Deep brain stimulation and simultaneous neurotransmitter detection

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Abstract

The goal of this work was to develop a method for time and position simultaneous deep brain stimulation (DBS) and neurotransmitter detection in an *in vivo* animal model. A simple chronic implant has been designed uniting both existing microdialysis probes and concentric microelectrodes for high frequency electrical stimulation (HFS). This implant enables HFS and microdialysis in one animal at the same time and at the same brain position. The method has been tested in vivo and samples of dialysate were analysed offline by HPLC and ECD. The detection of neurotransmitters in these samples was successful. While biochemical effects of HFS are still unknown, our method aims to answer this open questions.

1 Introduction

Deep brain stimulation (DBS) is well accepted in the treatment of symptoms of Parkinson's disease. However, the exact mechanism of DBS is still unclear [1]. Former in vitro experiments [2] in rat striatal slices showed evidence, that HFS with 130 Hz rectangle pulses has an effect on the GABAergic system. Here we present a method for time and position simultaneous HFS and microdialysis for neurotransmitter detection in freely moving rats.

2 Materials and Methods

We developed a simple device to chronically implant a microdialysis probe and stimulation probe under controlled conditions. For that purpose, we mounted a shortened 20 G canula to a commercially available microdialysis guide tube (CMA/11, CMA Microdialysis AB, Sweden). The canula was glued under microscopic and micromanipulator control to a custom made notch on the CMA/11with 2 component epoxy raisin (**Fig. 1**). The 20 G canula serves in the actual experiment as guide tube for concentric, 250 µm diam., 30 mm stimulation electrodes (CBCBG30, FHC Inc. Maine). We modified this electrode by a tappet to ensure proper positioning of the electrodes ellipsoidal stimulation plane towards the microdialysis probes membrane. That way we achieved a maximum overlap between the electrically stimulated and the chemical sampled tissue. The type of microdialysis probe was *CMA/11 14/4 Cupr* (CMA Microdialysis AB, Sweden). Its membrane has a diameter of 0.24 mm and its cutoff is 6 kD.

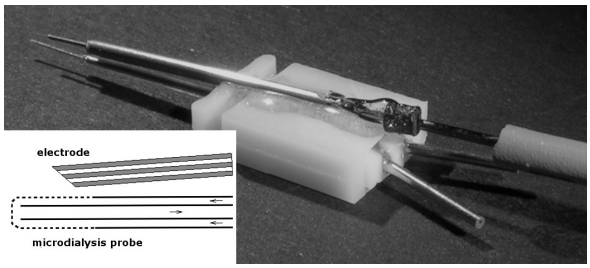

Fig. 1 Implant with inserted probes. The drawing on the left shows the positions of the tips to each other.

The combined guide tube stack (**Fig. 1**) is stereotactically implanted to anaesthetized Wistar rats, at least one week prior to the stimulation experiment.
Stereotactic animal surgery was performed on adult, male rats (av. 400 g, sourced from Charles River WIGA GmbH in Sulzfeld, Germany) preanaesthetized by brief CO_2 exposure and fully anaesthetized with Na-phenobarbital injection (35-30 mg/kg i.p.). Rats were placed in a stereotaxic frame and a single midsagittal skin incision was made on the head, the soft tissues retracted, and a minimal craniotomy (burr hole diameter 1.5 mm at Bregma +3mm mediolateral) drilled. The dura mater was punctured and the guide tube stack inserted with a micromanipulator to reach 3 mm below the skull surface. Two tiny bolts were screwed into the skulls surface and served as solid anchor for dental acrylic. Care was taken to have the dental acrylic float around the guide tube without penetrating the burr hole. The skin was finally sutured back to cover most of the incision, leaving the guide tube entrances accessible for later use.
After at least one week of recovery the first stimulation experiments took place. Rats were

exposed briefly to CO_2 and both the stimulation electrode and the microdialysis probe were threaded into the guide tube and hence to the right caudate nucleus. Firstly, the stimulation electrode was inserted. Afterwards a damaged microdialysis probe was threaded into the guidetube to perforate the target tissue. Successively this probe was replaced by the experimental one. Already at this time both experimental probes were connected to their electrical, resp. fluid circuits. See **Fig. 2** for a fully connected and awake animal.

All procedures with animals were reviewed and approved by "Ministerium für Umwelt, Natur und Forsten des Landes Schleswig-Holstein, Germany", and were conducted in accordance with the NIH guide for the Care and Use of laboratory animals.

After inserting the probes, animals were allowed to recover for 2 hours (closing of the blood-brain barrier). The caudate nucleus was perfused with artificial cerebrospinal fluid (aCSF) consisting of (in mM): 125 NaCl, 5 KCl, 2 $CaCl_2$, 1.14 $MgSO_4$, 1.29 KH_2PO_4, 25 $NaHCO_3$, 0.1 ascorbic acid; pH 7.4. A perfusion rate of 2 µl/min was selected. After a stabilisation period of 2 h, ton consecutive dialysis samples of each 20-min sampling period (40 µl) were collected. During this time two 30-min stimulation periods were conducted, 60 and 150 minutes after stabilisation.

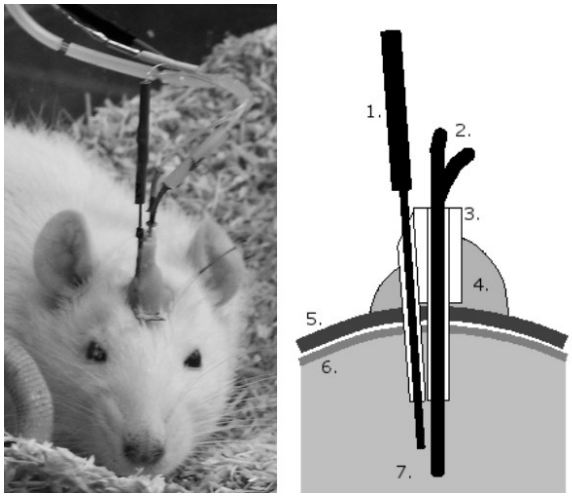

Fig. 2 Implant with probes. On the left: animal during experiment. On the right: Setup of the implant: **1.** stimulation electrode, **2.** microdialysis probe, **3.** guidetube, **4.** dental acrylic, **5.** scull, **6.** dura mater, **7.** target brain tissue.

After finishing the experiment both probes have been removed from the implant. That way the animals survived several experiments.

The electrical stimulation signal consisted of a monopolar positive rectangle pulse with the frequency of 124 Hz (60 µs pulse width, 8 ms delay). The current was set between 0.1 and 0.5 mA. The electrode used was bipolar, with a cathode at the center, concentrically surrounded by the anode.

Samples of dialysate were analysed using HPLC. In this test concentrations of neurotransmitters GABA and glutamate have been determined. The HPLC system is described in [2].

3 Results

Our chronical implant allows positioning of both the dialysis and stimulation probes in a target area with less then 1 mm distance from each other and minimaly damaging the brain. Sampling of microdialysis output during stimulation was successfully performed. HPLC detection of neurotransmitters GABA and glutamate gave clear and valid peaks. Glutamate outflow from microdialysis experiments are exemplary given in **Fig. 3**

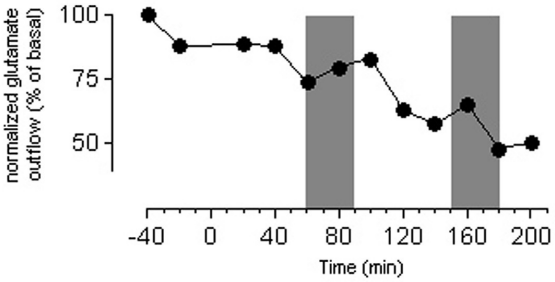

Fig 3. Glutamate outflow from a microdialysis experiment. The gray blocks shows the stimulation periods. Values are normalised (% of basal). Current 0.1 mA.

4 Conclusion

Our setup allows successful detection of neurotransmitters during stimulation in the freely moving rats. This permits future and ongoing research in the rationale behind deep brain stimulation in vivo.

Acknowledgment

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Literature

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[2] T. Li et al., *Neuronal electrical high frequency stimulation modulates presynaptic GABAergic physiology*, Neuroscience Letters 23 (2004) p117-120.