SUBCELLULAR ELECTRODE ARRAYS FOR MULTISITE RECORDING OF DOPAMINE IN VIVO

Helen N. Schwerdt, Minjung Kim, Ekin Karasan, Satoko Amemori, Daigo Homma, Hideki Shimazu, Tomoko Yoshida, Robert Langer, Ann M. Graybiel, and Michael J. Cima
Massachusetts Institute of Technology, Cambridge, USA

ABSTRACT

We report the fabrication and use of carbon fiber (CF) arrays with 8 recording electrodes having individual diameters of 8 – 10 µm (9 – 10 times smaller than conventional sensors and state of art) for electrochemical recording of dopamine from deep brain regions in a minimally invasive and high density manner. Accordingly, key advancements demonstrated are (1) radical reduction in size and (2) potential for wide spatial distribution of concurrent electrochemical measurement in the brain.

INTRODUCTION

Advancements in microfabrication have enabled the scaling of electrical brain interfaces for massive spatial recording densities and minimal form factors [1], [2]. These spatially resolved techniques have been imperative in mapping brain function, based on the electrical activity of neurons. Nevertheless, similar progress has yet-to-be-seen for recording neuroactive chemicals, which play a fundamental role in governing and modulating brain activity. Seminal work has been done to establish fast scan cyclic voltammetry (FSCV) for electrochemical monitoring of dopamine, a neurotransmitter that is heavily implicated in Parkinson's disease, movement disorders, and mood disorders [3]–[5]. FSCV allows monitoring of dopamine, and other electroactive chemicals, from the brain with *millisecond* temporal resolution and unprecedented chemical selectivity. The sensors used for FSCV are usually in the form of a carbon-based electrode that is manually constructed by threading a 7 µm carbon fiber (CF) into a pulled-glass (variable diameter) or fused silica capillary (nominal diameter of 90 µm) [5]. This carbon fiber microelectrode (CFM) is a single channel sensor and has been successfully implemented in numerous studies throughout the last 4 decades [3], [5]. Work has also been done to fabricate multichannel sensors by patterning of pyrolized photoresist films (PPF) to generate a linear array (100 µm diameter) that could be used to synchronously record dopamine from 4 channels [6].

We developed 8 channel arrays comprising cellular-scale probes (Fig. 1) in order to increase the number of recording sites and reduce the implant-incurred brain damage. The resulting probes have individual diameters of 8 – 10 µm, pitch of 250 µm, and lengths of 4.5 – 6 mm to reach deep brain structures (eg. striatum). Biodissolvable compounds were coated on the probes to transiently stiffen them so that the probes can penetrate brain tissue without deflection. Functionality of the fabricated sensors was evaluated by recording stimulation evoked dopamine in the striatum of anesthetized rats. The scientific benefit of the array was assessed by monitoring the spatial distribution of the intrastriatal dopamine signals as a function of the positioning of the stimulation electrode within the MFB. Ability of the probes to travel to subcortical structures was confirmed through observation of lesioned sites. We further examined the long term damage associated with the implantation of the dissolvable shuttle.

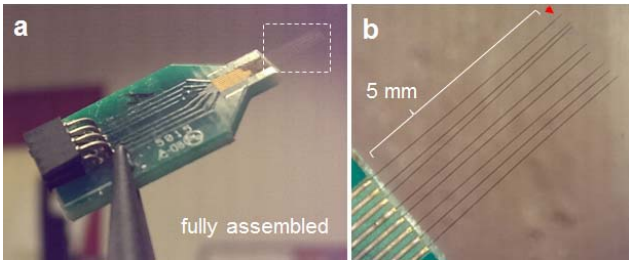

Figure 1. (a) An assembled 8 channel array with close-up of boxed area in (b) showing 5 mm long CF's (7 µm diameter) that have been encapsulated by parylene (700 nm) except at their apical tips (red arrowhead) where the CF's are exposed to provide the electrochemical sensing interfaces for dopamine measurement.

MATERIALS AND METHODS

FSCV Operation

FSCV is an electrochemical method whereby fast time-varying voltages are applied to a working electrode to induce and monitor electron transfer caused by redox reactions of electroactive compounds (ie. dopamine) at the electrode. A CFM is typically used as the working electrode whereby a triangular voltage waveform is scanned from −0.4 to 1.3 V against a Ag/AgCl reference electrode. High scan rates (400 V/s) are employed to enable rapid, *millisecond*, sampling according with the operation of these neurotransmitters in the brain. Dopamine chemical identity can be inferred by the generation of peak current changes at its redox potentials, typically, −0.2 and 0.6 V. Dopamine concentration is linearly proportional to the current change (I_{ox}) at its oxidation potential (ie. E_{ox} = 0.6 V) [5], [6]. The working electrode is held at −0.4 V in between each 8.5 ms scan, which is repeated at 100 ms intervals (ie. 10 Hz repetition frequency) to enhance dopamine adsorption. Current recorded at each scan are concatenated to generate data of the voltage-dependent current versus time. The data are background subtracted to remove the dominating capacitive current contributions, and extract and isolate changes in faradaic dopamine redox current. All data reported herein were collected with a LabView-based FSCV system (ordered from Scott B. Ng-Evans at the Lab of Dr. Paul E.M. Phillips at the University of Washington). Analysis was performed with scripts generated in Matlab (Mathworks, Matlab 2016A).

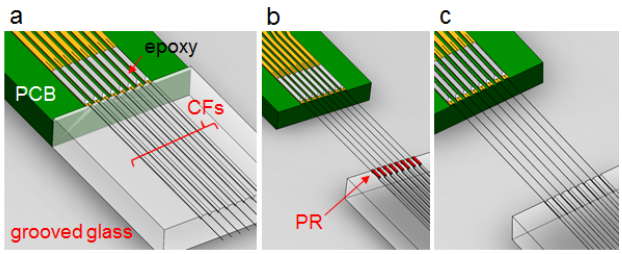

Figure 2. Illustrated fabrication process. (a) 7 µm diameter CF's placed and epoxy-bonded to PCB pads using a micro-groove array glass chip for alignment. (b) CF tips masked with PR followed by conformal parylene deposition (0.5 – 1.5 µm thick). (c) Lift-off PR release in acetone and isopropanol to expose CF sensing tips.

Fabrication

The fabrication process (Fig. 2) is as follows: (1) A printed circuit board (PCB) with conductive traces interfacing a mill-max connector (Mill-Max, 853-93-100-10-001000) is aligned to a glass substrate with trenches (250 µm pitch) to temporarily hold individual CFs (7 µm diameter and ~ 10 mm long) (Goodfellow, C 005722). Silver epoxy (Epo-tek, H20S) is applied to the individual traces to structurally and conductively connect each CF to the PCB. (2) The PCB-CF assembly is extended 4.5 – 6 mm so that only the CF sensing tips (100 – 200 µm) are anchored to the glass substrate, which are then masked with photoresist (PR) (AZ Electronics, AZ P4330). The assembly is heated at 120°C for 15 min to cure PR. The assembly is then immersed in isopropanol for 15 – 30 min, followed by an adhesion promoter consisting of A174 (Sigma-Aldrich, 440159), isopropanol, and distilled water at a volumetric ratio of 1:100:100 for 15 – 30 min. Followed by air drying, final rinse in isopropanol, and air drying, 0.5 – 1.5 µm parylene-C is uniformly deposited on the assembly. (3) CF sensing tips are exposed by dissolving PR masks in acetone, followed by rinsing in isopropanol. Fig. 1a displays the completed array. Polyethylene glycol (PEG) (molecular weight of 4000 – 8000 g/mol) is coated onto the sensors to temporarily stiffen electrodes and provide a dissolvable shuttle for brain insertion.

RESULTS

In Vitro Measurements

Probes were tested *in vitro*, in a flow cell, to measure their sensitivity to dopamine and build calibration standards for estimation of dopamine concentrations *in vivo*. Solutions used in the flow cell were phosphate buffered saline (PBS) buffer and dopamine hydrochloride (Sigma-Aldrich, H8502) as dissolved to concentrations of 0.25, 0.5, and 1 µM in PBS. An electronically actuated sample injection valve (Valco, Model 22Z) switched the samples flowing across the probes between PBS and dopamine solutions. The measured dopamine oxidation current is directly proportional to changes in dopamine concentrations. The measured sensitivity of the probes ranged 10 – 50 nA/µM for dopamine. For dopamine, the voltages at which peak current changes occur, or the redox potentials, are −0.2 and 0.6 V. Sensitivity of probes to dopamine is proportional to its measured background

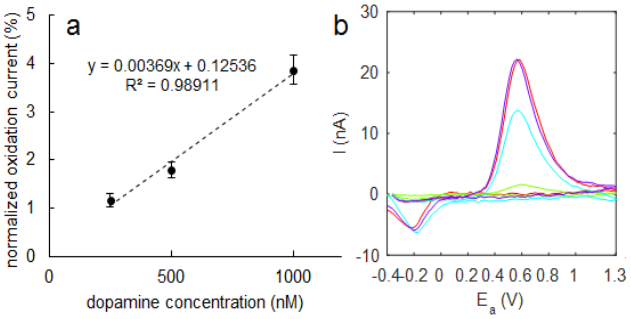

Figure 3. In vitro measurements of dopamine. (a) Normalized oxidation current measured (average of 8 probes) versus concentration of supplied dopamine analytes. Error bars represent ± standard deviation. Oxidation current represents current measured at the potential displaying highest current change, ~ 0.6 V, in response to dopamine. Normalized current is the percentage of oxidation current from the peak background current. (b) Background-subtracted voltammograms (ie. current, I (nA), versus applied voltage, E_a(V)) taken from measurements at 4 probes (overlaid traces) with 1 µM dopamine in the flow cell.

current (300 – 1900 nA). Calibration of individual probes to estimate dopamine concentration requires normalization to their individual sensitivities (ie. background current), which is done by taking the ratio of the measured oxidation current to known concentrations of dopamine to the background current (Fig. 3a). Current changes can, however, also occur at potentials outside of the targeted dopamine redox potentials. These interfering signals may be attributed to other molecules or ionic changes at the CF. The *in vitro* voltammograms (ie. plots of current vs. voltage) (Fig. 3b) are used as dopamine standards to assess selectivity of *in vivo* measurements using principle component regression to identify the amount of current corresponding directly to dopamine redox.

Implantation of Arrays

All animal procedures were approved by the Committee on Animal Care at the Massachusetts Institute of Technology and were conducted in accordance with the U.S. National Research Council Guide for the Care and Use of Laboratory Animals. Long-Evans male rats (Taconic, 350–450 g) were used in testing of the dopamine sensing CF arrays. Rats were anesthetized with

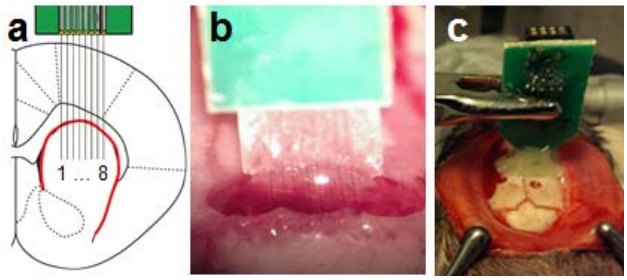

Figure 4. Implantation procedure: (a) Projected arrangement of implanted 8 probes in the striatum along coronal plane (AP 1.5 mm) with corresponding channel numbers. (b) The array lowered ~2 mm into brain with apical portions of PEG shuttle dissolved. (c) Array fixed to skull with bone cement.

isoflurane, and subcutaneously administered with meloxicam (2 mg/kg). Craniotomies were performed at the coordinates for the striatum (for arrays) at AP +1.5 mm and ML +2.1 mm, medial forebrain bundle (MFB) (for stimulation electrodes) at AP −4.1 mm and ML +1.7 mm, and contralateral supradural site (for reference electrode) at AP −2.3 mm and ML −3.5 mm (all coordinates relative to bregma). The array was lowered into the brain at a rate of ~ 0.1 mm/s (manually driven with a micromanipulator) while allowing basal portions of PEG to dissolve by application of PBS. The array was secured to the skull with acrylic cement (Ortho-Jet, 0206). The Ag/AgCl reference electrode was placed on the dural surface of the contralateral hemisphere. Bipolar stimulation electrodes (insulated Pt/Ir wires with 0.2 mm exposed tips and 125 µm diameters) were lowered to an initial depth of 6.0 mm and then lowered at increments of 0.1 to 0.2 mm to record evoked dopamine from striatal probes in response to stimulation at variable MFB depths (DV 6.0 – 8.7 mm).

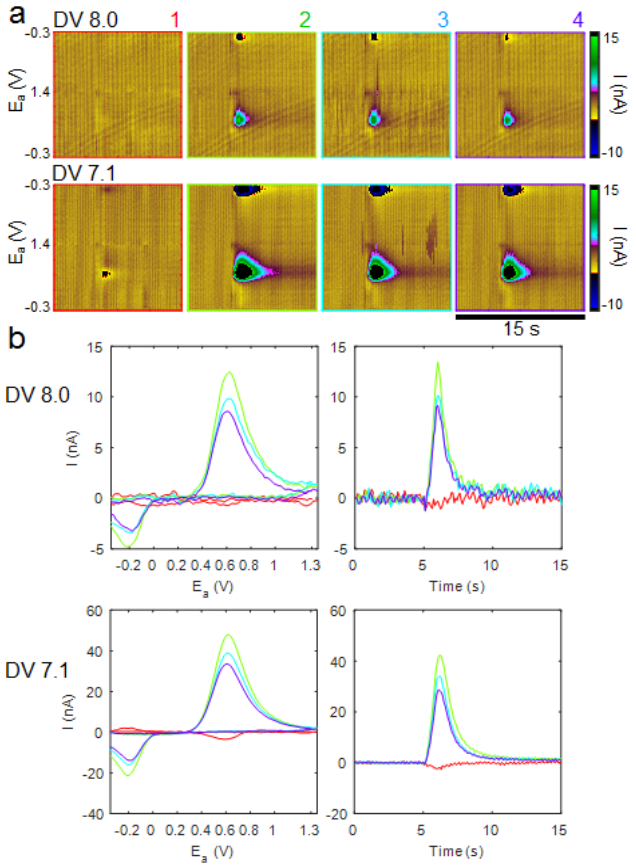

Figure 5. Measurements of dopamine at 4 channels of the arrays implanted in the rat striatum in response to MFB stimulation at two depths. MFB stimulation is applied at 5 s. (a) Background-subtracted color plots of measurements across 4 channels (channel numbers displayed at top of the plots). X- (time) and y- (E_a) axes along with color (I) scales are uniform for all plots. Measurements are shown for MFB stimulation applied at DV 8.0 mm (top panel) and DV 7.1 mm (bottom panel). Changes in current at the dopamine redox potentials (~ −0.2 and 0.6 V) are seen immediately following stimulation, and these changes are different across striatal sites, and at different stimulation depths. (b) Voltammogram (left) at 5.2 s and oxidation current versus time (right) plots (traces overlaid for each channel, with color scheme shown in (a)) at MFB DV 8.0 mm (top) and 7.1 mm (bottom). The oxidation current is linearly proportional to dopamine and is used to track changes in dopamine versus time (right plots).

In Vivo Measurements

Dopamine recording functionality was evaluated *in vivo* in anesthetized rats. Dopamine was recorded concurrently from 4 probes of the implanted array located in the striatum at DV 4.5 – 5.0 mm. Stimulation of the MFB was used to controllably evoke dopamine release in the striatum, which can then be detected by the intrastriatal probes. Stimulation was delivered to the MFB electrodes via a stimulus isolator (WPI, A365) that generated 48 biphasic pulses at 60 Hz with a pulse width of 2 ms, and amplitude of 200 µA, in response to computer software generated triggers. Dopamine release can be visualized in the background-subtracted current (Fig. 5a), which show clearly defined peaks centered around these redox potentials immediately following stimulation of the MFB. Evoked dopamine oxidation current amplitudes ranged up to 40 nA with the employed stimulation parameters. Examination of the voltammograms (Fig. 5b) demonstrates the chemical signature of dopamine, displaying clear peak current changes at the redox potentials. All channels showed positive correlation (Pearson's correlation coefficient, r, > 0.8) with the *in vitro* dopamine standards, except for channel 1 (most medial striatal site), which either did not display significant change in current (I_{ox} > 3 × standard deviation of noise) or showed a deflection in I_{ox} (r ~ −0.6) depending on the stimulation depth. The apparent change in dopamine concentrations was computed based on the sensitivity measurements made *in vitro*. The concentration change in dopamine across the 4 sites was heterogeneous and dependent on depth of MFB electrodes (Fig. 6). Channel numbers scale with laterality along the mediolateral axis of the striatum, as shown in Fig. 4a. Interestingly, the mediolateral distribution of peak evoked dopamine shifts most prominently when stimulation electrodes at the MFB are moved from DV 6.7 mm to DV

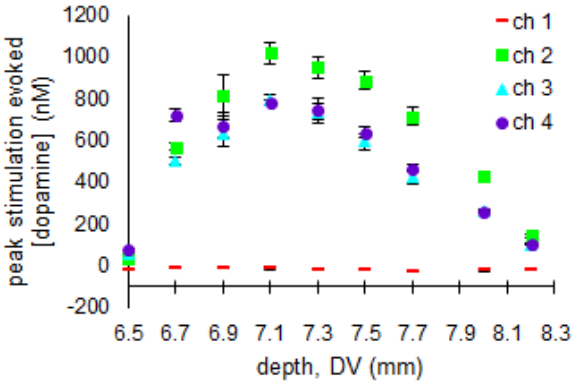

Figure 6. Peak stimulation evoked dopamine recorded in the striatum as a function of MFB stimulation electrode depth (DV) for the same rat represented in Fig. 5. Data were averaged over 2 – 5 stimulation trials for each depth. Stimulation parameters were fixed for each trial. Error bars represent ± standard deviation.

6.9 mm. These results demonstrate the potential of CF arrays in examining the spatiotemporal dynamics of dopamine and other neurotransmitters in the brain in a high throughput manner.

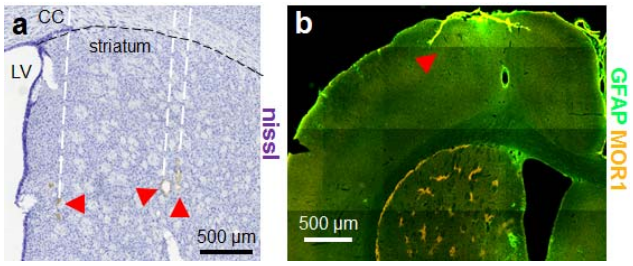

Figure 7. Histochemical examination of coronal brain slices estimated to coincide with implanted array. (a) Lesion marks are clearly visualized by appearance of brown color stains (arrowheads) and are inside striatum (LV is lateral ventricle, and CC is corpus collosum). (b) Brain implanted with rigid PEG shuttle, along with array, demonstrating increased GFAP expression (arrowhead) at superficial cortical layers. MOR1 principially stains striosomes: neurochemically and functionally distinct compartments residing striatum [4].

Histochemical Analysis of Implanted Brain Sites

Immunohistochemical examinations were made to evaluate extent of brain tissue response to implanted probes as well as verify the location of the sensing tips within the targeted striatal brain region. Electrolytic lesions were made at the tip of some of the probes to mark their locations in the striatum (cathodal DC current with amplitude of 20 – 30 µA applied for 20 – 30 s). Brains were blocked and cut at 30 µm intervals in the coronal axis, after probes had been implanted for at least 1 month. Lesioned sites were clearly identified and located within the striatum (Fig. 7a). Apart from the lesioned tips, individual probe tracks could not be visualized (no noticeable change in intensity of GFAP or CD11b/c) in any of the coronal slices suggesting a smaller tissue response in comparison to conventional ~ 100 µm diameter electrodes that can be clearly identified in similarly processed brain tissue [5]. Nevertheless, higher optical resolution (eg. confocal microscopy) can enhance visibility of the tracks made by the probes and the extent of their damage [7]. In a few rats, the undissolved and rigid PEG shuttle was inserted into the brain to evaluate long term response to this acute injury. This procedure produced noticeably heightened expression for astrocytes and microglia concentrated towards superficial layers where the shuttle had penetrated (Fig. 7b). Though this method facilitates surgical implantation, as it may obviate the need for a microscope and careful monitoring of probe travel, the amount of introduced damage may need to be carefully considered.

CONCLUSION

Results demonstrate the potential of microfabricated CF arrays for recording subsecond dopamine fluctuations at multiple sites in the brain, and their ability to mitigate inflammatory response of the brain tissue environment. The reduced inflammation around the probes may improve accuracy of the electrochemical measurements, and their functional longevity. Future work involves assessing overtime performance of the chronically implanted probes, and application in monitoring heterogeneous neurochemical signaling in behaving animals.

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CONTACT

*M.J. Cima; mjcima@mit.edu