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In Vivo Dopamine Detection and Single Unit Recordings Using Intracortical Glassy Carbon Microelectrode Arrays

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Abstract

In this study, we present a 4-channel intracortical glassy carbon (GC) microelectrode array on a flexible substrate for the simultaneous in vivo neural activity recording and dopamine (DA) concentration measurement at four different brain locations (220μm vertical spacing). The ability of GC microelectrodes to detect DA was firstly assessed in vitro in phosphate-buffered saline solution and then validated in vivo measuring spontaneous DA concentration in the Striatum of European Starling songbird through fast scan cyclic voltammetry (FSCV). The capability of GC microelectrode arrays and commercial penetrating metal microelectrode arrays to record neural activity from the Caudomedial Neostriatum of European starling songbird was compared. Preliminary results demonstrated the ability of GC microelectrodes in detecting neurotransmitters release and recording neural activity in vivo. GC microelectrodes array may, therefore, offer a new opportunity to understand the intimate relations linking electrophysiological parameters with neurotransmitters release.

INTRODUCTION

Dopamine (DA) is a key neurotransmitter that plays a pivotal role in a large variety of neurophysiological functions. Disruption of the secretion and uptake of this neurotransmitter is the cause of several psychiatric and neurological disorders [1], among which are Schizophrenia [2], Parkinson diseases [3] and drug addiction [4], To obtain a better understanding of how synaptic malfunctions lead to psychiatric and neurological diseases

and to take the current available clinical treatments to the next level, a fundamental understanding of the interplay between electrical and electrochemical signaling in the basic neurotransmission mechanisms is necessary. For this, the development of a device capable of precisely and selectively detecting neurotransmitters *in vivo* and simultaneously record electrical neural activity is needed. An ideal recording-detection platform has to fulfil many conflicting requirements in order to be able to (i) record informative and robust signals over several years and (ii) selectively and sensitively detect, in a reproducible way, small concentrations of neurotransmitters *in vivo*, at low levels that are typical of living systems. Despite the recent attempt to introduce new electrode materials [5,9] and develop novel microfabrication techniques to allow multi-site detection [10–11], the available techniques for *in vivo* DA detection often lack in stability and spatial selectivity and do not allow the simultaneous detection of electrical and chemical signals.

In this study, we address this problem by introducing a 4-channel glassy carbon (GC) microelectrode arrays on a flexible substrate to measure dopamine concentration *in vivo* and to simultaneously record neural activity at four different locations (220μm vertical spacing). We present preliminary *in vivo* validation of (i) the capability of GC microelectrodes to detect spontaneous DA concentration in the Striatum of a European starling songbird through fast scan cyclic voltammetry (FSCV), (ii) the capability to simultaneously record single-unit neural activity at four different depth locations from the Caudomedial Neostriatum (NCM) auditory area of a European starling songbird, compared with commercial penetrating metal microelectrode array.

EXPERIMENTAL DETAILS

Glassy Carbon Microarrav Fabrication

The fabrication of the thin-film devices used for this study is described in detail elsewhere [12,13], Here, we extend the microfabrication technology by adding a new reinforcing layer to allow brain penetration. An additional thicker layer of polyimide (Durimide 7520) (FujiFilm) was then patterned on top of the insulation layer to reinforce the penetrating portion of the device. Subsequently, the device was released from the wafer through the selective etching of the silicon dioxide with buffered hydrofluoric acid. The microelectrode array is made of 4 recording-detection sites with an area of 1500 μm^2 and a vertical inter-electrode distance of 220 μm.

Surgery, Neural Recording and Data Acquisition

Starling bird was anesthetized under isofluorane, and urethane and a craniotomy was made around Y-sinus with enough space for electrode implantation. Then the probe was attached to the head-stage and lowered down to 2000 μm by stereotaxic coordinates targeting Caudomedial Neostriatum NCM (Right hemisphere, posterior: 750 μm, lateral: 1600 μm from the bifurcation of the sagittal sinus). Finally, the reference/ground electrode was inserted under the skull opening.

After dropping the microelectrode implants to the desire area, a series of natural Starling bird songs were played in a random order to the anesthetized bird. The recordings were

performed by utilizing A-M recording systems (Sequim, WA) for 30 min. The neural recording was obtained from NCM auditory area of brain of a European Starling songbird. Brain response recording was started with sampling rate of 20 kHz from four electrode sites on both microelectrodes. The signals were filtered (300Hz – 5kHz) with gain of 5K. The recorded signals then were sent to a A/D converter (CED Power 1401) with 10X gain to be digitized. Recordings were performed both using our device and a commercial depth electrode as a reference (Neuronexus, A16-5mm-50-177, 15 μm diameter Ir-based microelectrodes).

In-vitro and *In-vivo* Electrochemical Detection

Fast-Scan Cyclic Voltammetry (FSCV) was carried out for dopamine detection using WaveNeuro FSCV Potentiostat System (Pine Research, NC). For *in vitro* DA calibration in phosphate-buffered saline solution (PBS; 0.1 M, pH 7.4) (Sigma Aldrich, USA), a waveform proceeding with a triangle scan at 400 V/s from −0.5 V to +1.3 V and back to −0.5V with respect to the reference Ag/AgCl electrode was used. The duration of each scan was 9 ms (900 data points) with a frequency of 10 Hz. The same voltage waveform was applied to microelectrodes at 60 Hz for 1 hour prior to the start of each experiment, to precondition the microelectrodes. Known concentrations of dopamine (10 nM – 400 nM) were then injected for 5 s and changes in current recorded for 40 seconds. The background current caused by the charging of the double layer was digitally subtracted, revealing the oxidation peak of DA. The *in vivo* detection of basal level of DA was obtained from the medial striatum (MSt) of European starling songbird without applying electrical stimulation.

RESULTS

Neural Recordings

We use songbirds as recoding model because their auditory area is similar to Superior Gyro area of the human brain which deals with speech and acoustic, making it a good candidate for neural recording [14]. Preliminary recording from GC microelectrode arrays has shown that the GC microelectrode array is capable of simultaneously record high quality single-unit neural activity at four different depth locations (220μm vertical spacing). The neural activity recorded using GC microelectrode array and commercial penetrating metal microelectrode array (Neuronexus, A16-5mm-50-177) were compared. Examples of bird song wave and recording activity for a GC microelectrode (top) versus a commercial microelectrode (bottom) are reported in Figure 1a and b. In both the cases, the stimulated neural activity is synchronized with the bird song wave. GC microelectrodes have shown to record greater spikes and lower base line activity compared with the commercial microelectrode. This means GC microelectrodes can pick up neural activity with a higher signal to noise ratio than the utilized commercial microelectrode.

FSCV Dopamine Detection

The GC penetrating microelectrodes are able to detect very clear DA peaks in a range of concentration from 10 nM, the lower detection limit, to 400 nM in PBS. Figure 2 shows an example of DA signal detected using a standard pyramidal FSCV waveform in which the applied voltage was ramped from −0.5 V to+1.3 V and then back to −0.5 V at 400 V/s scan

rate and 10Hz frequency. In particular, the color plot (Fig. 2a) shows the changes in current following 10nM DA injection in PBS in vitro. The changes in currents correspond to the reduction (−0.17V) and oxidation (0.63 V) peaks of DA, as visible from the correspondent background subtracted CV plot (Fig. 2b). The *in vitro* DA calibration curve is reported in Figure 2c. The microelectrode sensitivity to DA, defined as the normalized oxidation peak amplitude at the different DA concentrations, follows a logarithmic trend in the 10–50 nM concentration range and a linear trend (Pearson's r = 0.997) in the 50–400 nM concentration range. The functionality of the array was *in vivo* validated by measuring the spontaneous DA concentration in the MSt of an anesthetized European starling songbird, through FSCV. Figure 3 shows an example of the spontaneous DA signal detected; the oxidation peak amplitude corresponds to a 10nM concentration of the *in vitro* calibration results. The color plot (Fig. 3a) shows the changes in current due to a spontaneous DA release. The changes in currents correspond to the reduction (−0.17 V) and oxidation (+0.63 V) peaks of DA, as visible from the correspondent background subtracted CV plot (Fig. 3b).

CONCLUSIONS

In this study, we presented a 4-channel glassy carbon (GC) penetrating microelectrode array on a flexible substrate, able to (i) record high quality single-unit neural activity at four different depth locations (220μm vertical spacing) from NCM auditory area of brain of a European Starling songbird; (ii) *in vitro* measure 10 nM lower limit detection of DA through FSCV; (iii) *in vivo* detect DA spontaneous concentration in the MSt of an anesthetized starling songbird through the use of FSCV. To the best of our knowledge, this work represents the first report in the literature on the use of GC microelectrode array for *in vivo* DA detection in a songbird. These preliminary results show promising opportunity that GC microelectrode arrays may offer for real-time and simultaneous *in vivo* neural recording and neurotransmitter detection. Depending on the physiological and clinical goals, some of the microelectrodes of the probe could be used for electrical recordings while others could simultaneously be used for DA detection, helping in the understanding of the intimate relations linking electrophysiological parameters with neurotransmitters release.

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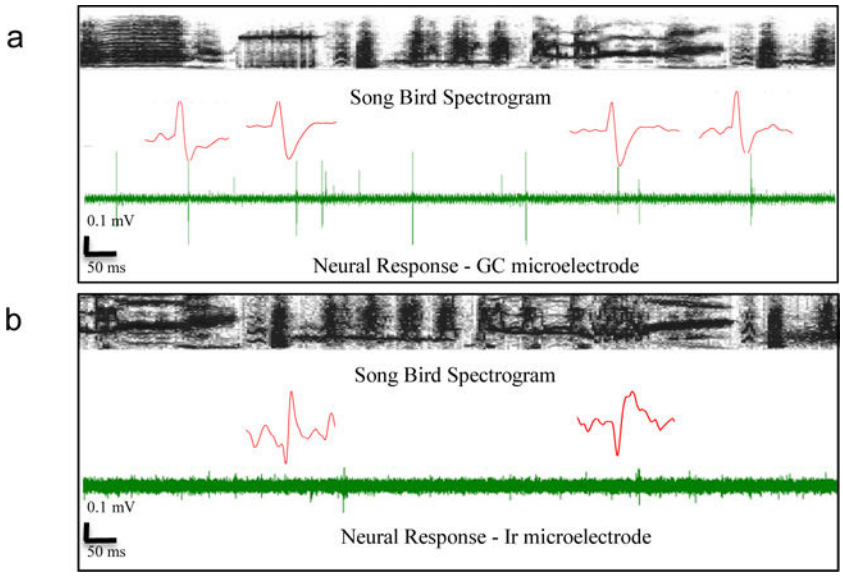

Figure 1.

Neural recording: comparative example of bird song wave and recording activity with a GC microelectrode (a) versus a commercial Ir microelectrode (b) Magnifications of single unit activities for both (a) and (b) are reported in insets.

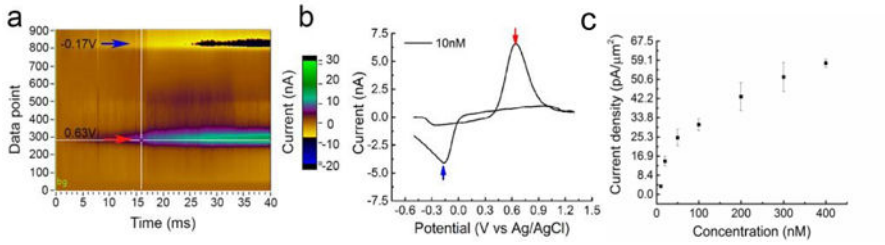

Figure 2.

example of 10 nM concentration dopamine signals detected using a standard pyramidal FSCV waveform in which the applied voltage was ramped from the holding potential of −0.5 V to the switching potential of+1.3 V and then back to −0.5 V at 400 V/s scan rate: color plot (a) and subtracted FSCV plot (b). (c) *In vitro* calibration curve in PBS solution.

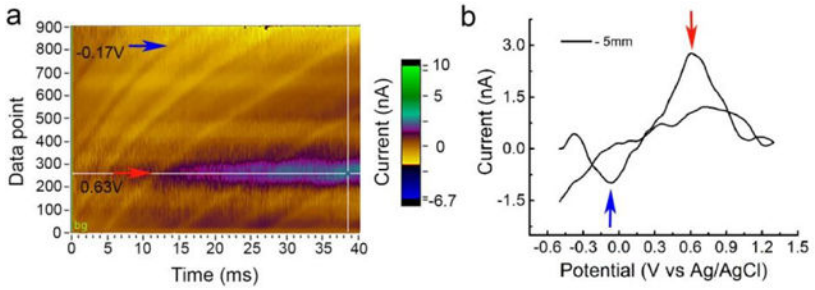

Figure 3.

example of spontaneous DA signal detected from the MSt of an anesthetized Starling songbird though the use of a standard pyramidal FSCV waveform in which the applied voltage was ramped from the holding potential of −0.5 V to the switching potential of +1.3 V and then back to −0.5 V at 400 V/s scan rate: color plot (a) and subtracted FSCV plot (b).