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A high sensitivity MEA probe for measuring real time rat brain glucose flux

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ABSTRACT

The mammalian central nervous system (CNS) relies on a constant supply of external glucose for its undisturbed operation. This article presents an implantable Multi-Electrode Array (MEA) probe for brain glucose measurement. The MEA was implemented on Silicon-On-Insulator (SOI) wafer using Micro-Electro-Mechanical-Systems (MEMS) methods. There were 16 platinum recording sites on the probe and enzyme glucose oxidase (GOx) was immobilized on them. The glucose sensitivity of the MEA probe was as high as 489 $\mu A\,mM^{-1}\,cm^{-2}$. 1,3-Phenylenediamine (mPD) was electropolymerized onto the Pt recording surfaces to prevent larger molecules such as ascorbic acid (AA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), and dopamine (DA) from reaching the recording sites surface. The MEA probe was implanted in the anesthetized rat striatum and responded to glucose levels which were altered by intraperitoneal injection of glucose and insulin. After the in vivo experiment, the MEA probe still kept sensitivity to glucose, these suggested that the MEA probe was reliable for glucose monitoring in brain extracellular fluid (ECF).

1. Introduction

Neuronal activity depends on ion gradients which are maintained by energy-requiring active transport. For this the brain requires a constant supply of glucose and oxygen, which were delivered by the cerebral circulation (Lowry and Fillenz, 1997). Glucose is a main nutrient in the brain and the well-documented effects of circulating glucose on cognitive function has suggested that glucose acts on brain systems important for memory formation (Dong et al., 2003). Understanding the glucose mechanisms, which underlies the functioning of the human brain, stands at the forefront of modern science.

Recently, different studies have been reported on glucose concentration detection in extracellular fluid (ECF) (Lowry et al., 1994, 1998a, 1998b, 1998c; Zilkha et al., 1994; Zhang et al., 2004; Fillenz and Lowry, 1998; Yang et al., 1995a; Yu et al., 2013; Vasylieva et al., 2011a; Shi et al., 2000) or on the relationship between ECF glucose and neuronal activity (Sanduskya et al., 2013; Fellows et al., 1992; Silver and EreciAska, 1994; Netchiporouk et al., 1996). Among these works, microdialysis has become one of the standard and widely accepted methods for in vivo monitoring (Sanduskya et al., 2013; Obrenovitch and Zilkha, 2001; Zhang et al., 2004; Yang et al., 1995a; Yu et al., 2013; Shi et al., 2000). However, this method is generally coupled to offline analysis by high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) or enzymatic electrode (Woitzik et al., 2001; Zhou et al., 1999; Malone et al., 1995; Zhang et al., 2004), and limited by its poor temporal resolution for most collection methods limited to the range of minutes. Though higher temporal resolution is possible with recent advances in dialysate collection and analysis (Morales-Villagrán et al., 2012), the real time data acquisition made by microdialysis for different brain region at the same time on-line analysis is not currently possible (Lowry et al., 1998a, 1998b, 1998c).

In comparison with microdialysis, implanted enzymatic electrode possesses simplicity of operation. Recent advances in biocompatible nano-materials and biotechnology make it possible to develop a biosensor with high sensitivity to detect the neurotransmitter and metabolites (Fillenz and Lowry, 1998; Heller and Feldman, 2008; Dale et al., 2005), and also be able to rapidly detect changes in cerebral glucose (Boutelle et al., 1986; Shram et al., 1997; Vasylieva et al., 2011b). Furthermore the electrochemical sensors are small, so that they can record at a sub-second temporal resolution and cause less disruption in surrounding tissue compared to microdialysis guide cannula and probes (Bungay et al., 2003; Borland et al., 2005). The high specificity and temporal resolution of sensors make them ideal for measuring the relationships between neurochemistry, metabolism and neural activity (Lowry and O'Neill, 2006).

Most of the implanted assays were carried out by using a carbon fiber (Netchiporouk et al., 1996; Harada et al., 1992;

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Michael and Adrian, 1995) or metal wire (Lowry et al., 1998b; Silver and ErreciAska, 1994; Fillenz and Lowry, 1998; Pernot et al., 2008; Vasylieva et al., 2011a) microelectrode based on the enzymatic method. In contrast to the manual construction of the micro-wire based arrays, the silicon-based microelectrodes are in mass production and also in a wide variety of shapes and sizes with reproducible submicron precision and with low restrictions in their electrode contact configuration (Grand et al., 2011). These structures are biocompatible with the brain tissue (Kotzar et al., 2002). Since the 1970s, a wide variety of probes have been constructed by using different silicon fabrication methods (Kotzar et al., 2002; Anderson et al., 1989; Bai et al., 2000; Campbell et al., 1991; Ghovanloo et al., 2003; Najafi et al., 1985; Rakwal et al., 2009; Wise et al., 1970). However, few of these were used as glucose biosensor.

In the present study, a novel implantable Multi-Electrode Array (MEA) probe made by MEMS methods was designed for continuously monitoring changes in ECF glucose concentrations. Calibration results showed the MEA probe had high sensitivity and good selectivity. Measurements were made in anesthetized rat brain when plasma glucose level was normal and altered by an injection of insulin or glucose.

2. Experimental

2.1. Reagents

Glucose oxidase (GOx, 233 U/mg) was purchased from Aspergillus niger in powder form, D-glucose (≥ 99.5%) and insulin from Sigma; ascorbic acid (AA, ≥ 99%), 3,4-dihydroxyphenylacetic acid (DOPAC, ≥ 98%) and serotonin (5-HT, 99%) from Alfa Aesar; dopamine (DA, ≥ 99%) from Acros Organics. Saline (0.9% NaCl) was purchased from Shuanghe company (Beijing, China), chloral hydrate from Sinopharm Chemical Reagent Co., Ltd. 1,3-Phenylenediamine (mPD, ≥ 99%) was purchased from Aldrich, bovine serum albumin (BSA, ≥ 99%) from Amresco, glutaraldehyde solution (25%) from Shanghai Chemical Reagent Company (Shanghai, China). The phosphate buffer saline (PBS, 0.1 M Na_2HPO_4–NaH_2PO_4–KCl, pH 7.4) was prepared from a PBS tablet (Sigma) with deionized water.

For in vitro calibrations, stock solutions of 1 M glucose were prepared, left for 24 h at room temperature to allow equilibration of the anomers and then stored at 4 °C. DOPAC, DA, 5-HT and AA solutions were prepared in a phosphate buffered saline (PBS) solution, 0.1 M, pH 7.4 just before use because of their gradual decomposition.

For the in vivo experiments, solutions of insulin (1 ml, 15 U/kg) were administered intraperitoneally (i.p.). Insulin solutions were prepared in normal saline and sonicated with heating for 5 min to ensure complete dissolution. 1 M glucose was prepared in normal saline.

2.2. Apparatus

All electrochemical measurements were performed on a Gamry electrochemical workstation (Gamry Reference 600, Gamry Instruments, USA). A Dell E5400 notebook PC was used to collect electrochemical data.

2.3. Fabrication of the MEA probe

Main steps of the MEA probe fabrication were referred to the paper (Wei et al., 2013). Each MEA probe contains four shafts capable of penetrating the brain and a bonding pads area which connects to external devices. The probe has 16 round recording sites (diameter of 15 μm), four on each shank. The distance between shanks is 200 μm. Every shank is 7 mm long or longer and 38 μm thick. Along the shank, the center to center distance between recording sites is 100 μm. After being assembled to a soft printed circuit board (PCB) holder the microelectrodes is applicable for recordings in rats.

2.4. Enzyme immobilization

The MEA probe was cleaned by reactive-ion etching first. The final enzyme solution used for biosensor preparation contained 0.5 U/μL glucose oxidase, 1% bovine serum albumin, and 0.125% glutaraldehyde in phosphate buffered saline solution (0.01 M, pH 7.4). Glutaraldehyde is one of the most widely used fixative reagents for enzymatic biosensor fabrication (Vasylieva et al., 2011a) and is easy to operate. The GOx layer was deposited by dipping the tip of the MEA probe in the enzyme solution. The electrodes were placed at room temperature for at least 72 h before their first use. The modified electrodes were stored at 4 °C when not in use.

2.5. Modification by 1,3-phenylenediamine

For in vivo experiments, mPD layer was deposited by electropolymerization for 15 min in 0.5 mM mPD solution in 0.1 mM PBS at pH 7.4 under a constant potential of +600 mV versus an Ag|AgCl reference electrode. At 15 min, the microelectrode tip was removed from the mPD solution, rinsed with deionised water, and stored at room temperature for 24 h prior to calibration (Michael and Borland, 2006). The process of eletropolymerization with 1,3-phenylenediamine was followed by immobilization of enzyme. When the 1,3-phenylenediamine was eletropolymerized before the enzyme immobilization, the sensitivity was too low to detect any glucose.

2.6. In vitro conditioning and characterization

For in vivo determinations using the glucose biosensor, regular calibration of the sensors with standard glucose solutions is crucial. Calibrations were carried out in standard PBS (0.1 mM, pH 7.4) solutions. An Ag|AgCl electrode was used as the reference electrode. The electrodes were held at a constant potential of +700 mV, which is the value generally used for H_2O_2 detection (Lowry et al., 1994). All calibrations were carried out at room temperature. Glucose and interfering chemicals were added according to the experimental protocol.

2.7. Surgical procedures

A male Sprague–Dawley rat weighing 200 g was anesthetized with chloral hydrate (350 mg/kg, i.p.) and placed in a stereotaxic frame. Electrodes were then implanted following a previously described procedure (Lowry and Fillenz, 1997). The glucose MEA probe was implanted into the striatum, coordinates with the skull leveled between bregma and lambda, were A/P +2.0 from bregma, M/L +2.5 and D/V −5.0 from dura (Lowry et al., 1998b). The glucose MEA probe was used as a working electrode to detect the glucose concentration in the rat brain. A silver wire which had electroplated AgCl (Ag|AgCl) was placed in the cortex used as the reference electrode. The electrodes were held at a constant potential of +700 mV. The electrodes and probe were fixed to the skull with dental screws and dental acrylate (Heraeas Kuizer Dental Ltd., Shanghai, China). An earth wire attached to one of the support screws. The sensors were tested using chronoamperometry responses. Once the background current for the MEA probe had stabilized (typically 30–45 min) experiments were begun. All signals