Design of a high-density multi-channel electrode for multi-structure parallel recordings in rodents

Nedjeljka Ivica*, Martin Tamtè*, Maruf Ahmed, Ulrike Richter, and Per Petersson

***Abstract*— In neurophysiology, investigating brain connectivity within and between different brain structures is of fundamental importance for understanding nervous system function and its relation to behavior. Yet, parallel recordings in multiple brain structures is highly challenging, especially in rodents, which are most commonly employed in neurophysiological research but rather small in size. In this study, the design and manufacturing of a high-density multi-channel electrode for chronic, multi-structure parallel recordings in rats is presented and exemplified with functional neuronal recordings from 128 recording channels, placed bilaterally in eight different brain structures, in an awake, freely moving animal.**

I. INTRODUCTION

More than 90% of today's medical research is done on rodents which, as mammals, share many similarities with humans in organ, structure and function. Because of their small size, high reproduction rate, low cost and easy handling, they are in several ways ideal for laboratory experiments and are commonly used within neurophysiological research. This field of research is concerned with understanding the function of the nervous system including the translation to behavior. Hence, a major branch within neurophysiology is specifically interested in deepening our understanding of how the brain controls different motor behaviors, and which changes that are occurring in pathophysiological states. For this and many other types of research, it is of specific interest to be able to correlate the behavior of awake, freely moving animals to simultaneously recorded neuronal activity.

It is known that the cortico-basal ganglia-thalamic circuit, comprised of a large number of different structures, is not only specifically active and involved during the planning, selection and execution of motor commands, but is also strongly affected by motor diseases and especially Parkinson's. Local field potential (LFP) oscillations in certain frequency bands and altered brain connectivity within this circuit are considered to be directly related to parkinsonian symptoms as well as levodopa-induced dyskinesia [1]–[3], the latter being a common side effect characterized by involuntary movements resulting from long-term medication with levodopa in parkinsonian patients. Thus, parallel and preferably chronic electrophysiological recordings from populations of neurons in all structures of the cortico-basal ganglia-thalamic circuit should be integral in addressing many of the high-level questions that remain for system level interpretations of motor behavior in both health and disease.

However, at present parallel chronic electrophysiological recordings in awake, behaving rodents have been restricted to just a subset of structures within the cortico-basal ganglia-thalamic circuit. A main reason is that the different structures within this circuit are anatomically adjacent within a relatively small volume in the rat's brain, see Fig. 1, such that it becomes technically highly challenging to acquire recordings from all these structures in parallel. In the current study, we have addressed these problems and report on a newly designed high-density multi-channel electrode for parallel bilateral multi-structure recordings in rats. The functionality is illustrated with the brain connectivity analysis of an example LFP recording from all structures of the cortico-basal ganglia-thalamic circuit in a freely moving animal.

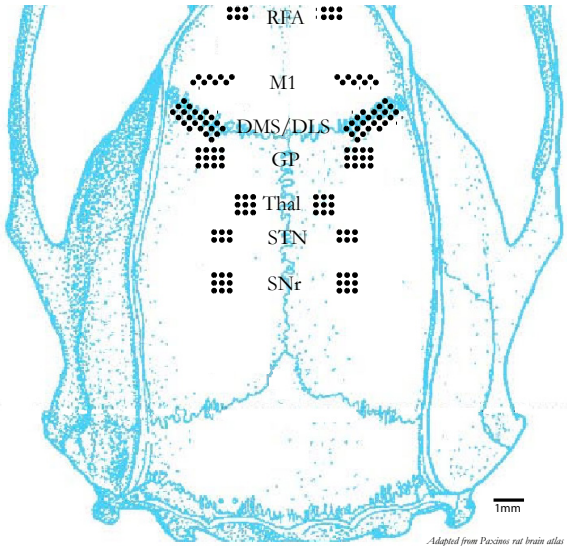

Fig. 1. Dorsal view of the rat skull with a constellation of electrode targets in the cortico-basal ganglia-thalamic circuit. (RFA: Rostral Forelimb area, M1: Primary Motor Cortex, DLS: Dorsolateral Striatum, DMS: Dorsomedial Striatum, GP: Globus Pallidus, Thal: Thalamus, STN: Subthalamic Nucleus, SNr: Substantia Nigra pars Reticulata.). Within structures, individual channels are placed at a high density (electrode spacing 250 μm).

II. DEVELOPMENT OF MANUFACTURING PROCESS

Several requirements had to be taken into account in the development of the manufacturing process of the novel

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electrode. In particular, it needs to 1) be compact and light, so that it can be mounted on the restricted surface of the rat's skull ($\sim$ 15$\times$10 mm) and be carried by the rat also during longer experiments, 2) be implantable in a single surgical procedure and thus needs to be built in one piece with high precision to reach all targeted structures, and 3) incorporate a sufficient amount of recording channels in each of the structures of interest, such that brain connectivity can be assessed not only between, but also within structures.

Currently, there are commercially available multi-channel recording systems that can handle 128 recording channels, while providing 16 dedicated reference channels, 16 ground channels, and 8 stimulation channels (giving 168 channels in total). Requiring a local reference in all implanted structures, using such a system allows for bilateral implantations in up to eight different structures. Furthermore, one might require at least five recording channels per structure. Using 128 recording channels allows this to be achieved with a margin and, depending on their anatomical size, recordings from certain structures might be augmented with additional channels. Figure 1 shows the constellation for bilateral implantation in eight structures of the cortico-basal ganglia-thalamic circuit which was employed in this study.

For a recording system with in total 168 channels, four head stages with one 44-pin Omnetics connector (0.64 mm pitch) each are required. However, two compact, passive 80-pin board-to-board connectors (Kyocera, 0.4 mm pitch; one for each hemisphere) were found superior in size to four Omnetics connectors. During experiments, the connection to the head stages was established via two custom-built adaptors (each linking one Kyocera to two Omnetics connectors). As the combination of two Kyocera connectors allows for in total 160 channels, in the current realization, it was chosen to reduce on the number of employed stimulation and ground channels.

Finally, to comply with the high demands on precision to reach the targeted structures, a manufacturing approach was adapted from [4], see Fig. 2. In the following, each step of the manufacturing process is described in detail.

A. Recording interface

The material of the recording interface needs to be stiff enough to be reliably implanted in deep and small brain targets, and provide a high yield in long-term (3-4 weeks) recordings of single cells [5]. For these purposes, tungsten wires (33 μm, impedance 300 kΩ at 1 kHz; California Fine Wire Co.) were chosen as recording, reference and stimulation electrodes.

B. Three-dimensional layout of recording interface

After deciding on the different targets, their anterolateral and vertical coordinates were retrieved from the literature. The approach employed a three-dimensional (3D) aligner (layout designed in SketchUp, Trimble, realized with 3D printer) with distinct apertures and depths, matching the anterolateral and vertical position of each implantation target, see Fig. 2. The anterolateral position of each tungsten wire was further assured by the use of a perforated two-dimensional (2D) array (polyethylene terephthalate), see Figs. 1, 2. Holes of 100 μm with 250 μm center-to-center distance within each structure were made by laser milling (355 nm, 50 Hz, 0-7 J/cm^2), allowing unrestricted passage of up to 50 μm insulated wires. The 2D array was aligned above the apertures of the 3D aligner such that the wires would pass through the array to the bottom of the 3D aligner, thus ending up at their correct relative vertical length. The wires were then secured with fast hardening UV-light hardening epoxy adhesive applied on top of the 2D array, such that it remained unaffected by bending of the wires in the next step.

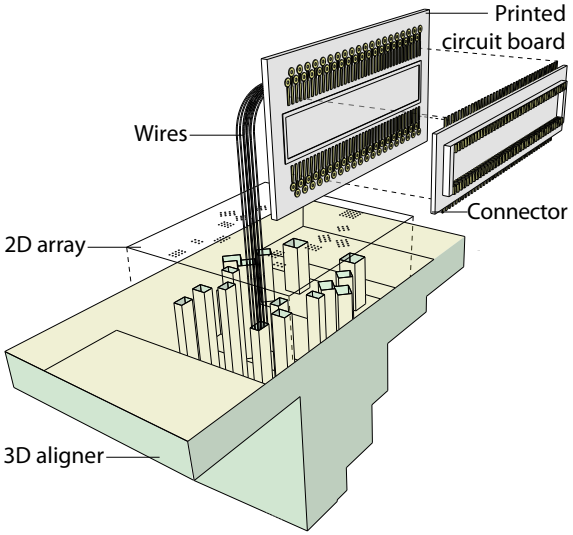

Fig. 2. Schematic overview of design assembly in its near-to-end stage. A three-dimensional (3D) aligner and a two-dimensional (2D) array assured the correct vertical and anterolateral positions of each of the individual channels. Connections to the compact Kyocera board-to-board connector were then established via a custom-designed printed circuit board (PCB).

C. Printed circuit board

Tungsten as a material has inherent properties that introduce difficulties when soldering it to another metal. This issue can be overcome for example by using two component silver conductive epoxy adhesive instead of the commonly used tin/lead solder. However, establishing a direct connection from the wires to the Kyocera connector employing this process still posed practical difficulties. To overcome these, a PCB with through-holes for each wire was custom-designed as a link to the connector. In a first step, the Kyocera connector was soldered onto the PCB. The wires were then threaded through the holes of the compound PCB-connector, see Fig. 2, and fixated on the back side of the PCB with UV-light hardening epoxy adhesive. The wires were then cut and de-insulated, and the conductive adhesive was applied and cured, establishing an electrical connection between the wires and the PCB. As a final step, all parts of the electrode were locked into one piece with UV-light hardening epoxy adhesive.

III. ANIMAL SURGERY AND DATA ACQUISITION IN VIVO

A. Animals

One adult female Sprague-Dawley rat (250 g; Taconic Inc.) was used in the present study. The animal was kept on a 12:12 h light cycle and received food and water ad libitum. All experiments were approved in advance by the Malmö/Lund ethical committee of animal experiments.

B. Surgical procedures

Unilateral Parkinson model Three weeks prior to implantation the animal received injections of 6-hydroxydopamine (6-OHDA) hydrochloride (3.0 μg/μl free base; dissolved in 0.02% ascorbate saline) into the medial forebrain bundle of one hemisphere [3]. Moderate motor impairments including asymmetric posture and gait and reduced forelimb dexterity were apparent two weeks after lesioning.

Implantation surgery The animal was anesthetized with Medetomidinhydrochloride/Fentanyl (0.3/0.3 mg/kg) and fixed in a stereotaxic device to secure a stable cranium position. The electrode was implanted by micromanipulator and fixated with dental acrylic attaching to screws in the skull. After surgery, the anesthesia was reversed by Atipamezolhydrochloride (0.5 mg/kg), and Buprenophine (0.05 mg/kg) was administered as postoperative analgesic. The rat was allowed to recover for 10 days after which experiments were initiated and pursued during a couple of months.

C. Experimental procedure

During recording sessions the animal was placed in a transparent cylinder (250 mm in diameter). The rat was first recorded for 30 min to establish baseline conditions. Subsequently, the rat was intraperitoneally injected with levodopa and benzerazide [3]. Dyskinesia developed 10 to 20 min post-levodopa injection and reached its peak severity $\sim$60 min post-levodopa injection. Recordings continued until the dyskinesia diminished spontaneously ($\sim$2 h post-levodopa injection).

D. Data acquisition

For recordings, the implant was connected to head-stages (unity-gain pre-amplifier with buffering capacity) and cables of the 128-multi-channel recording system (Neuralynx Inc.) via the custom-built adaptors. The cables were attached to a multi-channel commutator to allow the animal to move freely in the experimental environment. LFPs and single- and multi-unit activity were recorded in parallel. However, only LFPs were further analyzed in the current study (for detailed unit study using the same wire type see [3]). Reference channels were set in the software. LFPs were filtered 0.1–300 Hz and digitized at 1017 Hz.

IV. DATA ANALYSIS AND RESULTS

A. Preprocessing

After standardization of the raw LFP time series, local bipolar LFP time series were computed offline from all unique pairs of channels from the same structure. Occasional artifact periods were detected and rejected with a flatness criteria (thresholding of the median of the absolute value of the sample-to-sample difference). Further analyses were restricted to an arbitrarily chosen 1-min interval during baseline and around peak dyskinesia (i.e., 60 min post-levodopa injection), respectively. The baseline period is in the following referred to as healthy/parkinsonian state for the intact/lesioned hemisphere. The time period around peak dyskinesia will be referred to as the levodopa/dyskinetic state for the intact/lesioned hemisphere.

B. Frequency analysis

The spectral power in single LFP signals and the coherence between pairs of simultaneously recorded LFP signals was computed using a multitaper method [6] (non-overlapping 3-s windows, 5 tapers) implemented in Chronux 2.0 [7]. Each power spectrum was normalized to its pink noise background [3], allowing to describe power spectral deviations from the pink noise floor conveniently in terms of the unit dB_{pink}. As a final step, the mean power spectrum over time and all recordings channels was obtained for each structure. Because several recording channels were used in each structure, there were also several coherence measures for each pair of structures (i.e., one measure for each pair of LFP signals). In order to obtain one mean coherence for each pair of structures, the mean magnitude-squared coherence (MSC) was calculated employing the variance-stabilizing transform for the coherence (arc-tanh) [6].

C. Results

The results of the frequency analyses are illustrated in Fig. 3. For the MSC between, but also within structures, at least three frequency bands deserve to be specifically highlighted. The first interval includes frequencies from about 20 to 35 Hz. As pointed out by the black arrows for the coherence between RFA and DLS, an elevated coherence can be seen in this frequency band in the parkinsonian state when compared to both the healthy and the dyskinetic state. The second interval reaches from about 80 to 90 Hz, in which an elevated coherence can be seen for the dyskinetic state when compared to both the parkinsonian state and the levodopa state (highlighted by the red arrows in Fig. 3). Finally, elevated coherences can be seen, especially in subcortical structures, in a narrow frequency band around 6 Hz in all four observed states.

V. DISCUSSION AND CONCLUSION

Implanting electrodes into different brain structures most often implies different vertical positions of the implantation targets. In the manufacturing process, this may be achieved by manually cutting all wires to their desired length. However, this comes with the risk of a slightly variable wire length and the malformation of the thin electrode insulation. Thus, a 'bottom-up' approach as employed in the current study, which positions the tips of the wires at the desired relative depths from the start and thus avoids manual cutting, is clearly advantageous in this regard. It also deserves to

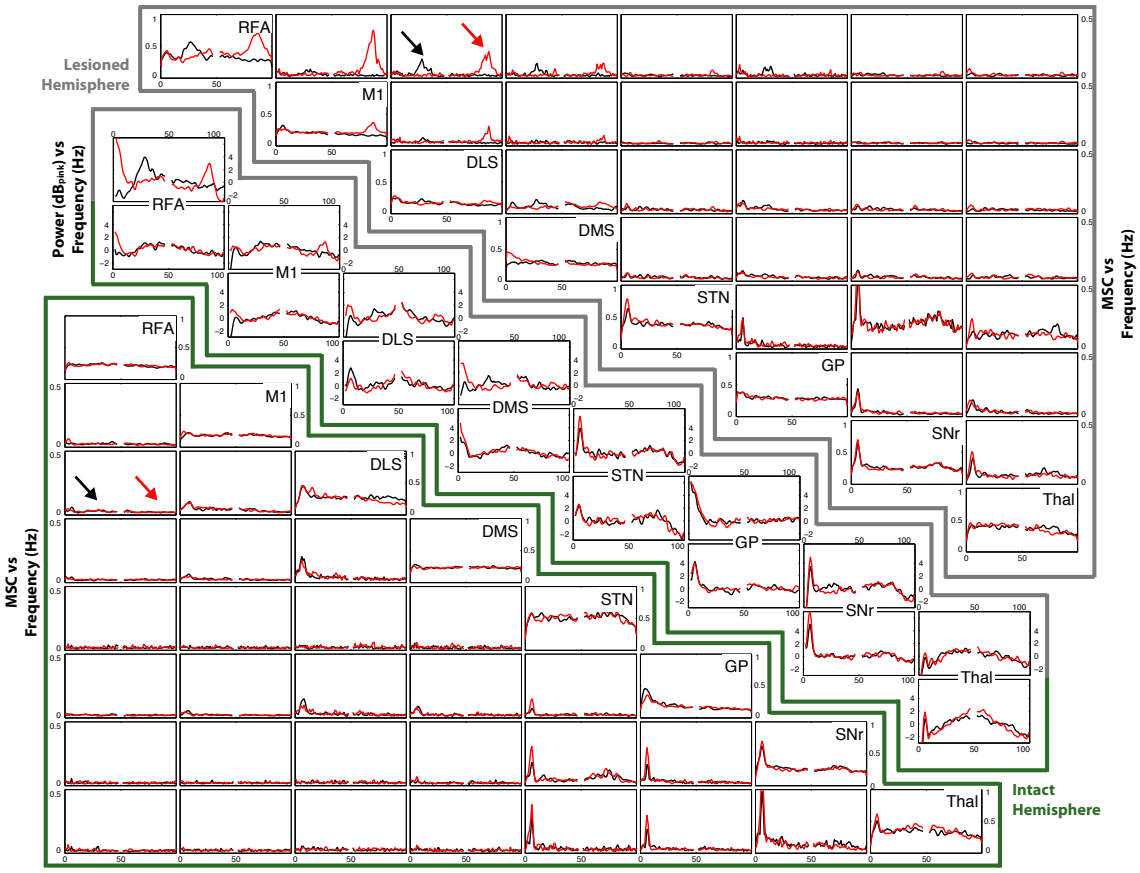

Fig. 3. Power and mean squared coherence (MSC) versus frequency displayed for the intact and lesioned hemisphere (lower left and upper right part of the figure, respectively) during baseline (healthy/parkinsonian state, black traces) and levodopa treatment (levodopa/dyskinetic state, red traces). For the MSCs between RFA and DLS two black and two red arrows in the intact and lesioned hemisphere point out a lower and higher frequency band, respectively, with notable differences in MSC between the four different states. (The narrow interval at 50 Hz is not shown because of power line interference. RFA: Rostral Forelimb area, M1: Primary Motor Cortex, DLS: Dorsolateral Striatum, DMS: Dorsomedial Striatum, GP: Globus Pallidus, Thal: Thalamus, STN: Subthalamic Nucleus, SNr: Substantia Nigra pars Reticulata.)

be highlighted that the presented manufacturing process has been developed such that it can easily be adapted to different target constellations by reproducing the employed 3D aligner and 2D array for the positions of the desired targets.

The choice of the targeted brain structures in the current study has been such that, for the first time, neural activity in all structures of the cortico-basal ganglia-thalamic circuit of an awake, freely behaving rat could be recorded in parallel. Such recordings open up for a large variety of studies on dynamic changes in brain connectivity between different states of activity in either healthy or diseased animals. In fact, all three frequency bands with elevated coherence have previously been associated with different aspects of Parkinson's and have even been suggested to have a direct pathogenic role for certain motor symptoms [1], [3]. Furthermore, the chronic implantation of the electrode allows to follow changes in neural activity within this circuit over several weeks, during which, e.g., a disease is progressing or a new motor behavior is learned.

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