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Temporal Resolution in Electrochemical Imaging on Single PC12 cells using Amperometry and Voltammetry at Microelectrode Arrays

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Abstract

Carbon-fiber-microelectrode arrays (MEAs) have been utilized to electrochemically image neurochemical secretion from individual pheochromocytoma (PC12) cells. Dopamine release events were electrochemically monitored from seven different locations on single PC12 cells using alternately constant-potential amperometry and fast-scan cyclic voltammetry (FSCV). Cyclic voltammetry, when compared to amperometry, can provide excellent chemical resolution; however, spatial and temporal resolution are both compromised. The spatial and temporal resolution of these two methods has been quantitatively compared, and the differences explained using models of molecular diffusion at the nanogap between the electrode and the cell. A numerical simulation of the molecular flux reveals that the diffusion of dopamine molecules and electrochemical reactions both play important roles in the temporal resolution of electrochemical imaging. The simulation also reveals that the diffusion and electrode potential cause the differences in signal crosstalk between electrodes when comparing amperometry and FSCV.

INTRODUCTION

We recently reported the development of a seven-electrode microarray (MEA) to image the exocytotic release of dopamine from single PC12 cells.¹ One of the unique properties of the MEA-based method is that the spatial heterogeneity of these exocytotic events can be directly observed at the single-cell level.¹ Although elegant experiments have been carried out with scanning-probe based electrochemical imaging methods to monitor neurochemical release,^{2,3} the electrode-array approach allows simultaneous measurement of spatially distinct dynamic events with high temporal resolution.

Neurons and other cells are heterogeneous systems owing to specialized protein machineries and lipid domains leading to heterogeneity in the cell membranes, nature and location of exocytotic release. For example, they form hotspots where neurotransmitters are released more frequently.⁴ A single cell usually secretes only one type of neurotransmitter. However,

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SUPPORTING INFORMATION AVAILABLE

The current-time response at the oxidation-peak potential for each color plot in Figure 1b, a representative cyclic voltammetric response in Figure 1b, three more typical amperometric traces. This material is available free of charge from via the internet <http://pubs.acs.org>.

certain neurons might secret more than one type of classical neurotransmitter upon stimulation.⁵ Some nerve and nerve-like cells release both classical and non-classical neurotransmitters, while others release multiple classical transmitters. For instance, PC12 cells are known to mainly release dopamine and acetylcholine. However, for some cell collections norepinephrine molecules are also detected from PC12 cells.⁶ It is thus of great interest to understand the spatial heterogeneity in both release kinetics and the chemical identity of neuronal secretion. Amperometry at carbon fiber electrodes provides excellent temporal and spatial resolution,⁷ but poor chemical resolution in electrochemical imaging. Voltammetry-based methods provide very good chemical information⁸ but only sub-second temporal resolution in single-cell studies.⁹ Proper utilization of these two methods for single-cell electrochemical imaging will therefore offer both kinetic and chemical information at the single-cell level. It is thus valuable to compare the performances of these two methods in electrochemical imaging on single neurons.

In this paper, we demonstrate that FSCV can be performed at individually addressable carbon-fiber MEAs to electrochemically image dopamine-release events from single PC12 cells. In addition, we compare the temporal resolution of FSCV measurements to amperometric measurements for imaging with the MEA. The results confirm that amperometry provides significantly better temporal resolution in MEA-based electrochemical imaging on single cells. We then use simulation models to show that the higher temporal resolution of amperometry results from the fast diffusive flux from the location of release, the fusion pore in the cell membrane during vesicle fusion, to the electrode surface and the electrochemical consumption of dopamine. In contrast, the temporal resolution in FSCV method is over longer time scales owing mainly to the regeneration of dopamine from the oxidized orthoquinone during cycling. This causes it to have a longer lifetime inside the cell/electrode nanogap. The current-time responses in both amperometry and FSCV have been modeled by simulating the diffusive flux of dopamine at the electrode/cell gap. The use of the electrode array on a cell introduces the concept of diffusive crosstalk in these measurements and the simulated results are in agreement with the recorded responses at single PC12 cells.

EXPERIMENTAL SECTION

Chemicals

Dopamine and methoxyferrocene (100%) were purchased from Aldrich. Potassium phosphate monobasic (99.8%), potassium phosphate dibasic (99.9%), KCl (99.9%) were purchased from Fisher. All chemicals were used as received. All solutions were made using 18 M Ω •cm water from a Millipore purification system.

Preparation of Carbon MEAs

The fabrication of seven-fiber MEAs has been previously described.¹ Briefly, the electrode-arrays were made in the following three steps. First, individual 5- μ m-diameter carbon fibers (Amoco, Greenville, SC) were inserted into a 7-barrel glass capillary (7B100F-4, World Precision Instruments). The multi-barrel glass capillary was then pulled using a horizontal glass-capillary puller (P-97, Sutter Instruments) to form two micro-tips. A freshly prepared epoxy (Epo-Tek, Epoxy Technology) was utilized to further insulate the carbon fibers. The electrode tip was cut after drying in an oven and polished at an angle of $\sim 60^\circ$ on a microelectrode beveller (BV-10, Sutter Instruments). Silver paint (Dupont) was injected into each glass capillary to make electrical connection with a tungsten wire placed in each barrel. Epoxy was applied to the capillary outlet to secure the tungsten rods.

Electrode Characterization

Both scanning electron microscopy (SEM) and steady-state voltammetry were typically utilized to characterize each electrode in the carbon-fiber MEAs. Steady-state voltammetric responses were obtained in a 2 mM dopamine solution (pH = 7.4) containing 0.1 M KCl and 10 mM phosphate buffer before and after the electrochemical imaging experiments to ensure that the carbon disk microelectrodes had well defined electrochemical response.

Electrochemical Imaging on Single PC12 Cells

All electrochemical imaging experiments were carried out in a Petri dish containing both the seven-fiber MEA working electrode and a Ag/AgCl reference electrode. The Petri dish was placed on an inverted microscope (CK30, Olympus) to facilitate placements of the microelectrode and a stimulation micropipette. Four bipotentiostats (EI-400, Ensman) were used to perform the multi-channel amperometric and voltammetric measurements. The potentiostats were used in two-electrode mode sharing the same reference electrode for all 7 electrodes. In amperometry experiments, a constant potential (800 mV) was applied at all carbon microelectrodes. The bipotentiostats were interfaced to a PC computer through a multi-channel data acquisition system (Digidata 1440A, Molecular Devices).

In the voltammetric imaging experiment, a triangle waveform, equal and opposite in sign to that desired at the working electrode, was generated by a PC and routed to the reference electrode of the controlling potentiostat. The potential was ramped from -0.4 V to 1.2 V and back at a scan rate of 1000 V/s. This waveform was repeated at 20 ms intervals.

Voltammetric responses were recorded using an in-house LabView program (TH 1.0, ESA). A glass micropipette containing high K^+ (100 mM) solution was positioned ~ 100 μm away to stimulate the cell secretion. Each stimulus was applied for 5 s at 45 -s intervals using 30 psi from a Femtojet (Eppendorf) microinjector. Other experimental details were performed as previously described.¹⁰

Finite-Element Simulations

The amperometric and voltammetric response of carbon fiber MEA on a single PC12 cell was simulated using Comsol Multiphysics® 3.4 software (Comsol, Inc.) on a Dell T7400 workstation (Quad core, 2.5 GHz CPU, 8GB Memory).

RESULTS AND DISCUSSION

Amperometric Imaging

In constant-potential amperometry, dopamine is rapidly and quantitatively oxidized after it is released under an electrode and the current is measured as a function of time. The resultant current vs. time trace is characterized for each release event.^{11,12} Amperometric detection yields both high temporal (~ 100 μs) and spatial resolution (~ 5 μm) due to the small physical size of the electrochemical probe. In our amperometric imaging experiments, the current-time response at each individual microelectrode is equivalent to those obtained at standard single carbon-fiber based electrochemical detection. Figure 1a shows a typical 15-s current-time response at a single PC12 cell. The current transients are detected after cells are stimulated using high K^+ (100 mM) solution. Figure 1a shows spatial heterogeneity in single-cell exocytosis. For example, electrode E in Figure 1a records more numbers of events than other electrodes in the same array covering different parts of the cell.

Fast-Scan Cyclic Voltammetry Imaging

FSCV has been extensively used to measure neurochemicals released at single cells and in the central nervous system with carbon-fiber microelectrodes. FSCV-based methods add

significant chemical resolution and sub-second temporal resolution to these measurements. In our experiments, FSCV has been carried out on individual PC12 cells using the seven individual carbon-fiber microelectrodes in the same array style. The advantage of utilizing FSCV with MEAs on a single cell is that it allows acquisition of chemical information in addition to high spatial resolution in electrochemical imaging.

Figure 1b is a 15-s time duration of a voltammetric imaging response (i.e. FSCV color plots) on a single PC12 cell using a carbon-fiber MEA. Each color plot represents a FSCV response from a 5-Sm-diameter area from the same PC12 cell. The current-time responses at the oxidation-peak potential (~ 0.7 V vs Ag/AgCl, at our scan rate, $v = 1500$ V/s) for each color plot are given in Figure S11a. A comparison of a representative cyclic voltammogram (Figure S11b) and a voltammogram obtained from a dopamine solution at the same experimental condition indicate that the secreted molecule is dopamine. Figure 1b also shows that all exocytotic events show the same oxidation and reduction potentials, indicating that dopamine molecules have been released during this recording period.

Importantly, a comparison of the FSCV responses from all seven microelectrodes also shows that some exocytotic events occur at the same time at different electrode locations on the cell. One possibility is that multiple exocytotic events are being co-detected at different electrodes; however, it is possible that separate events cannot be temporally separated from each other due to their relatively long time duration. For example, an exocytotic signal is observed in Figure 1b at channel B at about 4.5 s. Simultaneous signals are also detected on all the other channels except channel A. Similarly, a transient is observed from channel A at about 8 s, with signals simultaneously detected at channels E, F, and G. Overlapping signals are also observed at several other time spots.

Figure 2a and 2b display a comparison of the temporal resolution of amperometry and FSCV in MEA-based electrochemical imaging on single PC12 cells. The results for amperometry and FSCV imaging reveal several distinct differences in the MEA experiments. The temporal resolution in amperometric imaging is again significantly higher than in FSCV. The peak width in amperometry is about 5 ms for PC12 cells. However, FSCV signals under the conditions used here have typical half widths of about 200–300 ms, which are more than 10 times longer than in amperometry. As a result, there is a greater possibility in FSCV for concurrent or overlapping signals to occur at an individual electrode as well as crosstalk between electrodes (unoxidized dopamine molecules have a longer time to diffuse to other microelectrodes). Thus, there are more simultaneous signals observed in voltammetric imaging experiments than in amperometry. In amperometric imaging, it is very unusual to observe simultaneous exocytotic signals from three or more locations on the same cell. However, exocytotic events are often detected from more than three locations in FSCV-type imaging experiments.

Electrochemical signals are generated differently in amperometry and FSCV for obvious reasons. As illustrated in Figure 3, in amperometry, electroactive neurotransmitters are quantitatively oxidized by the microelectrochemical probe placed in close proximity to the cell. Thus, the effective surface concentration of redox neurotransmitters approaches zero at the electrode surface under amperometric conditions. This generates a large concentration gradient between the site of release and the electrode surface (about 0.1 M inside the vesicle to essentially 0 at the electrode over a distance of ~ 200 nm^{13,14}) resulting in a large flux of neurotransmitters perpendicular to the cell surface. The diffusion of neurotransmitters in parallel to the cell membrane is slow compared to the oxidative consumption. As neurotransmitters released from underneath the electroactive surface of a specific electrode are oxidized quickly, this results in no significant crosstalk at adjacent electrodes. Our numerical simulation results show that even when neurotransmitters are released from in

between two adjacent electrodes, they are mainly detected on the electrode closer to the fusion pore.

In FSCV experiments, however, electrochemical detection is obviously based on cycling oxidation and reduction of electroactive species, thus depletion does not occur as rapidly as for amperometry, but is dependent on diffusion out from under each electrode and degradation via coupled chemical reactions. Figure 3 shows released dopamine molecules being detected by repeated oxidation/reduction by FSCV at the individual electrode surfaces in the MEA. Here we assume any coupled chemical reactions are negligible so neurotransmitters are not consumed during electrochemical detection. This diffusion of neurotransmitters parallel to the cell membrane and electrode surface is generally slow compared to the time scale of the exocytosis event leading to significant broadening of FSCV signals. Additional diffusion resistance might result from adsorption of dopamine on carbon surfaces.¹⁵ In addition to broadening the response under an individual electrode, the lack of electrochemical consumption of the released transmitter in FSCV experiments means that molecules released from under one electrode can diffuse to the adjacent electrodes leading to significant crosstalk. This is an important issue in spatial resolution when using arrays of electrodes.

Numerical Simulation

In order to better understand the differences in the temporal resolution and potential crosstalk in both amperometric and voltammetric imaging experiments, we simulated the electrochemical current at microelectrode arrays resulting from molecular diffusion from release via an open fusion pore (intermediate of exocytosis). The simulation is based on three-dimensional diffusion in a defined geometry involving both the cell and a microelectrode array placed above the cell membrane.

A cross-section of the geometry of the electrochemical cell used for the simulation is shown in Figure 4a (not drawn to scale). For the simulation, a PC12 cell is represented by the black area measured as 20- μm in diameter and 10- μm in height. A cylindrical fusion pore is formed between an intracellular vesicle (150-nm-diameter¹⁰) and the cell membrane (5-nm thick). The position of the release site or fusion pore is measured about L nm away from the center of the PC12 cell, which is varied to study the crosstalk as a function of the position of the release site. A microelectrode array is positioned above the cell membrane with a fixed cell/electrode gap measured of about 200 nm.^{13,14} The microelectrode-array contains seven microelectrodes, with each microelectrode having 5- μm tip diameter. The distance between adjacent electrodes (edge to edge) is assumed to be 1 μm . The total diameter of the array and the height of the microelectrode-array have been set to be 20 μm . The dimension of the electrochemical cell is much larger than both the size of the fusion pore through which release occurs and the electrode/cell gap, thus semi-infinite diffusion prevails outside the gap. Here, the cell and the electrode are placed in a solution, which measures 100- μm in diameter and 60- μm in height. The concentrations of dopamine inside and outside the vesicle have been set to be 150 mM and 0 mM, respectively, at time = 0 of the simulation. The intravesicular concentration prior to release was then calculated based on the number of dopamine molecules detected after release in amperometry and the volume of the vesicle in the simulation.

Exocytosis is a dynamic and complex process involving Ca^{2+} induced protein-protein interaction, and the opening and the enlargement of the fusion pore.¹⁶ Neurotransmitters are released after the opening of the fusion pore, quickly reaching a maximum of diffusive flux followed by a decay of the flux to zero.¹⁷ We were unable to model the entire dynamic change in fusion pore opening. However, we have successfully modeled both the decay of the amperometric current, which is the major part of the entire amperometric signal, and the

shape of the FSCV signal using a fixed geometry of the fusion pore. We thus believe we can utilize the same simulation to evaluate the temporal resolution and potential crosstalk in the electrochemical imaging experiments. Our simulation results from both the amperometric signal and the FSCV signal give good agreement with the experimentally recorded signals.

Numerical Simulation of the Amperometric FSCV Signals

Although it is not the point of this paper, an important consideration in these simulations is the size of the fusion pore (the opening between the vesicle and the cell membrane during fusion). This was obtained by simulating a typical amperometric current-time response. We simulated the change in the flux of dopamine at the electrode surface as a function of time and then converted it into amperometric current using a 150 mM dopamine concentration. The diameter of the fusion pore was varied to make a comparison to a typical amperometric signal. Figure 4b displays different amperometric responses relative to the simulation when the diameter of the fusion pore was varied between 2 and 8 nm. The decaying part of the signal in the simulation was compared to the recorded signals as shown in Figure 4b and c. The simulated peaks shown in Figure 4b demonstrate that the amperometric current is dependent on the size of the fusion pore. The magnitude of the amperometric peak is larger when the fusion pore is larger corresponding to a larger diffusive flux. Figure 4c is typical signal recorded from amperometric recordings with the MEA at single PC12 cells (additional traces are provided in the supporting information, Figure SI2). When comparing the simulations in Figure 4b with the experiment, we found that most of the typical signals have peak shapes that fall between those simulated with the diameter of the fusion pore is between 4 and 6 nm. Hence, we used 6 nm for the subsequent simulations. This value is consistent with the work of Amatore and coworkers showing that the pore opening angle appears to be too small to represent full opening.¹⁸

The same geometric parameters and similar dopamine concentrations in and outside the fusion pore were used for the simulations of the FSCV peak for dopamine and the simulations of amperometry. The only difference was in the boundary conditions used at the microelectrodes. In the FSCV experiments, the dopamine molecules are not consumed. Thus, the boundary condition at the microelectrode surface was set to be a constant flux = 0 and the simulation of the voltammetric signals has been carried out using a thin-layer-cell type model.¹⁹ This approach is valid as the gap between the electrode and the cell membrane is much smaller than the diffusion distance during the time course of the voltammetric experiment.¹⁹ In the simulation, the dopamine molecules were allowed to diffuse freely after they are secreted from the fusion pore. The number of the dopamine molecules underneath each microelectrode can be integrated from the simulation at any given time whereby the FSCV peak current was calculated by using the number of the molecules present under each electrode,¹⁹

$$i_p = \frac{n^2 F^2}{4RT} N \nu \quad (1)$$

Here, i_p is the peak current in FSCV, n is the number of electrons transferred per dopamine molecule, F is the Faraday constant, R is the gas constant, T is the temperature, N is the number of dopamine molecules present underneath a single carbon fiber microdisk and ν is the sweep rate. Figure 4d is a simulated FSCV signal when the exocytotic release is located at the center of the electrode. Figure 4e shows an example of two typical FSCV signals with current at the voltammetric peak plotted vs. time. This approach must be qualified. We chose “typical” events here as average events will include those at different points from the electrode center. One can immediately notice that the peak current and the overall shape of the simulated response are in good agreement with the experimental recordings except that

the simulated data show transients that are much faster than observed in the experimental recordings. In fact, the peak width in the simulation is about 1/3 than that in the experiment (predicted diffusional transport is approximately 9 times faster than observed). There are two possible reasons for this observation, which might contribute to the faster diffusion of dopamine in the simulation. First, the roughness of the cell membrane and the electrode surface might increase the diffusion length, or decrease the apparent diffusion coefficient.²⁰ Second, it has been reported that dopamine strongly adsorbs at carbon electrodes,¹⁵ which might effectively slow down the apparent diffusion of the molecules.

The comparison between the recorded electrochemical data and the simulation reveals that the temporal resolution in electrochemical imaging can be qualitatively understood by release dynamics coupled to the diffusion of dopamine molecules in the electrode/cell gap. In amperometry, where dopamine is rapidly and completely oxidized to the orthoquinone, the temporal resolution is only limited by how fast dopamine molecules are released from the fusion pore. In FSCV, the temporal resolution mainly depends on the diffusion of the dopamine molecules away from the electrode/cell gap. When this diffusion is to the space under an adjacent electrode, then electrochemical crosstalk occurs.

Amperometric Imaging

To analyze the potential for crosstalk in amperometric imaging, the position of the release site (the star in the insets of Figure 5) was varied between the microelectrode in the center (black circle) and two adjacent electrodes (Red circle and blue circle), as shown in Figure 5. Figure parts 5a through 5c show the amperometric response at three adjacent electrodes as a function of the position of the release site. Figure 5a shows the responses at each microelectrode when the release site is present at the mid-point of the center electrode. One can see that there is no response at any other electrodes in the same array when release occurs at the center of a given microelectrode in the array. Figure 5b shows the response of three electrodes when release occurs under the center electrode, but ~50-nm away from the edge. The response at the adjacent electrode closest to the location of release shows about 2 percent the peak current observed under the center electrode and no current is observed at the other adjacent electrode on this current scale. Thus there is very little crosstalk in amperometric imaging when the release site is located underneath one microelectrode in the same array.

When release occurs under the insulating material in between two microelectrodes, the simulation shows that the current at each electrode is dependent on their distances to the release site. Figure 5c displays the simulated amperometric response at three electrodes when the release site is present in between the center electrode (black) and the edge (blue) one. The currents at the center electrode (black) and the edge electrode are comparable when release occurs in the middle of the two microelectrodes. The current at the other edge electrode (red) is negligible as compared to the other two. Another important fact is that the two signals are comparable only when the release location is very close to the middle of the two microelectrodes. Therefore, crosstalk only occurs when the release occurs right at the middle of two adjacent electrodes.

When the location of release is close to the center of three adjacent microelectrodes, the simulated signals are comparable but are much smaller than the signals recorded when the vesicle is underneath the electrodes. In fact, the average peak current in this case is only ~10% of the current when the vesicle is present under a single microelectrode.

FSCV Imaging

The simulations suggest that strong crosstalk is almost always present in FSCV imaging no matter where the location of release is found. Figure 6a shows the simulated FSCV peak current as a function of time when the release site is present at the center of the center electrode (black). It is immediately apparent that the signal at the adjacent electrode is 20–30% of the center electrode, indicating strong crosstalk. When the release site is not present under an electrode, the crosstalk is even stronger. Figure 6b shows the simulated signals when the release site is in between two adjacent electrodes. The signals are approximately the same for the two adjacent electrodes, which is about 25% of the signal when the vesicle is right under the microelectrode. Thus under the current conditions, the crosstalk can always be observed in FSCV type electrochemical imaging. This is in fact not surprising and has been reflected in our single-cell recording using FSCV, but we use the simulation to quantify and verify this effect here. Varying the sweep range of the applied potential could possibly eliminate the crosstalk and increase the temporal resolution in FSCV type experiments.

CONCLUSIONS

Carbon-fiber MEAs have been utilized to compare electrochemical imaging at single PC12 cells with amperometry and FSCV. Each method has advantages and shortcomings in single-cell electrochemical imaging. Amperometry offers better temporal resolution, but very little chemically specific information. Fast Scan voltammetry can be utilized to obtain better chemical resolution, but offers poorer temporal resolution and more crosstalk, which make it difficult to obtain high spatial resolution in microarray-based electrochemical imaging.

Numerical simulations have been utilized to understand the temporal resolution in electrochemical imaging at single cells. The results are aimed at single cells and would not be applicable to *in vivo* work where the electrode tip is not so close to the exocytosis source. The differences in temporal resolution can be qualitatively understood by diffusion of neurotransmitters inside the electrode/cell gap and the way electrode potential is applied in each method. Temporal resolution in amperometry is submillisecond; however, the resolution in FSCV is apparently more in the range of 300 ms in these experiments and the simulations also indicate that crosstalk is a negligible issue in amperometric imaging at microelectrode arrays.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) A 15-s amperometric imaging response of a single PC12 cell after a 5-s K^+ stimulation, and (b), a 15-s voltammetric imaging response on a single PC12 cell using a carbon-fiber MEA. Electrodes in the array are labeled A-G with G being the middle electrode.

Figure 2.

A comparison of the temporal resolution of FSCV and amperometry: (a) a 4-s amperometric response of a single carbon-fiber microelectrode on a PC12 cell, and (b), a 4-s trace of an FSCV recording on a single PC12 cell showing the peak current as a function of time. The inset in (b) shows the FSCV recording. The smaller spikes have been identified as noise based on their regularity and the lack of a chemical shape to the voltammogram.

Figure 3.

A schematic drawing of the concentration of released dopamine from a single vesicle in two different electrochemical recordings: FSCV and amperometry. The diagram shows the change in concentration of dopamine at early (μ s to ms) and later (ms to s) time scales following exocytosis.

Figure 4.

(a) The geometry of the electrochemical cell utilized for numerical simulations (cylindrical model through the electrode array with the cell below); (b) and (c), a comparison of the simulated amperometric response vs. a typical amperometric response. The size of the fusion pore for transmitter release was varied between 2 and 8 nm; (d) and (e), a comparison of the simulated FSCV response vs. a typical FSCV response, a fusion pore of 6-nm was used in the simulation.

Figure 5.

Simulated amperometric response at three adjacent microelectrodes in the MEA when the site of exocytotic release is localized at three different locations under the center carbon surface. The “*” in each figure indicates the location of release. The amperometric response (left side) is coded to the electrode by color.

Figure 6.

Simulated FSCV response at two adjacent electrodes when (a) the location of release is in the center of one electrode, and (b) when the location of release is between the two electrodes. The “*” in each figure indicates the location of release. The amperometric response (left side) is coded to the electrode by color.