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ARTICLE

Electrochemical responses of carbon fiber microelectrodes to dopamine *in vitro* and *in vivo*

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ABSTRACT Cylindrical, 7 µm in diameter carbon fiber (CF) microelectrodes were constructed and tested for their responses to dopamine in constant potential amperometry or fast scan cyclic voltammetry (FSCV). Amperometry was carried out in a miniature perfusion chamber whereas background subtracted FSCV was performed both *in vitro* and *in vivo*. For calibration performance of the microelectrodes, peak oxidation currents were determined using unmodified carbon tips of varying lengths and plotted against the tip lengths. A very close linear correlation (r = 0.997) was found between the two variables for tip lengths ranging from 25 to 300 µm. Also, a very close linear correlation was found between the oxidation current at a given carbon tip length in response to increasing dopamine concentrations measured by either amperometry or FSCV. *In vivo* experiments were carried out in the visual cortex of the anaesthetized rat to detect dopamine release in response to visual stimulation. Indeed, background subtracted cyclic voltammograms showed an increase in the current at 0.65 V which is the typical dopamine oxidation potential.
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KEY WORDS

carbon fiber
microelectrode
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Carbon fiber (CF) microelectrodes have been used to extracellularly record neuronal action potentials (Armstrong-James and Millar 1979) and to detect electrochemical signals *in vitro* and *in vivo* (Ponchon et al. 1979). They have been demonstrated to be very suitable for detection of catecholamines such as dopamine or norepinephrine and other oxidizable biological species including nitric oxide (Malinski and Taha 1992). Since the early times in CF applications for biorecording, a variety of enzyme-modified CF microbiosensors has been introduced for the *in situ* determination of biologically important compounds. Recently, the immobilization of DNA molecules or carbon nanotubes onto CF microelectrodes allowed the construction microsensors for a great variety of analytes. Another novel use of CF microelectrodes is sensing tissue oxigen levels at a micrometer scale. For review, see (Budai 2010).

The CF microelectrodes are graphite monofilaments and are about 7 µm in diameter. A basic CF microelectrode is an elementary carbon filament built in a mechanically supportive and electrically insulating borosilicate glass or plastic sheathing. The uninsulated carbon tip protruding from the sheathing by 10 µm to a few 100 µm provides an electroactive surface for picking up spikes from the near vicinity of neurons and/or surface for electron transfer in electrochemical measurements and microbiosensor applications. The chemically relatively inert CF has outstanding mechanical and electrical properties and provides an excellent base electrode for electrophysiological, electrochemical and biosensor applications on a micrometer or perhaps even on a submicrometer scale.

Miniaturization, however, has its own limitations. At a given current density, for example, an overly small electroactive surface may greatly diminish the electrode performance and shows a poor signal-to-noise ratio. When the electrochemical measurement is combined with single-unit recording a shorter tip of 20 to 30 µm is required, as longer carbon lengths give rise to multiple-unit recording (Stamford et al. 1993). For these reasons, and as longer carbon tip represents a greater active surface, we studied the electrochemical responses of CF microelectrodes as a function of the exposed carbon tip length. Experiments were performed using constant potential amperometry in a miniature perfusion chamber or employing fast scan cyclic voltammetry (FSCV) *in vitro* or *in vivo*.

Materials and Methods

Manufacturing CF microelectrodes

Single-barrel CF microelectrodes were made from borosilicate glass capillary tubing (1.50 mm O.D., 0.84 mm I.D., WPI, Sarasota, FL) containing a 7 µm in diameter carbon fiber monofilament as previously described in details (Budai and Molnár 2001). After pulling the blank, the excess length of the CF protruding from the tip of the glass was cut with a fine pair of scissors to about 5 mm. The exposed CF was finally trimmed to lengths of 25, 50, 75, 100, 200 or 300 µm using high voltage spark etching under a light microscope.

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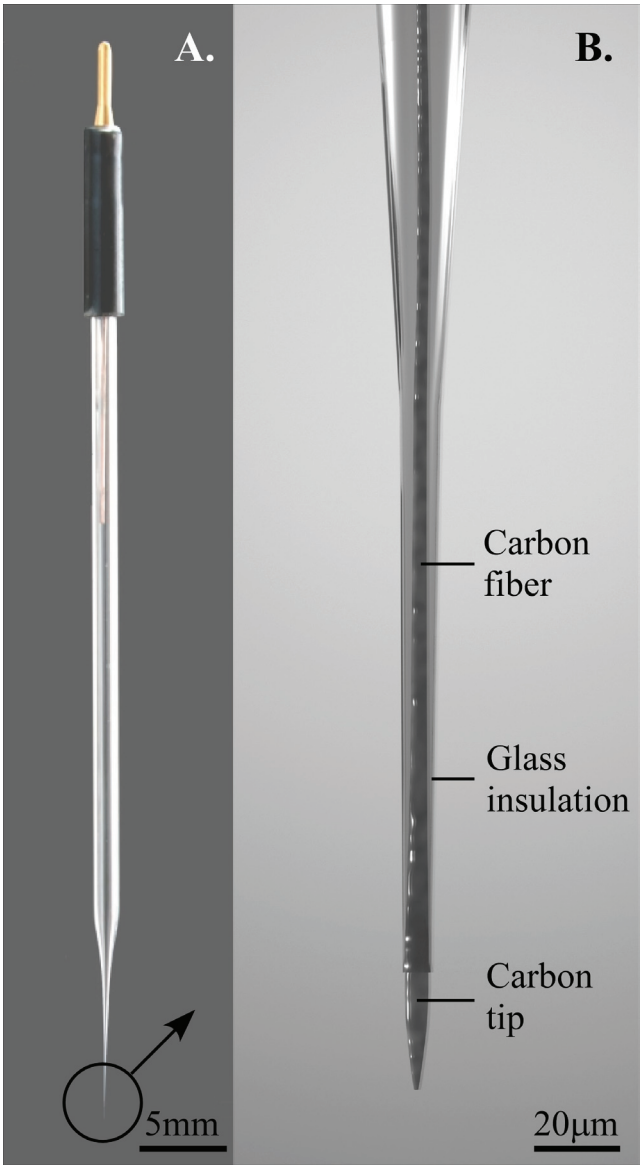

Figure 1. A: View of a carbon carbon fiber microelectrode. The gold-plated connector is galvanically connected to the 7 µm in diameter carbon monofilament that provides the lead element in the borosilicate glass micropipette. B: Light microscopic stucture of the tip. The carbon fiber protrudes from the electrically insulating glass support and ends in a sharp, conical tip. The length of the exposed carbon tip were selected between 25 µm (shown) and 300 µm in the present experiments.

The completed microelectrode is shown in Figure 1.

Electrochemical measurements

Constant potential amperometric experiments were carried out at room temperature (25ºC) using a 'Micro C' carbon fiber potentiostat (WPI, Sarasota, FL). The oxidization currents were measured using various lengths of the carbon tip as working electrodes that were kept at 0.65 V against a Ag/AgCl half cell in a miniature, 1 ml perfusion chamber. Physiological saline was continuously perfused at a rate of 2 ml/min from one of the syringes of a two-syringe infusion pump. The other syringe contained dopamine hydrochloride (Sigma, Saint Louis, MO) dissolved in saline at various concentrations and switchings between the two syringes were made using 3-port manifold. Data acquisition was performed using a NI PCI6221 multifunction data acquisition board placed in a desktop computer and programmed in LabView (National Instruments, Austin, TX). Fast scan cyclic voltammetry (FSCV) were performed using a custom-built analogue triangle waveform generator hardware (System Kellényi, BioPot V4, 2009) controlled by a LabVIEW driver developed in-house and the resulting signals were acquired by a PCI-6025E data acqisition card (National Instruments, Austin, TX). A CED 1401Plus data acquisition system (CED, Cambridge, UK) was also used to synchronize waveform application, data acquisition, and light stimulation delivery for *in vivo* experiments. A scan rate of 300 V/s was applied with a resting potential of -0.2 to -0.4 V. For reference Ag/AgCl electrode was used. In individual scans, the anodic limit was set to 1.0 V, and the measurements were typically repeated at 200 ms intervals. The collected raw data signal was on line background subtracted, signal averaged, and digitally filtered with a custom-built control software in a LabView environment. Dopamine solutions of various concentrations were measured to plot the calibration curves of CF microelectrodes. Dopamine was dissolved in 100 mmol/l phosphate buffer solution (pH= 7.4). Voltammograms obtained in phosphate buffer solution were subtracted from the curves of dopamine solutions to eliminate the background current (Howell et al. 1986). Maximum oxidation currents were plotted against the concentration of dopamine to find correlation between the two variables.

In vivo recordings

FSCV recordings were taken using 100 µm-long, unmodified CF microelectrodes in various areas of the visual neocortex of anesthetized rats to test the involvement of V1/V2ML cortical neurons in stimulation paradigm using light flash stimuli to induce related activity. Experiments were carried out in male Wistar rats (Charles River Laboratories, Gödöllő, Hungary) with the approval of the Animal Care Committee of the University of Pécs, Hungary and in compliance with international standards. Anaesthesia was induced with a single intraperitoneal injection of ketamine (100 mg/kg, SBH, Budapest, Hungary) and maintained with 20% of the initial dose administered in approximately every 45 min thereafter for a maximum of three times. Stereotaxic coordinates for the targeted regions were selected according to the atlas of Paxinos and Watson (1986) and were as follows: AP (from bregma): -5.5 to -6.5 mm; L: 2 to 3 mm; V (from brain surface): 0.5 to 3.0 mm.

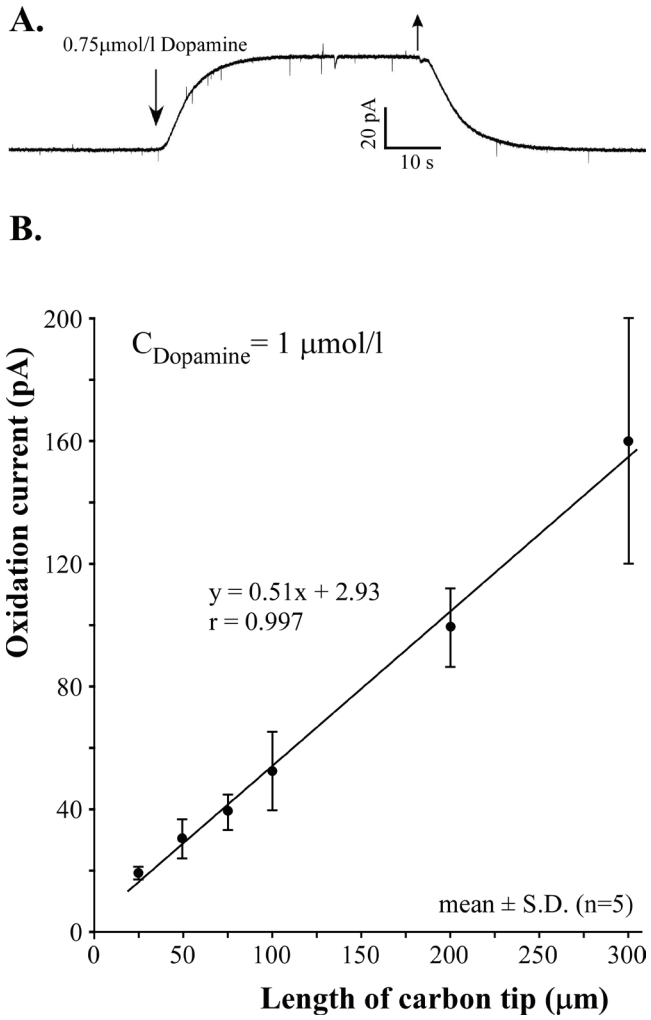

Figure 2. A: A representative constant potential amperometric response of a 100 µm long carbon tip to 0.75 µmol/l dopamine at 0.65 V in a miniature perfusion chamber. B: Linear relationship between the length of the carbon tip and the oxidation current at 0.65 V in response to 1.0 µmol/l dopamine. Data represent the mean ± SD of 5 experiments.

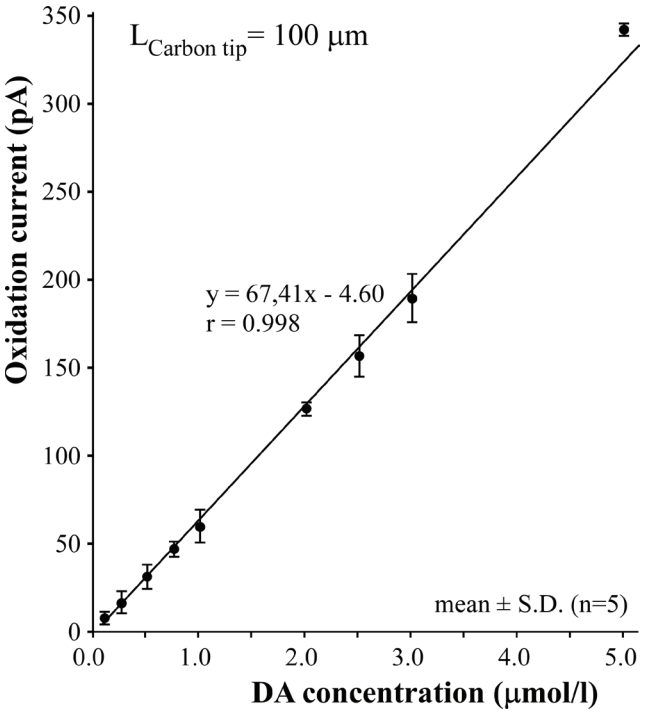

Figure 3. Linear correlation between dopamine (DA) concentration and the resulting oxidation current at a 100 µm-long carbon tip kept at 0.65 V. Data represent the mean ± S.D. of 5 experiments.

Visual stimulation were performed using two bright green LEDs (one on each side) served as whole field visual stimuli and were lit for 200 to 500 ms from a distance of 7 cm to the eyes of the subject. The frequency of visual stimulation was set between 0.2 and 0.5 Hz and a typical block of trials contained 20 to 50 trials to enable averaging of the electrochemical data. Extracellular multiple unit activity and micro-EEG was recorded through the carbon fiber of the microelectrode to ensure that the cortical area in the vicinity of the electrode was responding to visual stimulation. Neuronal signal was passed through a biological amplifier (BioAmp, Supertech Ltd, Pécs, Hungary) and an analogue-digital converter interface (Power 1401, CED, Cambridge, UK) and saved in a desktop computer.

Results and Discussion

Constant potential amperometry

In amperometric measurements, the CF microelectrode is held at a constant potential (e.g., 0.65 V for catecholamines) exceeding the redox potential of the substance of interest. When molecules such as epinephrine or dopamine hit the carbon surface, electrons are transferred and a current can be measured. For reviews, see (Kawagoe et al. 1993; Wightman 2006). Constant potential amperometry offers the best temporal resolution among the available techniques, however, it suffers from poor selectivity: any molecule that can be oxidized or reduced at the potential of the electrode is detected, so there is no way to differentiate between molecules. As expected, the surface area available for electron transfer will significantly influence the sensitivity and, hence, the signal-to-noise ratio in such electrochemical recordings (Kawagoe et al. 1991; Armatore et al. 2009). In case of the 7 µm in diameter CF microelectrodes the smallest possible electroactive area is formed when a glass-encased carbon monofilament is beveled to 90° or to other angles for studying dopamine release from single cells (Armatore et al. 2009; Adams et al. 2011). On the other end, up to 250 µm of the exposed carbon tip is usually left protruding from the sheathing when *in vivo* electrochemical recordings are taken from the brain (Fig. 1B) (Dugast et al. 2002; Clark et al. 2010). These larger cylindri-

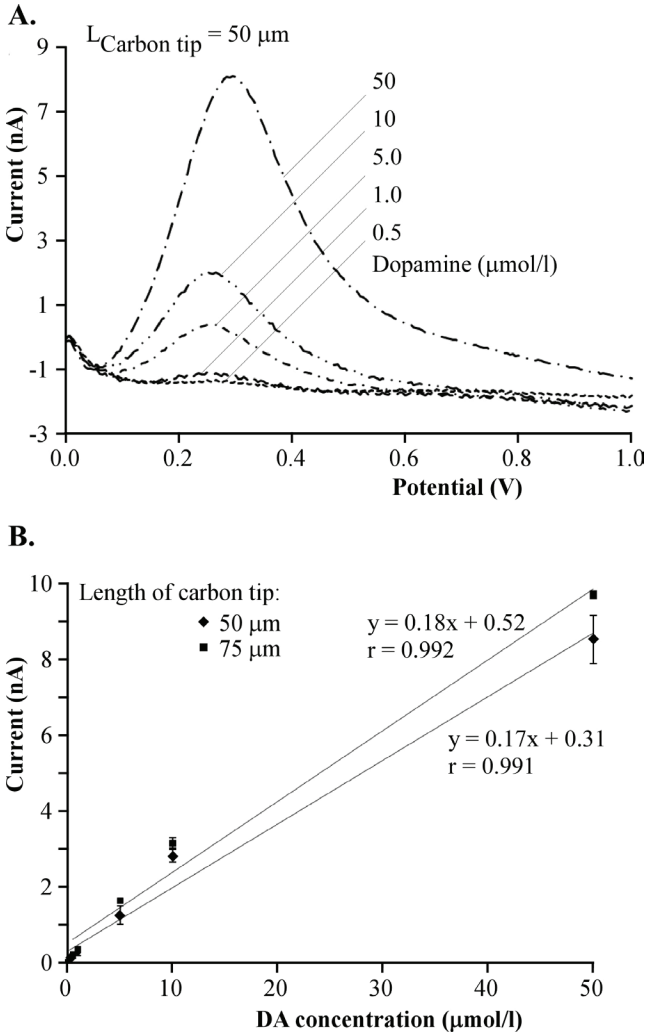

Figure 4. Fast scan cyclic voltammetry of dopamine (DA) in 0.1 mol/l phosphate buffer (pH= 7.4) using CF microelectrodes. A: Background subtracted voltammograms of dopamine plotted against the electrode potential. Measurements were performed using a 50 µm-long CF microelectrode. Only the oxidative phases of cycles are shown for clarity. B: Linear correlation between peak oxidation currents shown in panel A and the dopamine concentrations for CF microelectrodes having 50 µm or 75 µm tip lengths. Data represent the mean ± SD of 3 experiments.

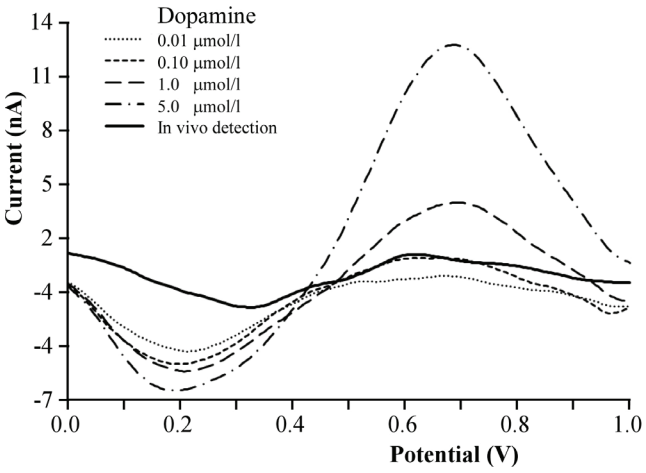

Figure 5. Fast scan cyclic voltammetry in the V2ML region of the visual cortex of rat brain using CF microelectrodes. Comparison of the background subtracted cyclic voltammograms obtained in dopamine solutions and in the V2ML region of the visual cortex in rat brain during light stimulation. Each curve is the average of 10 scans. The background signals to be subtracted were recorded in phosphate buffer solutions (for *in vitro* experiments) or in the V2ML without stimulation (for *in vivo* experiment). Oxidation peak currents for *in vivo* experiments appeared at the same electrode potential as in typical measurements of dopamine solutions *in vitro*.

cal microelectrodes are advantageous because the secreted molecules come from multiple terminals near the electrode, and the greater electroactive area leads to a larger signal that emerges from the Johnson noise of the current amplifier (Kovach et al. 1984). However, when the electrochemical measurement is combined with single-unit recording a shorter tip of 20 to 30 µm is required, as longer carbon lengths give rise to multiple-unit recording (Stamford et al. 1993).

In the present set of experiments, we studied the constant potential amperometric responses of cylindrical CF microelectrodes in a miniature perfusion cell to dopamine while the electrode potential was kept at 0.65 V (Fig. 2A). These electrodes were `pristine`, that is untreated and unmodified with any procedure, and their selected lengths were 25, 50, 75, 100, 200 or 300 µm. We found a linear relationship between the exposed carbon tip length and the dopamine oxidation current as shown in Figure 2B. For example, a 100 µm-long carbon tip produced about 50 pA oxidation current under these conditions. The linear regression analysis based on 5 measurements for each data point resulted in the equation of $y = 0.51x + 2.93$ with a correlation coefficient of $r = 0.997$. Also, a very close linear correlation was found between the oxidation currents measured at a 100 µm-long carbon tip in response to increasing dopamine concentrations (Fig. 3). The relationship between the two variables was $y = 67.41x - 4.60$ with a correlation coefficient of $r = 0.998$, based on 5 measurements for each data point.

Our primary goal was to find an exact relationship between dopamine oxidation current and the length of untreated carbon tip or dopamine concentration. It should be noted, however, that electrochemical pretreatment of the carbon tip or modifying the electroactive surface with Nafion (Kuhr and Wightman 1986, Brazell et al. 1987; Hashemi et al. 2009), 4-sulfobenzene (Hermans et al. 2006) or modified with an over-oxidized polypyrrole film (Zhang et al. 1996) can greatly enhance the selectivity and sensitivity of these CF microelectrodes. For review, see (Troyer et al. 2002).

Fast scan cyclic voltammetry

Fast scan cyclic voltammetry (FSCV) is performed by holding the microelectrode at a constant potential versus a reference electrode, followed by a rapid increase in potential and an immediate return back to the holding potential. The voltage limits are chosen so that the reduction and oxidation of the analyte of interest lies within this potential window. FSCV provides good chemical selectivity while retaining subsecond temporal resolution. Each measurement consists of a cyclic voltammogram that serves as a chemical identifier to provide chemical selectivity. It has been shown to be very useful for the detection of catecholamine fluctuations *in vivo* because of the high sensitivity and selectivity (Robinson et al. 2003, 2008).

CF microelectrodes are well-suited for use in FSCV measurements for the detection of monoamines (Baur et al. 1988). In the present experiments, 7 µm in diameter and 50 or 75 µm-long CF microelectrodes were used to measure dopamine oxidation currents in FSCV carried out in 100 mmol/l phosphate buffer (pH 7.4) at 300 V/s scan speed. As shown in Figure 4A, increasing dopamine concentrations led to increasing oxidation currents at a 50 µm-long CF microelectrode. When the background subtracted peak currents were plotted *versus* concentrations of dopamine, a close correlation between the two variables was revealed (Fig. 4B). The linear regression analyses based on 3 measurements for each data point resulted in the respective equations of $y = 0.17x + 0.31$ ($r = 0.991$) and $y = 0.18x + 0.52$ ($r = 0.992$) for 50 and 75 µm-long carbon tips.

In vivo measurements

Because fast scan cyclic voltammograms can be repeated every 100 ms, changes in dopamine concentration can be monitored with good chemical selectivity on the subsecond time scale. These characteristics make FSCV well suited for detecting phasic dopamine changes in the brain even in the freely moving animal (Robinson et al. 2003, 2008). By locating the potentials at which the maximum cathodic and anodic currents occur, one can distinguish many different neurotransmitters.

In our present study, FSCV was carried out in visual cortex of the rat to detect dopamine release in response to visual stimulation. Indeed, background subtracted cyclic voltammograms showed an increase in the current at 0.65 V which is the typical dopamine oxidation potential (Fig. 5). The background to be subtracted was recorded in the absence of light stimulation so the raise in oxidation current was clearly due to the visual stimulation afterward. It should also be noted, however, that there may be other electroactive substances which show oxidation peak at the potential similar to that of the dopamin. Hence, the measured oxidation peak can represent not only dopamine but other monoamines or dopamine metabolites. Through electrical stimulation of midbrain areas containing dopaminergic neurons (such as VTA) or local administration of dopamine can further justify the measured electrochemical signal (Phillips and Wightman 2003).

Acknowledgments

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