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Microfabricated FSCV-Compatible Microelectrode Array for Real-time Monitoring of Heterogeneous Dopamine Release

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Abstract

Fast scan cyclic voltammetry (FSCV) has been used previously to detect neurotransmitter release and reuptake *in vivo*. An advantage that FSCV has over other electrochemical techniques is its ability to distinguish neurotransmitters of interest (i.e. monoamines) from their metabolites using their respective characteristic cyclic voltammogram. While much has been learned with this technique, it has generally only been used in a single working electrode arrangement. Additionally, traditional electrode fabrication techniques tend to be difficult and somewhat irreproducible. Described in this report is a fabrication method for a FSCV compatible microelectrode array (FSCV-MEA) that is capable of functioning *in vivo*. The microfabrication techniques employed here allow for better reproducibility than traditional fabrication methods of carbon fiber microelectrodes, and enable batch fabrication of electrode arrays. The reproducibility and electrochemical qualities of the probes were assessed along with cross talk *in vitro*. Heterogeneous release of electrically stimulated dopamine was observed in real-time in the striatum of an anesthetized rat using the FSCV-MEA. The heterogeneous effects of pharmacology on the striatum was also observed and shown to be consistent across multiple animals.

Introduction

Electrochemical monitoring of neurotransmitters *in vivo* has contributed toward a greater understanding of the relationship between behavior and underlying neurochemistry, and has furthered the knowledge of neurological diseases and disorders ¹. The technique of background-subtracted fast scan cyclic voltammetry (FSCV) facilitates the subsecond detection of readily oxidizable analytes *in vivo*, such as catecholamines ². Additionally, the intrinsic ability of FSCV to distinguish catecholamines from common interferents (through a cyclic voltammogram) provides a necessary increase in selectivity, compared to other electrochemical techniques ³. The advantages of FSCV have recently allowed the technique to be applied to study the effect of environmental cues relating to drug seeking behavior ⁴ as well as the detection and identification of serotonin ⁵ and norepinephrine ⁶ release *in vivo*.

The technique of FSCV, however, is generally applied to a single electrode; limiting the scope of its experimental applicability in an integrative environment such as the brain. A microelectrode array (MEA) compatible with FSCV would facilitate such integrative studies encompassing multiple brain locations *in vivo* or *in vitro*. Furthermore, such technology could

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be used to study multiple neurotransmitters simultaneously. In fact, FSCV has recently been optimized (using various potential waveforms) to detect and monitor a variety of neurotransmitters and biological processes such as serotonin⁵, tyramine and octopamine⁷, oxygen fluctuations^{8, 9}, adenosine¹⁰, norepinephrine⁶ and epinephrine¹¹. The usefulness of a dual-waveform platform has been described previously and the ability to monitor both dopamine and oxygen fluctuations at an FSCV-MEA was demonstrated in a flow-injection analysis system¹².

We have recently shown that a FSCV compatible, carbon fiber based, MEA is a useful tool for monitoring such processes *in vivo*¹³; however these MEAs, like single carbon fiber microelectrodes, were difficult to fabricate reproducibly¹⁴. Unlike traditional electrode fabrication methods, microfabrication processes provide a platform upon which MEAs can be reproducibly batch fabricated. Microfabricated MEAs have been generally proven useful as enzyme modified biosensors to detect analytes such as glutamate¹⁵, glucose¹⁵ and acetylcholine^{16, 17}. Other such microelectrode arrays have been used to study neurobiological processes through the techniques of constant potential amperometry and electrophysiology^{18, 19}. These probes enabled simultaneous measurements of neurochemical transmission and action potentials, however the authors note that chemical identification using the amperometric technique is not possible¹⁸. The ability to identify electrochemical signals as actual neurotransmission is vital when performing any neurobiological experiment *in vivo*. However, despite the advantages of a microfabricated FSCV coupled MEA, one has yet to be applied towards the *in vivo* detection of neurotransmitter release and reuptake.

We have previously shown that a pyrolyzed photoresist film (PPF) microelectrode array coupled with FSCV maintained similar sensitivity and selectivity toward catecholamines as polyacrylonitrile (PAN) type carbon fiber microelectrodes in a flow injection apparatus¹². The advantages of these carbonaceous PPF arrays over those implementing noble metals include: lower cost, wider potential window, greater electrochemical inertness of the electrode surface²⁰. However, the overall size of these probes (~300µm x 200 µm) was unsuitable for the FSCV detection of neurotransmitters *in vivo*. Recent work by Michael and co-workers has illustrated the difficulty of detecting catecholamine release near a microdialysis probe of similar dimensions to our first generation probe^{21–24}. This research suggests that brain penetration injury is a plausible cause for the difference in observed performance between the two techniques of FSCV and microdialysis²². Since detection of catecholamine release with large probes *in vivo* is problematic, the design of the previous probes were substantially altered to decrease the overall dimensions of the MEA probes; thus enabling high quality *in vivo* measurements.

Here, we present a batch microfabricated FSCV-MEA capable of observing different neurochemical events within a single brain area or across brain areas (within 1 mm). The heterogeneity of electrically stimulated dopamine release was observed in the striatum of anesthetized rats. Additionally, the effect of pharmacological alterations on the stimulated dopamine release and reuptake was studied; specifically the effect of a D2 autoreceptor antagonist (raclopride) and a monoamine uptake blocker (cocaine). These pharmacological alterations produced results which support previous work while illustrating the need for a FSCV-capable microelectrode array *in vivo*.

Experimental

Chemicals

All chemicals and drugs used herein were obtained from Sigma-Aldrich (St. Louis, MO) and were used as delivered. Raclopride-HCl and cocaine-HCl dissolved in saline were administered intraperitoneally (i.p.). Post-*in vivo* electrode calibrations were done as previously described

^{12–14, 25} in a flow injection system with a Tris buffer solution (pH 7.4) consisting of 15 mM Tris, 140 mM NaCl, 3.25 mM KCl, 1.2 mM CaCl₂, 1.25 mM NaH₂PO₄, and 2.0 mM Na₂SO₄. Stock solutions of dopamine were made using 0.1 N HClO₄ and were diluted immediately prior to calibration.

Data Acquisition

A modified version of TH-1 software (ESA Inc., Chelmsford, MA) was used with a “Quad UEI” potentiostat (UNC Chemistry Electronics Shop, Chapel Hill, NC) in two electrode (vs. Ag/AgCl) mode for all electrochemical measurements *in vivo* and *in vitro*, as previously described ^{12, 13}. The triangular potential waveform was applied to the working electrode at 10 Hz and ranged from −0.4 V to 1.3 V at 400 V/s. This waveform was digitally generated and subsequently low-pass filtered at 2 kHz to remove digitization artifacts. Interestingly, Heien et al. has shown that this particular potential window increases the sensitivity of carbon fiber microelectrodes towards catecholamines ¹⁴. This sensitivity increase is most likely due to the regeneration of the carbon surface ²⁶. We have also previously shown that this waveform is compatible with the PPF working electrode material *in vitro* ^{12, 26}.

In Vivo Experiments

In vivo anesthetized preparations were done using male Sprague-Dawley rats (300–370 g; Charles River Laboratories, Wilmington, MA) anesthetized using urethane (1.5 mg/kg). Temperature was maintained at 37°C using a heating pad (Harvard Apparatus, Holliston, MA, USA). MEAs were placed in the caudate putamen using a stereotaxic frame with respective anteroposterior (AP), mediolateral (ML) and dorsoventral (DV) coordinates (AP +1.2 mm, ML +2.4 mm, DV −4.5 to −6.5 mm) ²⁷ orientating from bregma. An Ag/AgCl reference electrode was placed in the contralateral cortex and it was employed for all *in vivo* experiments.

Dopamine release was electrically elicited in the brain areas of interest using a bipolar electrode situated in the ventral tegmental area (VTA, location of the dopaminergic cell bodies) as previously reported ⁶. A bipolar stimulating electrode and voltage-to-current converter (NeuroLog System, Hertfordshire, UK) were used to apply stimulation pulses between voltammetric scans using the TH-1 software. Unless otherwise noted 60 pulses, 60 Hz biphasic stimulations were used ($\pm 300 \mu\text{A}$, 2 ms per phase).

For all experiments, after obtaining a stable stimulated release baseline, which was collected every 4 min. over a 30 min. timeframe, raclopride and cocaine were administered subsequently via intraparietal injection (i.p.). Following raclopride injection, steady-state evoked release was observed after approximately 20 min, 5 files were then collected every 4 min over a 20 min. window. Subsequent administration of cocaine followed, and files were collected in a similar manner.

Microfabrication of FSCV Compatible Probes

A schematic of the fabrication process is illustrated in Figure 1. The pyrolyzed photoresist arrays were manufactured using equipment in three separate clean rooms: the BMMSL (N.C. State), the NNF (NC State), and the CHANL facility (UNC-Chapel Hill). All fabrication was done on 3” 200 μm thick, [100], prime grade silicon wafers (Silicon Quest Inc., Santa Clara, CA). To prevent capacitive coupling to the conductive silicon substrate, a 3000 Å silicon nitride dielectric layer was deposited via low pressure chemical vapor deposition (LPCVD). This silicon nitride layer also served as a mask for the subsequent wet-etch of the silicon substrate.

The formation of the pyrolyzed photoresist film (PPF) was done as previously described ^{12, 28–30}. The PPF was used for the working electrode, interconnects and bonding pad; thus eliminating all metallization steps. Briefly, the process entailed spinning a 2 μm layer of

AZ1518 photoresist on the silicon nitride insulated substrate and subsequently patterned using standard photolithographic procedures (Karl Süss, MA55 Photoaligner). The photoresist was then pyrolyzed by placing the wafers in a quartz tube furnace (Sentro Tech, Inc., Berea, OH, USA) then ramping the temperature at 4°C/min to a final temperature of 1000°C. The temperature is held at 1000°C for one hour under a forming gas atmosphere (95% N₂, 5% H₂). The wafers were allowed to cool to room temperature under the inert atmosphere before removing them from the furnace.

Insulation of the PPF microelectrodes was accomplished in a non-conventional process to achieve a higher functional MEA yield per wafer. This process first involves the deposition of a 5000Å thick, low stress, silicon nitride layer using a plasma enhanced chemical vapor deposition (PECVD) system at 7.0 nm/min (Advanced Vacuum, Lomma, Sweden). The silicon nitride layer is then selectively “half etched” via capacitively-coupled reactive ion etching (RIE) (Semigroup, inc.), using photoresist as a mask. The entire probe-to-be is then remasked using photoresist and the extraneous silicon nitride is etched to the host silicon wafer. The half insulated probes are then anisotropically frontside etched at 1.6 µm/min using a 40% KOH solution at 80°C to a depth of 30 µm. Though the KOH etch produces 54° sidewalls, it produces negligible protrusion at the desired device thickness. Straight sidewalls are possible with [110] orientation, however this comes at the expense of the mechanical stability of the device due to undercutting³¹. The microelectrodes were then exposed (fully etched), again using the capacitively-coupled RIE system. The final insulation thickness of 2500Å was verified via interferometry (Nanometrics Inc. Milpitas, CA). The “half etch”-wet etch-“full etch” strategy allowed a vast increase in the amount of functional devices per wafer (5% to 90% functional devices), however convex corner etching at the [411] plane inhibited the complete release of the features in a wet etch solution³¹.

Although suitable corner-compensation methods might be possible³¹, there is no guarantee that a wet-etch release is compatible with the “half etch” strategy. Therefore, release of the MEAs was accomplished using backside deep reactive ion etching (DRIE) (Alcatel Inc., Annecy Cedex, France) at a reproducible rate of 3 µm/min. The etch stop for the DRIE process was easily optically determined through a viewport.

The desired final device thickness of the PPF-MEAs was between 15 and 25 µm, and although silicon is flexible at this thickness it is still quite fragile; therefore secure handling of the released devices became a high priority. A suitable adhesive and handle wafer are therefore necessary to handle these fragile devices in a vacuum system. Additionally, the adhesive needed to be compatible for not only the DRIE etch but it could also not foul the exposed carbon surface. The solution to these problems was the use of a double-sided Revalpha thermal release adhesive tape (Nitto Dinko Inc., Japan). The adhesive used on this tape was designed to be pressure sensitive on one side while the other side released at 150°C. This mounting strategy allowed the precise release and easier handling of the thin devices without fouling the electrode surface.

The final packaging of the PPF MEAs was done by mounting the devices on a printed circuit board and subsequent connections were made using silver epoxy and silver wire. DIP and Molex connectors were used to interface the arrays with the FSCV “Quad” headstage. It should be noted that this process is compatible with other electrode geometries as well as an increase in channel numbers. In fact, 16 channel electrodes were also successfully fabricated; however they are not supported by our electronics at this time (Figure 2b).

Results and Discussion

PPF Microelectrode Array Design

The fabrication considerations of manufacturing an *in vitro* microelectrode array compatible with FSCV were addressed in a previous publication¹². These probes, however, were not suitable for *in vivo* use due to the large overall size of the device. Scaling the probes down to the current size involved a considerably more intense fabrication process, but the small size is necessary to reduce tissue damage and enable *in vivo* neurotransmitter monitoring. It is known from previous research that measurements by voltammetric probes mounted on microdialysis probes are precluded by the tissue damage incurred from the size of the microdialysis probe (~220 μm in diameter)^{21, 23}. The final probe dimensions were designed to be 35 μm wide at the tip tapering to 100 μm after the sensing elements. These dimensions were verified after release using scanning electron microscopy (Figure 2a). While the sensing elements are located along a tapering substrate, the probe remains equally as thin throughout the entire length of the probe. Though much ongoing research is being dedicated to decipher the exact cause of both acute and chronic tissue damage, it is generally agreed upon that thinner probes should help reduce tissue damage over larger cross-sectional area implants^{32–34}. The released features were measured to be between 15 μm and 25 μm thick, within the desired range. The difference in the thickness was determined to be from non-uniform plasma density within the DRIE system, which was optically verified.

In addition to probe optimization, the microelectrodes themselves have been optimized to detect neurotransmitters *in vivo*. Though other electrode geometries are possible with this process, the electrode dimensions chosen for these experiments are 10 μm wide by 100 μm long. These dimensions yield a surface area which is comparable to the traditionally used cylindrical carbon-fiber microelectrodes used for *in vivo* electrochemical measurements; thus providing a basis for comparison.

Maximizing the surface area along the dorsal-ventral direction serves a dual purpose. The critical dimension for “band type” ultramicroelectrodes is the width of the electrode; therefore if the width is kept small enough a hemispherical diffusion profile can be maintained. This profile minimizes ohmic drop while maintaining a large enough surface area to maintain good sensitivity toward dopamine. The band electrodes described here are slightly recessed (0.25 μm) relative to the width of the band UME (10 μm) and the diffusion layer thickness (11 μm)¹²; therefore the proper diffusion profile should be maintained^{35, 36}. As the diffusion profile of these probes is critical to their *in vivo* functionality, future studies will, however, need to assess this topic in depth.

Additionally, dopamine has been shown to release in “microenvironments” within the brain, this heterogeneity causes varying catecholamine release along a dorsal-ventral track³⁷. Electrodes spanning longer in the dorsal-ventral dimension are therefore more likely to interrogate active terminals. While the spatial resolution of these probes can be improved by making the probes smaller, this would come at the expense of analytical sensitivity. We previously estimated the diffusion of dopamine to be around 11 μm for similar electrodes, and have shown that multi-site *in vitro* detection is possible with similar spacing between electrodes in a horizontal arrangement¹². The spacing for the sensing elements was chosen to be 100 μm to be well outside the estimated diffusion distance. This spacing also enabled simultaneous observation over a wider range of terminals within the desired brain region.

Electrochemical Characterization

PPF microelectrodes on a fused silica substrate are electrochemically comparable to T-650 carbon-fiber microelectrodes *in vitro*¹². Flow injection analysis of the current MEAs was done

to ensure that the electrode sensitivity remained consistent using the new device design. Figure 3a shows an average of cyclic voltammogram from 12 microelectrodes (from 3 different array devices) in response to a 500 nM dopamine bolus injection. In each case the voltammogram used was at the peak voltammetric current, and is shown to be relatively reproducible (± 0.75 nA standard deviation, $n = 12$ electrodes), compared to traditional carbon-fiber microelectrodes. The ability to reproducibly detect physiological concentrations of analyte is an advantage of the microfabrication process over traditional electrode fabrication methods, in which electrodes are often normalized to the response at a known concentration due to slightly irreproducible surface areas. The irreproducible surface areas occur as a result of manually cutting the electrode to length under an optical microscope ($\sim 100 \mu\text{m}$). Since the error in this surface area is largely researcher dependent it is difficult to make a quantitative comparison of the error associated with measuring dopamine at a carbon fiber microelectrode and the MEAs. The microfabrication process, however, should help to alleviate the irreproducibility due to human error; therefore allowing the electrodes to be consistent regardless of the experimenter.

Figure 3b shows a peak voltammetric current vs. concentration plot for *in vitro* dopamine injections. Consistent with our previous work, the silicon based MEAs maintain comparable sensitivity to T-650 carbon-fiber electrodes (of the same surface area) towards dopamine in a flow injection system^{12, 14}. Figure 3c shows the peak oxidation current vs. time traces for the MEAs ($n = 12$ electrodes, 3 different arrays) using both amperometric and FSCV techniques. No adsorption takes place during the amperometric oxidation of dopamine, while adsorption occurs during FSCV^{12, 38, 39}. As with a carbon-fiber electrode, the data indicates that the time response of the FSCV arrays was inhibited by the adsorption of dopamine, rather than a diffusion limitation. This adsorption related time delay has been observed previously on carbon fiber microelectrodes and PPF microelectrodes^{12, 39, 40}. Although the time response is limited by adsorption, the electrochemical sensitivity towards catecholamines remains much greater using the FSCV technique.

Crosstalk was assessed by applying a waveform to one electrode (channel 2) while holding an adjacent electrode at 0.0 V (channel 1). A bolus injection of 1 μM dopamine was injected onto the probe, resulting in signal only on channel 2 (Figure 4a). The electrochemical charging (“background”) current was negligible, and remained constant throughout the experiment (Figure 4b); therefore this current could be digitally subtracted. The peak current vs. time traces are shown in Figure 4c. This figure illustrates that in addition to sensitivity towards dopamine, the amount of crosstalk for these MEAs remained consistent with our previous work¹².

Simultaneous Observation and Identification of Stimulated Dopamine Release at Multiple Channels

A main advantage of a FSCV compatible microfabricated array is its ability to not only to detect *in vivo* catecholamine release, but also to identify the origin of the signal using its characteristic cyclic voltammogram. The ability of an MEA detect and identify neurotransmitters can be advantageous even in the same brain region. The heterogeneity of catecholamine release in the striatum has been demonstrated using FSCV^{37, 41, 42}. Researchers must compensate for this heterogeneity by using several animals and several electrodes. Although multiple animals will still be needed to establish a representative population, greater accuracy in a single animal can be achieved using an FSCV compatible MEA probe.

The first column in Figure 5 describes a representative data set ($n = 3$, animals and arrays) pertaining to dopamine which has been electrically elicited with a 60 Hz, 60 pulse, biphasic stimulation in the ventral tegmental area (VTA) ($\pm 150 \mu\text{A}$, 2 ms per pulse). The peak voltammogram vs. time traces (average of 5 trials) are provided along with the peak current voltammograms (insets) (average of 5 trials). The voltammograms allow for chemical

identification without the use of specific pharmacological agents or sentential electrodes to separate signal from physiological noise. Although dopamine cannot be distinguished from norepinephrine, previous studies have shown that the primary catecholamine released in the caudate is dopamine⁶. In this case, all four channels exhibited release, and provided a characteristic dopaminergic voltammogram. The average amount of electrically evoked dopamine release measured across all four channels was measured to be 137 ± 65 nM.

As expected, not every channel observed dopamine release for each experiment, although each channel was shown to be functional via post-calibration. This lack of observed dopamine release can be contributed to physiological heterogeneity within this brain region, as well as the small sampling hemicylinder (diameter of 12 μ m) around the FSCV probe¹². Additionally, heterogeneous time responses were qualitatively observed at different channels. It is possible that the presence of a longer diffusion distance between the release sites and some of the microelectrodes on the array could cause the delay in the time response at some of the channels^{43, 44}. As noted by other authors, placing the electrodes on the lateral edges of the probe might negate some of this delay¹⁸.

Although the MEAs allow for a better representation of neurotransmitter release in a single brain area, the concentration of dopamine release *in vivo* is less than expected (as measured by a carbon-fiber microelectrode). Several factors could contribute to this reduction in observed dopamine release. First, the arrays might be more prone to biofouling than a carbon fiber due to the planar electrodes being located on a flat silicon substrate, however post-calibration *in vitro* revealed only a slight change in electrode sensitivity (14.5 nA/ μ M precalibration vs. 11.5 nA/ μ M postcalibration (n = 12 electrodes, 3 arrays)). The planar geometry of the electrode itself might limit the diffusion of dopamine from surrounding terminals. Indeed, Figure 5 does illustrate characteristic signal “overshoot”, which is indicative of diffusion related artifacts^{45, 46}. Finally, the array’s orientation *in vivo* (currently facing the anterior direction) might also contribute to the reduction in observed dopamine release. Further testing will be needed to substantiate these theories and to optimize the sensitivity of these probes *in vivo*.

Heterogeneous Effect of Pharmacological Manipulation on Stimulated Dopamine Release

The FSCV coupled MEA can be used for observation and identification of electrically elicited dopamine release. Manipulating the system with pharmacology can provide further insight into neurotransmitter release mechanisms and the study of uptake kinetics. Drugs of abuse, such as cocaine, have also been used to study the relation between neurotransmission and drug seeking behavior. Recently two areas within the ventral striatum (nucleus accumbens core and shell) have been shown to respond differently to cocaine-reinforced stimuli in freely moving animals⁴⁷. Observing such a difference in real-time would be useful and could be accomplished using a FSCV-capable MEA that is able to monitor dopamine release and the effect of pharmacology in multiple brain regions with sub-millimeter separation.

The middle and right columns in Figure 5 describe a representative experiment (n = 3 animals) in which pharmacological agents were used to alter dopamine release and uptake using a D2 autoreceptor antagonist (raclopride) and a monoamine uptake blocker (cocaine). In these results, the heterogeneity of the drug(s) effect on dopaminergic terminals can be directly observed using one array in a single animal. The representative set of experiments shown in Figure 5 illustrates the heterogeneous effects of pharmacology across the four channels for both raclopride and raclopride-cocaine administrations. The pharmacological effect compared to the pre-drug stimulated release was significantly different across some channels ranging from 138% to 256% and 713% to 1357%, respectively.

The heterogeneous responses observed after pharmacological manipulation can be explained by both differences in dopamine terminal density⁴⁸ and/or heterogeneous autoinhibition

effects⁴⁵. The electrodes on the MEA are located at fixed dimensions along the dorsal-ventral direction. The electrodes in the array are, therefore, located in areas with varying terminal density affecting measurements at each respective recording site^{37, 42}. Traditionally with FSCV, it has been common practice to position the working electrode in an area with high dopamine terminal density⁴⁸. Measuring in these “optimized locations” is done to limit diffusional distortion caused by the electrode’s location further away from active dopamine terminals⁴¹. Recently, however, it has been shown that recordings done in “non-optimized” locations are largely affected by spatial variations in D2 autoinhibition, which can operate on multiple time scales⁴⁵. Although the channels with the most (Ch. 0) and least (Ch. 3) pre-drug dopamine release experienced the same drug effect from raclopride, other areas experience significantly different effects from raclopride (Ch.1 and Ch. 2). We assume that these results stem from both spatial differences in autoinhibition *and* dopamine terminal densities at the respective sites. The ability to probe both “optimized” and “non-optimized” locations simultaneously could prove to be a significant advantage over single channel recordings, even in a single brain region, to characterize these different pharmacological responses.

The information gained through using these MEAs allows for a better representation of the pharmacological effect of dopaminergic drugs at different sets of terminals within a single brain region while maintaining chemical selectivity for catecholamines. Figure 6 describes the average effect of both raclopride and raclopride-cocaine on all of the animals tested (separated by channel). As previously noted, not all channels exhibited release for each experiment (although the MEAs were verified to be functional via post calibration) therefore the graphs represent the average of trials done per specified animal. For multiple animals the pharmacological effect of raclopride and raclopride-cocaine was consistent across channels indicating that the variation found in a single animal was most likely caused by physiological heterogeneity rather than a property of the array itself. The average effect of raclopride and raclopride-cocaine for all channels was found to be significantly increased to 195 % and 1153%, respectively. Other studies involving raclopride and raclopride/ dopamine uptake blocker (cocaine, GBR12909) cocktail have found comparable results in the caudate putamen^{6, 49}.

Conclusion

This report describes a fabrication process for a microfabricated, FSCV compatible, microelectrode array which is functional *in vivo*. The ability to reproducibly batch-fabricate FSCV arrays is advantageous for high-throughput neurobiological measurements. Additionally the arrays were shown to be able to detect and identify electrically stimulated endogenous dopamine release and multiple spatial locations within the same brain region (dorsal striatum, caudate putamen). The real time observation of the physiological heterogeneity in this region was observed under normal and pharmacologically altered states.

This report has shown that FSCV compatible MEAs are advantageous for single brain area measurements, however measuring across multiple brain areas, and using the aforementioned potential waveforms to monitor multiple analytes simultaneously, will provide insight into the integrative nature of chemical transmission in the brain. Future research will be focused on the optimization of the probes for anesthetized and freely moving biological preparations as well as measuring neurotransmitters across brain areas.

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Figure 1.

Fabrication process of FSCV-MEAs. (a) LPCVD deposition of silicon nitride. (b) PPF formation. (c) PECVD deposition of low-stress silicon nitride. (d) Partial etch of silicon nitride. (e) full etch of silicon nitride to silicon. (f) Silicon etch in 40 % KOH at 80 °C and subsequent full etch of silicon nitride insulation layer. (g) Adherence of wafer to a handle wafer using thermal release tape. (h) backside DRIE thinning of wafer. (i) Thermal release of MEAs.

Figure 2.

Inspection of FCSV-MEAs. (a) SEM image of an MEA. The channels indicated here are used throughout the paper. Exposed pyrolyzed carbon is indicated as is the etched silicon nitride layer between the electrodes. (b) Optical image of both a 16 channel and 4 channel FCSV-MEA. PPF microelectrodes measured 10 x 100 μm with 100 μm spacing between electrode sites.

Figure 3.

In vitro characterization. (a) Average voltammetric response of PPF electrodes to a 500 nM bolus dopamine injection. (b) Peak voltammetric current vs. concentration plot for linear detection regime (c) Comparison of amperometric and FSCV measurements in response to a 5 second bolus injection of 500 nM dopamine. All experiments were done in TRIS buffer, pH 7.4 ($n = 12$ electrodes from 3 arrays). Error bars represent standard deviation.

Figure 4.

Electrical crosstalk analysis for FSCV-MEA. (a) background-subtracted fast scan voltammetric color plots for adjacent electrodes in response to a 5 second bolus injection of 1 μ M dopamine. Channel 1 (top) is held at 0.0 V while the -0.4 V to 1.3 V waveform is applied to channel 2 (bottom). (b) Charging currents of adjacent electrodes. (c) Peak voltammetric current vs. time traces in response to a 5 second bolus injection of 1 μ M dopamine. All measurements were done in TRIS buffer, pH 7.4.

Figure 5.

Simultaneously monitoring electrically excited dopamine release *in vivo*. Each trace is an average of five trials (4 min between trials) with the dashed lines indicating the standard error of these measurements. (insets) fast scan cyclic voltammograms of electrically stimulated dopamine release (scaled to highest release per experiment). Grey bars indicate stimulus start and duration (60 Hz biphasic VTA stimulation: 60 pulses, 2 ms, 150 μ A).

Figure 6.

Average drug effect across animals. Drug effect data was obtained by normalizing to electrically stimulated release with no pharmacological manipulation. All stimulations were 60 Hz biphasic VTA stimulations (60 pulses, 2 ms, 150 μ A) * indicates $p < 0.05$. The error bars represent the standard error. A microfabricated array of carbon microelectrodes was utilized for monitoring the neurotransmitter dopamine *in vivo* using fast scan cyclic voltammetry (FSCV). Heterogeneous release of electrically stimulated dopamine was observed in real-time in the striatum of anesthetized rats using the FSCV-microfabricated-microelectrode arrays.