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Development and Characterization of a Voltammetric Carbon-fiber Microelectrode pH Sensor

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Abstract

This work describes the development and characterization of a modified carbon-fiber microelectrode sensor capable of measuring real-time physiological pH changes in biological microenvironments. The reagentless sensor was fabricated under ambient conditions from voltammetric reduction of the diazonium salt Fast Blue RR onto a carbon-fiber surface in aprotic media. Fast-scan cyclic voltammetry was used to probe redox activity of the *p*-quinone moiety of the surface-bound molecule as a function of pH. *In vitro* calibration of the sensor in solutions ranging from pH 6.5 to 8.0 resulted in a pH-dependent anodic peak potential response. Flow-injection analysis was used to characterize the modified microelectrode, revealing sensitivity to acidic and basic changes discernable to 0.005 pH units. Furthermore, the modified electrode was used to measure dynamic *in vivo* pH changes evoked during neurotransmitter release in the central nervous system of the microanalytical model organism *Drosophila melanogaster*.

Introduction

Recently, there has been an interest in developing reagentless sensors to detect small pH changes in non-ideal environments.¹ Carbon-based sensing materials have been attractive substrates for this application since they are intrinsically biocompatible, conductive, and apt for surface modification. Indeed, ion-selective reporter molecules can be tethered onto a carbon surface through a variety of methods including chemical oxidation of the surface with corrosive acidic cocktails and plasma treatment,^{2, 3} physical adsorption of organic precursors,^{4, 5} and electrochemically-assisted covalent attachment via the oxidation of amines⁶⁻⁹ or the reduction of diazonium salts.¹⁰⁻¹⁶ Pioneered by Savéant and co-workers in the early 1990s, the reduction of aryl diazonium salts onto carbon surfaces is a well-characterized method for the selective *in situ* attachment of organic moieties.¹³ This mechanism involves the electrochemical generation of a solution radical from the diazonium modifier and subsequent covalent linkage to the carbon surface, which possesses marked stability to external stimuli.¹⁴

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Electrochemical measurements in the central nervous system (CNS) can be used to quantify redox-active chemical messengers such as catecholamines and indolamines, which are thought to play a fundamental role in the physiological and behavioral aspects of an organism. *In vivo* voltammetry with carbon-fiber microelectrodes has been used since the early 1970s to monitor chemical neurotransmission of these species in the CNS of various mammalian animal models.¹⁷⁻¹⁹ Neurosecretory events are often accompanied by a flux of endogenous species (e.g., H⁺, ascorbate, etc.) which can interfere with the voltammetric signature of targeted electroactive chemical messengers.^{5, 19-26} Of particular interest are pH fluctuations in the surrounding matrix, which are thought to occur as a result of metabolic processes that follow stimulated neurotransmitter release.^{20, 27-29} Indeed, Wightman and co-workers have reported on measuring very small acidic pH changes in rat brain slices subjected to electrically stimulated secretion with liquid membrane, ion-selective microelectrodes (ISMs).²⁰ With the emergence of new volume-limited, CNS-containing animal models such as the fruit fly, *Drosophila melanogaster*, comes the need to develop microanalytical tools capable of measuring pH fluctuations associated with neurotransmission.³⁰ We have previously reported on a system used to perform *in vivo* electrochemical measurements in the CNS of this exquisitely small animal model using fast-scan cyclic voltammetry (FSCV).^{31, 32} Herein we describe the development and characterization of a reagentless, biocompatible modified carbon-fiber microelectrode sensor for measuring dynamic physiological pH changes associated with *in vivo* neurotransmission.

Voltammetric pH-sensors measure changes in the redox-potential of a surface-bound, electrochemically active species as a function of pH. This methodology for measuring pH has been demonstrated with quinone-based surface modification of various electrodes.³³⁻³⁶ In a recent study by Tommos and co-workers, the formal potential of a surface-bound quinone on a gold electrode shifted to more negative potentials with increasing solvent basicity.³³ Although a variety of quinone-modified electrodes have been reported to respond to pH, few have been developed on biocompatible materials that exhibit activity in a physiologically relevant pH range.^{1, 37} In this work we have electrochemically grafted Fast Blue RR (FBRR) salt, a quinone-containing diazonium derivative, to a cylindrical carbon-fiber microelectrode. This resulted in a sensor capable of performing real-time, reagentless pH measurements in biological microenvironments. The redox response of the FBRR-functionalized electrode was characterized using FSCV in biological media set to a physiologically relevant pH range. We demonstrate that modification of a carbon-fiber surface with FBRR is a simple, reproducible, and cost-efficient method for fabricating a sensitive and stable sensor that can be used to measure small pH fluctuations in the CNS of *Drosophila* that arise as a result of stimulated release.

Experimental Section

Reagents

4-Benzoylamino-2,5-dimethoxybenzenediazonium chloride hemi(zinc chloride) salt (Fast Blue RR, FBRR, diazonium salt), tetraethylammonium tetrafluoroborate (TEABF₄), NaCl, KCl, CaCl₂, MgCl₂, NaHCO₃, NaH₂PO₄, sucrose, tris(hydroxymethyl)aminomethane (Trizma base®), and acetonitrile (ACN, anhydrous, 99.8%) were obtained from Sigma

Aldrich (St. Louis, MO). All chemicals were used as received. Adult hemolymph like (AHL) saline (108 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 8.2 mM MgCl₂, 4 mM NaHCO₃, 1 mM NaH₂PO₄, 5 mM trehalose (Fluka BioChemika, Buchs, Switzerland), 10 mM sucrose, 20 mM Trizma base®, pH 7.5) was made using ultrapure (18 MΩ cm) water and filtered through a 0.2 μm filter.³⁸ The pH of AHL solutions was adjusted with 0.5 M NaOH and HCl.

Electrode Preparation

Cylindrical carbon-fiber microelectrodes were fabricated as previously described.³¹ Briefly, a PAN-based 5 μm-diameter carbon fiber (T-40 12K, Amoco, Greenville, SC) was aspirated into a borosilicate glass capillary (1B 100-4, World Precision Instruments, Inc., Sarasota, FL) and sealed using a regular glass capillary puller (P-97, Sutter Instruments, Novato, CA). The carbon fiber was trimmed to a length of 50 or 200 μm measured from the glass junction. Electrical contact was made by back-filling the capillary with a silver composition (4922N DuPont, Delta Technologies Ltd., Stillwater, MN), followed by insertion of a tungsten wire, resulting in a 5 μm-diameter cylindrical carbon-fiber microelectrode. The 200 μm long cylindrical electrodes were used for all characterization experiments while the 50 μm long electrodes were used for the *in vivo Drosophila* application.

Chemical Modification of the Carbon-Fiber Microelectrode Surface

Fast Blue RR salt was electrochemically grafted onto the carbon-fiber microelectrode surface using diazonium attachment chemistry. Deposition of the diazonium salt onto the carbon-fiber microelectrodes was carried out using cyclic voltammetry on an Ensman Instruments EI400 microelectrode potentiostat (Bloomington, IN) operated in the two-electrode mode. A 2 mM solution of Fast Blue RR salt was prepared in acetonitrile containing 0.1 M TEABF₄. Solutions were purged with Ar (g) for 5 min prior to deposition in order to eliminate signal attributed to the reduction of O₂. Electrodes were electrochemically modified via reduction of the diazonium onto the carbon surface by scanning from +0.4 V to -0.8 V vs. Ag QRE (3 mm dia., Bioanalytical Systems, West Lafayette, IN) at 0.5 V/s. Data were collected and processed using LabView 8.0 software (National Instruments, Austin, TX) written in-house. Electrode surface coverage was calculated by subtracting the background current measured for a solution of 0.1 M TEABF₄ in acetonitrile from that due to deposition of the diazonium. The typical surface coverage obtained using the experimental conditions listed above was ~20 nmol/cm².

Electrochemical Measurements

Voltammetric responses of the diazonium-modified electrodes as a function of pH were collected using either a Dagan Chem-Clamp potentiostat (Dagan Corporation, Minneapolis, MN) or a flow-injection analysis apparatus with a current amplifier (428, Keithley Instruments, Inc., Cleveland, OH). Both systems were run by the TH 1.0 CV program (ESA, Chelmsford, MA)³⁹ coupled with two data acquisition boards (PCI-6221, National Instruments). A Ag/AgCl electrode, which served as the reference in all experiments following the initial deposition of FBRR, was made by chloridizing a silver wire (0.25 mm diameter, 99.999% purity, Alfa Aesar, Ward Hill, MA). Electrodes were positioned using

x,y,z-micromanipulators (421 series, Newport, Irvine, CA). All cyclic voltammograms were obtained using a triangular waveform scanned from -0.7 to +0.8 V vs. Ag/AgCl at 20 V/s and repeated every 200 ms unless otherwise noted. Electrochemical responses were plotted and statistical analysis performed using Prism 5.0 (GraphPad Software, La Jolla, CA). Anodic peak potentials (E_{pa}) were determined using a fifth order polynomial fit from LabView 8.0 software written in-house. Cyclic voltammetry was used to estimate the heterogeneous electron-transfer rate constant, k^0 , for this system via the method of Nicholson.⁴⁰

In vivo Drosophila Preparation

Female flies carrying Channelrhodopsin-2 (ChR2), a light activated ion channel, were crossed with male flies expressing tyrosine hydroxylase (TH) to produce mutant flies containing dopaminergic neurons that can be controlled through blue light stimulation (TH-GAL4/UAS:ChR2 genotype).⁴¹ Male mutant flies, 3-7 days old, were maintained at 25 °C in the dark and fed yeast containing 10 mM all-trans retinal (light sensitive chemical necessary for ChR2 function) for 2 days prior to experimentation. Blue light was applied through computer control of a 3-W Luxeon Star LED with a peak intensity of ~470 nm (LXHL-LB3C, Newark, Chicago, IL). Flies were prepared as previously described for *in vivo* FSCV measurements.³¹ Briefly, ice was used to temporarily immobilize flies before they were mounted in a homemade collar (38.1 mm diameter concave plexiglass disk with a 1.0 mm hole in the center) with low melting agarose (Fisher Scientific, Pittsburgh, PA). Microsurgery was performed on a stereoscope (Olympus SZ60, Melville, NY) to remove the cuticle from the top portion of the head, thus exposing the brain region. The head was covered with 0.1% collagenase solution for 30 min to relax the extracellular matrix in the brain then rinsed and bathed with AHL saline with the preparation maintaining its viability for 1.5 - 2.5 h.

Results and Discussion

Cyclic Voltammetry Deposition of the Diazonium Salt onto a Carbon-fiber Microelectrode Surface

FBRR was electrochemically grafted onto a carbon-fiber surface using cyclic voltammetry by scanning from +0.4 V to -0.8 V vs. Ag QRE at a rate of 0.5 V/s in a 2 M FBRR/0.1 M TEABF₄ –supported acetonitrile solution. A proposed mechanism for this reaction is presented in Scheme 1. A representative voltammogram of the diazonium salt reduction onto a cylindrical carbon-fiber microelectrode is shown in Figure 1A (blue trace). An irreversible reductive wave is observed around -0.5 V which is attributed to the solution radical formation of the diazonium derivative and its subsequent covalent linkage to the carbon-fiber surface as reported for a similar molecule.¹²

The charge (Q) of the diazonium deposited onto the surface is quantified from the current-time integral of the voltammetric trace. The slight charge observed from the solvent background (black trace) has been subtracted from the charge due to diazonium deposition (blue trace). Faraday's Law ($Q = nNF$) is used to convert Q to the corresponding number of moles of diazonium (N) deposited onto the carbon-fiber surface. In this equation the number

of electrons exchanged in the reduction reaction, n , is 1, and F is Faraday's constant (96,485 C/mol). The surface coverage of diazonium on the electrode is calculated by dividing the number of moles of FBRR by the geometric area of the 200 μm cylinder ($3.2 \times 10^{-5} \text{ cm}^2$). This results in a 20 nmol/ cm^2 surface coverage. As a point of reference, typical monolayer coverage for a surface-bound small organic molecule has been reported as 300 pmol/ cm^2 .⁴² Therefore, this suggests a multilayer deposition of FBRR onto our sensor, a result commonly observed for the reduction of aryl diazonium salts onto carbon surfaces.^{14, 43} The amount of FBRR deposited onto the carbon-fiber surface (Table 1) is dependent on both the scan rate and potential window of the voltammetric sweep. The voltammetric deposition of the diazonium is a time-dependent process; therefore, scanning at slower rates or to an extended negative waveform potential increases the amount of FBRR deposited onto the electrode surface. Interestingly, there is no significant effect in varying the concentration of diazonium in solution in increments from 0.5 to 5 mM on the resultant amount deposited onto the electrode (data not shown).

The presence of FBRR on the surface has been investigated using FSCV. In Figure 1B, cyclic voltammograms recorded at a bare carbon-fiber microelectrode (dashed black line) and the same microelectrode following modification with FBRR (solid blue line) show a clear indication of the presence of the electroactive diazonium salt on the electrode surface ($v = 20 \text{ V/s}$). The voltammogram of the FBRR redox system signifies quasireversible behavior with an apparent formal potential of -0.1 V in AHL saline at physiological pH (pH 7.5). Integration of the oxidative peak area from the redox-active molecule in Figure 1B results in an observed surface coverage of 40 pmol/ cm^2 . This is approximately two orders of magnitude smaller than that calculated from the diazonium deposition in Figure 1A. It is likely that the multilayer morphology of FBRR on the surface of the electrode prevents all of the deposited quinone groups from being electrochemically active. Scheme 2 illustrates a proposed mechanism for the oxidation-reduction reaction of the surface-bound quinone derivative during cycling of the applied potential. It is thought that voltammetric interrogation of this molecule initially induces a two-electron oxidation to convert the p -methoxy moiety on the conjugated ring to its p -quinone analogue. The quinone is then chemically reduced in a two-electron/two-proton exchange to form the hydroxy derivative of the molecule. Using the method of Nicholson,⁴⁰ the heterogeneous electron-transfer rate constant, k° , is determined to be 0.13 cm/s. This indicates that the FBRR undergoes outer-sphere electron transfer on the carbon-fiber surface, which is consistent with previous studies that have examined electron transfer kinetics over a wide insulating layer.⁴⁴

Electrochemical Characterization of the FBRR Microelectrode pH Sensor

The effect of scan rate on the electrochemistry of a FBRR-modified carbon-fiber microelectrode has been investigated using FSCV. Cyclic voltammograms of a FBRR microelectrode in pH 7.5 AHL saline solution at scan rates of 10, 20, and 50 V/s are plotted in Figure 2A. Because current is directly proportional to scan rate, the current scale on the y-axis has been divided by scan rate to allow for comparison of the peak positions at the different scan rates. Notably, neither the anodic peak potential (E_{pa}) nor the cathodic peak potential (E_{pc}) significantly shifts in value with varying scan rate in this range. At scan rates higher than 100 V/s (up to 350 V/s), the E_{pa} becomes more difficult to identify due to a

decrease in the ratio of the faradaic to the capacitive current. By inspection, the E_{pa} is well resolved from the background current at 20 V/s, a scan rate that should suffice for monitoring rapidly occurring neurosecretory events during *in vivo* applications. Therefore, this scan rate has been chosen to monitor pH changes in the remainder of this study. Furthermore, the anodic peak current vs. v (see Supporting Information) is linearly dependent for scan rates 10 – 350 V/s ($r^2 > 0.99$). This confirms that the oxidation and reduction of FBRR is a surface-confined reaction, as expected, and provides evidence that the diazonium compound is sufficiently tethered to the carbon-fiber surface.

The long-term stability of the FBRR microelectrode pH sensor has been studied by continuously cycling modified electrodes in pH 7.5 AHL saline solution for 2.5 h (-0.7 to +0.8 V vs. Ag/AgCl at 5 Hz with scan rate = 20 V/s). This corresponds to 45,000 voltammetric sweeps over the 2.5 h period. An 8% decrease in peak current is observed during the first 10 min of cycling (Figure 2B). During the remaining 2.5 h, the peak current remains fairly stable, decreasing an additional 13%. Therefore, the stability of the FBRR-modified microelectrode provides an ample time window for monitoring the pH in the CNS of *Drosophila* during *in vivo* electrochemistry measurements.

The selectivity of the sensor for H^+ has been investigated to determine if alternate ionic species present in the biological media interfere with the voltammetric response. To accomplish this, FBRR microelectrodes ($n = 3$) have been tested with FSCV in a series of AHL saline solutions that contained elevated concentrations of various inorganic cations. When the Na^+ concentration in the first AHL saline solution is increased by 40%, the E_{pa} remains unaltered. In addition, increasing the Mg^{2+} concentration by 45%, Ca^{2+} concentration by 50%, or K^+ concentration by 60% does not cause a shift in the E_{pa} . These studies validate that changes in the concentration of these four cations do not contribute to the shift measured in the E_{pa} , which suggests charged species, other than H^+ ions, in the AHL saline solution are not affecting the pH response of the FBRR microelectrode.

pH Response of Microelectrode Sensor Deposited with FBRR

To calibrate the voltammetric response of the sensor, the FBRR-modified carbon-fiber microelectrode has been investigated in AHL saline solutions of varying pH. The peak characteristics of cyclic voltammograms recorded over a pH range of 5.0 – 9.0 with a scan rate of 20 V/s have been examined. Figure 3 depicts representative voltammograms for the redox behavior of the FBRR microelectrode in three different pH solutions. The E_{pa} noticeably shifts to more negative potentials as pH is increased. The E_{pc} follows the same trend with pH as the E_{pa} ; however, the peak becomes difficult to distinguish from the background current in higher pH solutions (pH 8), as reported previously for chemically modified electrodes in physiological media.⁵ Therefore, the E_{pa} was chosen to calibrate the sensor and for subsequent *in vivo* studies instead of the E_{pc} or $E_{1/2}$.

FSCV has been used to determine the response of the sensor to pH changes. In a pH range of physiological relevance (6.5 – 8.0), a sigmoidal fit best describes the relationship between E_{pa} and pH (Figure 4, $n = 9$ electrodes). Linear regression of these data yields a slope of 38 mV/pH unit which is less than the theoretical value of 59 mV/pH unit for a reversible, two-electron/two-proton redox reaction at room temperature.⁴⁵ This deviation from the predicted

Nernstian value suggests the attachment of the FBRR to the carbon-fiber surface altered the electrochemistry of the quinone couple. pH-sensitive, glassy carbon electrodes tethered with alternative reporter molecules have been previously fabricated that exhibit expected Nernstian behavior, but practical limitations, such as high capacitive currents, larger diameters (millimeter), and lengthy time scales to obtain stable readings, have limited their biological usefulness.^{35, 36} For example, Shiu et al. have reported the development of a glassy carbon electrode (3 mm diameter) modified by adsorption of an anthraquinonesulfonate film that possessed a near Nernstian slope of 56.4 mV/pH unit in aqueous pH buffers.³⁵ However, it would not be feasible to use an electrode of this size to measure dynamic events associated with *in vivo* neurosecretion in volume-limited model systems such as *Drosophila*.

Microelectrode Response Time to a pH Change

Flow-injection analysis has been used to study the dynamic response of the sensor by introducing plugs of AHL saline solution of varying pH to a FBRR-modified microelectrode. Ideally, a fast electrode response time to a minor change in pH of the surrounding solution would produce a square-shaped E_{pa} vs. time trace. Figure 5 shows the E_{pa} response of the modified electrode sensor to 0.2 pH unit changes. After initial immersion in an AHL saline solution of pH 7.4, the electrode is exposed to a bolus of AHL saline solution of pH 7.2 (Figure 5A). Likewise, AHL solution of pH 7.6 is introduced to the electrode in pH 7.4 solution in Figure 5B. By inspection, the modified electrode response to a 0.2 change in pH is square-like and consistent for measurement of either an acidic or a basic pH change. Indeed, flow injection calibration of the sensor revealed marked sensitivity for H^+ , capable of detecting pH changes as small as 0.005 (based on $S/N > 3$) with a time response equal to 1.6 s (τ determined from exponential decay).

Measuring dynamic *in vivo* pH Changes in the *Drosophila* CNS

Previously, we have developed a method for placing a carbon-fiber microelectrode into the CNS of *Drosophila* to electrochemically measure neurotransmitters *in vivo* with FSCV.^{31, 32} Here, we utilized the pH electrode to monitor a dynamic pH change associated with neurotransmitter release in the brain. Blue light stimulation has been used on the mutant fly TH-GAL4/UAS:ChR2 to evoke dopamine neurosecretion as demonstrated previously in *Drosophila* larvae by Venton and co-workers.⁴⁶ This mutant expresses blue light sensitive cation channels which are specific to dopaminergic neurons, allowing dopamine release to be controlled through timed blue light stimulations. Following microsurgery, a micromanipulator is used to insert the cylindrical FBRR-modified electrode into the CNS region of an adult mutant fly. We use blue light stimulation to induce neurotransmitter release and were able to subsequently measure a change in E_{pa} , which corresponds to an ~ 0.034 acidic pH change in the mutant fly following a 5-s stimulation with blue light (Figure 6, solid red line). This value is in agreement with pH fluctuations observed as a result of stimulus-coupled secretion in rat brain slices (0.047 unit pH change in the cortex) reported by the Wightman lab using ISMs.²⁰ To ensure the response is due to a biological change in the fly, the experiment has been repeated with the electrode in the surrounding solution outside of the fly brain (solid black line). This experiment demonstrates the high temporal sensitivity of the FBRR sensor and highlights its utility for real-time analyses of

pH fluctuations associated with neurosecretion in new, emerging volume-limited biological microsystems.

Conclusion

A carbon-fiber microelectrode pH sensor was developed via the voltammetric reduction of the diazonium salt FBRR. The stability and sensitivity of the sensor for H^+ was characterized in biological media set to physiologically relevant pH ranges. FSCV was used to probe the surface-bound diazonium derivative as a function of pH. The peak corresponding to E_{pa} for the FBRR-modified electrode was correlated to small changes in pH. Flow-injection analyses were used to characterize the temporal response of the sensor for solutions of varying pH, resulting in a limit of detection to 0.005 pH units. Furthermore, direct *in vivo* measurements of pH were carried out in the *Drosophila* CNS after stimulated neurotransmitter release, revealing an acidic change in a brain region dominated by dopaminergic neuron innervations. These data demonstrate the utility of this easily fabricated sensor for measuring dynamic changes in extracellular pH in this and other new emerging microanalytical animal models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Cyclic voltammograms of a carbon-fiber microelectrode before and after FBRR attachment. (A) Cyclic voltammogram of the electrochemical reduction and subsequent covalent attachment of FBRR onto the carbon-fiber surface. Background charge of the carbon-fiber electrode in solvent only (black trace). Reduction of FBRR at the carbon-fiber surface (blue trace). Diazonium concentration = 2 mM in 0.1 M TEABF₄/ACN. Potential window = +0.4 V to -0.8 V vs. Ag QRE. Scan rate = 0.5 V/s. (B) Cyclic voltammograms (average of 5 scans each) of a bare carbon-fiber microelectrode (dashed black line) and a carbon-fiber

microelectrode modified with FBRR (solid blue line) in AHL saline. Potential window = -0.7 V to +0.8 V vs. Ag/AgCl. Scan rate = 20 V/s.

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Figure 2.

Electrochemical characterization of the FBRR microelectrode pH sensor in pH 7.5 AHL saline solution. (A) Cyclic voltammograms (average of 5 scans each) of the FBRR redox couple at 3 scan rates. The current scale on the y-axis has been divided by scan rate so the peak positions at the different scan rates can easily be compared. In this scan rate range, neither the anodic peak potential (E_{pa}) nor the cathodic peak potential (E_{pc}) significantly shifts in value. (B) The effect of continuous cycling of the electrode on anodic peak current (i_{pa}). Error bars are SEM ($n = 3$).

Figure 3.

Cyclic voltammograms of a microelectrode modified with FBRR in AHL saline solutions of different pH measured with scan rate = 20 V/s. Asterisk (*) corresponds to the E_{pa} for each voltammogram (average of 5 scans) with the dashed vertical line included for comparison. As the pH increases, the E_{pa} visibly shifts to more negative potentials. (A) pH 6.5 (B) pH 7.5 (C) pH 8.0.

Figure 4.

The anodic peak potential, E_{pa} , as a function of AHL saline solution pH for FBRR-modified electrodes. The E_{pa} has a sigmoidal relationship with changing pH in a physiological relevant pH range (6.5-8.0). Error bars are SEM ($n = 9$ electrodes).

Figure 5.

Plot of E_{pa} vs. time during flow injection changes past the electrode of 0.2 pH units in AHL saline. The electrode is able to consistently measure either an acidic or a basic pH change. (A) Initial AHL saline solution of pH 7.4 is decreased to pH 7.2. (B) Initial AHL saline solution of pH 7.4 is increased to pH 7.6.

Figure 6.

Physiological pH measurements in adult *Drosophila* CNS. A representative trace of a dynamic, acidic pH change associated with neurotransmitter release is measured in a mutant fly CNS (solid red line). A control stimulation of the same electrode in AHL saline solution only is plotted for comparison (solid black line). The black arrow corresponds to a 5-s stimulation with blue light.

Scheme 1.

Scheme 2.

Table 1

Effect of varying voltammetric deposition parameters of the FBRR on the carbon-fiber surface.

Scan Rate (V/s)	Potential Window (V vs. Ag/AgCl)	Γ (nmol/cm ²)
0.500	+0.4 $\rightarrow$ -0.2	2.7 $\pm$ 0.9
0.500	+0.4 $\rightarrow$ -0.4	9.9 $\pm$ 3.5
0.500	+0.4 $\rightarrow$ -0.6	14.5 $\pm$ 1.4
0.500	+0.4 $\rightarrow$ -0.8	21.5 $\pm$ 2.7
0.500	+0.4 $\rightarrow$ -1.0	25.0 $\pm$ 2.7
0.050	+0.4 $\rightarrow$ -0.8	29.4 $\pm$ 6.4
0.100	+0.4 $\rightarrow$ -0.8	24.4 $\pm$ 1.2
0.500	+0.4 $\rightarrow$ -0.8	20.0 $\pm$ 2.1
1.0	+0.4 $\rightarrow$ -0.8	19.7 $\pm$ 0.6
5.0	+0.4 $\rightarrow$ -0.8	9.9 $\pm$ 1.4

[FBRR] = 2 mM in 0.1 M TEABF₄/ACN. Error is SEM with $n = 3$ electrodes for each measurement.