Published in final edited form as:

Analyst. 2017 August 07; 142(16): 2912–2920. doi:10.1039/c7an00508c.

An Implantable Multimodal Sensor for Oxygen, Neurotransmitters, and Electrophysiology during Spreading Depolarization in the Deep Brain

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Abstract

Brain tissue injury is often accompanied by spreading depolarization (SD) events, marked by widespread cellular depolarization and cessation of neuronal firing. SD recruits viable tissue into the lesion, making it a focus for intervention. During SD, drastic fluctuations occur in ion gradients, extracellular neurotransmitter concentrations, cellular metabolism, and cerebral blood flow. Measuring SD requires a multimodal approach to capture the array of changes. However, the use of multiple sensors can inflict tissue damage. Here, we use carbon-fiber microelectrodes to characterize several aspects of SD with a single, minimally-invasive sensor in the deep brain region of the nucleus accumbens. Fast-scan cyclic voltammetry detects large changes in oxygen, which reflect the balance between cerebral blood flow and energy consumption, and also supraphysiological release of electroactive neurotransmitters (i.e., dopamine). We verify waves of SD with concurrent single-unit or DC potential electrophysiological recordings. The single-unit recordings reveal bursts of action potentials followed by inactivity. The DC potentials exhibit a slow negative voltage shift in the extracellular space indicative of wide-spread cellular depolarization. Here, we characterize the multiple modalities of our sensor and demonstrate its utility for improved SD recordings.

Graphical abstract

Implantable 5- μ m sensor is characterized for simultaneous measurements of oxygen, dopamine, and electrophysiology in the deep brain during spreading depolarizations.

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Author contributions

C.N.H carried out data collection and analysis and wrote the manuscript. J.A.J developed the convolution-based method and the associated analysis software. M.D.V. made hardware adjustments, enabling DC potential recordings. M.D.V. developed software for data acquisition and data analysis of DC potentials. R.M.W. conceived the sensor. All authors read and approved the final version of this manuscript.

Notes

The authors declare no competing financial interest.

Keywords

spreading depolarization; voltammetry; convolution; dopamine; oxygen; DC potential

Introduction

Spreading depolarizations (SD) are waves of widespread cellular depolarization that occur with pathologies such as traumatic brain injury, stroke, hemorrhage, and migraine with aura.¹ These waves slowly propagate through neural tissue (2–6 mm/min) and cause neurotransmitter release, ion gradient imbalance, and increased energy demand.^{2,3} The quantity and duration of SD waves following the initial injury positively correlate with final infarct size.^{4–7} Conversely, treatments that minimize the occurrence of these waves prove to be neuroprotective in rodent experimental models.^{4,7–10} As such, SD events in compromised tissues can be both a marker for delayed cell death and a possible target for therapeutic intervention.

SD waves are accompanied by ~10 fold increases in extracellular potassium ions, as well as a movement of sodium, calcium, and chloride ions, along with water, into the cell.¹¹ This ion flux results in cellular depolarization and subsequent release of excitatory amino acids and monoamines.^{12,13} As a consequence, neurons increase metabolic rates to both restore ion gradients and also buffer the extracellular space against excitotoxic neurotransmitters. In healthy tissue, blood vessels dilate to increase blood flow, remove toxic buildups of species, and deliver oxygen and glucose to replenish energy substrate stores.³ However, under pathological conditions SD is accompanied by decreased cerebral blood flow. This prolongs the duration of ion imbalance and oxygen starvation leading to cell death.³

Multimodal recordings are extensively used for SD studies due to the wide array of chemical, morphological, and hemodynamic changes that occur.¹⁴ Thus, many current methodologies rely on multiple invasive sensors that may contribute to tissue damage.^{15–17} Improved, single-implant devices recently detected simultaneous changes of glucose and lactate in real time.^{18,19} However, these sensors do not provide parallel electrophysiological measurements, which verify SD. As such, there remains a need in the field of SD research for a sensor that can detect both chemical and electrophysiological phenomena with minimal invasiveness that permits access to the entire brain.

Here, we describe a single carbon-fiber microelectrode sensor to simultaneously record real-time changes in oxygen, dopamine, and electrophysiological activity. The 5- μ m dimension

of the electrode allows for real-time characterization of SD in both superficial and deep brain regions. In agreement with previous studies, single-unit activity revealed that bursts of cell firing occurred at the onset of the SD wave, followed by a period of inactivity, allowing verification of the SD waves.^{20, 21} We also separately recorded a negative shift in DC potential of the extracellular space arising from widespread depolarization of cells, traditionally used to verify SD.^{22–24} Further, using FSCV we detected large oxygen changes and supraphysiological dopamine release in the nucleus accumbens (NAc). Coupling FSCV with electrophysiology provides a minimally-invasive, multimodal recording system, capable of monitoring SD waves in traditionally studied cortical areas as well as deeper brain regions.

Methods

Chemicals

All chemicals were used as received from Sigma-Aldrich (St. Louis, MO). Urethane was dissolved to 50/50% w/v in bacteriostatic 0.9% NaCl. KCl was dissolved in deionized water. A physiological buffer was prepared for calibration experiments containing 3.25 mM KCl, 145 mM NaCl, 1.25 mM NaH₂PO₄, 1.2 mM CaCl₂, 1.2 mM MgCl₂, 2 mM Na₂SO₄, and 15 mM Trizma® HCl.

Simultaneous Electrochemical and Electrophysiological Data Acquisition

A switched headstage controller and two-electrode system consisting of a carbon-fiber working electrode (~75- μ m exposed length) and Ag/AgCl reference electrode were used for all studies, as described previously.²⁵ HDCV (High Definition Cyclic Voltammetry, UNC-Chapel Hill, NC, USA) software program controlled voltage applied to the carbon-fiber microelectrode and recorded resulting currents. Electrochemical data was displayed as a color plot where the x-axis is time, the y-axis is the applied voltage, and current is shown in false color. Cyclic voltammograms (CVs) or current-over-time traces are obtained from the color plot by taking either a vertical or horizontal section, respectively.

To detect both dopamine and oxygen with FSCV, an oxygen-sensitive waveform was employed (Fig. 1A). The applied potential scanned from 0 to 0.8 V, then to -1.4 V before returning to a 0 V holding potential at a rate of 400 V/s every 200 ms. The holding potential was applied to the electrode for 5 ms preceding and following the application of the waveform to allow amplifier settling. Between CV recordings, the amplifier connected to the carbon-fiber electrode was switched to a voltage follower to record electrophysiology. Digitizer software (Plexon, Dallas, TX) recorded single-unit action potentials and Offline Sorter (Plexon, Dallas, TX) was used to remove interfering signals and group units using principal component analysis.

DC potential electrophysiological recordings required modifications to the headstage design (UNC Electronics Facility) so that the voltage at the carbon-fiber electrode first passed through a unity gain voltage-follower, then a constant voltage-following amplifier (Tektronix AM 502 Differential Amplifier, Beaverton, OR), and finally a low-pass filter the data from DC to 100 Hz (gain $\times 100$). In-house software (UNC Electronics Facility) was temporally

aligned to voltammetry data by a TTL-pulse. We continuously recorded the voltage output from the amplifier at 10 kHz. A MATLAB (Natick, MA) script averaged 30 data points occurring 20 ms before application of the waveform to obtain one voltage value every 200 ms. This minimized noise and allowed the amplifier to settle after applying the voltammetric waveform.

Signal Verification

The extensive redistribution of ions that occurs during SD produces significant capacitive peaks in the recorded CVs, which occur simultaneously with signals of interest. To verify the electrochemical measurements of oxygen, we compared FSCV responses to traces obtained with amperometry, a technique that is immune to capacitive changes.²⁶ We recorded two SD signals obtained at the same location with FSCV using the oxygen-sensitive waveform, and then made amperometric measurements, at the same location, of an additional two SD waves by holding the carbon fiber at -1 V to reduce oxygen.

To verify dopamine signals we used a convolution-based method to measure and remove non-faradaic components from the CVs.²⁷ For this method, the waveform was also scanned at 400 V/s. The scans were preceded by a 1.5-ms step from -0.4 to -0.3 V and back to -0.4 V for 1.5 ms. This potential step was used to compute the impedance of the carbon fiber immediately before collection of the cyclic voltammogram generated by a triangle ramp from -0.4 V to $+1.3$ V that returns to -0.4 V (Fig. 1B). The UEI filtering was set to 10 kHz to avoid filtering artifacts during application of the step function. An in-house LabVIEW (National Instruments, Austin, TX) program uses the electrode's current response to the $+0.1$ V step to predict and remove interfering impedance effects from the measured CVs, revealing the faradaic signal.²⁷

Animal Care and Stereotaxic Surgery

All procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill. Male Sprague-Dawley rats (350–550 g, Charles River Laboratories) were anesthetized by intraperitoneal injection of urethane (1.5 g/kg) and placed in a stereotaxic frame (Kopf, Tujunga, CA). The scalp was excised to expose the skull. Burr holes were drilled for insertion of the electrode and the stimulus to generate SD. All coordinates are in reference to bregma (Paxinos and Watson, 2007). Coordinates for the carbon-fiber recording electrode were $+2.2$ mm anterior-posterior (AP) and $+1.7$ mm medial-lateral (ML). The recording electrode was lowered -0.8 to -2.1 mm dorsal-ventral (DV) for motor cortex studies and -6.9 to -7.4 mm DV for those in the NAc. For single-unit recordings, the DV coordinate of the electrode was optimized for a location where spontaneous neuronal firing was detected. A chloridized silver wire served as the reference electrode and was fixed in the contralateral hemisphere with a stainless steel screw. Burr holes were drilled at -0.8 AP, $+0.8$ ML; -0.8 AP, $+3.2$ ML; and -2.8 AP, $+1.7$ ML to allow for pinpricks or microinjections of KCl to stimulate waves of SD 3–5 mm away from the recording site. Pinpricks were delivered first with 27- or 22-G hypodermic needles to 7.5 mm DV. If this technique failed to produce a SD recorded at the carbon-fiber electrode, a stronger stimulus of a KCl microinjection was used. A 33-G infusion needle (Plastics One Inc., Roanoke, VA) attached with plastic tubing to a 10- μ L Hamilton syringe was used to

manually inject 2 μ L of KCl, at a concentration of 0.25 or 1 M, using the lowest required for initiating a SD. Stimulations were repeated every 20 minutes to allow for recovery between SDs.

Calibrations

Electrodes were post-calibrated in an air-impermeable flow-injection analysis system to obtain sensitivity values for oxygen and dopamine. We determined calibration factors of 1.12 nA/ μ M for dopamine at its oxidative peak (+0.65 V) and -0.3 nA/ μ M for oxygen at its reductive peak (-1.33 V) for the oxygen sensitive waveform; 7.95 nA/ μ M dopamine for the convolution-based method waveform; and -0.059 nA/ μ M for oxygen using amperometry at -1 V. The current values recorded are converted to concentrations with the calibration factors. The factors are negative for oxygen because its reduction current is used for quantification.

Resistance recordings of electrodes were made with Axopatch 200B patch clamp amplifier (Axon Instruments, Foster City, CA).

Statistical Analysis

Two-tailed, paired *t*-tests and Pearson's correlation tests were performed with GraphPad Prism 4 (GraphPad Software, San Diego, CA) and $p < 0.05$ was considered statistically significant.

Results and Discussion

Simultaneously obtained oxygen, dopamine, and DC potentials during SD

Recordings of widespread cellular depolarization serve as the gold-standard for identifying SD events.^{22, 23} Cells fire bursts of action potentials at the onset of the depolarizing wave and then remain in a quiescent state for several minutes as energy stores are replenished and ion gradients are reestablished.^{20, 21} In compromised tissue, cells may be electrically silent, but still exhibit a depolarization. In both instances, a negative shift in the DC potential occurs, indicative of widespread cellular depolarization.^{14, 22–24} Additionally, recordings of DC potential shifts during SD events provide a quantitative measure of the duration of the tissue depolarization. Therefore, we modified our system to record constantly the voltage at the carbon-fiber electrode through a separate DC-coupled amplifier, permitting measurement of extracellular DC potentials.

Fig. 2 shows the results of our multimodal sensing capability, with simultaneous oxygen, dopamine, and DC potential recordings. Oxygen and dopamine concentration traces are obtained from the currents displayed in false color in the color plots at their respective reduction or oxidation potentials. DC potentials are recorded simultaneously, using a separate hardware filter. Our combined approach realizes a need in the field of SD research for an improved characterization method, amenable to recordings in compromised or healthy tissue in all brain regions. Additionally, the carbon-fiber microelectrode is much smaller (5- μ m diameter) than traditionally used microdialysis sensors^{13, 15}; probes of this size do not elicit SD upon implantation, which can alter the tissue's responsivity.²⁸ The use of multiple

sensors not only damages tissue, but complicates the temporal alignment of measurements. Other studies with combined electrophysiology and dopamine recordings have been unable to discern the difference in onset time for the two signals.^{12, 29, 30} With our time-locked recordings at high temporal resolution (5 Hz), we found that the DA signal onset followed the DC potential onset by 6.8 ± 0.9 s (average $\pm$ SEM, $n = 8$ subjects).

Investigators in the voltammetry field use both conventional carbon-fiber electrodes as well as epoxy-sealed electrodes with improved glass-fiber seals.³¹ The latter are preferred for voltammetry because they have lower background currents. However, the epoxy-sealed electrodes distorted the shape of the DC potential changes because of their stronger capacitive coupling to the solution (Fig. 2A,C,E). Thus, the DC shifts appeared multi-phasic and derivative-like, similar to AC-filtered recordings.²³ We evaluated the capacitive and resistive characteristics of both electrode types by applying potential steps (Fig. 2C,D,E). Non-epoxy sealed electrodes preserved the shape of the negative DC shifts during *in vivo* SD recordings due to their decreased “leak” resistance, caused by imperfections in the glass-fiber seal (Fig. 2B,D,E). However, the magnitude of the recorded potentials is attenuated due to the capacitive elements selecting against slow frequency changes. The epoxy-sealed electrodes ($n = 7$) recorded shifts of 5.7 ± 0.6 mV with typical RMS noise of 133 μ V; and, the non-epoxy electrodes ($n = 6$) recorded shifts of 2.3 ± 0.7 mV with noise levels of 19 μ V. Though the two electrode types were unable to record SD events at the same location in any given subject, the electrochemical and electrophysiological data for both are consistent with expected SD patterns (*vide infra*).

Repeated waves of SD yield highly reproducible signals

A pinprick or KCl stimulus, delivered at 20-minute intervals, elicited SD waves 3–5 mm away from our recording electrode. Consistent with the slow speed of travel of SD waves, the electrochemical and electrophysiological signals appeared at our sensor about 2 minutes after stimulation.^{2, 3} The initial event observed was an increase in single-unit firing or decrease in DC potential. This was followed by an array of large, overlapping signals in the color plot of the cyclic voltammetry recordings, lasting ~1–2 minutes (Fig. 3A,B). These results conform to the expected supraphysiological surge of chemical and electrical activity that radiates from a traumatized brain region, which includes concomitant shifts in ions, pH, oxygen, and neurotransmitters.^{2, 3, 11, 12, 32–34} For a given location, successive SD signals were very similar (Fig. 2B), consistent with the “all-or-none” characteristic² of the depolarizing wave. This allows for within animal controls. The time to SD onset following stimulation typically varied by ~20s; the concentration traces in Fig. 2B are aligned to the SD onset.

Interpretation of SD signals in FSCV measurements

FSCV produces multivariate data sets, which allow for identification of multiple signals and interfering currents. The most prominent features of SD in the background-subtracted CVs are capacitive peaks following the switching potentials (SPs) of the applied waveform (+0.8 V and –1.4 V for the oxygen-sensitive waveform, Fig. 4). These tend to obscure the current from the faradaic reactions of oxygen and dopamine. A large background current is generated by charging the electrical double layer when using high scan rates, as in FSCV.³⁵

However, the massive change in ionic concentrations during SD and the adsorption of ions to the electrode alters the double-layer capacitance, thus changing the background current.^{27, 36} The altered capacitance manifests most strongly as peaks at the switching potentials of the background-subtracted CVs because the polarity of the scan reverses, necessitating the discharge and recharge of the capacitance. The capacitive peaks are then time-delayed because of the low-pass filtering of the UEL.

Another component of the electrochemical SD signal arises from pH-dependent redox of quinone moieties on the carbon-fiber surface (Q-peak, Fig. 4).^{36, 37} The electrochemical reaction of quinone surface groups involves electrons and protons, and contributes current to the background signal. Consequently, the change in pH that occurs during SD events^{32, 33} shifts the oxidation and reduction potentials of this process, producing prominent signals in the background-subtracted color plot. The first peak of the redox couple overlaps the dopamine oxidation current and the second peak is more well-defined at -0.1 V on the reductive scan.^{36, 38}

SD produces capacitive and pH signals of a confounding magnitude, not seen in previous FSCV literature.^{38–40} Interfering currents in color plots are commonly resolved from catecholamine signals through principal component regression analysis (PCR).⁴¹ However, the large magnitude and temporal overlap of the various responses in SD precludes obtaining the independent standards required to build a training set for PCR.^{41–43} Therefore we employed other methods to verify dopamine and oxygen currents.

Varied temporal evolution of the overlapping signals allows for dopamine identification

CVs taken during the wave of SD (Fig. 4B) do not resemble those for dopamine, due to the overlapping ionic- and pH-based signals. However, we took advantage of the different temporal characteristics of the signals for dopamine oxidation and the interfering currents to obtain an improved CV. The background signal in FSCV is typically set at a time where there are no significant changes (i.e., quiescent baseline). In Fig. 5, the background for subtraction is instead set at a time during the SD where the interfering currents (SPs and Q-peak) are near maximum and the dopamine signal has partially decayed. Most of the interfering signal is subtracted, generating a CV with the characteristic oxidative and reductive peaks assigned to a dopamine concentration increase (Fig. 5B, black CV). Although this approach yields an improved CV, the concentration trace for dopamine still contains other signal components.

Convolution-based prediction improves dopamine CVs and concentration profiles

We employed a convolution-based method with enhanced dopamine sensitivity and selectivity to verify dopamine release currents during SD.²⁷ The negative holding potential (-0.4 V) pre-concentrates dopamine at the electrode surface, and the extended positive limit ($+1.3$ V) prevents fouling of the electrode surface and enhances adsorption of dopamine.⁴⁴ The $+0.1$ V potential step probes the impedance changes occurring at the time of each waveform application, and convolution of the triangular wave with the derivative of this response predicts the corresponding background signal (or generated charging current) in the existing impedance state. This enables removal of the interfering, capacitive (nonfaradaic)

currents that arise from ionic changes that are a hallmark of SD (Fig. 6). After subtraction of convolution-based predictions of non-faradaic current, the color plot and CV clearly show the redox peaks indicative of a large increase in dopamine (compare CVs in Fig. 4B and Fig. 6C to dopamine CV in Fig. 3 of REF 44).⁴⁴ We obtained peak values of $8.2 \pm 1.7 \mu\text{M}$ dopamine ($n = 7$ subjects), which agrees with recordings made during SD in the striatum with less selective voltammetric techniques and, more recently, with microdialysis.^{12, 29, 30} It is important to keep in mind that calibration curves for dopamine with FSCV deviate from linearity above $\sim 5 \mu\text{M}$,⁴⁴ which could lead to an underestimation of the concentration. Also, during SD, the extracellular space shrinks, causing an enhanced increase in concentration for a given increase in quantity of a species.³ However, this concentration is orders of magnitude larger than physiological transients (100–200 nM),^{45–47} highlighting the supraphysiological nature of this phenomenon.

The difference in dopamine peak current before and after convolution-based data treatment was $14.8 \pm 2.2\%$, revealing non-trivial contribution from capacitive currents. The quantification accuracy over the full time course is improved (Fig. 6D), which is vital to investigations of release dynamics. Few studies examine the effects of monoamines in SD, though dopamine is implicated in ischemic damage of catecholaminergic nerve terminals^{48–50} and serotonin receptor activation contributes to the hyperemic response during cortical SD.⁵¹ FSCV is ideal for future studies investigating these electroactive neurotransmitters.

Amperometry verifies selectivity for oxygen measurements in FSCV during SD

FSCV is a more selective electrochemical technique than amperometry, as it records currents generated over a range of potentials instead of one potential. The CV thus aids in differentiating between the array of reversibly and irreversibly oxidized species in the brain such as dopamine, norepinephrine, serotonin, ascorbic acid, DOPAC, and uric acid.⁴⁴ However, as shown here, the FSCV measurements during SD are complicated by the nonfaradaic currents generated by ionic changes. Amperometric detection of oxygen at carbon electrodes is highly selective due to the absence of *in vivo* compounds reducing at similar potentials (-0.6 to -1.0 V), the immunity to nonfaradaic signals, as well as its insensitivity to changes caused by pH or temperature fluctuations.^{26, 52, 53} For these reasons, amperometry is widely used to measure *in vivo* oxygen.^{17, 26, 54, 55} Therefore, we employed amperometry to confirm that oxygen currents were devoid of interference.

The same carbon-fiber electrode was used for both FSCV and amperometry recordings during SD within subjects. Epoxy-sealed electrodes must be used for amperometric recordings to avoid generating current due to the change in resistance of the extracellular media. We compared two oxygen traces obtained by FSCV during SD to two consecutive oxygen traces recorded with amperometry (Fig. 7). The peak concentrations recorded with the two methods were not significantly different (two-tailed, paired *t*-test, $p = 0.40$, $n = 5$), while the concentration-over-time traces exhibited a significant correlation (Pearson's $R = 0.87$, $p < 0.0001$, $n = 5$), confirming that FSCV oxygen traces were free from interfering currents.

Conclusion

SD is involved in pathological processes and promotes lesion growth in compromised neural tissue. Characterizing SD waves is crucial to improve diagnostics and develop therapeutic interventions. Due to the array of changes occurring during SD, multimodal sensing is employed which often requires multiple probes, complicating experimental procedures and producing greater tissue damage. We have optimized FSCV at carbon-fiber microelectrodes for minimally-invasive recordings of oxygen and neurotransmitters during SD with simultaneous single-unit or DC potential electrophysiological verification. Substantial, overlapping signals are produced due to the extensive ionic, pH, oxygen, and neurotransmitter changes during SD. Though we were unable to use PCR for signal authentication, we verified the recordings of oxygen and dopamine by amperometry and a convolution-based method, respectively. As proof-of-concept, we recorded signals of SD in the NAc, revealing large oxygen changes and supraphysiological release of dopamine during the depolarizing wave. FSCV is a powerful technique for minimally-invasive, multimodal recordings of chemical and electrophysiological changes during SD in all brain regions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Douglas C. Kirkpatrick for electrode resistance data used in Figure 2C,D,E.

Funding

This research was supported by a grant from the NIH (DA032530 to R.M.W.).

Abbreviations

CV	cyclic voltammogram
DA	dopamine
FSCV	Fast-scan cyclic voltammetry
NAc	nucleus accumbens
PCR	principle component regression
O₂	oxygen
SD	spreading depolarization
SPs	switching potentials

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.