits at the output pins on which the connector of the lead system is plugged. Each PWM-signal has an own duty cycle, i.e. a period consisting of two different parts, an active (time of current flow) and an inactive part (time of no current flow). A duty cycle of 50% means that the active and non-active time during one period is equal. According to the chosen stimulation parameters, one period at the output pins is 7.74 ms and the duty cycle which is regulated by auxiliary switches is 0.66 %. The above mentioned transistor circuits can deliver monophasic (i.e. positive and negative) or alternating pulses along the output pins with a maximum resistance of 20 kΩ. For bench top testing different current amplitudes (50, 100, and 200µA) were applied against a load resistance (18 to 20 kΩ) to prove the stability and reliability of the programmed pulses, those current amplitudes were 52.84 ± 7.80 µA (for 50µA), 115.86 ± 13.82 µA (for 100µA), and 203.19 ± 15.61 µA (for 200µA) of stimulation and were associated with a duty cycle time of the batteries ranging from 18 (minimum) to 34 (maximum) days for all current amplitudes applied.

(ii) The programmer allows the communication with the IPG by sending and receiving stimulation protocols within an electromagnetic field generated by an inductance and amplified by an inductive coil, in which the rat has to be placed. As for the IPG a microcontroller is the central control unit and is directly connected to a sender, whose inductance is powered by a switching regulator and to a receiver. The switching regulator transforms the battery power (~ 9V) to ~ 40V to produce the field intensity necessary for the inductance of the sender. The receiver uses the inductance of the electromagnetic emitter attached to the DC-to-DC converter of the IPG.

(iii) Monopolar stimulating electrodes are custom made by cutting defined lengths of pure iridium wire (length 12mm, diameter 150µm) following electrochemical etching of the tip (length ~ 350µm to 400µm, mean surface area 0.0012cm²) and gluing the wire into a polyimid insulated silica capillary (inner and outer diameter 180µm and 340µm, respectively) using an epoxy resin. At the proximal end, a lead wire (MP35N alloy) is laser welded followed by activating the electrode by repetitive cycling voltammetry to increase their charge storage capacity. The lead system (kindly provided by Medtronic Inc.) comprises two sub-assemblies (i.e. an electrode lead wire and an extension lead) that can be interconnected during surgery. The distal end of the lead wire is attached to the female connector sleeve of the extension lead which comprises the indifferent electrode given by an electrical coiling (MP35N) around the exterior surface.

III. The Feasibility of Long Term DBS in Rats

For *in vivo* use the DBS device (IPG) was encapsulated in a silicon rubber, connected to the siliconized extension lead, and sterilized in a plasmatic atmosphere followed by its implantation in rats (n=10) weighing 300 to 350g. The electrode was stereotaxically implanted in the STN as the target structure for DBS. The total duration time for surgery was ~ 1h and was well tolerated by the animals. After a recovery from surgery STN-DBS was initiated 36h later with 0, 50 or 100µA of alternating pulses (0.0nC/phase, 2.6nC/phase, and 5.2nC/phase, respectively) and maintained for 3 weeks. During this observation period neither inflammatory reaction within the cavity of the implant nor hardware failures of the DBS device were observed. The biocompatibility of long term STN-DBS by meaning of a sustained brain integrity was investigated semi-quantitatively using different histological staining procedures (i.e. *Nissl's* staining, Hematoxylin and Eosin, *Klüver-Barrera* staining *van Gieson's* staining, GFAP and NeuN-immunoreaction, respectively) in defined horizontal brain slices along the trajectory of the electrode (including the active tip). As a result, all rats showed a minimal to mild tissue reaction adjacent to the tip and the shaft of the electrode, irrespective of the stimulus protocol (i.e. 0, 50, or 100µA) and were restricted to a distance ranging between 39µm to 61µm around the electrode. Importantly, neurons in the vicinity of the tip of the electrode had a vital aspect indicated by the presence of typical neuronal morphology without karyolysis, hyperchromic or pyknotic nuclei, cytoplasmic agglutinations, eosinophilic cytoplasm or membrane rupturing. There was no evidence for phagocytosis of degraded material derived from the tip of the electrodes.

IV. Discussion

Here we describe a completely implantable and programmable microstimulation system allowing the application of long-term DBS in rats. This system has been shown to be reliable in bench top testing by meaning of the stability of various stimulus protocols delivered by the impulse generator up to five weeks. Most importantly, *in-vivo* testing demonstrated the biocompatibility of the implant

and the safety in the application of continuous DBS through monopolar activated iridium electrodes with regard to the integrity of brain tissue.

In this regard, our system fulfills the requirements of a stimulation system for continuous DBS in small laboratory animals comprising (i) the respect of the animal's biological, physiological and behavioral integrity, and (ii) the technical reliability of the stimulation hardware during the entire stimulation period [6]. Implants and electrodes were well tolerated by all rats as reflected by their good biocompatibility and the miniaturized size of the implantable assemblies. At the brain level only minor reactive changes in the neuronal tissue (e.g. reactive astrocytes, glial proliferation and isolated monocytes) were observed along the trajectory of the electrode (within a radius of less than 60µm) comparable to those described for clinical DBS as well as experimental stimulation [7, 8] and are likely to reflect reactive tissue changes as a result of an ongoing repair within the brain parenchyma due to the insertion injury. Most importantly at the level of the electrode - tissue - interface no disturbances in the integrity of the brain were observed. The safety of electrical brain stimulation depends on various factors and is predominantly dependent on the charge density (QD) and the properties of the stimulating electrodes [9, 10]. In our study QDs ranged from 2.17 ± 0.36 µC/cm²ph for 50µA and 4.53 ± 0.60 µC/cm²ph for 100µA, which is far below the threshold of 4mC/cm²ph to induce irreversible *Faradaic* reactions at the electrode-electrolyte-interface *in-vitro* [11].

Despite the majority of advantages when using an implantable DBS device several practical aspects have to be considered, such as assembling steps to ensure fluid resistance of the system and the complex surgical implantation procedure. Specifically, the IPG is currently only programmable for the stimulating waveform and the current amplitudes, but not for pulse width and frequency. Advances in programming the microcontroller will allow the generation of more flexible stimulation protocols in the near future. Moreover, further efforts are undertaken in establishing a comparable system for mice. Due to the availability of transgenic models for different disease entities the application of chronic DBS in mice models will offer a new dimension to prove new indications for DBS.

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