In-Vitro Real-Time Coupled Electrophysiological and Electrochemical Signals Detection with Glassy Carbon Microelectrodes

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The ability to carry out coupled and simultaneous electrical and electrochemical signals detection in the context of central and peripheral nervous systems has been of major research interest for sometimes now. This real-time capability could significantly help in developing more complete insight and understanding of the underlying mechanism of the interplay between electrical and neurotransmitter signals in neural communications at synapses, particularly in diseased states. Further, with increasing clinical interest in the use of electrical stimulation as therapeutic platform for variety of disease states from spinal cord injury to Parkinson’s disease and essential tremor, there is pressing need for understanding the actual mechanism of its efficacy. In this study, therefore, we present an enabling platform that consists of a novel polymeric probe supported on a flexible substrate with microelectrode array specifically targeting simultaneous detection of neurotransmitters and electrophysiological signals. This probe consists of an array of patternable glassy carbon (GC) microelectrodes which have superior electrochemical performance due to their wide electrochemical window and surface attachment chemistry tailorability along with excellent and stable conductivity. In this study, we report that these microelectrodes can detect— in real time— serotonin, a key neurotransmitter involved in mood and sleep regulations, in in-vitro environment within 25 nM - 1 μM concentration range with resolution of 25 nM while simultaneously recording ECoG (electrocorticography) electrical signals. The probes are also capable of stimulating at a current density of 2.5 A/cm² (360 μA) and wide voltage range of at least −0.6 V to +1.2 V with remarkable stability.

With growing research and clinical interest in the use of electrical stimulation treatments for central and peripheral nervous systems for diseased states such as stroke, spinal cord injuries, Parkinson’s disease, and essential tremor, there is a pressing need for understanding the exact underlying complex molecular mechanisms of action that could induce neuroplasticity.1,2 In particular, there is a strong need for research focusing in the interplay between electrical and electrochemical signaling at neural synapses in the context of direct electrical stimulation of the cortex or/spinal cord for restoring neurological function after injury such as stroke. While there is increasing evidence supporting the efficacy of such electrical stimulations in neuroplasticity, fundamental questions regarding mechanisms underlying the effects of electrical stimulation on synaptic connections and what molecular mechanisms are involved in the process largely remain unanswered. This fundamental understanding is critical for designing better hardware as well as defining a rationale and evidence-supported stimulation protocol for each of the diseased states. Currently, the most commonly used method for correlating neuroplasticity to serotonin is microdialysis which essentially consists of tubular dialysis membrane that mimic capillaries through which a solution is constantly perfused and small solutes like neurotransmitters cross the semipermeable membrane.2,3 This procedure, however, has a serious drawback as it allows only electrochemical detection and is typically carried out post-treatment ex-vivo in a non-live animal model, thus resulting in a lack of real-time data. Recently, in-vivo fast-scan cyclic voltammetry (FSCV) on implanted wires (such as carbon fibers) and arrays to obtain real-time neurotransmitter data has been investigated with some level of success.2,4-6 While this has represented significant progress in the field, the arrays used typically have a rigid substrate backing and often have large foot-print. Consequently, this makes them impractical for long-term implantation, particularly in the soft and flexible spinal cord.2,5-7 Furthermore, only a few of these arrays were made specifically for neurotransmitter detection applications2,9 with none of them used for simultaneous and coupled neurotransmitter and electrophysiological recording.2,5,8

Further, the literature, in the context of simultaneous electrical and electrochemical detection of neurotransmitters in the spinal cord, is almost non-existent to the best of our knowledge. Reported research in microelectrode arrays specifically dealt with only spinal cord implantation that were generally placed in the epidural space for surface recording and stimulation.10-12 A recent example consists of a flexible stimulating epidural microelectrode arrays designed for implantation in a rat’s spinal canal and was tested in-vitro.13 This array contains 12 platinum-iridium (PtIr) microelectrodes supported on polydimethylsiloxane (PDMS) substrates and had its own power source capable of delivering up to 1 mA pulses at 100 pulses.13 Another recent microarray has rigid tungsten electrodes supported on PDMS substrate which was maintained in-vitro to stimulate the surfaces of rat spinal cords.13 Therefore, while these microelectrode arrays can be used for surface stimulation and recording, they cannot, however, sense neurotransmitter activity near neuronal junctions.13 As an alternative, very thin wires with few microns of diameter implanted in the spine have been used.14 The limitation with these wires, however, is that they must be individually placed and the spacing and depth would vary substantially between experiments, leading to difficulty in extrapolating spatial from temporal resolution.

Therefore, a clear and pressing need exists for a platform that can be used to detect both neurotransmitter and electrophysiological signals in a real-time and simultaneous fashion, particularly one that can be used in the spinal cord. To address these key shortcomings, we introduce a new, compact and flexible penetrating neural probe with patternable glassy carbon microelectrodes array designed for a long-term implantation in a rat spinal cord. The use of GC microelectrodes offers a number of compelling advantages over existing microelectrode materials, namely (i) it is the gold standard for...
electrochemistry due to its inertness, hence ideal for detecting neurotransmitters with an acceptable sensitivity and detection limit,\(^4\) (ii) its mechanical and electrical properties are tunable to respond to the needs for tissue penetration and stimulation,\(^9\) (iii) it has long-term stability under electrical stimulation,\(^16\) (iv) has faster electrokinetics and hence faster response time as compared to thin-film metals, and (v) it is amenable to surface modification such as coating with protective polymers such as Nafion due to carbonyl and carboxyl functional groups that typically are present on its surface.\(^17,19\)

We believe that these unique advantages offer a compelling case for a new and novel platform for simultaneous electrophysiological and electrochemical detections in the cortex as well as spinal cord.

### Methods

In this section, we report in detail the device architecture, microfabrication and pattern transferring techniques, along with detailed electrochemical characterizations and description of a custom-built breadboard with electrical signal recording, stimulation and cyclic voltammetry capabilities.

**Neural probe device design.**—The determination of the shape of the probe is driven by considerations for implantation in the spinal cord of an animal model (rat) where mechanical flexibility and ability to comfortably access the cords without causing damage are important.\(^15,19\) As shown in Figure 1, each device has a total length of 1.5 cm, and consists of 12 probes 2.0 mm in length. In turn, each probe has a single oblong-shaped GC microelectrode with 40 \(\mu\)m width and 500 \(\mu\)m length and supported on 2 mm long polyimide base that is capable of bending so as to wrap around the spinal cord during implantation. Once bent, these probes have a penetrating depth of about 1 mm to enable detection in motor pools of the cervical spine of the rat. These probes all fan out of the tip of a center shaft that in turn has 4 suture holes for stabilization during implantation and a 3 mm wide bump-pad base for interconnect to a 13 channel ZIF (zero insertion force) connector to data acquisition and signal generation hardware. At the top of the probe is a single reference microelectrode made of Pt with an area of 0.075 cm\(^2\). Platinum traces are used with chromium as adhesion layer and then insulated with polyimide (HD4100). Note that the Pt microelectrode is on the opposite side of the GC microelectrode array. Openings at the end (bump-pads) allow for access to data recording, cyclic voltammetry and stimulation electronics. Out of the 6 microelectrodes on one side of the probe, one microelectrode will be used for stimulation for serotonin detection (CE1 in Figure 2a) while another microelectrode (WE in Figure 2a) will be used to record electrical current response in the area which includes serotonin oxidation and the electrophysiological signal. Further, when coupling the ECoG signal with the serotonin input, the same microelectrode (WE) will be used as the working (reading) electrode in both electrochemical circuits, while two separate electrodes, one adjacent to the working electrode (i.e., CE1 in Figure 2a) and another one on the opposite side of the device (CE2) for the ECoG input will be used as counter-electrodes. While not all the 12 channels in a given probe are used in these current in-vitro experiments, the presence of multiple electrodes will allow more complete temporal and spatial resolution and content on serotonin detection by offering additional sets of arrayed microelectrode sites.

**Microfabrication of device.**—The fabrication of the devices is described in detail in our earlier publications.\(^17,18\) In summary, the glassy carbon microelectrodes were fabricated by patterning negative photoresist SU-8-100 (Microchem, Westborough, MA) on a silicon dioxide wafer, which was then pyrolyzed in inert atmosphere under nitrogen using a PECVD furnace (MTI Corp, Richmond, CA) at a ramp rate of about 10°C/h and held at 1000°C for one hour. The pyrolysis process was followed by spin-coating and curing of HD4100 photo-patternable polyimide (HD Microsystems, Wilmington, DEL) that will eventually act as a flexible substrate. Subsequently, electrical vias through the back of the microelectrodes were opened and thin-film metals (Cr and Au) then deposited through lift-off process on the substrate to create conductive traces, bump-pads, and the Pt reference electrode. Finally, a second layer of polyimide was spun on the traces for electrical insulation.\(^17,18\) The final device was lifted-off the oxide wafer using buffered Hydrofluoric acid (BHF) (Fisher Scientific, Waltham, MA) and then soaked in deionized water, cleaned and dried. Figure 1a shows a picture of one such released probe.

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**Figure 1.** Penetrating neural probe for spinal cord applications (a) whole device (b) close view of probes with glassy carbon electrodes (c) horizontal cross section of probe. Traces are typically 40 \(\mu\)m.
Electrochemical characterizations.—To validate the electrical integrity of the microelectrodes, electrochemical impedance spectroscopy was performed by superimposing a sine wave (5 mV RMS amplitude) onto the open circuit potential from 1 M Hz to 1 Hz frequency sweep before and after each test using a frequency analyzer and potentiostat (Solartron Analytical, Ametek, San Diego, CA). Electrochemical tests were performed in standard 0.01 M phosphate buffer solution (PBS) (Fisher Scientific, Waltham, MA) and a standard Ag/AgCl reference electrode (Ametek, San Diego, CA). Thus, all potentials are referred to Ag/saturated AgCl.

Nafion deposition on microelectrodes.—Serotonin, the neurotransmitter of interest in this study, has a tendency to adsorb onto the glassy carbon microelectrodes and hence negatively impacting the ability to obtain an accurate and repeatable linear calibration curve. Thus, a barrier is required between glassy carbon microelectrode and the solution containing serotonin. In this study, Nafion (a conductive copolymer of tetrafluoroethylene (Teflon) and perfluoro-3,6-dioxo-4-methyl-7-octene-sulfonic acid) is used as a barrier.22,23 One possible method of Nafion application includes using an ethanol based solution. The solution will dry at room temperature leaving a thin Nafion film on the electrode. This method, however, produced a film with poor adhesion and potentialistat (Solartron Analytical) produces a film with poor adhesion by itself.24 To form a stable bond between the Nafion and the glassy carbon microelectrodes, Nafion was applied to the surface directly and electrochemically. For this, 0.1 mL of Nafion solution (5%) in ethanol (Fuel Cell Earth, Woburn, MA) was placed on the microelectrode array of the device and dried with a heat gun until all solvents were evaporated. Subsequently, this device was then placed in a 100 mL electrochemical cell with 100 mL of PBS and 1 mL of Nafion solution. The device then underwent cyclic voltammetry at a scan rate of 100 mV/s for about 350 cycles in voltage range of −0.6 V to 1.2 V with Pt as a counter-electrode. Standard Pt counter-electrode was used while all the 12 GC microelectrodes on the device were electrically connected to each other (and hence acting as a single working electrode) to allow simultaneous deposition of Nafion on all microelectrodes.

Hardware for ECoG and serotonin detection.—The experimental set-up for the simultaneous detection of serotonin and ECoG signal required designing and building a circuit board that integrates components for inputting recorded ECoG waveforms, generating stimulating electrical signals for microelectrodes, recording electrical signals, and performing FSCV. The complete set-up is shown in Figure 2 and consists of three major components: (i) a potentiostat electrometer to input the raw in-vivo recorded ECoG data (recorded at 30 kHz) and remove noise actuated through the device, (ii) a potentiostat from Solartron Analytical to input high frequency triangle wave for FSCV and also record voltage data from the working electrode in the electrochemical cell at a sampling rate frequency of 500 kHz, and (iii) an electrochemical cell (Figure 2b).

The potentiostat electrometer in turn consists of three operational amplifiers: (a) the first one operating as control amplifier (Control Amp) that reads the difference between the reference electrode and the counter and the working electrode, (b) the second one acting as an I/E converter (ECoG current signal to voltage converter),25 and (c) the third one acting as an electrometer to filter out noise and adjust signals to desired high frequency low amplitude ranges. The electrometer also creates a feedback system within the potentiostat between the Control Amp and the electrometer amplifiers to filter out noise. It further keeps the signal within desired ranges and is designed to specifically target high frequency low amplitude ranges (i.e., 5000 Hz and 200 nV), making it compatible with fast-scan cyclic voltammetry.26 Feedback loop is accomplished by comparing the desired ECoG input signal between the control amplifiers output signal and the electrometer, thus canceling any gain that may have accumulated within the circuit and driving down the voltage amplitudes. This potentiostat electrometer design allows output of ECoG signals to remain within desired frequency and voltage amplitude ranges by filtering out any accumulated circuit noise. The electrochemical cell consists of a standard Ag/AgCl reference electrode along with the neural device (Figure 2a). Three sets of microelectrodes of the device were used in these experiments, where one microelectrode was used to send the signal from the electrochemical cell to the electrochemical cell (CE2) while the other two adjacent microelectrodes were used for FSCV, one working as a counter (CE1), the other working to read the electrochemical signal as well as the ECoG signal (WE).

In a typical experiment, the first step was conditioning each microelectrode designated for stimulation or recording by performing 100 cycles of cyclic voltammetry at a scan rate of 100 mV/s in the electrochemical cell. Conditioning activates the carbon surface by eliminating a variety of artifacts attached to the electrodes and also stabilizes the electrochemical cell for repeatable measurements. If the microelectrodes were determined to be in good condition (impedance at 1 kHz is less than 200 kΩ after conditioning), two adjacent electrodes on the same side of the probe were then used as the counter and working electrodes in the serotonin detection experiments.
In the literature, Nafion has typically been used to prevent adsorption on surfaces.21,22 The Nafion film attracts cations to the surface of the electrode and the net positive charge caused by the cation layer attracts anions to the surface, forming a second layer. Subsequently, a double layer capacitor is formed around the microelectrode which generates a hydrophobic environment allowing electrons access to the electrode through electron hopping.23 One possible method of Nafion application includes using an ethanol based solution. The solution will dry at room temperature leaving a thin Nafion film on the electrode. This method, however, produced a film with poor mechanical properties.21

To form a stable bond between the Nafion and the glassy carbon microelectrodes, Nafion was applied to the surface directly and electrochemically. When applied electrochemically, an oxidation peak due to the Nafion oxidation appears at 1.1 V and reduction peaks appear at 0.4 and −0.4 V. After about 100 scans, as shown in pink in Figure 4, the oxidation and reduction peaks reach maximum amplitude. At this point the impedance, as shown in Figure 5, reaches a minimum. Figure 5 also shows that, at the minimum impedance Nafion generates a larger phase with a phase shift, indicating a large capacitive effect. At 100 scans, we expect the coating to be much more uniform and homogeneous; however, the large capacitance implies a presence of significant space between the Nafion and the glassy carbon that creates extra capacitance. In this case, the unbalanced oxidation and reduction peaks in Figure 4 imply an unstable coating which would result in Nafion degradation and, hence, an unstable electrode. Thus, to ensure that only reversible oxidation and reduction reactions were occurring at the surface of the electrode, scans were continued until

![Image](https://example.com/image.png)

**Figure 3.** Optical Images of the oblong-shaped GC microelectrodes on a probe (a) Zoom of nafion coated GC microelectrode on the probes (b) Device after bending with one end inserted into ZIF (zero-insertion force) connector (c) Zoom of the device head after bending (d) bent device penetrating 1% agar.

**Results and Discussion**

After fabricating the device, a series of optical and electrochemical characterizations were carried out to validate the capability of these microelectrodes to penetrate the spinal cord and detect both electrophysiological signals and 5-HT concentration.

**Penetrating test.**—The probes were individually inspected using a light microscopy (Hirox, Hackensack, NJ) as shown in Figure 3a. After inspection, probes on the device were bent and held in position with Sylgard 184 poly dimethyl siloxane (PDMS) (Fisher Scientific, Waltham, MA), shown in Figures 3b and 3c. After bending, penetrating test through a plate of 0.1% agar that is typically used as a good representative model for neural material was performed.21,22 As seen in Figure 3d, the probes were able to penetrate down 1 mm through the agar without bending, making the device appropriate for implantation in rat spinal cord. The probe stayed in the bent position not only for the duration of the experiments, but also over a period of several weeks. This has a clinical relevance as it offers an encouraging data on the long-term viability of the device architecture.

**Performance of nafion coating.**—In the literature, Nafion has typically been used to prevent adsorption on surfaces.21,22 The Nafion film attracts cations to the surface of the electrode and the net positive charge caused by the cation layer attracts anions to the surface, forming a second layer. Subsequently, a double layer capacitor is formed around the microelectrode which generates a hydrophobic environment allowing electrons access to the electrode through electron hopping.23 One possible method of Nafion application includes using an ethanol based solution. The solution will dry at room temperature leaving a thin Nafion film on the electrode. This method, however, produced a film with poor mechanical properties.21

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**Figure 4.** Cyclic voltammetry showing increase in Nafion oxidation peaks with increasing number of scans.
oxidation and reduction peaks disappeared. For most electrodes, this occurred at about 350 scans. This increased the impedance slightly but decreased the phase indicating closer contact between Nafion and the carbon electrode. Figure 6 shows how the Nafion coating changes the sensitivity and charge storage capacity of the electrode. Before Nafion coating (red) the area of the curve is small, indicating a small charge storage capacity of 14 mC/cm², and the oxidation peak of 5-HT at about 0.7 V cannot be seen without background subtraction. However, with the Nafion coating, after 350 scans, area of the green curve is much larger, giving a charge storage capacity of 119.4 mC/cm² resulting in better sensitivity to the 5-HT oxidation which can be seen as a small inflection can be seen at 0.7 V even without background subtraction.

**Calibration.**—Calibration of peak current vs concentration was done with on-device glassy carbon microelectrodes. To predict how this would change the electrochemical interaction, impedance readings between GC-to-GC microelectrode (working electrode – GC; counter electrode – GC) were compared with traditional large platinum counter electrode to GC reading. Figure 7 shows that the difference in impedance is very small and statistically negligible. However, the Nyquist plot shows higher imaginary impedance, especially at lower frequencies, indicating a more capacitive effect in GC microelectrodes. These differences are most likely due to decreased distance between the counter and the working electrode and an additional capacitive effect on the GC-to-GC readings from the GC and the Nafion.

Figure 6 shows two additional effects of Nafion. One is an increase in charge storage capacity, from 14 mC/cm² to 119.4 mC/cm². The other is an increase in serotonin oxidation sensitivity; the red graph, before Nafion, shows no oxidation peak when scanned at 1.5 kV/s, while the Nafion coated electrodes show a slight inflection at about 0.7 volts, the oxidation of 5-HT was extrapolated.
After conditioning, GC-to-GC readings were used to calibrate the probes. As described under the Hardware for ECoG and serotonin detection section, cyclic voltammograms were taken at various 5-HT concentrations. As shown in Figure 8, the background current was subtracted from these cyclic voltammograms and the measurement of the peak current density at the oxidation voltage of 5-HT (~0.7 V) was plotted against concentration for a variety of input frequencies (i.e., 715 Hz, 940 Hz, and 1.1 kHz). The 715 Hz input, for example, with a scan rate of 1 kV/s and a range of −0.6 to 1.2 V per cycle gave a reasonable sensitivity and a lower detection limit of about 2.5 μM, which is clinically relevant concentration for human applications, but not low enough for serotonin concentrations in a rat model. As a result, the scan rate was then increased to 1.5 kV/s to improve the sensitivity. However, decreasing the voltage range to −0.4 to 1 V per cycle while running a scan rate of 1.5 kV/s lowered the detection limit to about 25 nM. Working with a scan rate at this higher frequency not only enables detection at lower limits but also allows for a more straightforward method of separating electrophysiological signals which are typically in the range below 100 Hz from the FSCV signal through frequency analysis.

It is important to note that, although we could detect 25 nM, the difference between 25 nM and 50 nM was very small and did not follow a linear trend. Thus, despite the linear regression predicting better sensitivity, in practice, the background subtraction only resolves a difference of 25 nM. While having sensitivity close to the lower detection limit is not ideal, these results are still comparable to the detection limits reported by others in literature. The Mayo Clinic’s WINCS device meant for human intraoperative cortical neurotransmitter detection reported detection of concatenation of serotonin from 250 μM - 10 μM in-situ. Other in-situ experiments reported a lower detection limit of 0.1 μM in mouse brain tissue. In-vitro experiments by Swamy et al., reported detection of 130 nM in vitro with single carbon fiber electrodes with a coating of single walled carbon nanotubes. Zachek et al., reported in-vitro results of 5 μM detection of serotonin with pyrolyzed carbon microelectrodes followed by another study.
where they reported detecting 500nM of serotonin in a rat cortex. Since the rat brain tissue and rat brain serotonin concentrations were detected between 100 nM and 500 nM, concentrations much higher than our lower detection limit, we expect that our microelectrode array will still sufficiently detect serotonin in-vivo. We, therefore, submit that these sets of data points, despite noise and artefacts, represent an important improvement in serotonin detection limits.

**Coupled electrophysiological and neurochemical detection.**—Figure 9 shows coupled detection FSCV results before and after background subtraction at 5 μm. This concentration was selected as a representative one and the data generated for all other concentrations varying from nanomolar to micromolar ranges showed similar responses. As a natural consequence of the nature of ECoG signals, coupled detection resulted in noisy cyclic voltammograms, an example of which is shown in Figure 9a. A basic low-pass filtering was first carried out on the raw data to extract the ECoG over several cycles. This was followed by background subtraction that revealed a peak current at about 0.7 V as shown in Figure 9b. In the case of nanomolar range concentrations, the noises generated were significant enough to make it difficult to discern the oxidation peak with high confidence. This highlights the fact that a more refined filtering may be required in-vivo in order to detect concentrations in the nanomolar range. However, at the same time, it is important to remember that a good part of the noise and artefacts are a consequence of the ECoG signal read separately in-vivo *apriori* and subsequently applied as an external voltage input. In other words, directly read in-vivo ECoG signals will be clearly devoid of some of the additional noises introduced in these sets of experiments suggesting the noise problem could be substantially lower than what these in-vitro experiments indicate. Between Figures 9 and 10, it is clear that the hardware and GC microelectrodes used in this study enable simultaneously and real-time recording of electrochemical and electrophysiological signals through the same microelectrode. Further, while some refinement in the filtering may be needed, the data still confirms the premises that these two signals that were recorded simultaneously through the same microelectrode can be further manipulated to break them down to both components.

A key test for establishing the validity of the approach reported in this study is to determine if the ECoG data collected directly in-vivo...
from an animal model and the one re-read through cyclic voltammetry during simultaneous electrical and electrochemical recording are similar. For this, we carried out the following. The ECoG data extracted from the working electrode was filtered with a lowpass filter at 30 Hz, which corresponds to the range for alpha and beta waves. It has to be noted that, since most ECoG signals occur below 100 Hz, only a small amount of ECoG spectrum is included in the 1ms long FSCV that was run. This corresponds to about 100 cycles of FSCV that were run at 1.5 kV/s scan rate, simultaneously with ECoG input. Since the impedance is nearly linear at low frequency, the current density was then multiplied by the area and impedance to obtain the voltage. In parallel, the same filter was applied to the original in-vivo ECoG data and focused on the 1 second. These two sets of data were then plotted in Figure 10 where the filtered ECoG data collected directly in-vivo and re-read through cyclic voltammetry are demonstrated to be essentially similar except a small shift due to noise indicating that FSCV and the sampling rates used did not inhibit the capture of the ECoG data. This is an important observation validating the approach of this experiment despite the small artifacts from the raw CV data (label a & b) that show up in the filtered ECoG data. We suspect that these artifacts are due to small drifts in the electrochemical cell; but on such a small scale, the analysis of the ECoG would likely not be altered.

In addition to these artefacts, we observed that a 10x fold increase in amplitude did occur due to the amplifier settings in the electrometer potentiostat (the raw ECoG data was multiplied by 10 to show a better comparison); this makes the data noisier than what we would expect in-vivo. Furthermore, for in-vivo applications, we do not expect to continuously run the FSCV, so drifts in the electrochemical signal could potentially be much smaller and can be extrapolated from time data if necessary. In general, the effect of noise and artefacts in such relatively complicated system of simultaneous recordings along with potential stimulations is expected and will always be a concern. However, we think that the results reported in this paper are encouraging given that GC microelectrodes have higher signal-to-noise than typical thin-film microelectrodes and will, therefore, be potentially less affected by noise than thin-film metal microelectrodes.

Further, while not performed in this experimental set-up, the microelectrode array design allows for at least 6 sets of electrochemical data to be obtained offering a richer data set that offers more complete and statistically significant information. In this arrayed set-up, if every other electrode is used as a counter electrode, then each adjacent electrode can be used to detect serotonin and electrophysiological data giving spatial and temporal data about the serotonin and its effect on the electrophysiological signal. In addition, through immobilization of enzymes through hydrogen or covalent bonding of functional groups to the surface of these GC microelectrodes, additional neurotransmitters such as GABA (gama-aminobutyric acid), glutamate dehydrogenase, and epinephrine can be detected amperometrically by the same probe introduced in this study. In this case, selective immobilization can be done on different microelectrodes, allowing for detection of multiple neurochemicals and, hence, offering much more insight into the electrophysiological signals.

**Conclusions**

The results presented here prove that glassy carbon microelectrodes generated from a polymer precursor can be used to detect serotonin at concentrations as low as 25 nM and with 75 μM resolution, resolution lower that what is presented in the current literature. The following summarize the key achievements reported in this paper:

1. We introduced a GC microelectrode probe that is supported on a flexible polyimide base that allows easy bending of the electrode shafts for easy and safe placement at the spinal cord at a depth of 1 mm, a depth necessary to reach motor pools.
2. This device’s ability to bend and penetrate marks the first reported work in the literature of penetrating GC microelectrodes. Previous reports were limited to ECoG readings from the surface of the cortex.
3. The use of Nafion prevents surface adsorption during electrochemical detection; thereby allowing repeatability and stability of results.
4. The ability to detect serotonin in real-time within 25 nM - 1 μM concentration range with resolution of 25 nM while simultaneously recording ECoG electrical signals.
5. The ability to obtain a linear calibration curve using electrodes from a single array indicates that not only can the device stimulate electrically and detect neurotransmitters, but it can also stimulate at a current density of 2.5 A/cm² (360 μA) and voltage range of at least −0.6 V to +1.2 V with remarkable stability.
6. Furthermore, at a scan rate of 1.5 kV/s, we were able to detect 5H-T in the presence of noise and electrophysiological signal. This same recording has also been able to obtain low frequency electrophysiological signal.

Further, in a recently published paper, we had further shown a direct comparison between thin-film and GC microelectrodes and demonstrated that – due to a higher charged ions diffusion in GC – the Warburg impedance in GC is higher giving rise to faster response time and electrokinetics. Taken together, therefore, these outcomes provide a compelling case for the use of probe made of GC microelectrodes as an ideal real-time coupled electrical and electrochemical detection platform.

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