

Flame Etching Enhances the Sensitivity of Carbon-Fiber Microelectrodes

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Small sensors are useful for *in vivo* measurements and probing small spaces. In this paper, we compare two methods of fabrication of small, cylindrical carbon-fiber microelectrodes: flame-etching and electrochemical etching. With both methods, microelectrodes can be fabricated with tip diameters of 1 to 3 μm . Electrodes were tested with fast-scan cyclic voltammetry. Flame etching resulted in electrodes that have larger S/N ratios and higher currents per unit area for 1 μM dopamine than normal carbon-fiber microelectrodes or electrochemically etched electrodes. Therefore, the increased sensitivity is not just a property of size. The flame-etched surfaces had nanometer-scale surface features that were not observed on the other electrodes and exhibited increased sensitivity for other electroactive compounds found in the brain, including ascorbic acid, DOPAC, and serotonin. Faster kinetics and a faster response to a step change in dopamine were also observed, when the applied waveform was -0.4 to 1.0 V and back at 400 V/s. The sensitivity of the flame-etched electrodes was enhanced by overoxidizing the surface. The flame-etched electrodes were used to detect dopamine release in anesthetized rats after a single stimulation pulse. The small flame-etched electrodes will facilitate measurements of low concentrations in discrete brain regions or small organisms.

Small probes are essential for making *in vivo* measurements of neurotransmitters in order to disturb the tissue as little as possible. Microdialysis sampling has been one of the most popular techniques for *in vivo* chemical measurements.¹ However, the implanted microdialysis probes are 250 to 500 μm wide and can cause considerable tissue damage and disrupt the blood–brain barrier.^{2–4} Microelectrodes have also been widely used for neurochemical monitoring.⁵ Because they are normally 7 to 30 μm in diameter, microelectrodes cause less damage to neurons and blood vessels in the brain upon implantation than dialysis

probes.⁶ Smaller diameter electrodes might damage fewer cells upon implantation and would be beneficial for making spatially resolved measurements and measuring neurotransmission in smaller organisms. In rodents such as mice, brain regions may be only tens of microns wide and single cells are only a few microns.⁷ Lower order model organisms such as the sea slug (*Aplysia californica*), worm (*Caenorhabditis elegans*), and fruit fly (*Drosophila melanogaster*) also have very small nervous systems. For example, the brain of fruit fly is less than 100 μm wide⁸ and contains only picograms of each neurotransmitter.⁹

Carbon-fiber microelectrodes have been a popular type of microelectrode for making measurements in the brain because of their easy fabrication and good electrochemical properties.⁵ The diameter of carbon-fiber microelectrodes has been decreased by electrochemical etching⁷ and flame etching¹⁰ to achieve tip diameters of 1 μm or less. Disk microelectrodes have been made from these etched fibers by insulating the fibers with a polymer coating and removing the end with a scalpel to expose a small disk of carbon. This method of fabrication does not expose any of the etched surface as a sensor. Only the tip is electroactive, but the measured currents are low because of the small electrode area. These tiny disk electrodes are excellent for single cell measurements, where the electrode can be positioned over the cell and quantal release detected.¹¹ For studies in intact animals, cylindrical geometry electrodes are preferred. Cylinder microelectrodes have a length of fiber that is not insulated so they sample from a larger area and provide greater sensitivity.¹² A larger sampling area is helpful in an intact brain because the location of nerve terminals is unknown. Therefore, it would be useful to develop a cylindrical electrode with a smaller diameter that would allow better spatial resolution.

Electrochemical detection of dopamine and other positively charged neurotransmitters has been shown to be limited by the kinetics of adsorption at carbon electrodes, and larger electrodes would be expected to have more adsorption sites.¹² The sensitivity of carbon electrodes is also very dependent on surface chemistry. For example, surface defects that expose edge plane graphite sites

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have been shown to be more active¹³ and the adsorption of analytes depends on surface functional groups.^{14,15} Indeed, a number of techniques used to improve the sensitivity of carbon fibers function by changing the surface chemistry. Overoxidation of a carbon fiber increases sensitivity by increasing surface roughness and adding oxide groups that act as adsorption sites for cationic neurotransmitters.^{16,17} Laser ablation cleans the carbon surface and exposes graphite edge sites.^{14,18} Carbon nanotube treatments have been developed to increase sensitivity by adding catalytic sites.^{19,20} Polymer coatings, such as Nafion²¹ and over-oxidized polypyrrole,²² have also been used to improve sensitivity by preconcentrating analyte near the electrode, but they slow the time response of the electrode. A method for fabrication of smaller electrodes without compromising sensitivity would be valuable for making neurochemical measurements.

In this paper, we describe how flame etching creates small, sensitive electrodes for *in vivo* use. Flame-etched electrodes showed an increased sensitivity per area and S/N ratios for many neurotransmitters when compared to normal carbon-fiber electrodes and similar size electrochemically etched electrodes. The flame-etched electrodes also had increased kinetics and a faster time response. These highly sensitive, small electrodes were used for *in vivo* measurements of single pulse dopamine stimulations and will be useful for measurements in discrete brain regions.

EXPERIMENTAL SECTION

Electrode Construction. Carbon-fiber microelectrodes were fabricated by aspirating a single T-650 fiber (7 μ m diameter, Cytec Engineering Materials, West Patterson, NJ) into a glass capillary (1.2 mm \times 0.68 mm, A-M systems, Carlsburg, WA). The glass capillary was then pulled into two separate electrodes using a vertical pipet puller (Narishige, model PE-21, Tokyo, Japan). The fiber extending from the glass was cut with a scalpel under the microscope so that it extended 100 to 150 μ m from the glass seal.

To flame etch, the electrodes were held in a butane flame (Lenk butane torch, Kinston, NC) for about 3 s. The flame temperature was approximately 2100 °F, and the electrode was held just on the edge of the blue part of the flame. Positioning was important, as placing the electrode in the hottest part of the flame tended to destroy the glass seal. As the very end of the tip became red, the electrode was rotated in order to ensure even etching on all sides.

Other electrodes were electrochemically etched in a solution of 0.5 mM K₂Cr₂O₇ in 5 M H₂SO₄.⁷ This solution was suspended in a small platinum loop and the electrode positioned in the drop

by a micromanipulator. A 6 V, 60 Hz sine wave was applied between the electrode and platinum wire for 20–30 s to etch. Some electrodes were not etched and were tested for comparison (these are referred to as normal electrodes). All electrodes were soaked for at least 10 min in isopropanol immediately before use.

To compare the geometric areas of the electrodes, normal and electrochemically etched electrodes were treated as cylinders with surface area = $2\pi rl + \pi r^2$ where r is the radius and l is the length. This accounts for the cylindrical surface area and the one exposed end. The flame-etched electrodes were estimated as conical, because the tip radius (r) was smaller than the radius at the glass seal (R). The surface area was $\pi(r + R)((R - r)^2 + l^2)^{1/2} + \pi r^2$ where l is the length of the electrode. These equations give an estimate of the geometric surface area but would not account for any microscopic surface roughness.

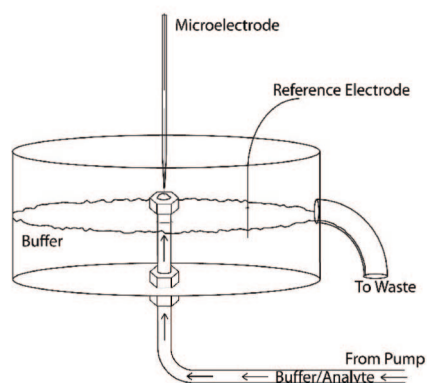
Chemicals. Dopamine, norepinephrine, epinephrine, serotonin, ascorbic acid, and DOPAC (3,4-dihydroxyphenylacetic acid) were purchased from Sigma-Aldrich (Milwaukee, WI). The rest of the chemicals were purchased from Fisher Scientific (Suwanee, GA). Stock solutions were prepared in 0.1 M perchloric acid. Dopamine, norepinephrine, and epinephrine were made as a 10 mM stock, ascorbic acid and DOPAC were made as a 100 mM stock, and serotonin was made as a 1 mM stock solution. The stock solutions were diluted to the desired concentration in tris buffer on the day of the experiment. The tris buffer consisted of 15 mM tris(hydroxymethyl)aminomethane, 140 mM NaCl, 3.25 mM KCl, 1.2 mM CaCl₂, 1.25 mM NaH₂PO₄, 1.2 mM MgCl₂, and 2.0 mM Na₂SO₄. The pH of the buffer was adjusted to 7.4. All solutions were made in deionized water (Milli-Q Biocel, Millipore, Billerica, MA).

Electrochemical Instrumentation. The fast-scan cyclic voltammograms were collected using a ChemClamp potentiostat (Dagan, Minneapolis, MN, with custom-modified gain settings). The data was acquired using Tar Heel CV software (gift of Mark Wightman), which is written in LabVIEW (National Instruments, Austin, TX). The data acquisition hardware was the same as previously described (Heien et al., 2003). National Instruments PCI 6052 and PCI 6711 boards were used to apply the triangle waveform and collect the data. For most experiments, an applied potential waveform of -0.4 to 1.0 V and back at 400 V/s every 100 ms was used. To test the effects of overoxidation, the electrode was scanned from -0.4 to 1.5 V and back at 400 V/s. The reference electrode was silver-silver chloride.

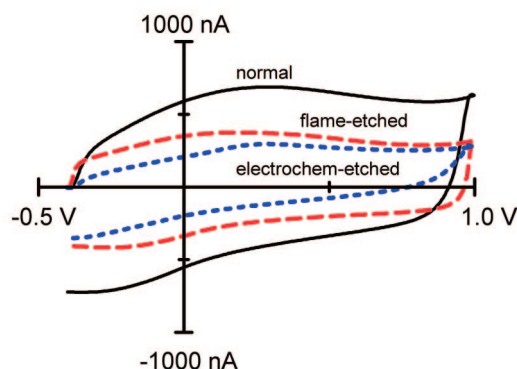
Flow Injection Analysis. Flow injection analysis was used to test the response of electrodes to fast changes. A bath type flow cell was used, with the electrode placed in the top of a drop, formed on 1 mm diameter tubing placed vertically, that constantly overflowed into the bath (Figure 1A). The reference electrode was placed in the bath, about 1 cm away. The flow cell was positioned onto a two-position air actuator. The sample was loaded into a 500 μ L loop before the experiment, and the air actuator turns the valve allowing the sample to flow by the electrode. The buffer was pumped through the flow injection apparatus by using a syringe pump at 2 mL/min (1.06 cm/s, Low RFI Syringe Pump 22, Harvard Apparatus, Holliston, MA). Injections of the sample were only 3 s long in order to simulate fast changes in neurotransmitters.

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A. Flow injection set-up



B. Background



C. 1 μ M Dopamine

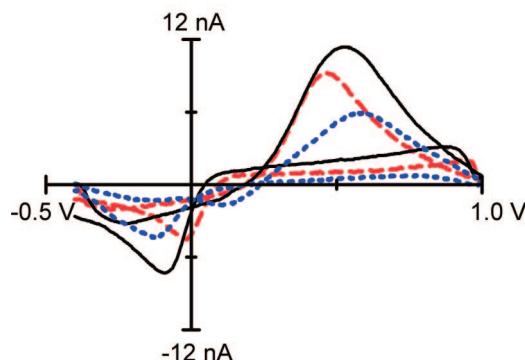


Figure 1. Fast-scan cyclic voltammetry. (A) Setup for flow injection analysis. The working microelectrode was positioned in the droplet that formed on the top of the tubing and the reference in the overflow bath. (B) Background charging currents for 125 μ m long normal (solid line), flame-etched (red dashed line), and electrochemically etched (blue dotted line) carbon-fiber microelectrodes. Electrodes were scanned from -0.4 to 1.0 V and back at 400 V/s every 100 ms. (C) Background-subtracted cyclic voltammograms for 1 μ M dopamine. Colors are the same as in panel B.

Scanning Electron Microscopy. Scanning electron micrographs were collected on a JEOL JSM-6700F instrument (Japan). Before imaging, electrodes were sputter coated with a thin layer of Pd–Au to make them more conductive (PECS 682, Gatan Inc., U.K.). Images were collected in secondary electron mode.

Animal Experiments. All animal experiments were approved by the University of Virginia Animal Care and Use Committee. Male, Sprague–Dawley rats (250–325 g) were purchased from Charles River (Wilmington, MA) and anesthetized with urethane (1.5 g/kg i.p.). Holes were drilled in the skull for the placement

of the microelectrodes. The working electrode was lowered into the nucleus accumbens (coordinates in mm from bregma: anteriorposterior (AP), $+1.2$; mediolateral (ML), $+2.0$; dorsoventral (DV), -9.0). The bipolar stimulating electrode was implanted in the substantia nigra/ventral tegmental area (AP, -5.6 ; ML, $+1.2$; DV, -8.0). The dorsoventral placement of the stimulating electrode was adjusted to maximize dopamine release. The working electrode was lowered in increments of 100 μ m until a site with robust release was found, and then the electrode was kept in place as repeated stimulations were applied. Stimulations were generated by Tar Heel CV and applied using a stimulus isolator (Dagan, BSI-950 Biphasic Stimulus Isolator). Either a single stimulation pulse or a train of 12 pulses (60 Hz) was applied. Each stimulus pulse was biphasic, 2 ms per phase, 300 μ A of current.

Statistics. All statistics were performed in GraphPad Prism (GraphPad Software Inc., San Diego, CA). t tests were used to test statistical significance. p values were considered statistically significant at the 95% confidence level ($p < 0.05$). Values are given as mean \pm standard error of the mean (SEM) in tables for n number of electrodes.

RESULTS AND DISCUSSION

Fabrication of Flame-Etched and Electrochemically Etched Electrodes. Cylindrical electrodes were flame etched by inserting the tips in a butane torch for a few seconds. It was important to not hold them too close to the hottest part of the flame or the glass seals would be ruined. The etching process produced electrodes that varied in size, but with some practice, electrodes with tip diameters between 1 and 2.5 μ m were reliably fabricated. Submicron electrodes were sometimes made, but they were more fragile and might not be as suitable for *in vivo* applications. The flame-etched electrodes tended to be conical after etching because the tips of the fibers were etched more than the fiber at the glass seal. To electrochemically etch, a cylindrical microelectrode was placed in a drop of oxidizing solution in a platinum loop and a sine wave applied. This evenly etched the entire exposed fiber, so the resultant electrodes were cylindrical in geometry. To create electrodes with approximately the same area as the more conical flame-etched electrodes, the electrochemically etched electrodes were fabricated with 2 to 3.5 μ m diameter tips. All electrodes were 100 to 150 μ m long. The average length for each group was similar, 135 ± 3 μ m for normal electrodes ($n = 22$), 133 ± 5 μ m for flame-etched electrodes ($n = 22$), and 129 ± 7 μ m for electrochemically etched electrodes ($n = 8$).

Background Currents. Smaller background currents were expected for the etched electrodes because the double layer charging current is proportional to surface area. The background currents of the etched electrodes are about half that of a normal cylinder electrode of equal length using fast-scan cyclic voltammetry (Figure 1B). To compare background currents, the maximal current for the outgoing scan (usually around $+0.2$ V) was averaged. The background current for normal electrodes was 830 ± 70 nA compared to 460 ± 60 nA for the flame-etched electrodes and 420 ± 50 nA for electrochemically etched electrodes. The electrochemically etched electrodes were slightly larger in geometric area than the flame-etched but had slightly smaller background currents (see Table 1). Apparent capacitance values were calculated from the background currents (using the formula $i = vC$, where v is scan rate and C is capacitance) and then divided

Table 1. Averaged Electrochemical Parameters for 1 μ M Dopamine Detection, When Scanning from -0.4 to 1.0 at 400 V/s^a

	i_{pa} (nA)	geometric area (μm^2)	normalized i_{pa} (pA/ μm^2)	S/N	ΔE_p (V)
normal electrodes ^b	10.5 ± 0.7	3260 ± 70	3.0 ± 0.2	160 ± 12	0.69 ± 0.01
flame-etched electrodes ^b	8.5 ± 0.8^c	1430 ± 90	5.4 ± 0.5^c	250 ± 25^c	0.61 ± 0.01^c
electrochemically etched electrodes ^d	5.2 ± 0.6^c	1570 ± 120	3.3 ± 0.2	150 ± 20	0.72 ± 0.03

^a i_{pa} is oxidative peak current; ΔE_p is the difference between the oxidative and reductive potentials. ^b $n = 22$. ^c Data are significantly different than normal electrodes ($p < 0.05$). ^d $n = 8$.

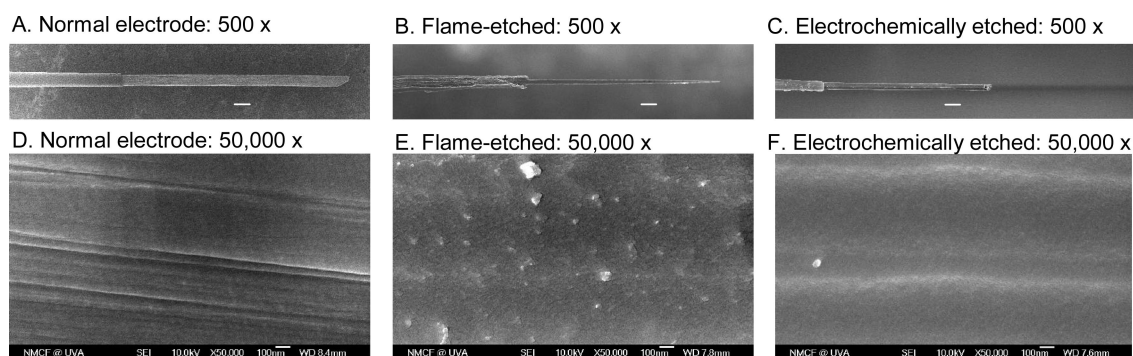


Figure 2. Scanning electron microscopy images. The top images are at $500\times$ magnification with $10\text{ }\mu\text{m}$ scale bars. The carbon fiber extends from the glass insulator, which is on the left of the images. The bottom images are magnified $50,000$ times, and the scale bars are 100 nm . (A) The normal carbon-fiber cylindrical electrode is $7\text{ }\mu\text{m}$ in diameter and $140\text{ }\mu\text{m}$ long. (B) The flame-etched electrode shown has a $1\text{ }\mu\text{m}$ wide tip, and the diameter near the glass seal is $2.5\text{ }\mu\text{m}$. This electrode is $125\text{ }\mu\text{m}$ long. (C) The electrochemically etched electrode is $3\text{ }\mu\text{m}$ in diameter and $105\text{ }\mu\text{m}$ long. (D) The highly magnified normal carbon fiber is striated but has an otherwise smooth surface. (E) The flame-etched electrode surface has bumps and surface features but not striations. (F) The electrochemically etched electrode is fairly smooth.

by the calculated geometric area. The apparent capacitance per unit area for flame-etched electrodes, $79\text{ }\mu\text{F}/\text{cm}^2$, was larger than normal and electrochemically etched electrodes, with values of $63\text{ }\mu\text{F}/\text{cm}^2$ and $66\text{ }\mu\text{F}/\text{cm}^2$, respectively. This discrepancy might indicate a difference in surface roughness that is not accounted for in the geometric area calculation or an increase in surface groups that can also contribute to the charging current.

Sensitivity. For detecting changes in neurotransmitter levels, background-subtracted cyclic voltammetry is used. Figure 1C shows example background-subtracted cyclic voltammograms for $1\text{ }\mu\text{M}$ dopamine at $125\text{ }\mu\text{m}$ long electrodes. The flame-etched electrode current was about 20% smaller than for the normal cylindrical electrode, even though the geometric area of the flame-etched electrode was over 50% smaller. The signal for the electrochemically etched electrode was about half that of the normal electrode, and it was also 50% smaller by area. The peak oxidative currents (i_{pa}) were averaged for all electrodes, and the oxidative current for the flame-etched and electrochemically etched electrodes were significantly different than the normal electrodes (Table 1, $p < 0.05$). However, normalized currents (current divided by geometric surface area) were significantly larger for the flame-etched electrodes but not for the electrochemically etched electrodes.

Table 1 also shows that signal-to-noise ratios (S/N) were significantly greater for flame-etched electrodes, but similar for normal and electrochemically etched electrodes. Noise is principally due to variance in impedance and is proportional to capacitance, which for the same electrode material should be proportional to electrode area. In potential sweep experiments, studies have shown that noise correlates better with background current than area, reflecting the noise contributions of properties

such as surface roughness and the seal of the electrode.²³ Etched electrodes are smaller in area and have smaller capacitances. Indeed, the average noise value for flame-etched electrodes was 52% of normal electrodes, similar to the decrease in background current (55%). Because the noise was smaller but the signal did not decrease proportionally to the area, the S/N ratios increased for flame-etched electrodes. The electrochemically etched electrodes had similar S/N ratios to normal electrodes because both the signal and noise decreased by 50%.

Electrode Surfaces. To investigate the differences between the electrode surfaces, scanning electron microscopy images were taken (Figure 2). In the top images, the whole electroactive area of the fiber is pictured, with the carbon fiber protruding from the pulled glass. The normal T-650 carbon fiber is $7\text{ }\mu\text{m}$ in diameter (Figure 2A). The flame-etched fiber pictured is $1\text{ }\mu\text{m}$ in diameter at the tip and $2.5\text{ }\mu\text{m}$ at the glass seal (Figure 2B). The electrochemically etched electrode is $3\text{ }\mu\text{m}$ in diameter (Figure 2C). Figure 2D, E, and F show the surface of the cylinder electrodes magnified $50,000$ times. The normal carbon fibers are striated, but the surface otherwise looks smooth. The flame-etched electrodes are not striated; however, the surface has bumps and other surface features on the scale of 100 nm . The electrochemically etched electrodes are not striated and have a smoother surface with fewer surface features.

Kinetics and Response Times. Because the flame-etched electrodes showed increased sensitivity, their properties were further investigated. Figure 1C shows that the oxidation and reduction peaks for the flame-etched electrode are closer together

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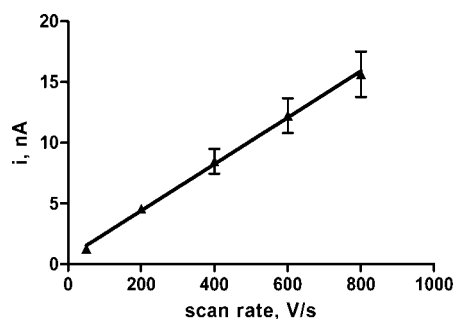


Figure 3. Peak current vs scan rate. The plot of oxidative peak currents vs scan rate is linear ($r^2 = 0.998$, $n = 4$) for flame-etched electrodes, indicating that kinetics are adsorption controlled.

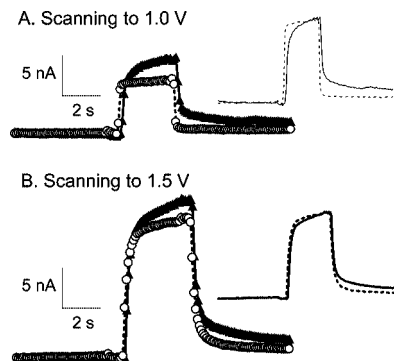


Figure 4. Temporal response of electrodes. Response of normal (triangles/solid line) and flame-etched (circles/dashed line) electrodes to a 3 s injection of 1 μM dopamine during flow injection analysis. The insets show the responses with normalized peak currents to enable comparison of the peak shapes. (A) The applied cyclic voltammetry waveform was from -0.4 to 1.0 V and back at 400 V/s. (B) The applied potential was scanned from -0.4 to 1.5 V and back at 400 V/s every 100 ms.

when the electrode is scanned from -0.4 to 1.0 V. Table 1 shows that the average ΔE_p , the difference between the oxidation and reduction peak potentials, for the flame-etched electrodes is significantly lower than normal. Figure 3 shows that a plot of peak current vs scan rate is linear for flame-etched electrodes. The correlation coefficient is $R^2 = 0.998$ ($n = 4$). The error bars show differences in sensitivity between electrodes, but peak current was highly linear with scan rate for each individual electrode. This plot shows that the kinetics are adsorption controlled.

Figure 4A shows the temporal response of electrodes to a 3 s injection of dopamine. The response should be square, as a square pulse flows by the electrode in the flow cell. However, the slow kinetics of adsorption and desorption have been shown to result in a nonideal shape for dopamine.^{12,24} For the normal cylinder electrodes, the peak response continues to increase during the exposure to dopamine. The current is slow to return to baseline and is still elevated a few seconds after the injection of dopamine is finished. The response for the flame-etched electrode is much sharper upon injection of dopamine, and it comes back to baseline quickly. The fast temporal response is an indication of fast adsorption/desorption processes.¹² Therefore, the flame-etched electrodes respond faster to a concentration change and would

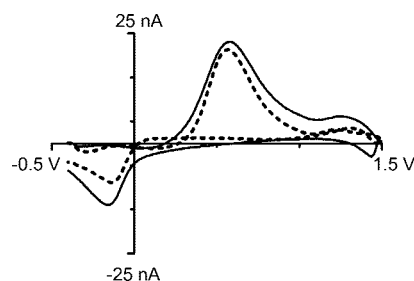


Figure 5. Effect of electrochemical overoxidation. Background-subtracted cyclic voltammograms for 1 μM dopamine are picture for normal (solid line) and flame-etched (dashed line) electrodes. The potential waveform was from -0.4 to 1.5 V and back at 400 V/s at 10 Hz.

Table 2. Average Electrochemical Parameters for 1 μM Dopamine Detection, When Scanning from -0.4 to 1.5 at 400 V/s^a

	i_{pa} (nA)	normalized i_{pa} ($\text{pA}/\mu\text{m}^2$)	S/N	ΔE_p (V)
normal electrodes	27 ± 3	4.8 ± 0.3	180 ± 40	0.710 ± 0.009
flame-etched electrodes	23 ± 2	8.1 ± 0.8^b	290 ± 40^b	0.708 ± 0.007

^a $n = 11$ for both normal and flame-etched electrodes. ^b Data are significantly different from normal electrodes ($p < 0.05$).

be expected to better follow the dynamics of transient neurotransmitter changes.

Electrochemical Overoxidation. Another method for improving electrode sensitivity is to overoxidize the electrode surface. Several strategies for overoxidation have been developed, including repetitive excursions to 3.0 V at 70 Hz before use²⁵ and scanning continuously to a more positive potential.^{16,17} Scanning carbon-fiber microelectrodes to 1.4 V instead of 1.0 V has been shown to increase the peak currents for dopamine 3- to 7-fold,^{16,26} presumably by oxidizing the carbon surface and creating more surface roughness.²⁷ We changed the switching potential from 1.0 to 1.5 V to investigate whether flame-etched electrodes would show greater sensitivity with electrochemical overoxidation.

Increasing the switching potential increased the background currents of the electrodes. However, the flame-etched electrodes had a larger increase in background current with the overoxidized waveform. Consequently, the mean background current for the normal electrodes, 1160 ± 130 nA, was not significantly different from that of the flame-etched electrodes, 900 ± 100 nA ($p = 0.15$).

Figure 5 shows background-subtracted cyclic voltammograms of 1 μM dopamine for flame-etched and normal electrodes with a scan limit of 1.5 V. The peak currents are larger than when the scan limit was 1.0 V (compare Figure 5 to Figure 1C) for each of the electrode types. The current magnitudes of the flame-etched and normal electrodes are about the same for the oxidative waveform. Table 2 gives average data for 1 μM dopamine with a 1.5 V scan limit. The peak oxidative currents were not statistically different, but the normalized current per unit area was larger for

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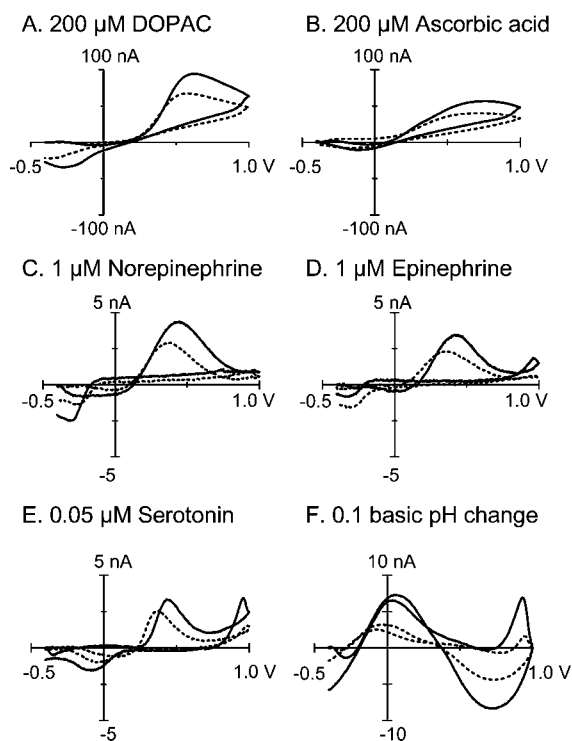


Figure 6. Response to other electroactive compounds. Background-subtracted cyclic voltammograms are plotted for flame-etched (dashed line) and normal (solid line) electrodes. The applied waveform was -0.4 to 1.0 V and back at 400 V/s. Compounds tested were (A) 200 μ M dihydroxyphenylacetic acid (DOPAC), (B) 200 μ M ascorbic acid, (C) 1 μ M norepinephrine, (D) 1 μ M epinephrine, (E) 0.05 μ M serotonin, and (F) a $+0.1$ basic change in buffer pH.

flame-etched electrodes. S/N ratios were also significantly greater for flame-etched electrodes. Therefore, similar currents can be obtained at the smaller diameter flame-etched electrodes without proportionally increasing the noise. This experiment shows that the gains in sensitivity of flame etching and electrochemically pretreating an electrode can be combined to produce an electrode with even greater sensitivity.

With an extended oxidation limit, ΔE_p was no longer different after flame etching (Table 2). ΔE_p increased slightly for normal electrodes after extending the scan limits, but increased by 0.1 V for flame-etched electrodes. The increase may be due to the changes in surface chemistry expected with electrochemical overoxidation,¹⁶ including the addition of oxide groups, which increases sensitivity but slows kinetics. Figure 4B also shows that the time response of both flame-etched and normal electrodes is slowed after overoxidation, with flame-etched electrodes having similar responses to normal electrodes.

Sensitivity to Other Compounds. Figure 6 compares cyclic voltammograms for flame-etched and normal cylinder electrodes for other electroactive compounds in the brain. Dihydroxyphenylacetic acid (DOPAC) and ascorbic acid are two anionic compounds that can interfere with dopamine detection in the brain. DOPAC is a main metabolite of dopamine, and ascorbic acid is a ubiquitous antioxidant, and both are present in high concentrations in the brain.^{24,28} Figure 6 A and B show cyclic voltammograms of 200 μ M DOPAC and 200 μ M ascorbic acid. The peak currents for DOPAC and ascorbic acid were larger at

Table 3. Peak Oxidative and Normalized Currents for Other Compounds^a

	i_{pa} (nA)	normalized i_{pa} (pA/ μ m ²)
norepinephrine		
normal	4.2 ± 0.7	1.3 ± 0.2
flame-etched	2.7 ± 0.3^b	2.2 ± 0.3^b
epinephrine		
normal	3.5 ± 0.4	1.0 ± 0.1
flame-etched	2.3 ± 0.4	2.0 ± 0.3^b
DOPAC		
normal	110 ± 10	36 ± 3
flame-etched	73 ± 6^b	63 ± 6^b
ascorbic acid		
normal	61 ± 4	20 ± 1
flame-etched	43 ± 3^b	36 ± 3^b
serotonin		
normal	2.8 ± 0.5	0.9 ± 0.1
flame-etched	2.3 ± 0.3	2.0 ± 0.2^b
basic pH change ^c		
normal	-7.6 ± 1.1	2.7 ± 0.3
flame-etched	-4.3 ± 1.1^b	3.0 ± 0.6

^a $n = 11$ for normal electrodes and flame-etched electrodes. ^b Value is statistically different than normal electrodes ($p < 0.05$). ^c For the pH change, the currents were measured at 0.6 V, the potential for dopamine detection. These currents are not Faradaic, but arise due to changes in the background charging current.

normal than flame-etched electrodes, but the normalized currents were larger for DOPAC and ascorbic acid at flame-etched electrodes (Table 3). This indicates that the flame-etched electrode surface is more active for anions as well as cations. Similar increases in sensitivity for both dopamine and DOPAC have been observed at fractured glassy carbon electrodes and were attributed to an adsorption mechanism that was not charge specific.²⁹

Norepinephrine and epinephrine are catecholamine neurotransmitters with similar electrochemistry to dopamine.³⁰ Serotonin is an indolamine neurotransmitter with a similar oxidation potential to dopamine.³¹ All three are positively charged at physiological pH. The flame-etched electrodes show a slightly smaller signal for 1 μ M norepinephrine (Figure 6C), 1 μ M epinephrine (Figure 6D), and 0.05 μ M serotonin (Figure 6E). However, when peak oxidation currents were averaged, this difference in peak current was only significant for norepinephrine (Table 3). Flame-etched electrodes had higher sensitivities per unit area for norepinephrine, epinephrine, and serotonin (Table 3). ΔE_p also decreased for each of these compounds at flame-etched electrodes.

Carbon-fiber microelectrodes are also sensitive to changes in concentrations of ions such as Ca^{2+} ³² or pH changes.³³ A pH change can alter the background charging current, causing background subtraction errors that can be confused with Faradaic peaks. Basic pH changes have been observed after neuronal activity *in vivo*,³⁴ and the response to a $+0.1$ unit basic pH change is shown in Figure 6F. The electrodes exhibit a negative current around the oxidation potential for dopamine. Therefore, a pH

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change can be erroneously reported as decrease in dopamine concentration. The size of the pH current at flame-etched electrodes is about half that at normal electrodes (Figure 6F), a significant difference (Table 3). This difference in current was roughly the same as the difference in total background charging currents (1.8-fold greater for normal electrodes). Therefore, the currents normalized for electrode area were not significantly different (Table 3). Flame-etched electrodes do not exhibit a higher sensitivity to pH changes than expected due to their area, in contrast to the rest of the compounds tested. This would be beneficial for *in vivo* studies because the flame-etched electrodes show a higher sensitivity for dopamine but not pH, so pH should be less of an interferent at flame-etched electrodes.

Mechanism of Sensitivity Enhancement. Several mechanisms could be proposed to explain the increased sensitivity of flame-etched electrodes. Decreasing the size of a cylindrical electrode results in a higher diffusional flux, leading to higher current densities for diffusion controlled species.³⁵ However, the increased sensitivity for flame-etched electrodes is not simply due to higher diffusional flux because it is not observed at the electrochemically etched electrodes that were approximately the same size. This suggests that the enhanced signal-to-noise is due to either increased area for adsorption of molecules or faster kinetics at the surface. Our data present evidence for both mechanisms. The SEM images (Figure 2) show more surface bumps and features on the flame-etched electrodes that could increase the area. In addition, the decrease in ΔE_p for flame-etched electrodes indicates faster surface kinetics and the fast temporal response (Figure 4) is an indication that the adsorption/desorption rate constants are faster for flame-etched electrodes.¹²

The enhanced sensitivity and kinetics are similar to those observed after the polishing of graphite electrodes. Graphite has two different surfaces: a basal plane and an edge plane. The planes display very different electrochemical properties, with faster adsorption rate constants and faster electron transfer kinetics of redox couples at edge plane graphite.^{13,36} Defects in the surface create step edges, where some of the edge plane of the graphite is exposed. For highly ordered pyrolytic graphite, peak to peak separation is dependent on surface defect density, with more defects leading to lower ΔE_p values.³⁷ Therefore, any treatment of the carbon fiber that causes defects that expose edge plane sites should be catalytic. For example, laser ablation has been shown to increase the electron transfer kinetics of carbon electrodes.^{18,38} We believe that the defects and bumps on the flame-etched surface could provide additional edge plane sites that offer increased area for adsorption. In addition, more edge plane sites could also lead to increased electron transfer kinetics or faster adsorption rate constants.

In Vivo Detection of Dopamine after a Single Stimulation Pulse. The utility of the flame-etched cylinder electrodes was demonstrated by measuring stimulated dopamine release in anesthetized rats. The working electrode was implanted in the nucleus accumbens, a region highly innervated with dopamine

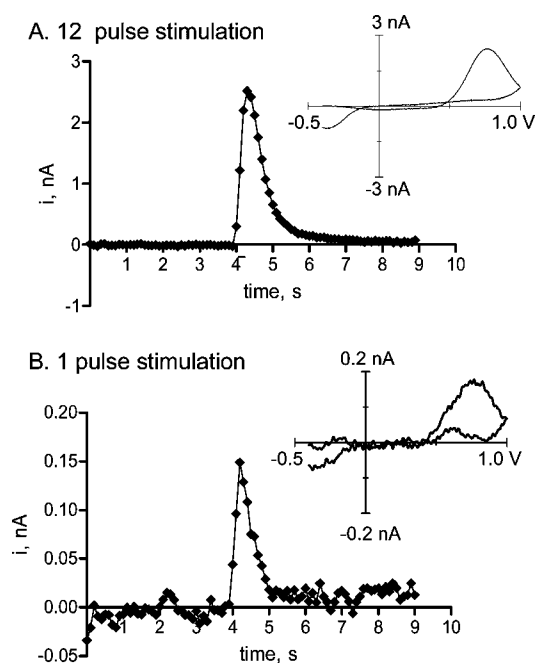


Figure 7. *In vivo* measurements of dopamine with flame-etched electrodes. A 1 μm tip diameter flame-etched electrode, 130 μm long, was implanted in the nucleus accumbens. A stimulating electrode was implanted in the substantia nigra/ventral tegmental area. (A) A train of 12 stimulation pulses was applied at 60 Hz (biphasic, 300 μA , 2 ms per phase) at 4 s. The oxidative current vs time is shown as well as the background subtracted CV. Using precalibration values for the electrode, this is approximately 310 nM dopamine. (B) A single stimulation pulse (biphasic, 2 ms per phase, 300 μA) was applied at 4 s. From precalibration values, the maximum concentration of dopamine is approximately 25 nM.

neurons that mediate reward and addiction. Dopaminergic cell bodies were stimulated in the substantia nigra/ventral tegmental region. Traditionally, stimulated release experiments *in vivo* have used short trains of electrical stimulations because dopamine could not be observed with a single stimulation.³⁹ An example of dopamine release evoked by a train and detected at a flame-etched electrode is shown in Figure 7A. Twelve biphasic stimulation pulses were applied at 60 Hz. The traditional waveform for dopamine detection, scanning -0.4 to 1.0 V and back at 400 V/s, detects a robust dopamine signal. However, in this study we were also able to detect dopamine after a single stimulation pulse (Figure 7B). Dopamine release was clearly observed, even though the signal lasts only about 1 s. Using precalibration values, the concentration for the one pulse stimulation was estimated to be about 25 nM dopamine. While dopamine detection can vary with placement of the electrode *in vivo*, presumably due to the geometry of release sites,⁴⁰ one-pulse release was detected at different electrode placements within this animal and similar results were obtained in 3 other animals. Repeated measurements of 1 pulse stimulations were made for over 2 h, so the sensitivity of the electrode did not decrease over that time.

One-pulse stimulations have been measured after dopamine uptake inhibition,³⁹ but these are the first known published measurements of one-pulse stimulations in an intact, normal

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animal. Dopamine transients have been measured in freely moving animals without stimulations, and these behaviorally evoked concentrations are on the order of 50–100 nM.^{41,42} However, those measurements required scanning to high potentials to increase the electrode sensitivity, which slows the temporal response of the electrodes.¹⁶ Our one-pulse stimulations were collected using the traditional waveform to prove that small amounts of dopamine could be observed without scanning to high potentials. The limit of detection for our measurements was 10 nM. Higher sensitivity would also be achieved at the flame-etched electrodes if the switching potential was increased. The flame-etched electrodes are robust enough to be implanted in the brain and can be used to detect single-pulse stimulations. This method might prove valuable in determining how dopamine released by a single pulse facilitates or depresses subsequent dopamine release.

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CONCLUSIONS

We have shown that flame etching activates the surface of carbon-fiber microelectrodes, leading to increased sensitivity and faster kinetics. In addition, higher sensitivity can be obtained by combining the benefits of flame-etching and electrochemical overoxidation. The electrodes were sensitive enough to detect dopamine after a single stimulation pulse in an anesthetized rat. The flame-etched electrodes should be useful for making measurements of low concentrations of neurochemicals in small environments.

ACKNOWLEDGMENT

This research was supported by the National Science Foundation (CHE 0645587) and the University of Virginia. We thank Sylvia Cechova, Megan Huffman, and the Nanoscale Materials Characterization Facility at UVA for taking the SEM pictures.

Received for review January 17, 2008. Accepted March 17, 2008.

AC8001275