Semichronic, Collocated Deep Brain Stimulation and Multisite Recording in Rats

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Abstract

Multisite microelectrode recording represents a suitable procedure to study microphysiology and network interactions in the central nervous system on a short time scale. This enables a deeper understanding of recent neurochemical studies to investigate changes in the neurotransmitter GABA caused by deep brain stimulation at the same position. We describe in the following a semichronic procedure to implant a miniature system bringing both stimulation and monitoring probes in close proximity in the caudate putamen of freely behaving rats. New multisite microelectrodes to replace existing microdialysis probes are built from spun wires, characterized and described. The design of a closed loop recording and stimulating system is discussed.

Keywords:
Deep Brain Stimulation, animal model, microdialysis, multisite microelectrode, micro wires, Digital Signal Processor

1. INTRODUCTION

Parkinson's syndrome represents with an annual incidence of 10 new cases among 100000 inhabitants one of the most abundant neurodegenerative diseases. In an increasing number of cases this disease is symptomatically treated by so called high frequency (130 Hz) functional and permanent stimulation of sensitive brain regions in the basal ganglia [1, 2]. This procedure is principally reversible and adaptable, it only requires the implantation of stimulation electrodes inside the basal ganglia [3].

Unfortunately, even though this Deep Brain Stimulation (DBS) proves to be very beneficient in 80% of the treated cases, there is no stringent and complete scientific rationale, on how this functional stimulation reaches this effect [4].

To shed some light on this question we investigated the effects of brain micro stimulation in a model region of the rats brain, the caudate putamen [5, 6]. We developed a procedure, to implant some type of chronic guidance system onto the rats skull [7]. This guide wire system permitted every other week the acute and daylong implantation of one stimulating microelectrode very closely located to one microdialysis probe in the brain of a freely behaving animal. Due to the fact, that microdialysis sampling from the target region took 20mins, we were only able to determine an increased GABA outflow as a result of short term electrically stimulating this brain region [8]. However, the real cause for this stimulated increase in neurotransmitter outflow must lie in a change of electrical activity of the originating neuronal network [9].

Consequently, we present with the following work in progress to develop a low latency, electrophysiology-based monitoring of electrical stimulation in the caudate putamen of the rat. In fact, the multiunit activity of cell ensembles should manifest the change of network activity by stimulation notably.

2. MATERIALS AND METHODS

In order to investigate in vivo stimulation effects in a an area as small as possible in a rats brain, a microstimulation electrode has to be realiably positioned in close proximity to two different types of monitoring probes, either microdialysis or micro-recording probes. Both probes are to e implanted semi-chronically, i.e. on the day of the experiment they are inserted in the predetermined position and are recovered at the end of the experimental session.

2.1. Collocating probes in vivo

In order to achieve this goal, a small guide tube stack was developed to be implanted prior to actual experiments. The

guide tube stack consists of a commercially available microdialysis guide tube (CMA/11, CMA Microdialysis AB, Sweden) and a 20G cannula, trimmed to size and fixed to the microdialysis guide tube. The second cannula serves as guide for a concentric bipolar stimulation electrode with 250 µm outer diameter and 30 mm total length (modified bipolar, concentric platinum-iridium (Pt/Ir) electrodes CBCBG30, FHC Inc., Maine). The guide tube stack is later cemented on

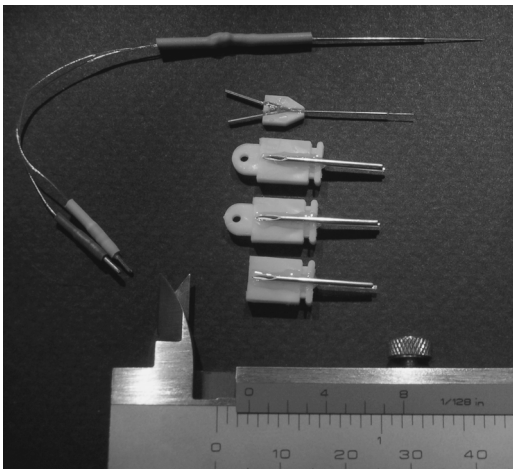

Figure 1: Manufacturing picture of collocated probe stacks. From top: Stimulating electrode, microdialysis probe and different guide tube stacks.

the skull of an anesthetised rat.

2.2. Semi-chronic animal procedure

Male Wistar rats (400g) are used in this study. All procedures with animals are reviewed and approved by the Ethics Committee of the University of Lübeck and the competent government agencies and were conducted in accordance with the NIH guide for the Care and Use of laboratory animals.

Details of this procedure are described elsewhere [8], but in short preparatory guide cannula implantation is performed on rats pre-anesthetized with CO_2 followed by full anaesthesia with sodium pentobarbital (53 mg/kg i. p.). Using standard stereotaxic techniques under microscopic and micromanipulator control, our intracerebral double guide cannula is placed just above the right caput nuclei caudati and fixed to the skull with dental cement. The coordinates for implantation of the double guide cannula are: AP -0.26 mm, ML -3.0 mm relative to bregma, and DV -3.0 mm from the dural surface [10]. Animals are allowed to recover from surgery for at least 5 days, before microdialysis experiments are performed.

On the day of the experiment, a microdialysis membrane (CMA/11, cut-off of 6kDa, outer diameter 240 µm and membrane length 4 mm, Carnegie Medicine, Stockholm, Sweden) or the Niotrode and a stimulation electrode are inserted into the guide cannula, under the condition of CO_2 and isoflurane anaesthesia to prevent animals from distress or pain. The monitoring probe (microdialysis or Niotrode) uses the medial and the stimulation electrode the lateral guide. The microdialysis probe and the stimulation electrode are successively lowered via their respective guide cannula into the right caudate nucleus to achieve a minimal distance between the monitoring probe and the stimulation electrode. For microdialysis, perfusate was collected with 1,2µl/min flowrate. GABA and glutamate were offline analysed by means of HPLC with electrochemical detection after pre-column derivatization of sampled perfusate (20min collecting time, 24µl volume) [11].

2.3. Multiste recording probes

One of the boundary conditions for these microelectrodes is to cause minimal trauma to the target region. Therefore their maximal outer diameter has to be not more than 100µm in order to fit into a 33G stabilizing cannula (Figure 4 B). Recording microelectrodes are made from eight insulated micro-wires (Ø 25µm from 18µm Ni-Chrome and 3.5µm insulation) spun in a circle around a ninth Platinum/Iridium wire (Ø 25µm plus 6.5µm insulation). Spun wires form a helical and stable strand around the immobile center wire and are ultimately glued with low viscous epoxy (Epo-Tek 302-3M, Polytec PT GmbH, Waldbronn, Germany) to the center (see Figure 2 and Figure 4 A.). The spun assembly is cut and beveled under an angle of 45° to remove excess glue and insulation in order to expose the inner metal cores. The resulting asymmetries, whether in electrode construction or relative geometry, ensure that different electrodes record neural signals under slightly different geometrical boundary conditions, supposedly facilitating non-ambiguous detection

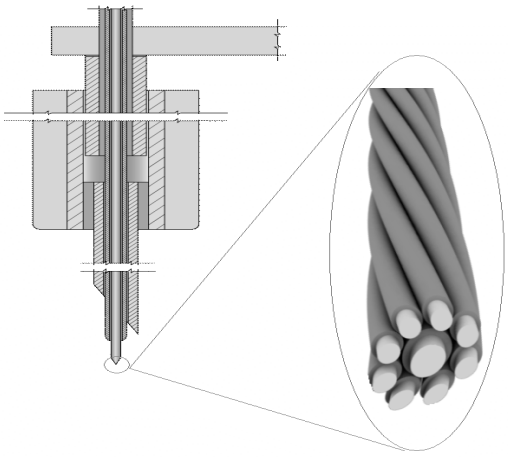

Figure 2: Design sketch of the microwire assembly (right) in a series of concentric guide tubes (left).

of neuronal action potentials [12].

The spun "Niotrode" itself is mechanically quite stable, as long as their length does not exceed a few Millimeters. Stable insertion and smooth movement of the Niotrode along its z-axis is achieved by a "Matryoshka"-type guide tube design: The wire assembly is supported by the mentioned 33G carrier tube, which runs in a 27G dura penetrating tube (Figure 4 C), being guided by two more tubes (24G and 20G).

Niotrodes are characterized by a fast three-point impedance measurement, kindly provided to us by K. Yoshida, SMI,

Aalborg University and described in detail in [13, 14]. In brief, band-limited noise current is passed through the test electrode and a large counter electrode immersed in physiologic saline. Currents through and voltage drop over test electrode and a separate reference electrode is measured. Fast Fourier Transforms are calculated of adequately sampled and windowed voltage and current waveforms and the empirical transfer function estimated. This estimate leads to the impedance spectrum of the electrode under investigation. A typical set is shown in Figure 5.

2.4. Complete setup

Figure 3 shows an overview over the complete, closed-loop recording setup.

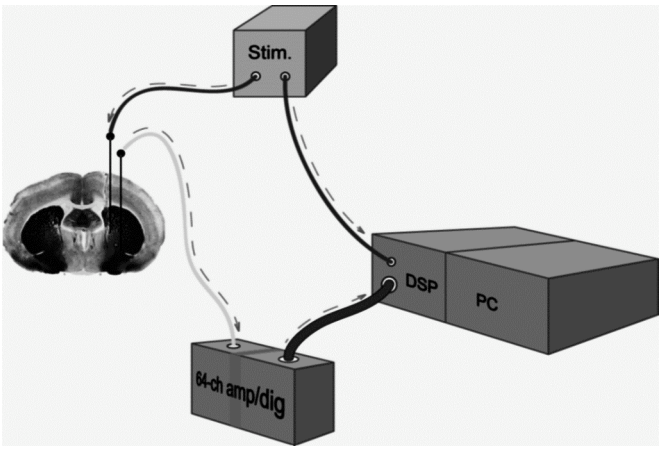

Figure 3. Schematic overview of the electronic set-up. Stimulation is achieved through one microelectrode, while the multisite Niotrode is used to record resulting stimulation patterns from the network.

Stimulating currents from a monopolar stimulator (Isostim A320D stimulator, WPI, Berlin, Germany) will cause severe artifacts in the recording setup, which is one of the reason why we perform very early advanced signal processing on a DSP-board.

A battery operated, custom made, combined amplifier/digitizer (to be described elsewhere) will perform digitization of neuronal signals with a variable gain between 1k and 100k, a wide bandwidth of 0.1Hz to 12kHz and 16bit resolution on potentially 64 channels. The controlling host-PC is connected to the amplifier/digitizer by glass-fiber, thus fostering classification of the whole system to a medical device with CF protection class.

The front-end device is based on a PIC micro-controller, which controls amplification gain and 64 A/D converters in banks of eight. A receiving DSP-board for buffered acquisition and pre-processing is based on the TMS320C6701 TI processor house in a M67 board from Innovative Integration (Thousand Oaks, California) [15].

3. Results and Discussions

The principle feasibility of semichronic, collocated stimulation and micro-monitoring in a freely moving animal to investigate deep brain stimulation was already shown in [7, 8]. The extension of this work is aimed at higher time resolution with the same procedure. For this purpose, we developed metal-based multisite microelectrodes to replace the successful microdialysis probes in vivo.

The following results document the technology of this work in progress.

Figure 4 shows a micrograph of one of our simple Niotrodes. Clearly visible are the spun wires in a successive, concentric arrangement of guide tubes. Electrode sites are to small on this scale to be visible.

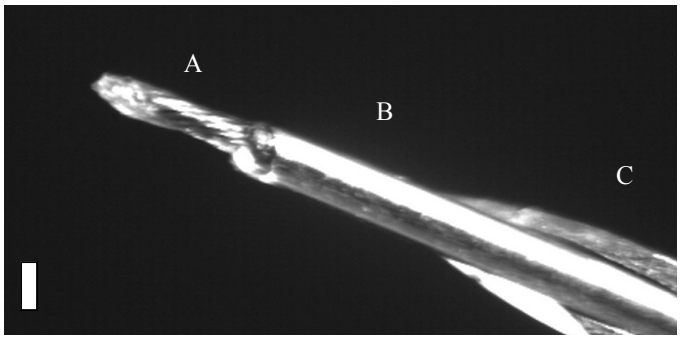

Figure 4. Nine-wire recording electrode: **Niotrode**.
Bar depicts ca. 100µm. A is the actual Niotrode, B a 33G stabilizing tube and C the dura penetrating 27G cannula.

Figure 5 illustrates the complete complex spectrum over a wide frequency range of several recording sites. Curves are taken as averages of at least 10 different measurements of each electrode site. It is clearly visible, that measured electrode sites feature a complex impedance in the range of 300kOhm@1kHz, thus promising the ability to really record from single and multiple neuronal units.

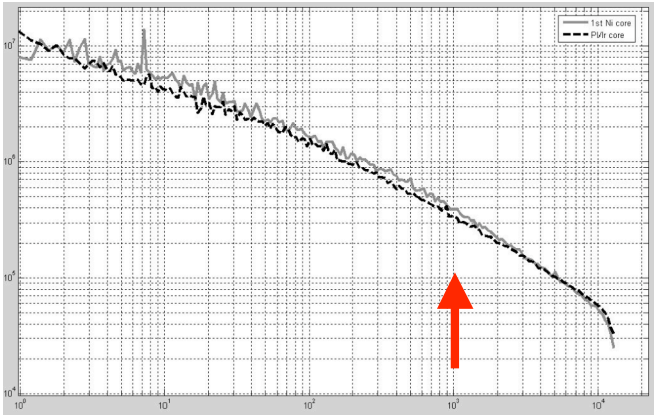

Figure 5: Averaged estimates of impedance spectrum of recording sites Ni1 and the Pt/Ir core of an exemplary Niotrode. Arrow depicts the physiologically relevant impedance at 1kHz.

4. Outlook

Although this work is in progress and awaits its first complete experimental evaluation, its components are very promising and may lead to a short latency on-demand deep brain stimulation based on the patients own neuronal activity pattern.

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