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Selective and Mechanically Robust Sensors for Electrochemical Measurements of Real-Time Hydrogen Peroxide Dynamics *In Vivo*

Leslie R. Wilson, Sambit Panda, Andreas C. Schmidt, and Leslie A. Sombers*

Department of Chemistry, North Carolina State University, Raleigh, North Carolina 27695, United States

Abstract

Hydrogen peroxide (H_2O_2) is an endogenous molecule that plays several important roles in brain function: it is generated in cellular respiration, serves as a modulator of dopaminergic signaling, and its presence can indicate the upstream production of more aggressive reactive oxygen species (ROS). H_2O_2 has been implicated in several neurodegenerative diseases, including Parkinson's disease (PD), creating a critical need to identify mechanisms by which H_2O_2 modulates cellular processes in general, and how it affects the dopaminergic nigrostriatal pathway, in particular. Furthermore, there is broad interest in selective electrochemical quantification of H_2O_2 because it is often enzymatically-generated at biosensors as a reporter for the presence of non-electroactive target molecules. H_2O_2 fluctuations can be monitored in real time using fast-scan cyclic voltammetry (FSCV) coupled with carbon-fiber microelectrodes. However, selective identification is a critical issue when working in the presence of other molecules that generate similar voltammograms, such as adenosine and histamine. We have addressed this problem by fabricating a robust, H_2O_2 -selective electrode. 1,3-phenylenediamine (mPD) was electrodeposited on a carbon-fiber microelectrode to create a size-exclusion membrane, rendering the electrode sensitive to H_2O_2 fluctuations and pH shifts, but not other commonly studied neurochemicals. The electrodes are described and characterized herein. The data demonstrate that this technology can be used to ensure the selective detection of H_2O_2 , enabling confident characterization of the role this molecule plays in normal physiological function, as well as in the progression of PD and other neuropathies involving oxidative stress.

Graphical abstract

*CORRESPONDING AUTHOR: PHONE: lasomber@ncsu.edu, 919-515-0320.

ORCID

Leslie A. Sombers: 0000-0002-0978-9795

Author Contributions

L.A.S. conceived of the work and designed the experiments. A.C.S., L.R.W., and S.P. collected and analyzed the data. All authors contributed to preparation of the manuscript.

Notes

The authors declare no competing financial interest.

Keywords

Oxidative Stress; Fast-Scan Cyclic Voltammetry; Carbon-Fiber Microelectrode; Phenylenediamine; Striatum

INTRODUCTION

ROS such as the superoxide radical ($O_2^{\cdot-}$), nitric oxide ($NO^{\cdot}$), and the hydroxide radical ($\cdot OH$), play important physiological roles in brain function. For instance, they serve as important byproducts of cellular metabolism under normal conditions, can serve as neuromodulators, and can become dysregulated in pathological conditions.^{1–5} Indeed, oxidative stress has been implicated in the initiation and progression of several neurodegenerative disorders, including the slow destruction of dopamine (DA) neurons in Parkinson's disease (PD).^{5–13} Direct measurements of ROS in brain tissue can significantly advance our understanding of the role that these molecules play in modulating brain function and dysfunction; however, the measurements are challenging due to the instability and relatively low concentration of many of these short-lived species. H_2O_2 is more stable than other ROS, it can accumulate to relatively high concentrations, and can readily diffuse through biological membranes. It can serve as a measure of oxidative stress, because more reactive species (i.e. $O_2^{\cdot-}$) are readily converted to H_2O_2 by enzymes such as superoxide dismutase. Furthermore, there is broad interest in quantitative detection of this molecule, because it is often enzymatically-generated at biosensors to serve as a reporter for the presence of non-electroactive species.^{14–19}

There are few analytical methods available for dynamic measurements of H_2O_2 and other ROS *in vivo*. Florescence imaging probes offer low limits of detection, but these generally suffer from poor chemical selectivity and irreversible activation.^{20,21} Additionally, quantitative analysis with this method can be problematic, due to the challenges of calibrating fluorescent dye intensity. Second-generation dyes for ROS are promising due to improved chemical selectivity; though, they are also limited by kinetics and temporal resolution.^{22,23} ROS in intact brain tissue can be measured using microdialysis sampling, by including spin trap reagents in the dialysate.²⁴ These are then separated offline by high-performance liquid chromatography and subsequently quantified. However, microdialysis is a diffusion-based (steady-state) sampling technique that typically operates in the range of minutes, preventing a second-by-second assessment of molecular dynamics. Furthermore, the microdialysis probe is generally on the order of 0.5–1 mm in diameter and 3–4 mm in

length. These are dimensions that can span multiple brain regions and cause significant damage to tissue in the sampling region, confounding results.

Electrochemical methods are perhaps the most promising analytical tools available to measure rapid chemical dynamics in the brain. These methods offer excellent temporal resolution and electrodes can be made on the micrometer scale²⁵, allowing specific brain regions to be targeted. FSCV is a differential technique frequently used with carbon-fiber microelectrodes to quantitatively monitor rapid neurotransmitter fluctuations in brain tissue.^{25,26} This method also offers qualitative information, because characteristics such as peak shape and position can be used to identify a given class of molecules. Real-time detection of H₂O₂ with FSCV has recently been accomplished in brain tissue, generating voltammograms with a single oxidation peak at ~1.4 V.^{27,28} However, care must be exercised when using voltammetry to detect H₂O₂ in live brain tissue, because other endogenous molecules such as adenosine^{29,30} and histamine^{31,32} also oxidize near 1.4 V, confounding the direct measurement of H₂O₂ when using this approach (Figure 1). Each of these molecules has an inherent and requisite function in the brain and, as such, selective measurements of each are essential. Many times, pharmacology can be used to verify analyte identity – administration of known pharmacology should alter the signal in a predictable way.³³ However, this approach to signal validation is precluded when distinguishing H₂O₂ from adenosine or histamine, as the dynamics of these molecules are presumably interlinked.^{3,34} Any molecule that increases cellular activity in the brain drives energy demand, which drives cellular pathways of energy metabolism and mitochondrial respiration to produce adenosine triphosphate and ROS.

In order to address this issue, we have developed a reproducible technique for the electrodeposition of 1,3-phenylenediamine (mPD) onto the carbon-fiber sensing surface. Electrodeposition of this polymer creates a size-exclusion membrane to reject larger molecular interferents that could be falsely identified as H₂O₂. The mPD membrane has been used extensively as a coating in both microdialysis sampling^{35–37} and electrochemical sensors^{38–40}, typically coupled with amperometric methods. The goal of this project is to fully characterize mPD-coated carbon-fiber microelectrodes for voltammetric measurements, and to then apply this tool *in vivo* to selectively measure real-time H₂O₂ fluctuations in the rat dorsal striatum.

EXPERIMENTAL SECTION

Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used as received, unless otherwise specified. *In vitro* electrochemical experiments were carried out in 0.1 M phosphate buffered saline (PBS) at a physiological pH of 7.4 and at room temperature. mPD solutions were prepared on the day of electrodeposition. Brain slice experiments used artificial cerebral spinal fluid (aCSF) saturated with 95% O₂ and 5% CO₂, at pH 7.4. aCSF consisted of 124 mM NaCl, 26 mM NaHCO₃, 3.7 mM KCl, 2.4 mM CaCl₂, 1.3 mM MgCl₂, 1.3 mM NaH₂PO₄, and 10 mM glucose. All aqueous solutions were made from double deionized water >18 MΩ·cm (Millipore, Billerica, MA).

Microelectrode Fabrication

Fused silica tubing (164.7 μm outer diameter/98.6 μm inner diameter) with a polyimide coating (Polymicro Technologies, Phoenix Arizona) was cut to 2 cm in length and placed in a bath of 70% isopropyl alcohol. A T-650/35 polyacrylonitrile carbon fiber was inserted through the tubing under a stereoscopic microscope. After insertion, the carbon fiber and silica were allowed to dry for 24 h. A seal was created using fast-hardening epoxy (McMaster Carr, Atlanta, GA) at one end of the silica tubing. An electrical connection with the carbon fiber was made using highly conductive silver epoxy (MG Chemical, Thief River Falls, MN) and a gold pin (Newark Element 14, Palatine, IL). This was allowed to dry for at least 24 h. The connection was insulated using fast-hardening epoxy and electrodes were subsequently placed in a 100° C oven to cure for 20 min. After drying, a second layer of insulation around the connection was made using liquid insulating tape (GC Electronics, Rockford, IL) and allowed to dry. Exposed carbon fibers were then cut to 100–150 μm under a stereoscopic microscope. Dual-microelectrode devices (DMEDs) were created by placing two microelectrodes less than 200 μm apart under a stereoscopic microscope and cementing them together using fast-hardening epoxy. For brain-slice experiments, the fused silica insulation was cut to 4 cm. All other aspects of the microelectrode fabrication protocol remained unchanged. “Injectrodes” were made under a stereoscopic microscope by positioning a 26 gauge, 10 mm long, microinjector cannula (Plastics One, Roanoke, VA) 0.5 mm above the exposed carbon fibers in a DMED and cementing with fast-hardening epoxy. After positioning, injectrodes were allowed to dry for at least 24 h before use.

Flow-Injection Apparatus

In vitro calibration was performed using a flow-injection apparatus. Individual microelectrodes were lowered into a custom electrochemical cell (North Carolina State University, College of Science Machine Shop) using a micromanipulator (World Precision Instruments, Inc., Sarasota, FL). A syringe pump (New Era Pump Systems, Inc., Wantagh, NY) supplied a continuous flow (1 mL/min) of PBS across both the working and reference (Ag/AgCl) electrodes. Three-second bolus injections of analyte were accomplished using a six-port HPLC valve and air actuator controlled by a digital valve interface (Valco Instruments Co., Inc., Houston, TX).

Electrochemical Data Collection

A waveform optimized for the electrochemical detection of H_2O_2 ²⁷ was applied to the carbon-fiber microelectrode. The potential window ranged from –0.4 V to +1.4 V, applied at 10 Hz using a scan rate of 400 V/s. The electrodes were conditioned with this waveform for 30 min prior to data collection. Potential application and current transduction were performed using a Universal Electrochemistry Instrument (UEI, University of North Carolina - Chapel Hill, Department of Chemistry Electronics Facility) for brain slice experiments or a WaveNeuro (Pine Research Instrumentation, Durham, NC) for *in vitro* and anesthetized experiments. These instruments were operated using HDCV software (University of North Carolina - Chapel Hill, Department of Chemistry Electronics Facility) to control waveform input and output. A 6363 PCIe card (National Instruments Corp., Austin, TX) was used to interface to a computer. Signal processing (background subtraction,

signal averaging and digital filtering (2-pole Sallen-Key Filter, 2 KHz)) were software controlled. All electrochemical data collection was performed within a custom-built Faraday cage.

Animal Subjects and Care

Drug-naïve, male Sprague–Dawley rats (275–300 g, Charles River Laboratories, Raleigh, NC) were allowed to acclimate to the facility for several days before experiments commenced. Animals were individually housed on a 12:12 h light/dark cycle with free access to food and water. Animal care and use was in complete accordance with the NC State University institutional guidelines (IACUC) and the *Guide for the Care and Use of Laboratory Animals*.

Brain Slice Preparation

Rats were deeply anesthetized with urethane (1.5 g/kg, intraperitoneal administration), decapitated, and the brain was rapidly removed (< 2 min). The brain was mounted and placed in a bath of cold aCSF saturated with 95% oxygen/ 5% carbon dioxide gas. Tissue was sliced (400 μ m) on a vibratome (World Precision Instruments, Sarasota, FL) and coronal slices containing the striatum were allowed to rest in the aCSF for at least 1 h before the start of an experiment. Brain slices were subsequently placed in a recording chamber (Warner Instruments, Hamden, CT), and superfused with continuously oxygenated aCSF buffer maintained at 34°C for at least another hour. DMEDs and bipolar tungsten stimulating microelectrodes (FHC, Neural micro Targeting Worldwide, Bowdoin, ME) were positioned about 100 μ m below the surface of the slice with the aid of a microscope (Nikon Instruments, Inc., Melville, NY) and micromanipulators (Scientifica Ltd., United Kingdom). Biphasic electrical stimulation consisted of five 500 μ A pulses at 60 Hz with a pulse width of 4 msec, generated with a DS-4 Biphasic Stimulus Isolator (Digitimer Ltd., Welwyn Garden City, England). Local drug application (microinfusion) was achieved using a 33 gauge microinjector needle (Plastics One, Roanoke, VA) positioned 100–200 μ m away from the working microelectrode(s) using a syringe pump (KD Scientific, Holliston, MA) with a flow rate of 26.5 μ L/min over 3 sec.

Anesthetized Animal Surgery

Rats were deeply anesthetized with urethane (1.5 g/kg, i.p.), and positioned in a stereotaxic frame (Kopf Instrumentation; Tujunga, CA). A heating pad (Harvard Apparatus, Holliston, MA) was used to maintain body temperature at 37°C. Holes for electrodes were drilled in the skull according to coordinates from the rat brain atlas of Paxinos and Watson⁴¹, relative to bregma. The DMED was placed in the dorsal striatum (anterior-posterior: +1.5 mm, medial-lateral: + 2.5 mm, dorso-ventral: –5.0 mm from the skull surface). The Ag/AgCl reference electrode was placed contralateral to the working electrode. Electrodes were cemented to the skull using acrylic dental cement (Lang Dental Manufacturing Company, Wheeling, IL). All microinfusions (saline and mercaptosuccinic acid, MCS) were performed as described above, but at a flow rate of 0.5 μ L/min for 1 min.

Data Analysis and Statistics

All data are shown as the mean $\pm$ standard error of the mean (SEM). To determine calibration factors, injections of four concentrations spanning a physiological range were performed in triplicate and peak oxidative currents were averaged. Differences between slopes were assessed using an analysis of covariance (ANCOVA) with Tukey's post-hoc test. Principal component regression (PCR) is a multivariate statistical method that was performed using HDCV Analysis software for quantitative determination of individual chemical contributors to the voltammetric data.^{42,43} Training sets consisted of four clean cyclic voltammograms collected for 2-sec bolus injections of DA (250–1000nM), H₂O₂ (20–80 μ M), and acidic shifts in pH (0.05–0.20 pH units), collected *in vitro* in the flow-injection apparatus. Paired t-tests were used to compare data collected before and after drug administration. One-way analysis of variance (ANOVA) with a Bonferroni's multiple-comparison post-hoc test was used to assess electrodeposition time. All statistical and graphical analyses were carried out using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA). In all cases, significance was designated at $p < 0.05$.

RESULTS AND DISCUSSION

Electrodeposition of mPD

Microelectrodes were individually placed into a solution of 5 mM mPD in PBS, and a triangular waveform (0.0 to +1.0 V versus Ag/AgCl, 5 V/s) was applied at 1 Hz to electrodeposit the mPD membrane on the carbon-fiber surface (Figure 2 A and B). Ideally, the mPD coating should exclude molecules found *in vivo* that are larger than H₂O₂, such as adenosine. To optimize electrodeposition time (1–60 secs), bolus injections of 80 μ M H₂O₂ and 2 μ M adenosine were run in a flow cell using a triangular waveform. The potential was scanned from –0.4 V to +1.4 V at 400 V/s and a frequency of 10 Hz, and the ratio of the peak oxidation currents was calculated. The data demonstrate that a 5 sec electrodeposition time was most effective at facilitating the preferential detection of H₂O₂ (Figure 2 C). Thus, this deposition time was used for the remainder of the study. At shorter electrodeposition times, adenosine wasn't effectively excluded from the electrode surface. With deposition times longer than 5 sec, adenosine was completely attenuated but the current generated in the oxidation of H₂O₂ was also substantially decreased. This was most likely due to increased polymer thicknesses that hindered diffusion of H₂O₂ to the electrode surface. Scanning electron micrographs of the uncoated and mPD-coated microelectrodes provide visual verification that a thin coating of the mPD polymer was electrodeposited onto the carbon surface with this approach (Figure 3 A and B).

Characterization of mPD membrane

A DMED allows two parallel electrodes to simultaneously experience the same solution (Figure 3C), permitting direct comparison of electrode performance. Physiological concentrations of adenosine⁴⁴, DA⁴⁵, histamine^{46,47} and H₂O₂²⁷ were sampled *in vitro*, along with a pharmacologically relevant concentration of mercaptosuccinic acid (MCS, Figure 4D–F). MCS is an irreversible inhibitor of glutathione peroxidase, an enzyme that protects the brain from oxidative damage by reducing free H₂O₂ to water. It is redox active and oxidizes at a potential close to that of H₂O₂ oxidation. The mPD coating effectively

excludes adenosine, histamine, DA, and MCS from the electrode surface, but allows H₂O₂ to permeate the polymer for detection. Notably, sensitivity to H₂O₂ at the coated electrode is decreased by about 30% as compared to detection at the uncoated electrode, likely due to the loss of active sites on the microelectrode surface and restricted diffusion through the polymer.

Voltammograms collected using the mPD-coated electrode exhibit a concentration dependent, acidic pH shift in response to MCS (Figure 4E), but the majority of the current due to inherent redox activity is eliminated. The mPD membrane cannot exclude a shift in pH, since both the hydronium (H₃O⁺) and hydroxide (OH⁻) ions are sufficiently small to diffuse through pores in the membrane and affect charging current generated at the electrode surface. A small acidic pH-shift is also evident in the voltammogram for H₂O₂ when using an mPD-coated microelectrode (Figure 4N). Protons are generated in the oxidation of H₂O₂ (Reaction 1), and their diffusion from the electrode surface appears to be slowed by the membrane.

Fortunately, this pH signal does not interfere with H₂O₂ detection, because it can be quantified and subtracted using PCR (Figure 5). A training set consisting of voltammograms for acidic pH shifts can be used to remove the pH signal from the raw data (black trace), leaving the residual signal corresponding to H₂O₂ (red trace). Overall, these results demonstrate that the mPD membrane acts as a size-exclusion polymer, allowing smaller species such as H₂O₂, H₃O⁺, and OH⁻ to diffuse to the electrode surface while effectively excluding larger molecules.

Membrane Stability

The stability of the mPD membrane was evaluated *in vitro* over the course of 4 h with a flow-injection apparatus using uncoated and mPD-coated microelectrodes bundled in a DMED. The peak current obtained in the oxidation of a bolus injection of 2 μM adenosine was plotted every 20 min (Figure 6). The currents collected across the 4 h recording session did not significantly deviate for either electrode type, demonstrating stable electrode performance. Color plots of raw data collected at the end of the experiment are shown (Figure 6B,C). These demonstrate both the integrity of adenosine detection on the uncoated electrode after extended use, and the ability of the mPD-coated electrode to continuously exclude this species (Figure 6B,C). The uncoated microelectrode maintained a mean current response of 38.2 ± 0.3 nA over the 4 h period; whereas the mPD-coated electrode recorded significantly less current in response to adenosine, with a mean of 1.3 ± 0.1 nA (Unpaired two-tailed t-test, ****p<0.0001, n=4).

H₂O₂ Measurements in Live Tissue

The capacity of the mPD coating to ensure selective measurements of H₂O₂ in tissue was evaluated by using a DMED to investigate the extracellular environment in a rat brain slice encompassing the striatum. A mild electrical stimulation was used to induce striatal DA

release and to increase local cellular activity, which should generate H_2O_2 by way of cellular respiration. As anticipated, the uncoated microelectrode detected a rapid increase in DA concentration as a result of vesicular release, as well as a small signal that was putatively assigned to H_2O_2 (Figure 7A). The mPD-coated microelectrode detected a small increase in H_2O_2 in response to the stimulation, but a DA signal was not observed (because DA is effectively excluded by the membrane, Figure 7C).

To pharmacologically validate the voltammetric signal attributed to H_2O_2 , 5 mM MCS was microinfused in the vicinity of the working electrodes (DMED). An electrical stimulation delivered 15 min later elicited significantly more current at the peak oxidation potential for H_2O_2 (~1.4V) at both microelectrodes, as compared to the stimulation before MCS, validating H_2O_2 identification (uncoated: paired two-tailed t-test, *** $p < 0.001$; $n = 5$; mPD-coated: paired two-tailed t-test, *** $p < 0.001$; $n = 5$). DA release was recorded only with the bare electrode. The mean DA concentrations recorded before and after application of MCS were not significantly different (data not shown, paired two-tailed t-test, $p > 0.05$; $n = 5$). Previous studies have reported a decrease in evoked DA release when H_2O_2 levels are amplified by MCS.^{28,48–51} However, those were completed in other preparations using different MCS concentrations and electrical stimulation parameters over a range of timescales.

A representative H_2O_2 concentration versus time trace collected at an mPD-coated microelectrode is shown in Figure 7E, and an overall analysis of amplitude, area, duration, and decay time (τ) for the H_2O_2 events recorded on both electrode types is shown (Figure 7F–I). Microinfusion of MCS, a glutathione peroxidase inhibitor, significantly increased event area. Analysis of event duration and τ demonstrates that this treatment increased the lifetime of H_2O_2 in the extracellular space. Overall, these data confirm that electrical stimulation locally elicits the generation of H_2O_2 , and that the mPD membrane ensures its selective detection by excluding larger species also elicited by the stimulation, including DA.

H_2O_2 Measurements in the Intact Animal

A DMED injectrode was used to selectively detect H_2O_2 and DA dynamics in the dorsal striatum of an intact rat. Sterile saline was microinfused into the recording environment and voltammograms were simultaneously collected at both bare and mPD-coated electrodes for 30 min. Next, 200 mM MCS was microinfused for 1 min at the same rate, and data were collected for another 30 min. H_2O_2 generation was recorded at both electrodes, with significant increases in the amplitude and area of H_2O_2 events clearly evident in the first 5 min after MCS administration (Figure 8A–C). Importantly, much of the current that was generated at the oxidation potential for H_2O_2 on the uncoated electrode is likely due to the oxidation of MCS itself. MCS generates substantial current at ~1.4 V (Figure 4D), it was directly introduced to the vicinity of the electrode, and attempts to distinguish the H_2O_2 contribution to the signal from that of MCS using PCR⁵² failed because the residual error tolerance threshold, Q_{α} , was greatly exceeded (Figure 8D). These data directly demonstrate the utility of the mPD coating when using pharmacological agents, such as MCS, which can directly interfere with the measurement.

CONCLUSION

The results presented herein clearly demonstrate that caution should be executed when analyzing data collected in the complex environment of the brain. FSCV allows for the direct quantification of endogenous H₂O₂ dynamics, and it is also useful for detecting enzymatically generated H₂O₂ using enzyme-modified carbon-fiber microelectrodes.^{14–17} There are certainly many instances in which PCR analysis is sufficient to distinguish H₂O₂ from interfering species evident in the collected data; however, selectivity is always a primary concern which using electrochemistry. Additional measures must be taken to ensure selectivity when there is not a clear distinction between electroactive species potentially contributing to the signal (i.e., when PCR fails). In this work, we have described a straightforward approach to modifying the electrode surface with a size-selective, mPD membrane to provide optimized sensitivity to H₂O₂ while excluding larger molecular interferents. We have determined that the mPD membrane is stable on the surface of the fiber for at least 4 h, and we have demonstrated its utility *in vivo*. This advance is important, because it will enable confident measurements describing the role that H₂O₂ plays in normal physiological function, as well as in the progression of neuropathies involving oxidative stress.

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Figure 1.

Multiple neurochemicals found endogenously oxidize at ~1.4V, the peak oxidation potential for H_2O_2 . Voltammograms are presented for (A) H_2O_2 (40 μM), (B) adenosine (2.0 μM), and (C) histamine (7.5 μM).

Figure 2. Optimization of mPD electrodeposition. (A) Electrodeposition waveform. (B) Representative current vs. time trace collected at +1.0 V during a 5 sec electrodeposition. (C) Ratio of peak oxidation currents collected for H_2O_2 and adenosine (ADO) with electrodes fabricated using various electrodeposition times. The electrodes created using the 5 sec electrodeposition performed significantly better than those created using other electrodeposition durations (One way ANOVA, Bonferroni's Multiple Comparison Test, $F(7,81)= 14.06$, **** $p<0.0001$, *** $p<0.001$, * $p<0.05$, $n=4$).

Figure 3. A dual-microelectrode device (DMED). (A) Scanning electron micrograph of an uncoated carbon fiber, and (B) a fiber after electrodeposition of the mPD polymer. (C) Graphical representation of a DMED.

Figure 4.

The mPD membrane ensures selective detection of H_2O_2 . Representative color plots with CVs inset (white) for various species detected on uncoated (left) and mPD-coated (middle) microelectrodes bundled in a DMED. mPD-coated microelectrodes effectively exclude all of these analytes except for H_2O_2 (M-O), as quantified with the calibration curves (right). The slopes (coated vs. uncoated) were significantly different for all panels (ANCOVA with Tukey's post hoc test, C: adenosine (ADO), $F(1,36)=306.07$, I: DA, $F(1,35)=418.75$, F: MCS, $F(1,36)=332.28$, L: histamine (HIST), $F(1,26)=325.33$, O: H_2O_2 , $F(1,36)=44.79$, $***p<0.0001$, $n=3-4$).

Figure 5. mPD-coated microelectrodes record an acidic shift in pH when monitoring H_2O_2 (black). A training set for acidic pH shifts was created (inset) and PCR was used to remove the pH contribution from the voltammogram, leaving the signal from H_2O_2 intact (red).

Figure 6. mPD membrane stability. (A) Maximum currents recorded simultaneously at bare and mPD-coated electrodes for the oxidation of 2 μ M adenosine, plotted every 20 min over a 4 h recording session. (B) Adenosine detection at the uncoated electrode was stable, and (C) the mPD-coated electrode consistently excluded adenosine. An unpaired two-tailed t-test was used to compare data collected at the last time point, (**** $p < 0.0001$, $n = 4$ DMEDs).

Figure 7.

Quantification of endogenous H₂O₂ in striatal brain slices. (A–D) Representative color plots showing H₂O₂ (*) and DA (‡) fluctuations simultaneously recorded on uncoated (top), and mPD-coated (bottom) electrodes in response to electrical stimulation (60 Hz, 60 pulses, 500μA) before (left) and after (right) local microinfusion of MCS. (E) The concentration vs time trace for H₂O₂ extracted from the representative data shown in (D). (F–I) Summary of H₂O₂ event amplitude (Δ [H₂O₂]), area, duration, and tau. MCS reliably increased the amplitude and duration of H₂O₂ events while slowing H₂O₂ clearance, pharmacologically validating the measurement. (Paired t-tests. Uncoated electrode: Amplitude, ***P<0.001; Area, p<0.05; Duration, **p<0.01; Tau, *p<0.05; mPD Coated electrode: Amplitude, ***p<0.001; Area, **p<0.01; Duration, **p<0.01; Tau, *p<0.05.)

Figure 8. Quantification of H_2O_2 events in intact brain tissue. Representative color plots simultaneously recorded on (A) bare and (B) mPD-coated microelectrodes upon local microinfusion of MCS (green arrow). Note the different scale bars for current (color). (C) Representative H_2O_2 concentration vs time traces extracted from (B) to demonstrate the effects of local microinfusion of saline (black) and MCS (red). MCS significantly increased the area and amplitude of the currents generated at ~ 1.4 V on the mPD coated electrode (see Table 1 for statistics). (D) At the bare electrode, the residual error tolerance threshold, Q_{α} , was greatly exceeded (note logarithmic scale).

Table 1

Local microinfusion of MCS increased the amplitude and area of H₂O₂ events recorded on the mPD-coated microelectrode, pharmacologically validating the measurement.

	Amplitude (μM)	Area (μM*s)
Saline (control)	4.9 ± 0.4	357 ± 111
MCS	48 ± 3	8608 ± 1606
t-statistic	t(3)=20.00	t(3)=4.820
p-value	***p<0.001	*p<0.05