Biomed Tech 2012; 57 (Suppl. 1) © 2012 by Walter de Gruyter · Berlin · Boston. DOI 10.1515/bmt-2012-4437

An implantation technique for polyimide based flexible array probes facilitating neuronavigation and chronic implantation

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Abstract

Flexible, polyimide based probes for chronic implantation provide a promising option for biocompatible neural interfaces minimizing foreign body response in the tissue and preventing electrode micromovements. Low mechanical stability is an issue for insertion of flexible probes. Solutions for precise intracerebral implantation are required to provide transient stability during insertion without obstructing functionality. We used flexible, polyimide based probes with lateral arrangement of recording and stimulation sites and a reinforced punch hole at the probe tip. Via the punch hole at the tip, the probe was mounted to a support rod and was then navigated to the insertion site. While the probe was lowered into the tissue, neuronal activity was recorded via the flexible probe in steps of 200 µm. Characteristic multi-unit neuronal activity was recorded on the trajectory to the subthalamic nucleus. After insertion, the support rod was removed and neuronal activity was measured from the subthalamic nucleus in the awake and freely behaving animal. The reported implantation technique facilitates precise insertion while preserving probe functionality.

1 Introduction

Current development in neuroprosthetics is focused towards small sized MEMS based microelectrodes which facilitate extracellular recordings in neuronal tissue via multiple contact sites [1, 2]. Advanced fabrication methods allow for small electrode contact sites, a large number of contact sites per unit surface area and a wide spectrum of electrode and substrate materials. Yet, the performance of rigid microelectrodes based on silicon, ceramics or metals which are commonly tethered to the skull seems to be hampered due to brittleness of the material, electrode micromovements and glial scarring [3, 4, 5]. The use of flexible, polyimide based probes promises to prevent the problems occurring due to brittleness and rigidity of the material to a great extent. Further, polyimide has been shown to be biocompatible and alleviation of the inflammatory response to probe implantation has been reported [6, 7]. Thus, polyimide based probes have been used already as a substrate material for bioelectronic interfaces [8]. The application of polyimide based neural probes has, however, been limited since the lack of mechanical strength makes precise intracerebral implantation difficult [9]. By increasing the thickness of the polyimide layer, introduction of a silicon backbone layer, or microfluidic channels filled with biodegrabable polymer intracortical implantation has been achieved while paying the cost by more complicated manufacturing processes and increased probe size [10].

Stiff biodegradable polymers or crystals have been used to coat flexible probes to facilitate insertion [11]. Still, biodegradable coatings and most shuttle systems prevent the validation of the targeted brain area since recording of neuronal activity patterns during insertion is impossible when the electrode contact sites are covered [12]. In the current study, we present a simple approach for intracerebral implantation of flexible, polyimide based probes, facilitating target validation by neuronavigation and precise chronic implantation. To compare signal quality and long term performance with a standard method we used metal based linear array probes in a parallel approach.

2 Methods

Flexible array probes for intracerebral insertion, neuronavigation and long term implantation were microfabricated from 20µm thick electro-spun polyimide. Three stimulation sites with 3600 µm² in the middle of the shaft and six off-centered recording sites with 400 µm² were produced by gold vapour deposition and were galvanically reinforced.

The probes feature alternating recording and stimulation sites as well as large surface ground contacts and a punch hole at the tip to facilitate tissue insertion. Ten reinforced gold contacts with 0.5 mm pitch for connection were inserted into a ZIF connector, soldered onto a small PCBoard.

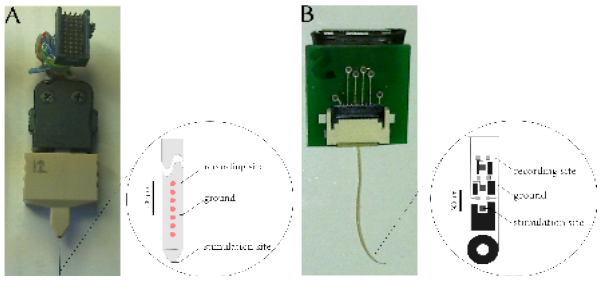

Figure 1: Probes for intracerebral insertion, neuronavigation and long term implantation. **A)** Metal based rigid probe with gold micro-wires embedded in a stainless steel shaft. Seven recording sites (3850 µm²) are arranged along the probe shaft serving as ground. A stimulation site (11300 µm²) is located at the probe tip. **B)** Polyimide based flexible probe featuring gold contact sites. Six off-centered recording sites (400 µm^2), three stimulation sites (3600 µm^2) in the middle of the shaft as well as large gold patches serving as ground.

Rigid probes, used for comparison of signal quality and long term performance, were produced from a hollow stainless steel shaft and gold microwires. A lateral array of contact sites was formed by the ends of the gold leads, embedded into the shaft. The rigid probes feature seven recording sites with 3850 µm² in a lateral array along the probe shaft over a distance of 750 µm with a centre-to-centre distance of 125 µm, starting 400 µm from the tip. A 11300 µm² stimulation site is located at the probe tip (Fig 1). A 10-channel-mobile-phone-connector was used for both probe types to provide the connection interface to the commercial hardware (RZ5 BioAmp, RA16PA Medusa preamplifiers, ZIF-Clip digital headstage, ZCA-NN32 headstage adapter, AC 32 motorized commutator, TDT, US) which was used to measure neuronal activity.

The probes were inserted and implanted into the rat brain by performing a stereotaxic surgery. All procedures with animals were reviewed and approved by the University of Lübeck and the Ministry for Agriculture, the Environment and Rural Areas, Schleswig-Holstein in Germany, and were conducted in accordance with the NIH guide for the Care and Use of laboratory animals. Male Wistar rats were used and housed separately under standard lighting conditions (12 h light-dark cycle, lights on at 06:00 am), 22 °C and 40 % humidity with free access to food and water. Before the surgery, animals received initial inhalation anaesthesia with isoflurane, followed by i.p. injection with 80 mg/kg ketamine (Ketavet®, Pfizer) and 1 mg/kg xylazine. Trepanation points were determined according to standard stereotaxic protocol under microscopic control (0.7-4.5X Zoom lens NT53-374, Edmund Optics and CCD Camera DFK41BF02.H, The ImagingSource). Trepanation holes with d = 2.3 mm were placed at AP+0.56 and ML-0.26 (in cm) relative to the inter-aural point. Around the trepanation, four fixation screws were placed in 0.3 mm distance. Another skull screw was placed at AP+0.25 (in cm) and ML-0.25 (in cm) relative to the inter-aural point and was later connected to the amplifier's ground.

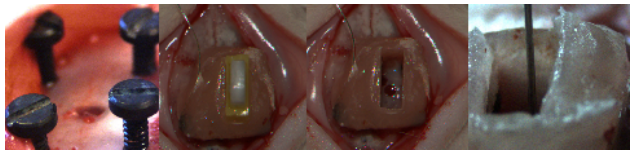

Figure 2: Implantation procedure for metal based, rigid probes facilitating re-use after cleaning. Using a silicone spacer, a dental resin housing was built around the fixation screws. The rigid probe was inserted and the housing was filled with silicone oil before sealing with dental resin.

Re-usability of implanted rigid probes was ensured by building a dental resin housing around the fixation screws, which was later filled with high viscosity silicone oil (Fig. 2). Rigid probes were inserted into the cerebral tissue in steps of 200 µm to a maximum depth of 0.84 cm from the skull surface without further complication using a stereotaxic frame (Stoelting) and a piezo-driven micropositioning stage (PILine M-633 Linear Motor Stage and C-867 Controller, PI, Germany). After insertion, the housing was closed using dental resin. The wound was sutured and treated with polyvidone iod solution (Betaisodona, Mundipharma).

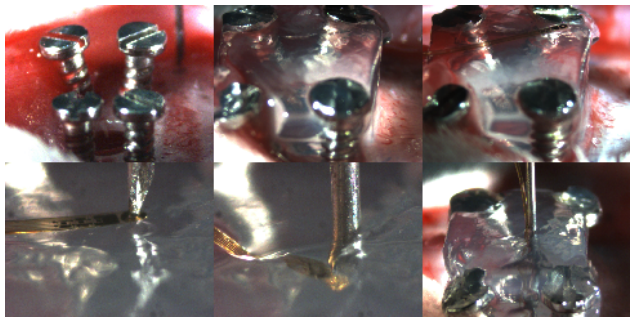

Figure 3: Implantation procedure for flexible, polyimide based probes facilitating recording of extracellular neuronal potentials during insertion. A 0.5 % agarose matrix was placed onto the fixation screws. The flexible probe was placed onto the agarose and adhered. The punch hole at the probe tip was picked up by a sharpened support needle and was navigated to the precise insertion coordinates using stereotaxic equipment.

For flexible probe insertion, a matrix of 0.5 % agarose in

0.9 % NaCl was placed around the fixation screws. The flexible probe was loosely placed onto the agar and adhered due to capillary forces to the water bound in the agarose matrix. The sharpened support needle was mounted to the stereotaxic frame and the micropositioning stage was used to pick up the probe tip at the reinforced punch hole. Then, the support needle was navigated to the insertion coordinates and lowered through the agar matrix into the tissue in steps of 200 µm to a maximum depth of 0.84 cm below the skull surface (Fig. 3). After insertion to the target depth, the support needle was rotated by approx. 90° and was then slowly retracted, whereas the flexible probe remained in the tissue. After insertion, the housing was closed and the agar matrix was covered using dental resin. The connecting PCB holding the mobile connector was embedded in the dental resin as well. Finally, the wound was sutured and treated with polyvidone iod solution (Betaisodona, Mundipharma). Extracellular neuronal potentials were recorded at 24.4 kHz and were bandpass filtered at 300-3000 Hz. At each depth step, signal wavetrains with 60 s duration were recorded. Patterns of neuronal activity occurring in the wavetrain signal were assigned to the anatomical nucleus where the recording took place and compared to neuronal activity patterns described in the literature. Spike detection was performed using a median threshold filter [15].

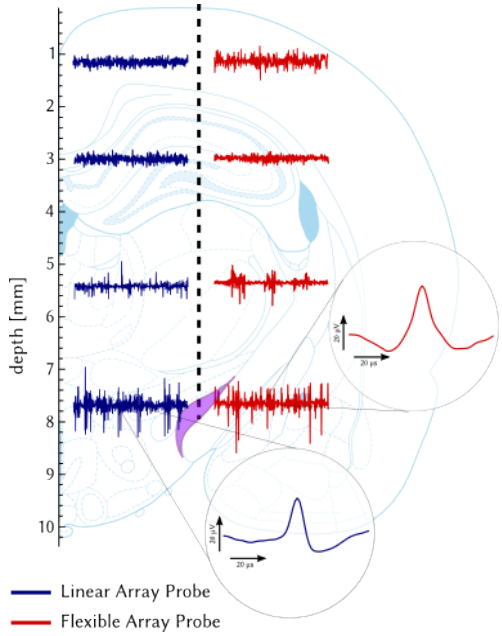

Figure 4: Wavetrain signals, recorded during probe insertion in the respective area of the rat brain along the trajectory to the subthalamic nucleus. The recordings, performed with flexible, polyimide based probes (red) show similar signal qualities and patterns as the recordings obtained with rigid, metal based probes (blue). Single unit extracellular potentials can be detected from wavetrains measured with flexible (red) and rigid (blue) probes.
Spike shapes occurring in each region were compared. After implantation chronic recordings were performed in the awake and freely moving animal after a recovery period of five days up to 30 days post implantation.

3 Results

We present a simple approach for intracerebral implantation of flexible, polyimide based probes. During probe insertion, neuronal activity was recorded and representative activity patterns for each larger region are shown (Fig. 4) In the current approach, the performance of flexible, polyimide based probes was directly compared to the performance of rigid, metal based probes.

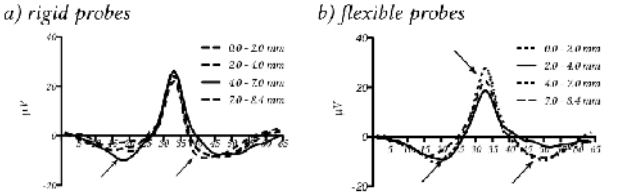

Figure 5: Mean spike shape of detected spikes in cortex, hippocampus, thalamus and subthalamic nucleus. a) Rigid, metal based probes. b) Flexible, polyimide based probes.

While performing neuronavigation, we recorded generally low spiking activity in the cortex and hippocampus in anaesthetized rats. Spiking activity increased in the thalamus and rhythmic bursting was observed as well as tonic firing. Flexible probes seem to be especially suitable to record thalamic rhythmic activity. In the subthalamic nucleus large regular spikes were observed followed by rhythmic bursting. These findings are in line with reports about the neuronal activity recorded in these regions using extracellular probes [13, 14].

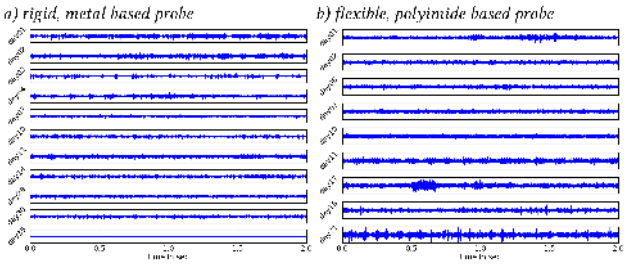

Figure 6: Probe performance during measurements in the awake and freely moving animal after chronic implantation. a) Activity was observed with probes for up to 35 days after implantation. b) Flexible, polyimide based probes were used until day 21 after implantation.

The mean spike shapes of detected spikes in each region do not differ much. Mean spike shapes obtained from recordings with rigid probes differ in the amplitude of the hyperpolarization peak before and after the spike. Using flexible probes, slight differences are also observed in the

spike amplitude.

After implantation, the probe performance was examined by neuronal recordings in the awake and freely moving animal. A signal of good quality has been recorded with rigid, metal based probes up to day 30 after the implantation. Flexible, polyimide based probes have been examined up to day 21 whereby signal quality remains constant.

4 Conclusion

In the current study we presented an implantation technique for flexible, polyimide based probes facilitating acute recordings during probe insertion. Thus target validation by neuronavigation becomes possible with flexible probes. We compared the obtained recordings to meaurements made with comparable rigid, metal based probes and found similar characteristic while performing neuronavgation and during chronic implantation.

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