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In vivo iontophoretic BDA injection through a buried microfluidic channel of a neural multielectrode

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Abstract

This paper presents in vivo local iontophoretic release of a neuronal tracer, biotinylated dextran amine (BDA) in the rat brain using monolithically integrated microfluidic channel buried in a neural multielectrode. The tracer injection is controlled by iontophoresis using Pt electrodes in the vicinity of the outlet of the microfluidic channel. The successful injection is evaluated through histological maps of the labelled nerve cells in 3D. Together with previous electrophysiological studies we conclude that the presented device is capable of simultaneous in vivo multichannel neural recording and controlled tracer injection for mapping neuronal pathways of the brain.

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Keywords: silicon microfabrication; neuronal tract tracing; electrophoresis; cerebral cortex, rat

1. Introduction

Thorough investigation of the brain's wiring diagram is essential for understanding how the central nervous system[CNS] processes information at both local and global scales [1]. Anterograde and retrograde tracing

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techniques are fundamental means of connectional neuroanatomy allowing the mapping of neuronal pathways [2]. Biotinylated dextran amine (BDA) is a widely used tracer, for labelling the origin as well as the termination of neural connections [3]. High molecular weight BDA (10 kDa<) yields sensitive and detailed labelling of axons and terminals (target regions), while low molecular weight BDA (3 kDa>) yields sensitive and detailed retrograde labelling of neuronal cell bodies (origin of pathways) [4].BDA labelling revealed projections from numerous regions in the CNS, e.g. from the cortex to the substantia nigra [5], from the brainstem [6], medulla [7], thalamus [8], etc.
The two point source diffusion based real time techniques for drug delivery are iontophoresis and pressure ejection, both from a micropipette [9]. In iontophoretic delivery charged molecules are released by passing a small current through the micropipette. The advantage of iontophoresis over pressure injection is that ions and not the solution can be released in a precise amount by controlling the iontophoretic current [9].
If recording of neural activity is also needed, an additional electrode is positioned into the investigated brain area close to the micropipette or glass capillary used for neuronal labelling [10]. However, there are several drawbacks of such an approach including the precise alignment of the different electrodes/syringes, crowded instrumentation and the invasive manner of the surgery is also notable.
In our work, we propose a method of using silicon multielectrode with monolithically integrated microfluidic channel to control tracer delivery by iontophoresis in vivo, which is also capable of simultaneous signal recording. Detailed description of the device fabrication and application for convection enhanced delivery of drugs through the blood brain barrier can be found in our previous work [11].

2. Experimental

2.1. Design of the channel and the microelectrodes

Here we demonstrate the iontophoretic tracer injection capability of the silicon multielectrode, using a Pt site as the counter electrode. The monolithically integrated microfluidic channel is 15 mm long, and 314 μm^2 in cross-section. The fluidic outlets are on the side of the probe as presented on Fig. 1.a. The counter Pt electrode is 30x30 μm^2 and the distance between the fluidic outlet and the Pt counter electrode is 125 μm. Schematic layout of the probe tip is shown in Fig 1.b.The fluidic connection to the probe is described in our previous work [11]. The fluidic channel was filled with the BDA solution prior to the surgery.

2.2. Surgery

Two Wistar rats (600 g, 520 g) were anesthetized with mixture of ketamine-xylazine (dosage: 1,6 ml/body weight kg and 0,8 ml/body weight kg). After craniotomy and durotomy the somatosensory cortex was exposed and the injection was made in a vessel free area. The targeted depth of the injection was 1000 μm below the surface of the cerebral cortex. We used a 1:1 mixture of high and low molecular weight 10% BDA (10.000K and 3000 K, final cc 5 % each) (Molecular Probes, Inc. Eugene, OR, USA) dissolved in 0.01M phosphate buffer (pH 7.4) and injected via iontophoresis for 15 min. The counter Pt electrode was negatively biased relative to the channel containing the BDA, while the electrical current was measured. A positively biased silver electrode was placed into the fluidic channel to repel the positively charged BDA molecules out of the drug delivery channel. A control experiment was carried out without any voltage bias allowing passive diffusion from/into the microfluidic channel. The injection parameters of the different experiments are shown in Table 1.

After 7 days survival the animals were anesthetized (same as before) and transcardially perfused with 150 ml of physiological saline solution (0.9 % NaCl in distilled water) and 500 ml fixative (4% paraformaldehyde in 0.1 M pH 7,4 PB). After removing the brain the region of interest was dissected and postfixed for 1 hour in the sama fixative solution on room temperature. Series of 60 μm thick horizontal, vibratome sections were collected and the standard ABC protocol (Elite kit, Vector Laboratories, Inc. Burlingame, CA, USA) was used to visualize BDA labeling with Nickel enhanced diaminobenzidin (DAB) (Sigma-Aldrich Kft, Budapest, Hungary) as the chromogen (for more details about the procedure see Négyessy et al.,[12]).

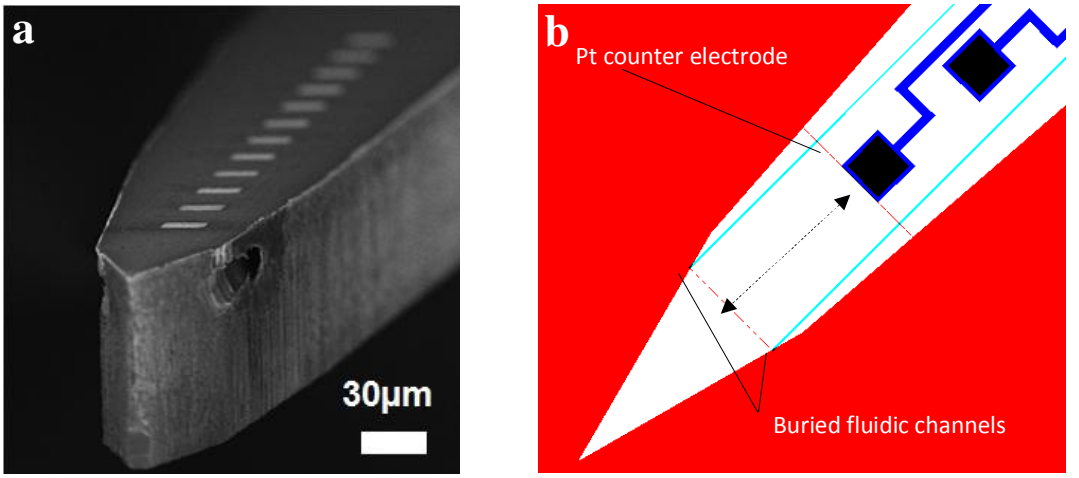

Figure 1.a: SEM image of a multichannel silicon neural electrode with 12 Pt contact sites on the top surface and two buried microfluidic channels. The outlets of the channels are on the sidewalls. b:Schematic layout of the probe. Light blue lines represents the center of the buried microfluidic channels. Dark blue regions are the Pt recording sites and wiring. The first Pt electrode was used as counter electrode during iontophoretic injection of BDA. The Pt sites are 30x30 μm^2.

Experiment	Target depth (µm)	Measured current (µA)	Length of ON/OFF cycles (sec)	Total time of delivery min
E0	1000	0	0	15
E1	1000	4	7/7	15
E2	1000	7-19	1/1	15
E3	1000	1-2	1/1	15
E4	1000	1-2	12/5	15

Table 1: Parameters of the BDA injections. E0 is the control experiment without bias on the Pt electrode

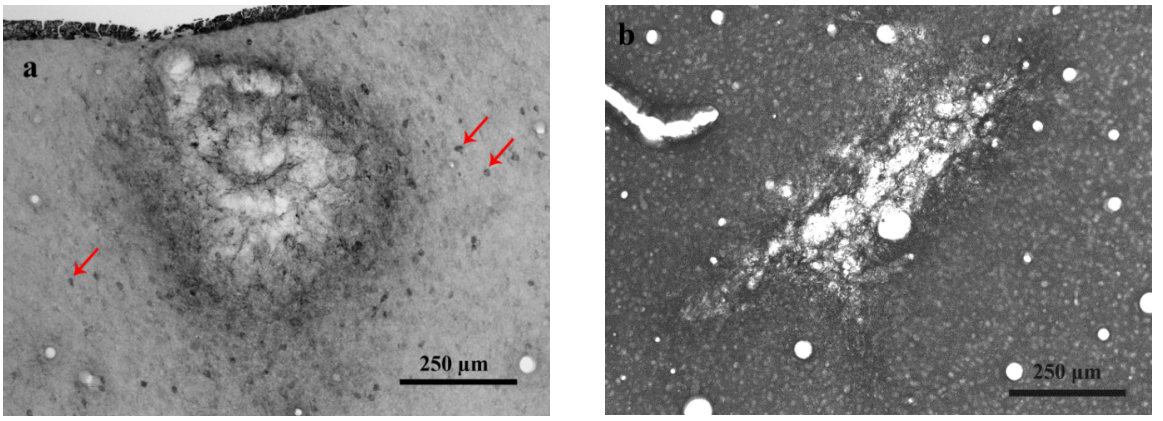

Figure 2. BDA labeling of neurons after iontophoretic injection. Greyscale images taken with a light microscope. a, In case of *E1, BDA injection resulted in the retrograde labeling of neuronal perikarya (red arrows) all around the injection site shown by the tissue damage at the electrode track b. In control (E0) there was no BDA labeled neuron. However, the electrode track is clearly visible*

3. Results

Comparison of the control experiment and the iontophoretic injection is shown in Fig.2.a and b. Labeling of the neurons and fibers in the somatosensory cortex can be seen on Fig.4 in higher magnification. The best retrograde and anterograde labeling were obtained in E1.The injury caused by the electrode was around 300 µm in each case, the size of the electrode shank. In the control case, where no current was used we did not find any labeled structures. In the other cases we found anterogradely labeled axons and retrogradely labeled neurons. Although, in bidirectional tracing we have to consider some methodological drawbacks, most notably backfilling axons of retrogradely labeled neurons, which could be mistakenly identified as anterograde labeling from the injection site. However, we found dens axonal labeling distant from retrogradely labeled neurons, which suggest that our bidirectional tracing worked effectively (see Fig. 3.a and b). More detailed discussion on this methodological issue can be found in [12].

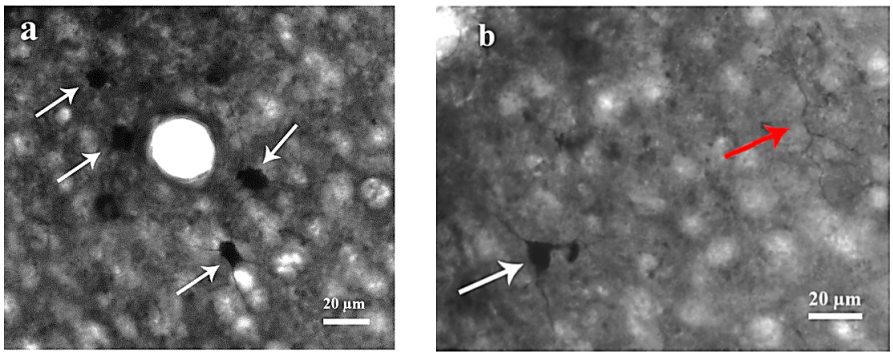

Figure 3. Light microscope greyscale images of the labeled structures. a, Labeled neurons (white arrows) around a vessel. b, Labeled neuron (white arrow) and a labeled axon (red arrow).

4. Conclusion

Iontophoretic injection of the mixture of high and low molecular weight BDA using a monolithically integrated drug delivery channel of a silicon multichannel brain electrode was presented. A Pt electrode site of the probe was used as a counter electrode. The successful delivery resulted in anterograde and retrograde labeling of neurons in the vicinity of the implantation site. The advantage of our method is the low diffusion of the tracer around the electrode track as compared to traditional iontophoretic injections resulting in a core and a halo region of dark labeling.

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