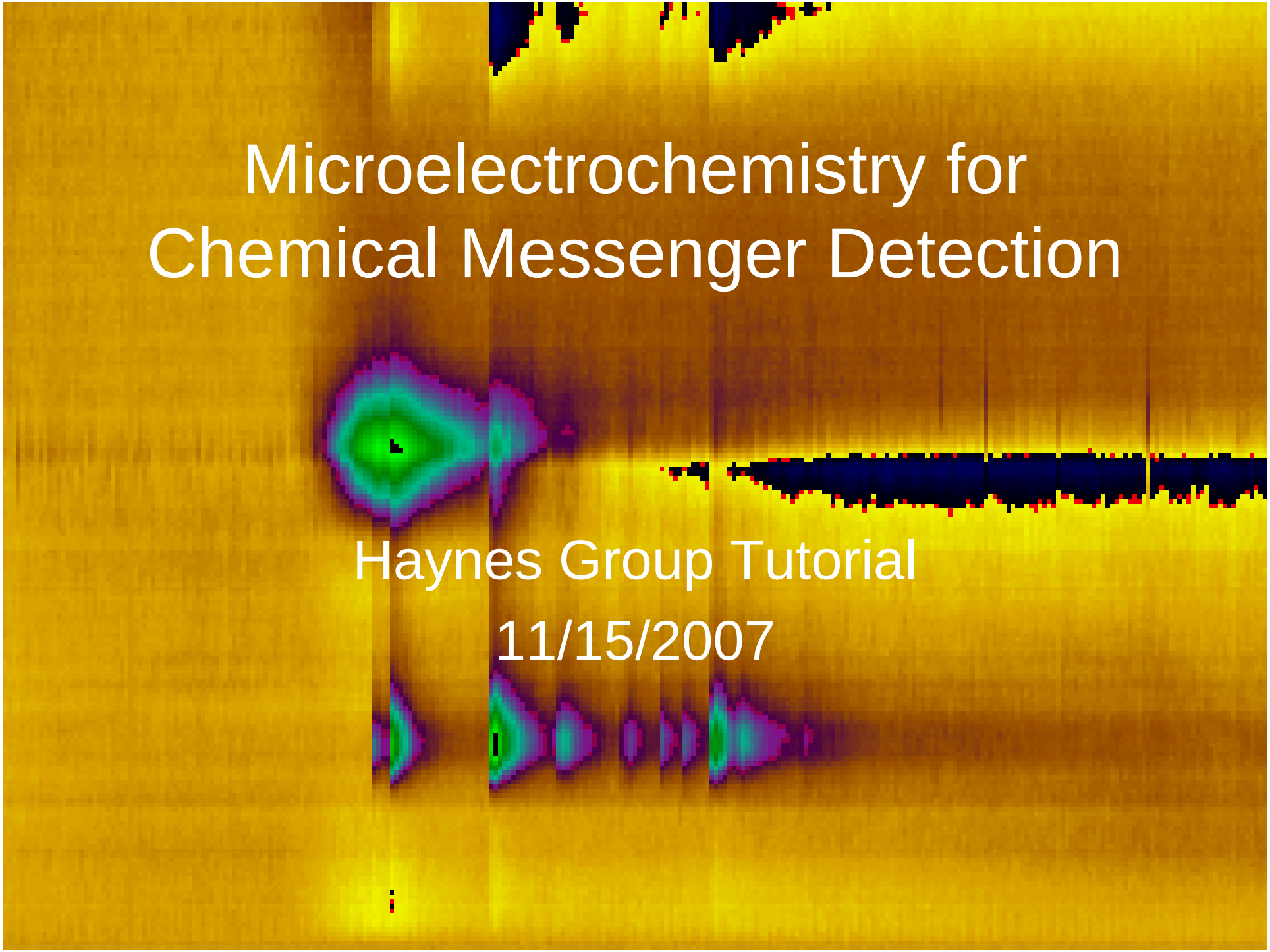


Microelectrochemistry for Chemical Messenger Detection

Haynes Group Tutorial

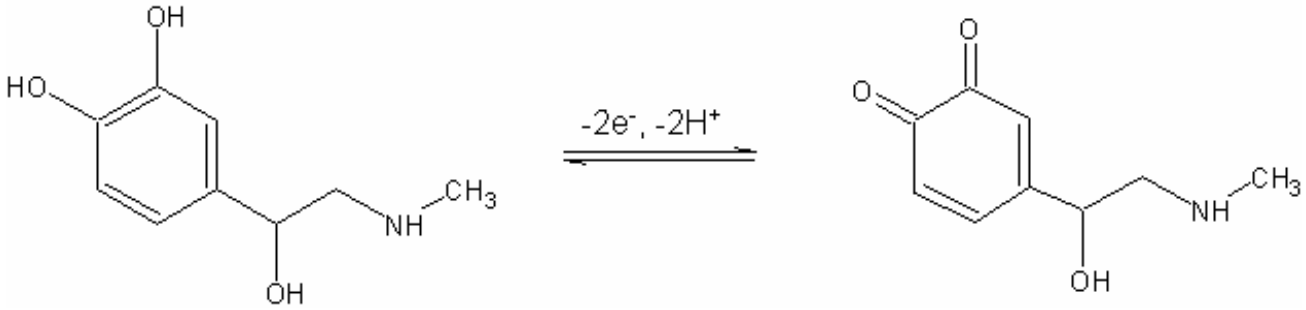
11/15/2007

Tutorial Outline

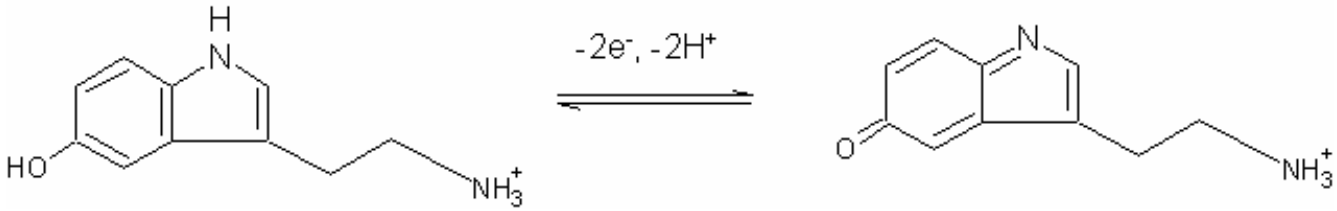
- Introduction to microelectrodes
- Fast-scan cyclic voltammetry
- Constant potential amperometry
- Bioanalytical applications of microelectrochemistry

Relevant Redox Reactions

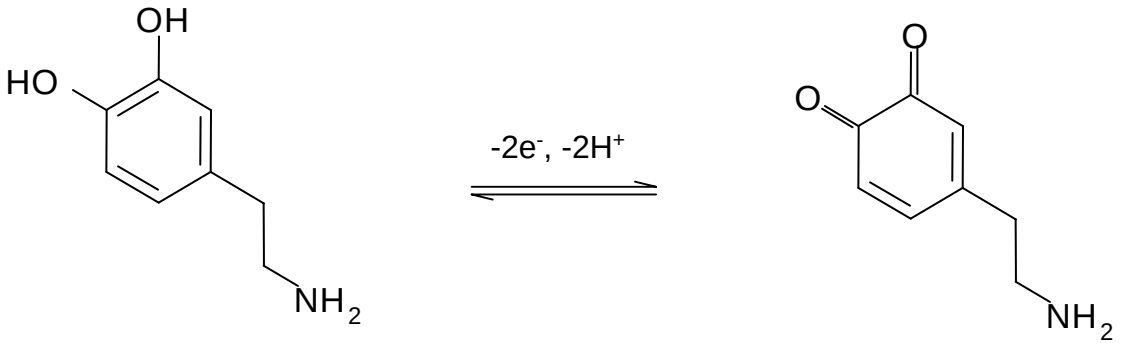
Epinephrine
Eapp = +650 mV
Chromaffin Cells



Serotonin
Eapp = +650 mV
Mast Cells

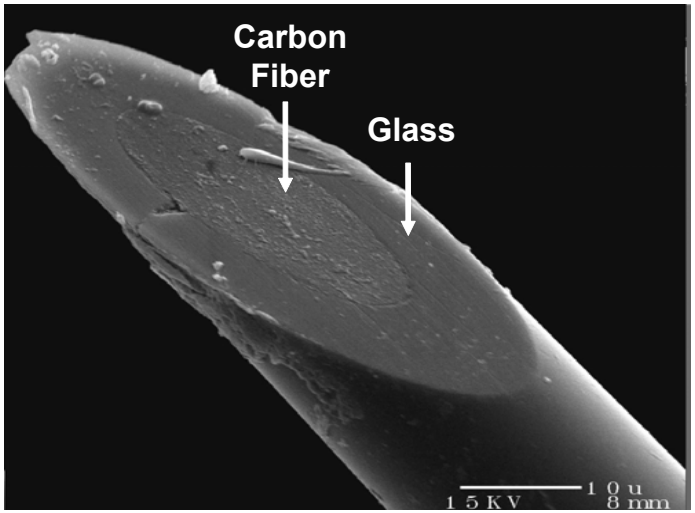


Dopamine
Eapp = +650 mV
Striatal Neurons



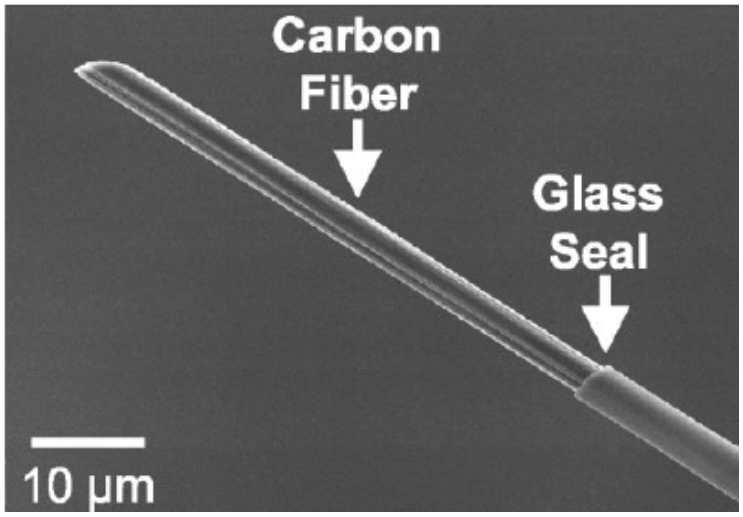
Carbon-Fiber Microelectrodes

Disk

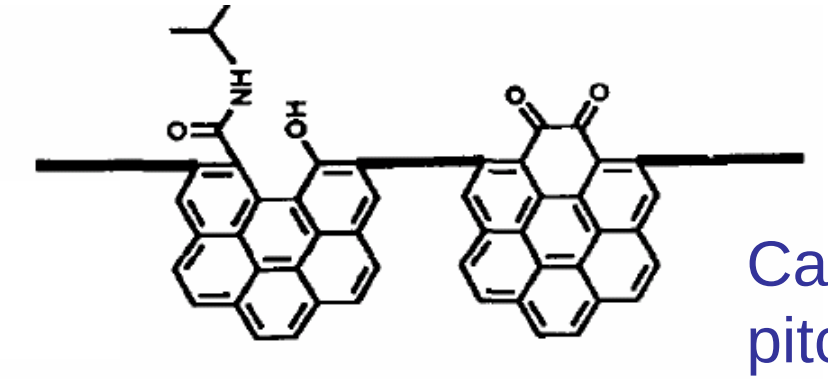


SA ~ 50 μm²

Cylinder

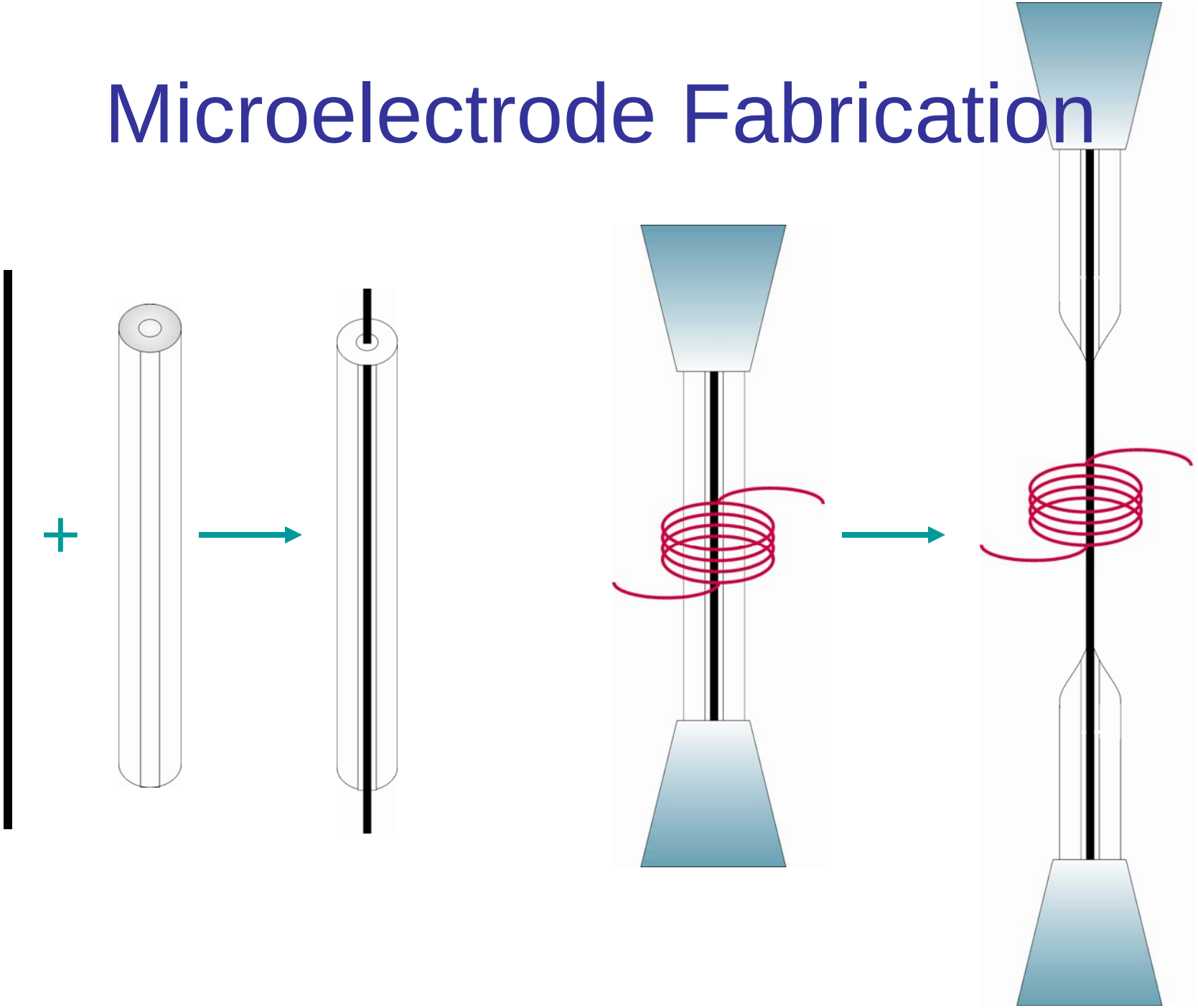


SA ~ 1000 μm²

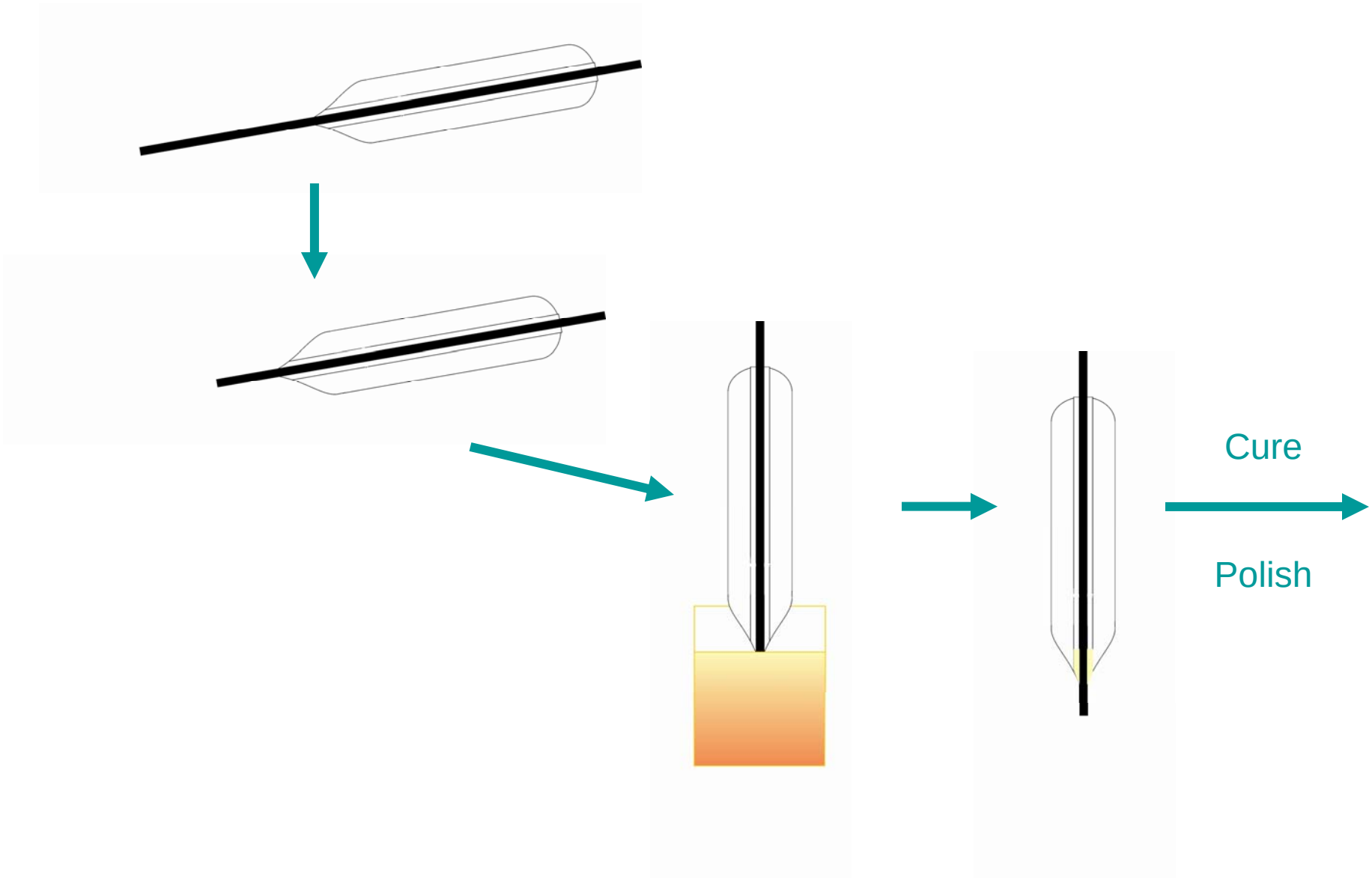


Carbon fibers made from either pitch precursor or polyacrylonitrile.

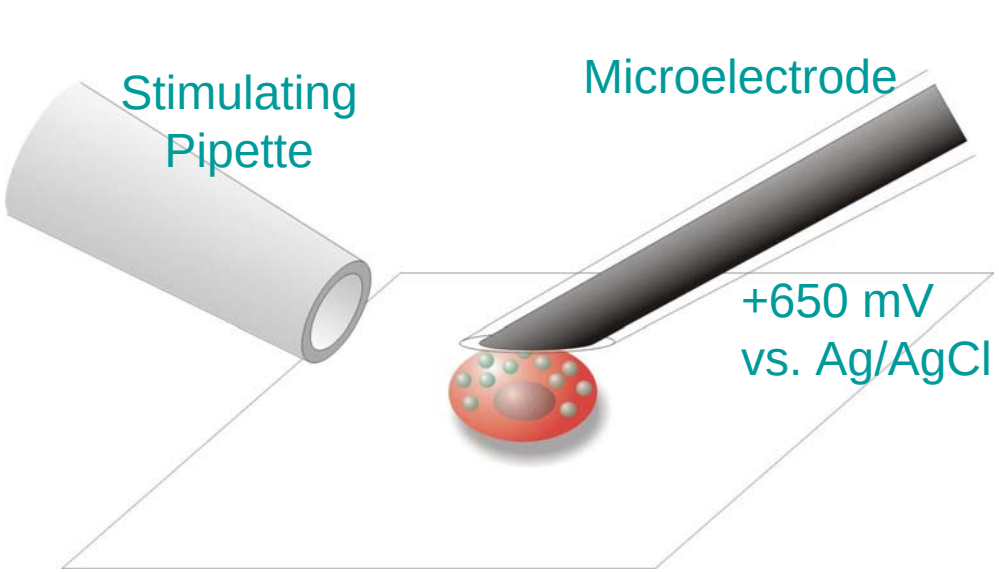
Microelectrode Fabrication

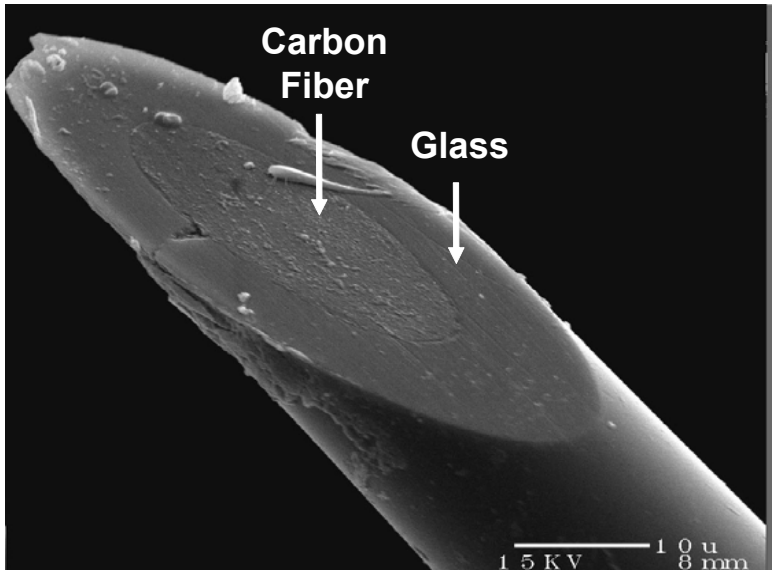


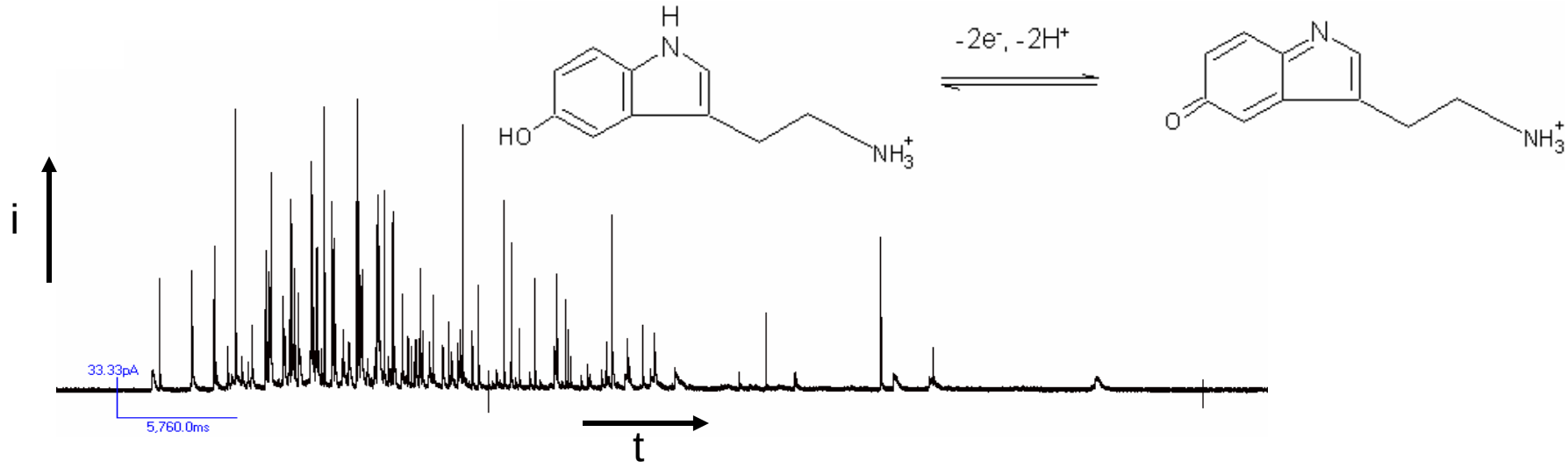
Microelectrode Fabrication



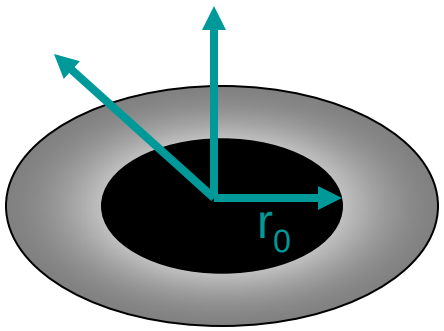
Amperometry







Diffusion at Disk Microelectrodes



Diffusion occurs in two dimensions

*radially wrt the axis of symmetry

*normal to the plane of the electrode

So, current density is not uniform across the face of the disk (greater at the edge).

Current-time relationship has 3 regimes:

1. Short time scale (diffusion layer << r_0): diffusion has a semi-infinite linear character
2. Intermediate time scale (diffusion layer ~ r_0): radial diffusion becomes important and the current is larger than for a continuation of pure linear diffusion
3. Long time scales (diffusion layer >> r_0): the current approaches a steady state

Diffusion-Limited Current

The Cottrell equation describes change in current with respect to time at a planar electrode in a controlled potential experiment under diffusion control.

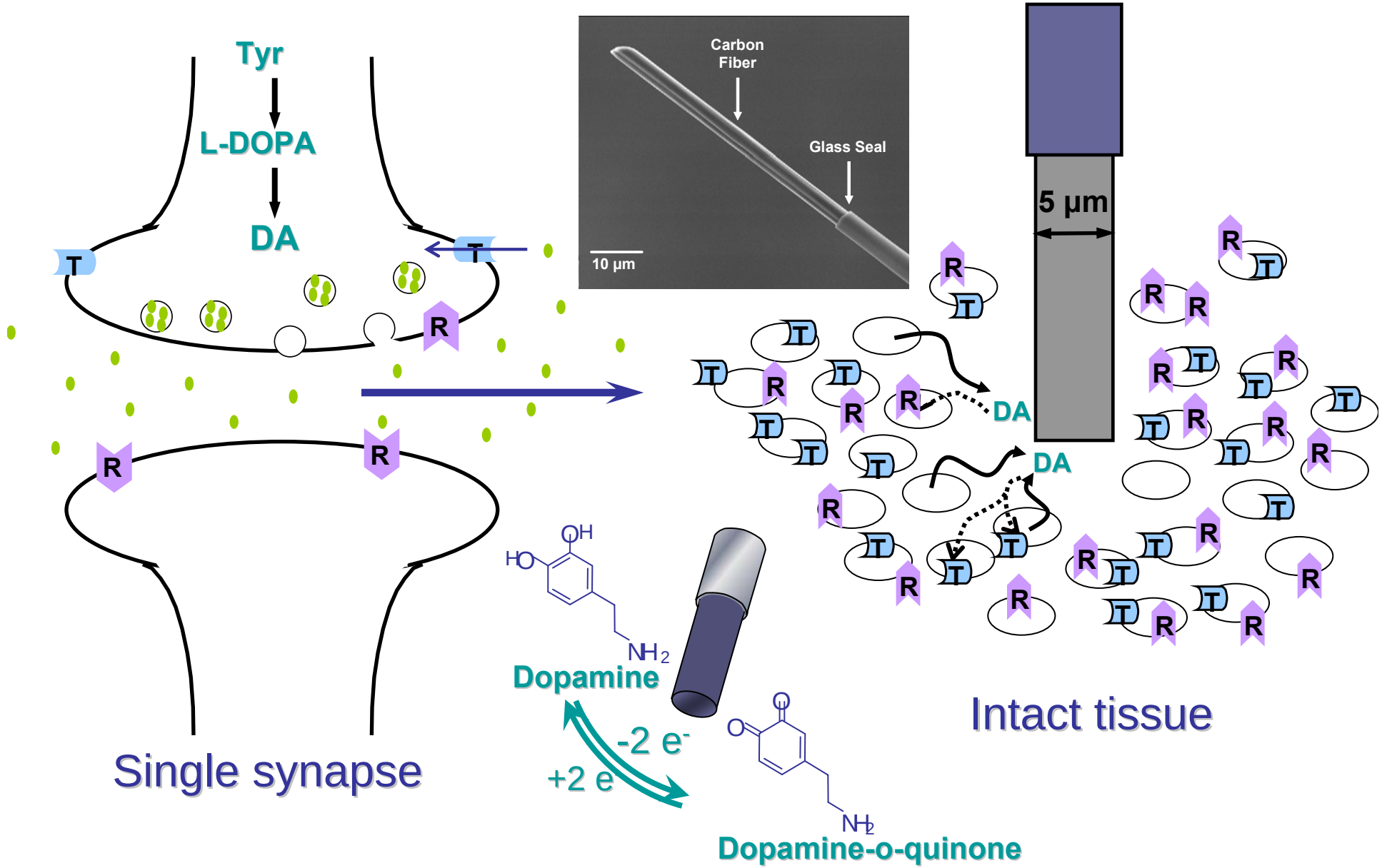
$$i_d(t) = \frac{nFAD_O^{1/2}C_O^*}{\pi^{1/2}t^{1/2}}$$

n = number of electrons exchanged
F = Faraday's constant (96,485 C/mole)
A = electrode area (cm^2)
D_O = diffusion coefficient for species O (cm^2/s)
C^*_O = initial concentration of the reducible analyte O (M)

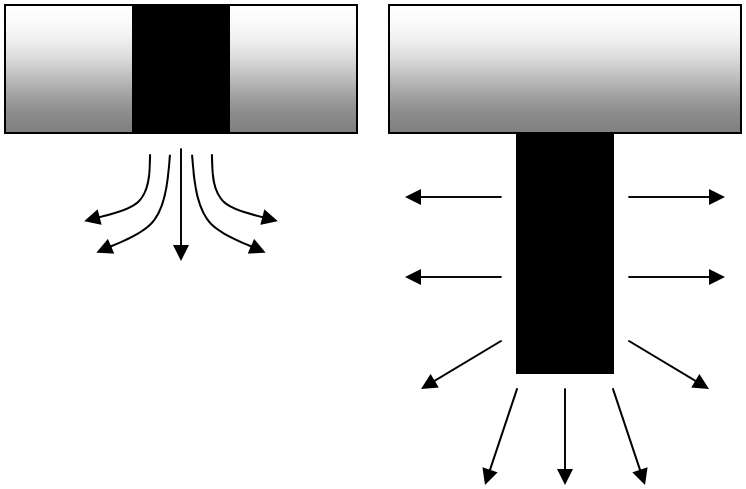
Modified Cottrell equation for disk microelectrode:

$$i_{ss} = \frac{4nFAD_OC_O^*}{\pi r_0} = 4nFD_OC_O^*r_0$$

Dopamine Synthesis, Release, and Uptake



Diffusion at Cylinder Microelectrodes



Diffusion occurs in one dimension

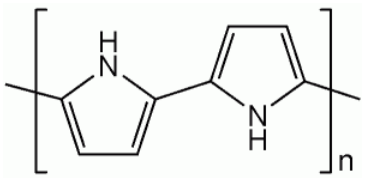
Current-time relationship has 2 regimes:

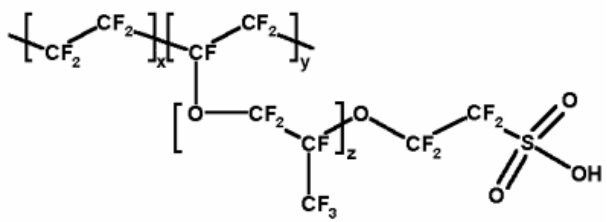
1. Short time scale (diffusion layer << electrode curvature):
   diffusion follows Cottrell equation
2. Long time scales:

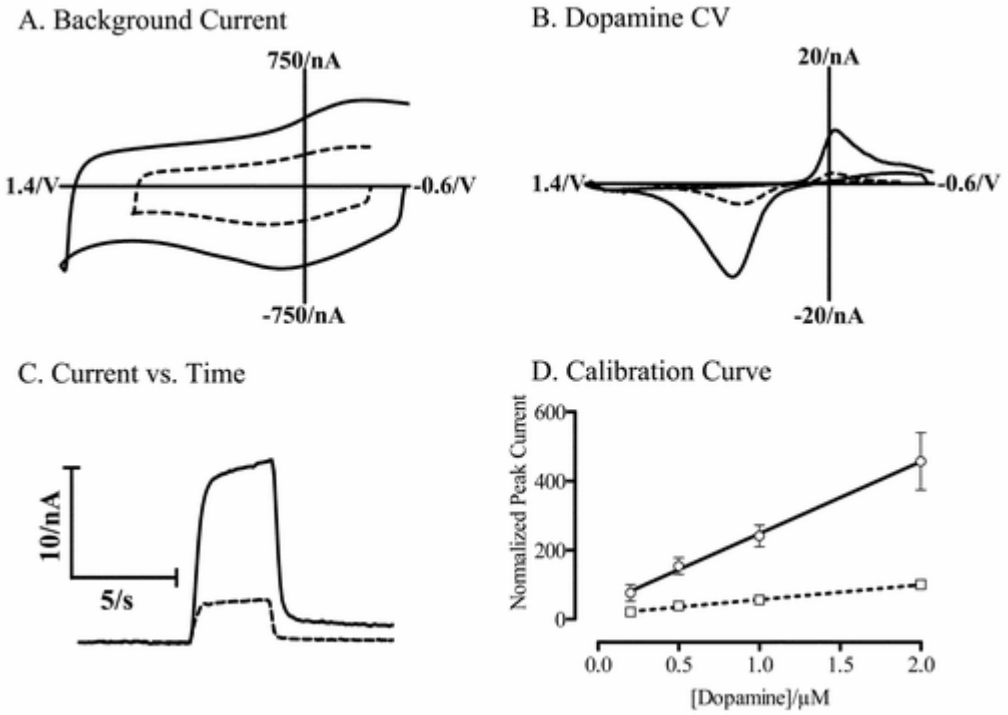
$$i_{qss} = \frac{2nFAD_O C_O^*}{r_0 \ln \tau} \text{ where } \tau = \frac{4D_O t}{r_0^2}$$

Modifying Carbon-Fiber Microelectrodes

- Combating fouling of the carbon surface
  - Apply CV in 0.1 M NaOH
- Treatments for increased sensitivity/selectivity
  - overoxidation
  - nafion
  - polypyrrole
  - 4-sulfobenzene



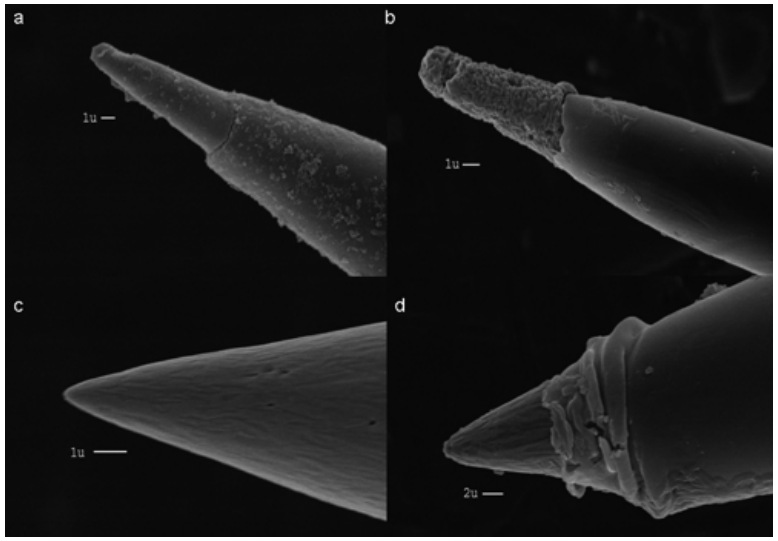




Heien et al, *Analyst*, 2003.

Other Electrode Materials

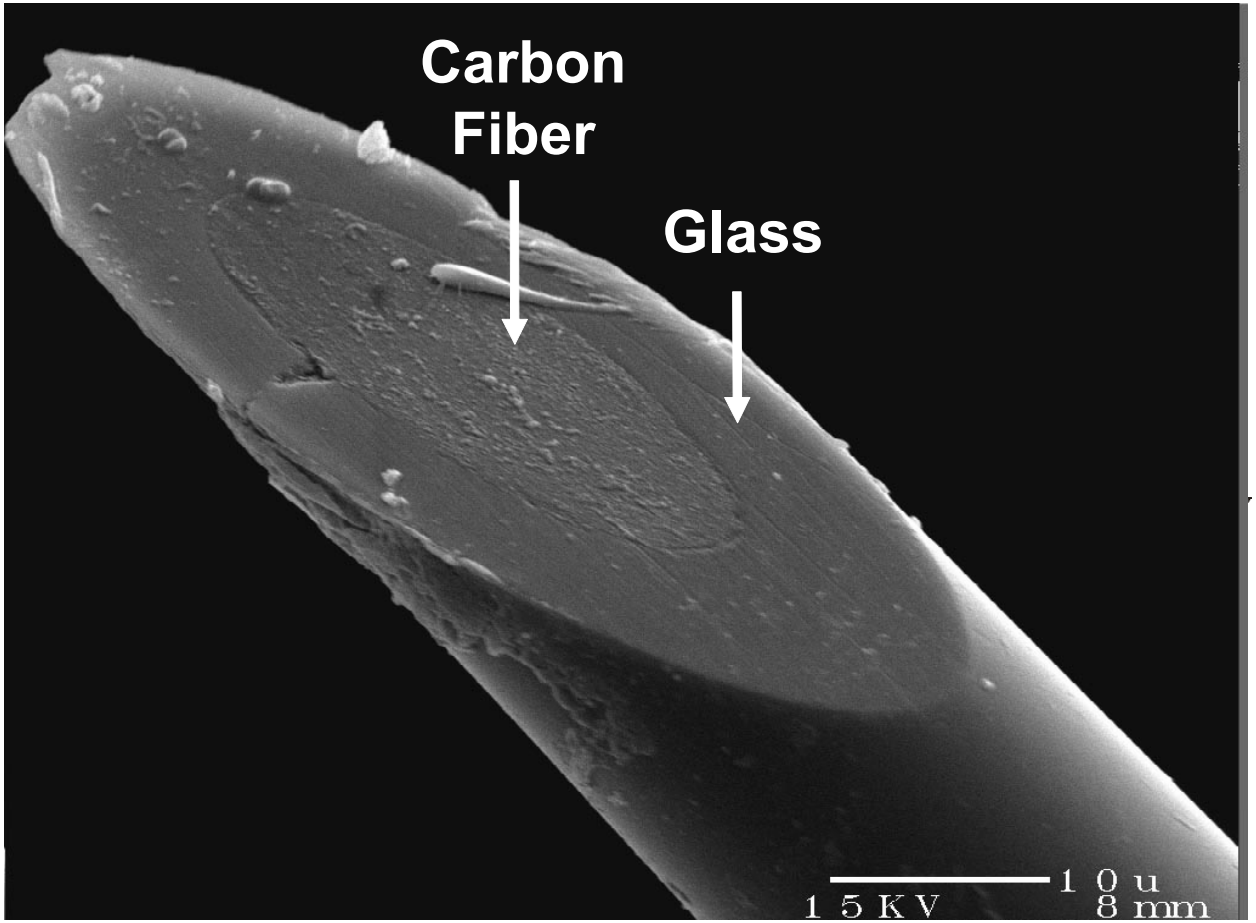
- Other microelectrode materials
  - Pt
  - Au: harder to keep clean
  - BDD: decreased fouling
- Plate materials onto W
  - increased rigidity
  - bend without damage



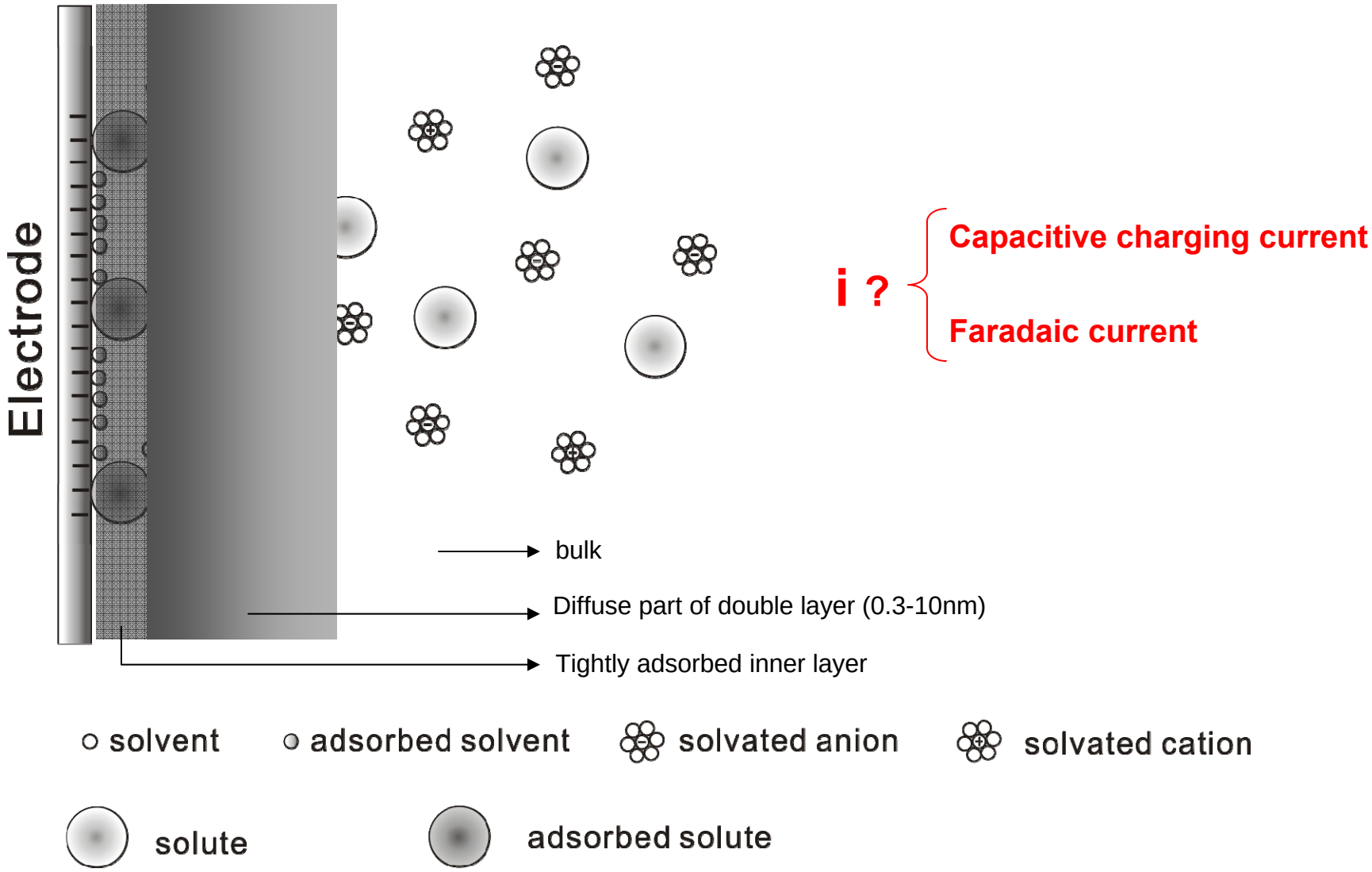
Hermans et al, *Langmuir*, 2006.

Fast-scan cyclic voltammetry

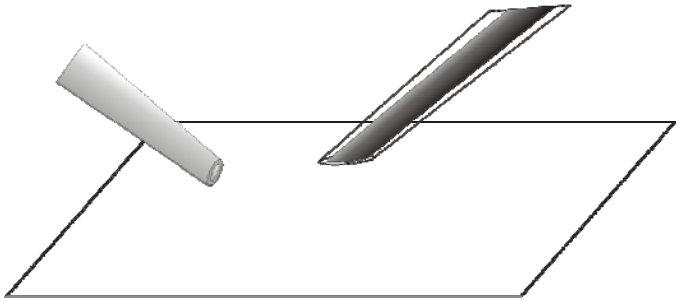
An electrochemical method to collect current signal derived from an applied waveform, typically triangular waveform.

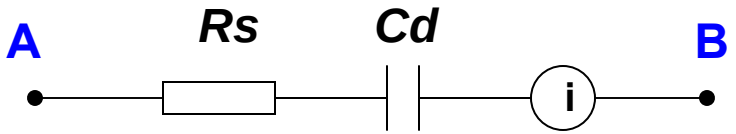


The Electrical Double Layer on a carbon-fiber microelectrode

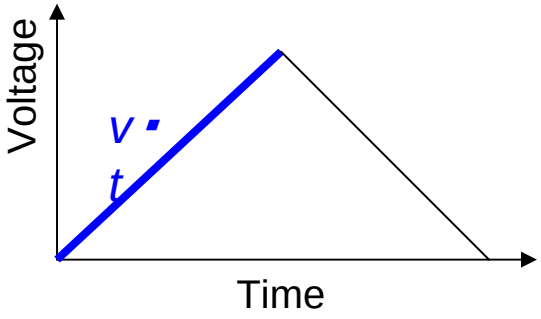


Capacitive charging current





Circuit Model with Rs representing solution resistance and Cd for double layer capacitance at the electrode surface

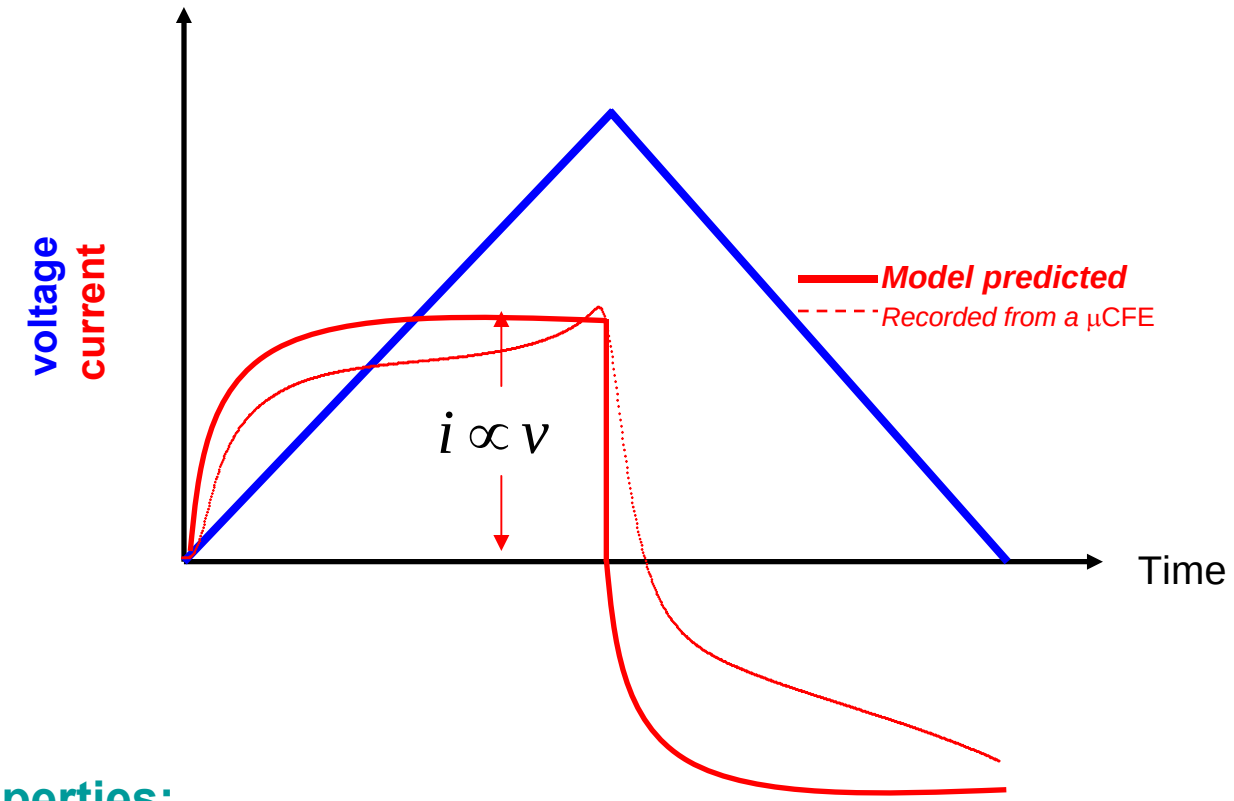


$$E_{AB} = E_{Rs} + E_{Cd}$$

$$v \bullet t = Rs(dQ/dt) + Q/Cd$$

$$i = v \bullet Cd \bullet [1 - e^{-\frac{t}{Rs \cdot Cd}}] \qquad \propto v \quad \textbf{\textit{scan rate}}$$

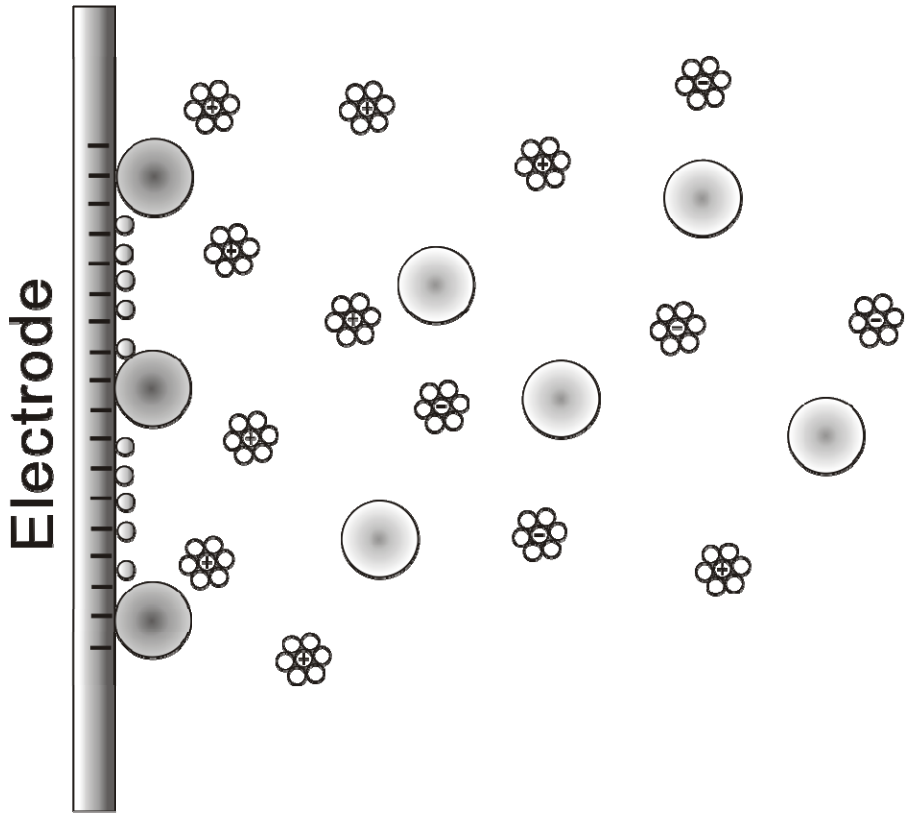
Capacitive charging current



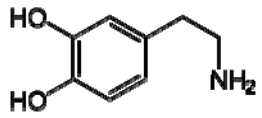
Properties:

- Induced by voltage scanning
- Current amplitude is proportional to scanning rate (viz., $i \propto v$)
- Negligible e.g. 10mV/s at a regular macro-size electrode
- Substantial e.g. 1000v/s at a micro-size electrode,such as μCFE

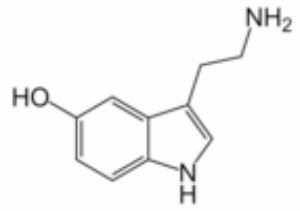
Faradaic current



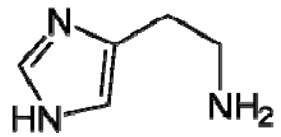
Electroactive Solutes



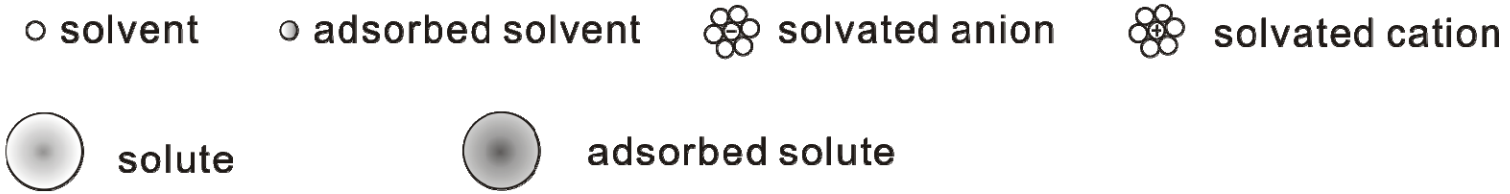
dopamine



serotonin

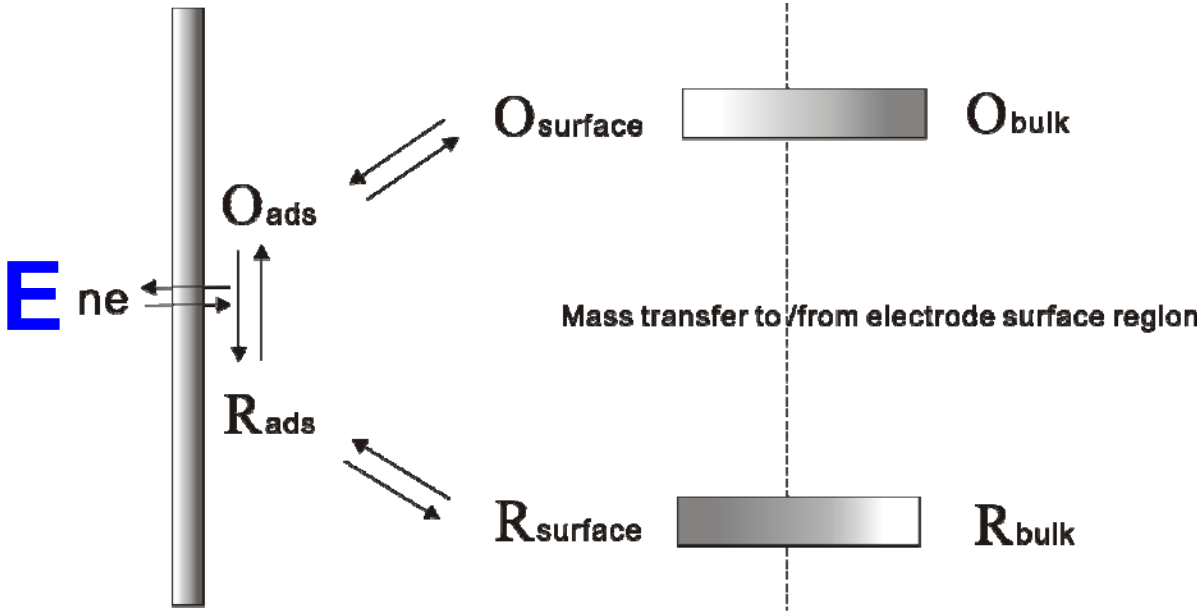


histamine



Faradaic current

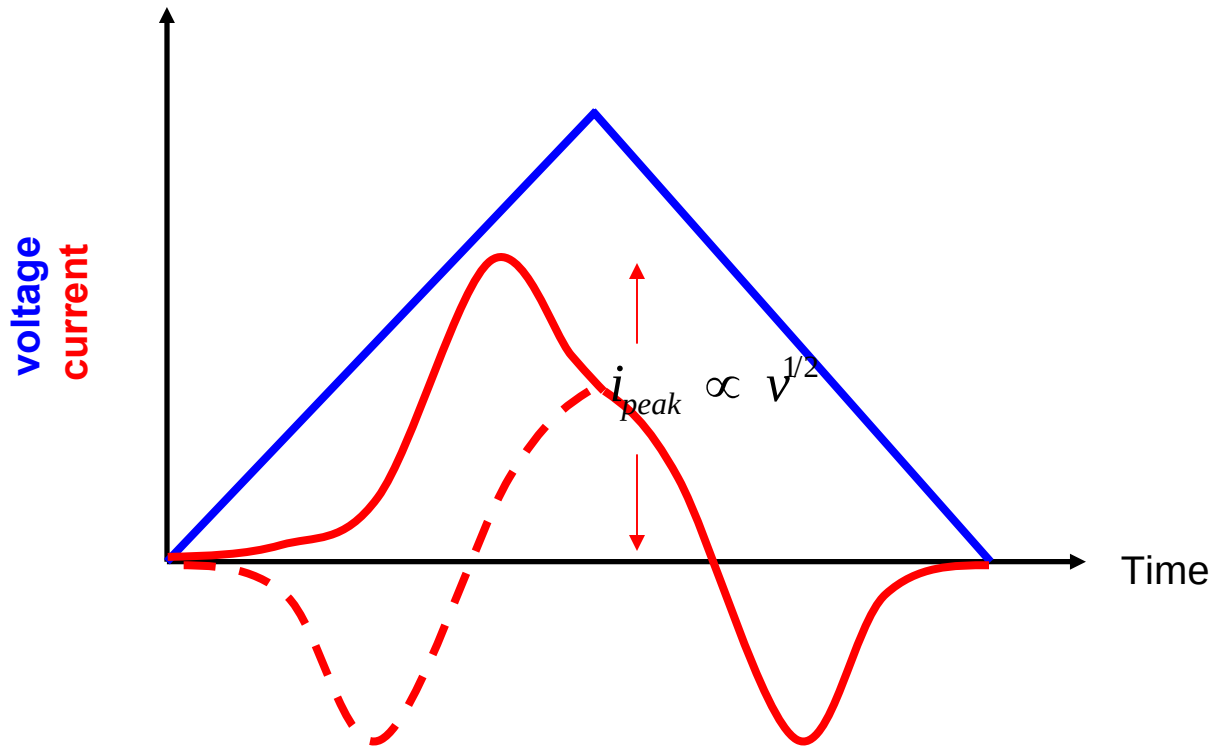
i proportional to flux



$$\mathbf{E} = E^o + \frac{RT}{nF}\ln\frac{C_o(surf,t)}{C_R(surf,t)}$$

$$\mathbf{i} = nFAD_o$$

Faradaic current



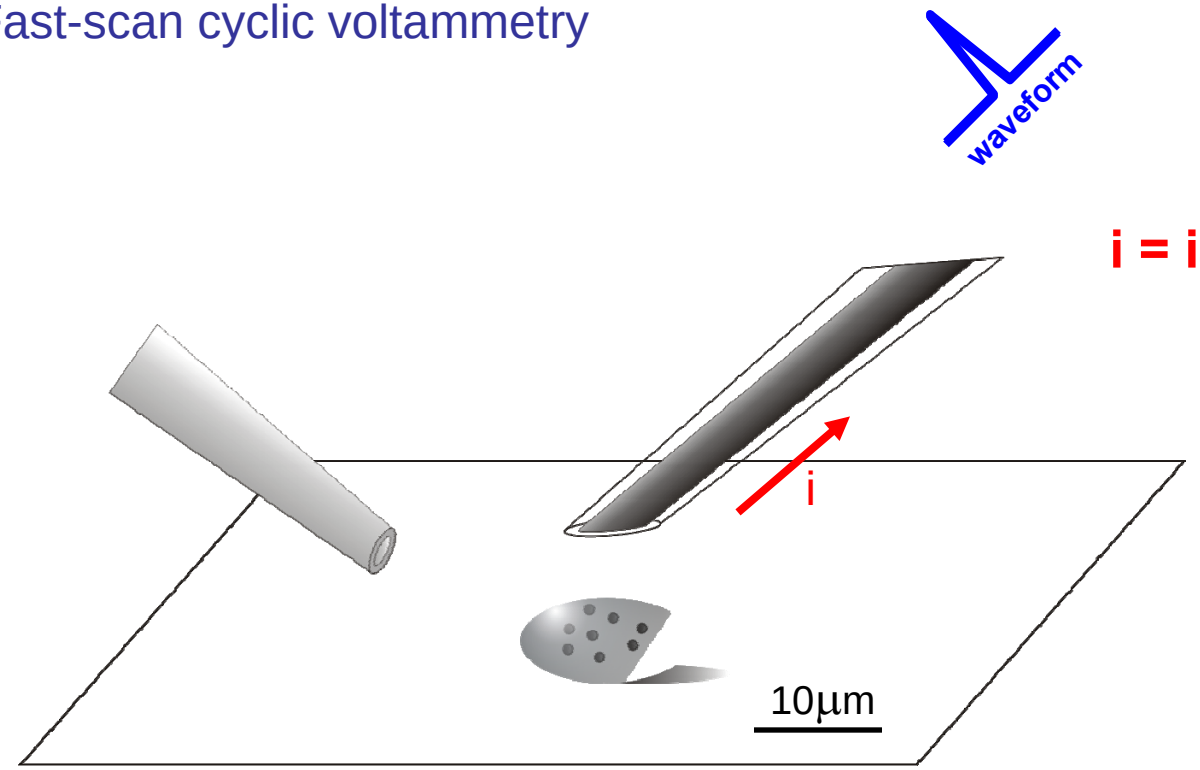
Properties:

- generated by redox couple
- $i_{peak} = 2.69 \times 10^8 n^{3/2} A D^{1/2} \bullet v^{1/2} \bullet C \quad \propto \quad v^{1/2}$

  $A: area(m^2); \quad C: bulk \quad concentration(mol/L);$

  $D: diffusion \quad coefficient(m^2/s); v: scan \quad rate(V/s); T: 25^o C$

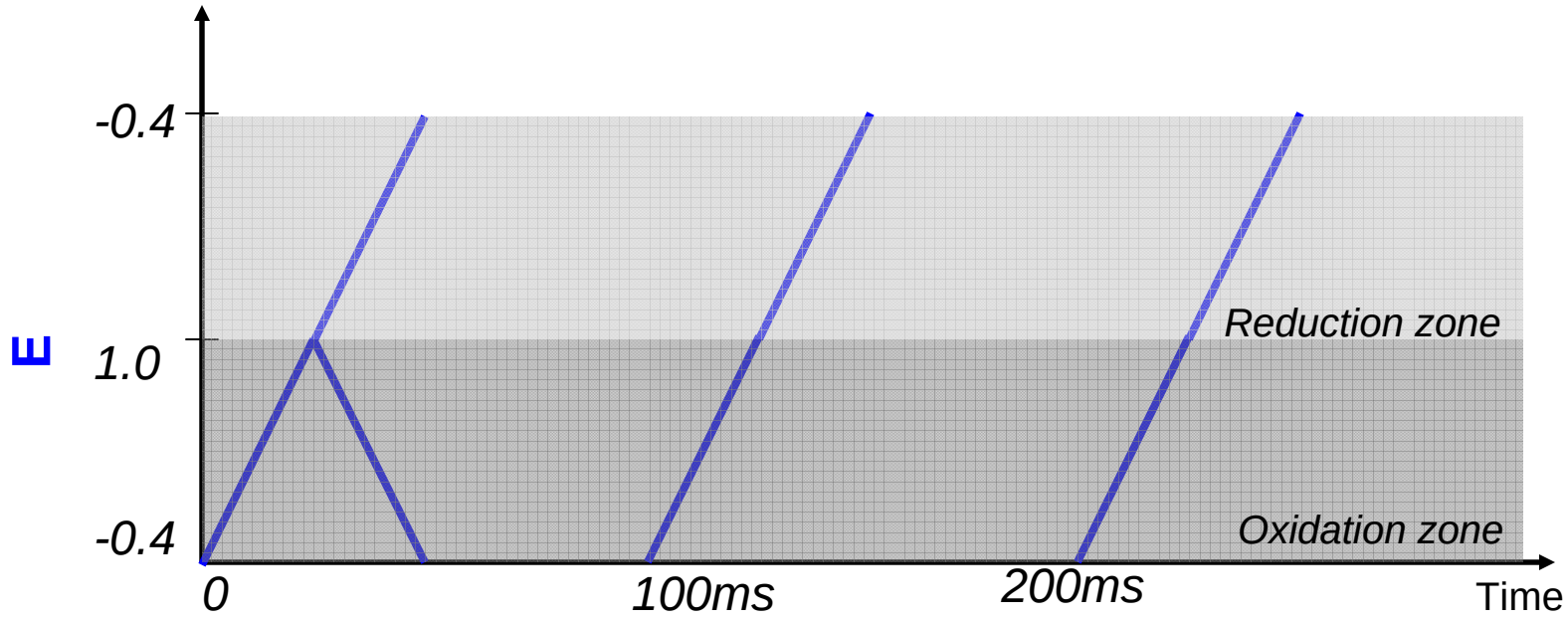
Fast-scan cyclic voltammetry



$$i = i_{charging} + i_{faradaic}$$

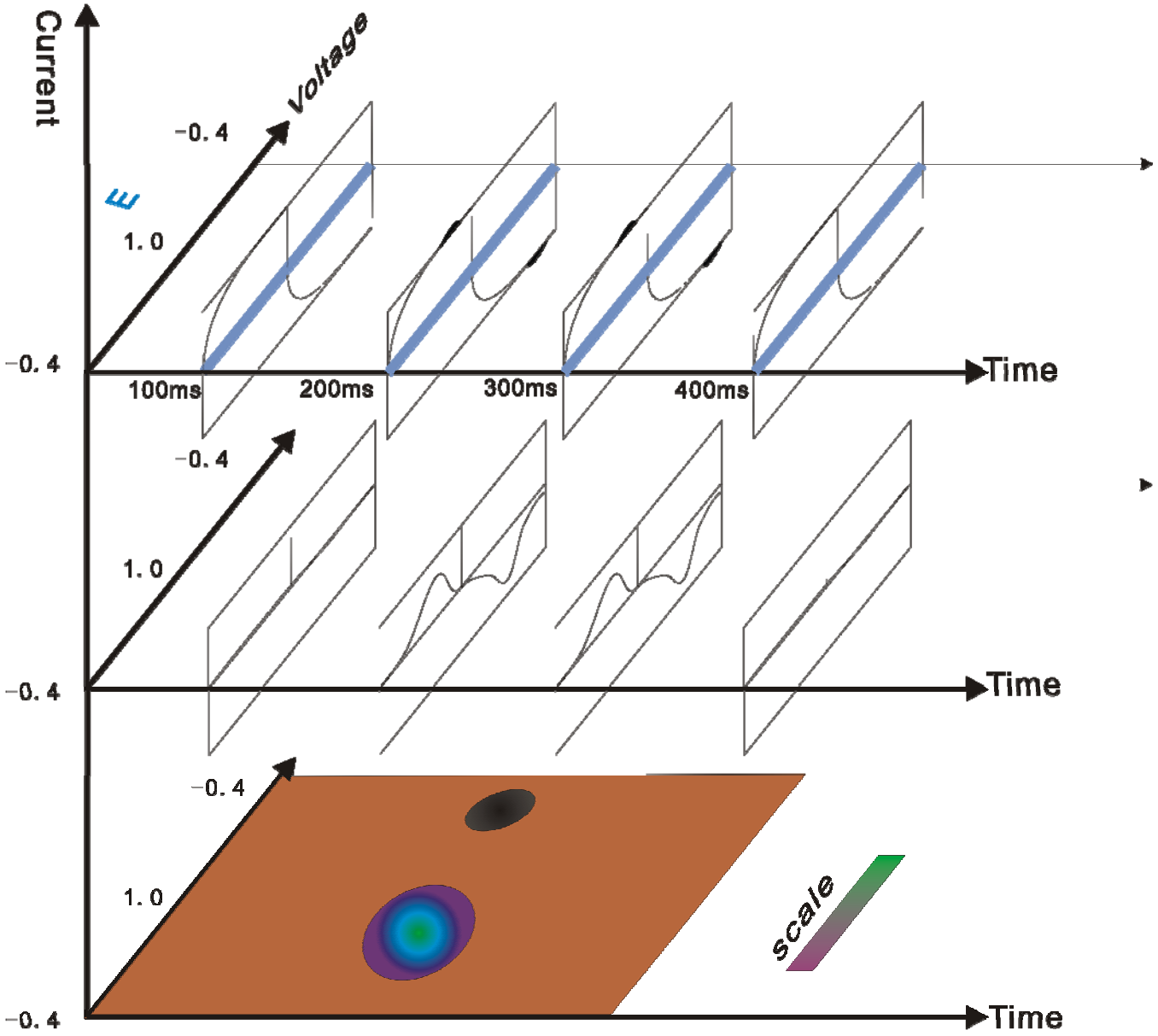
Measurable

Fast-scan cyclic voltammetry also known as ***background-subtracted cyclic voltammetry***

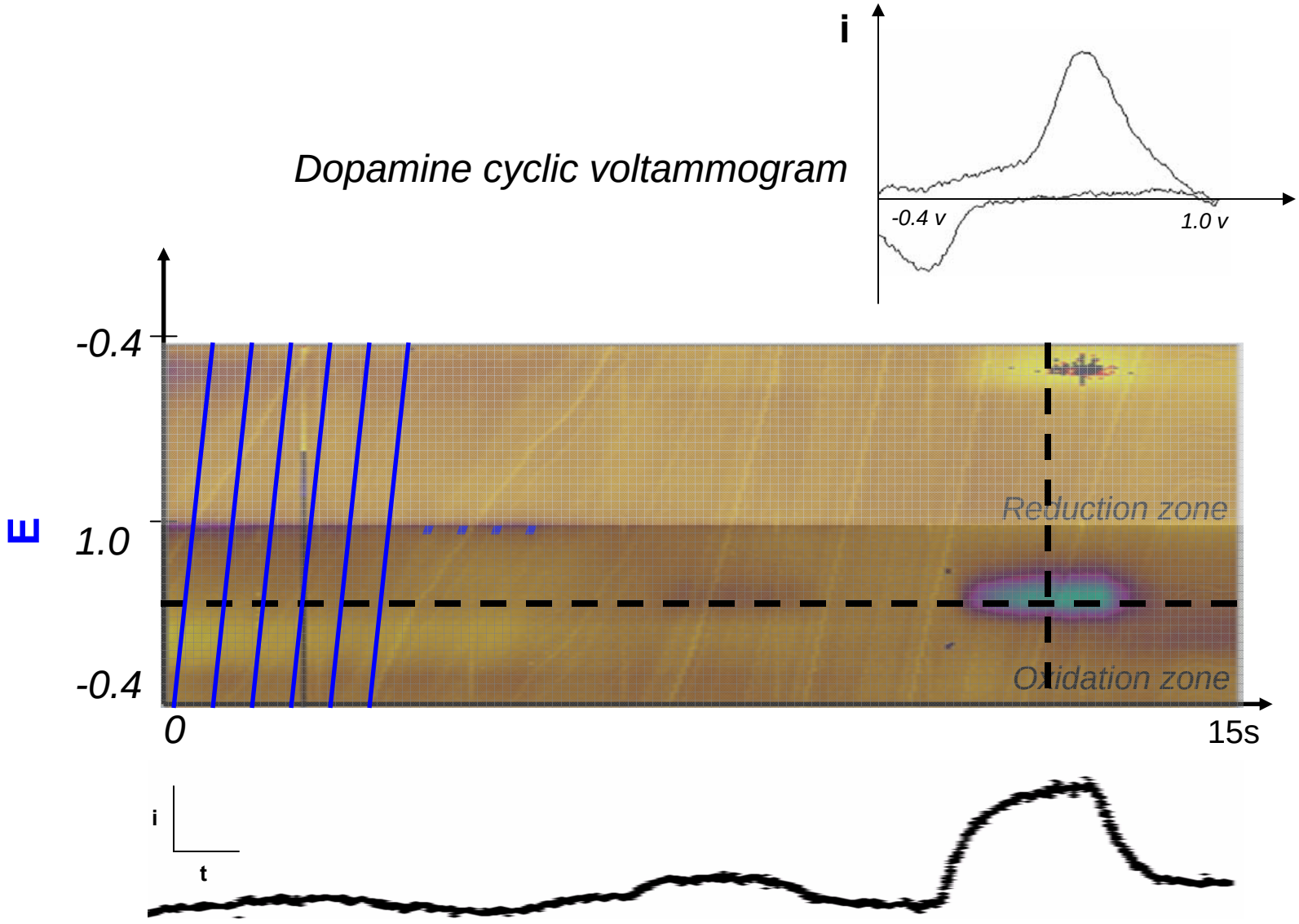


Parameters to define a waveform pattern:

- Positive/Negative limits i?
- Scanning rate (e.g. at a typical μCFM, it takes 10ms to finish a cycle at about 300v/s)
- Applying frequency (time delay between two consecutive cycle, e.g. 10Hz shown above)


Current
Voltage
−0. 4
E
1. 0
−0. 4
Time
100ms
200ms
300ms
400ms
−0. 4
1. 0
−0. 4
Time
−0. 4
1. 0
scale
−0. 4
Time

Dopamine cyclic voltammogram



Dopamine transients

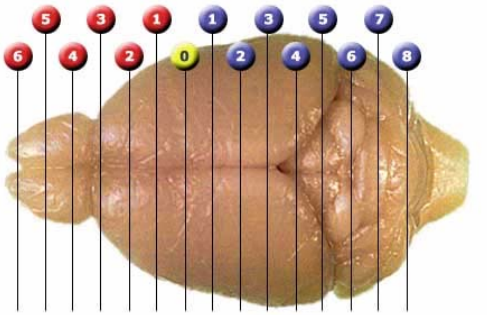
Advantages:

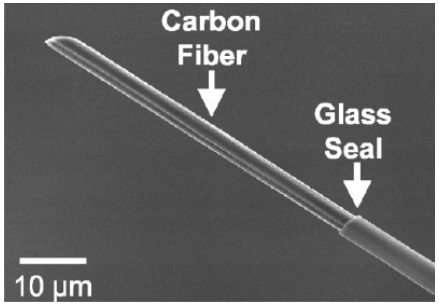
- *high spatial resolution due to micro-scale electrode, particularly useful in studying exocytosis on single cell level and physiological transients of chemical messengers in slice preparation.*
- *high temporal resolution due to extremely high scanning rate, offering the capability to follow milli-second transient in physiological environments.*
- *Offering molecular identity*

Disadvantages:

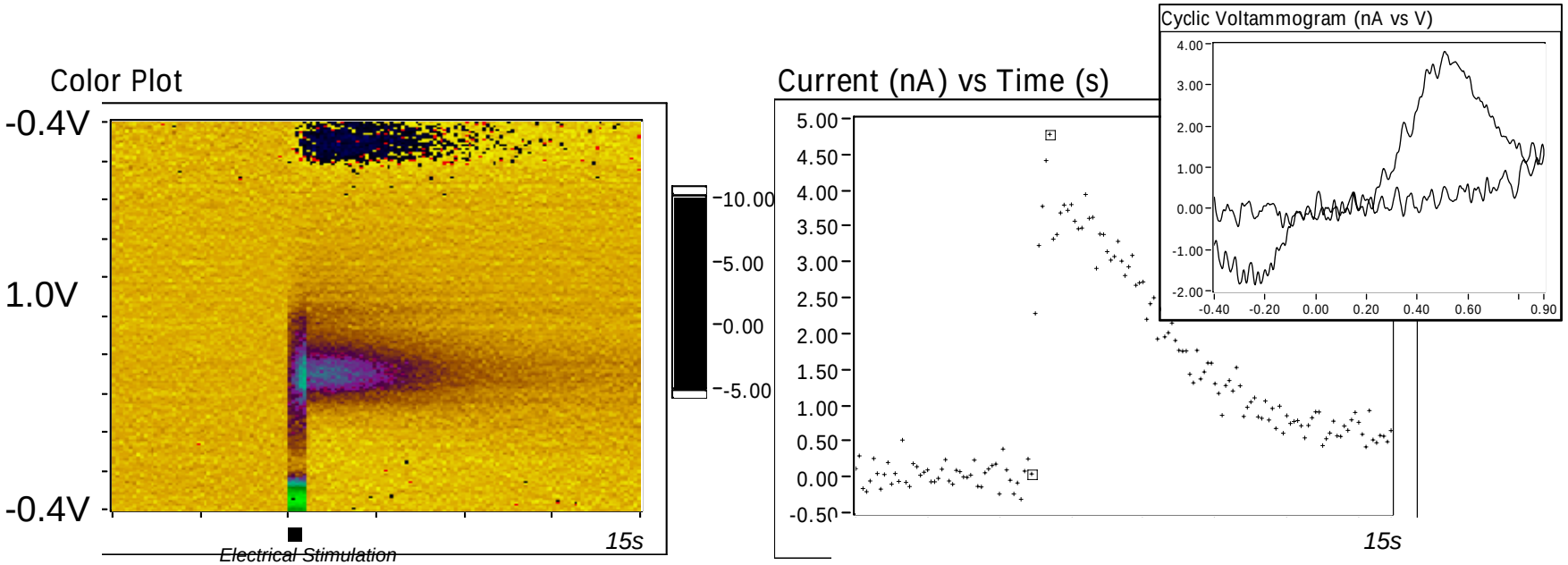
- *Sensing targets limited by electroactivity*
- *fouling effects by cellular debris, protein etc.*

Chemical messenger transients in brain slice

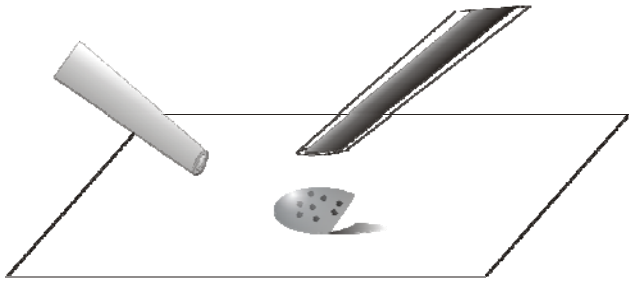


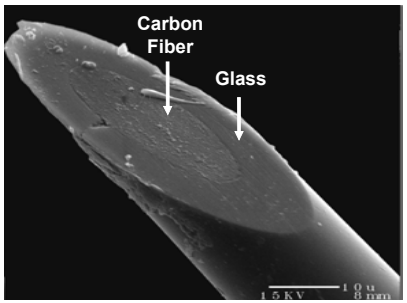


Following dopamine transient in a mouse brain

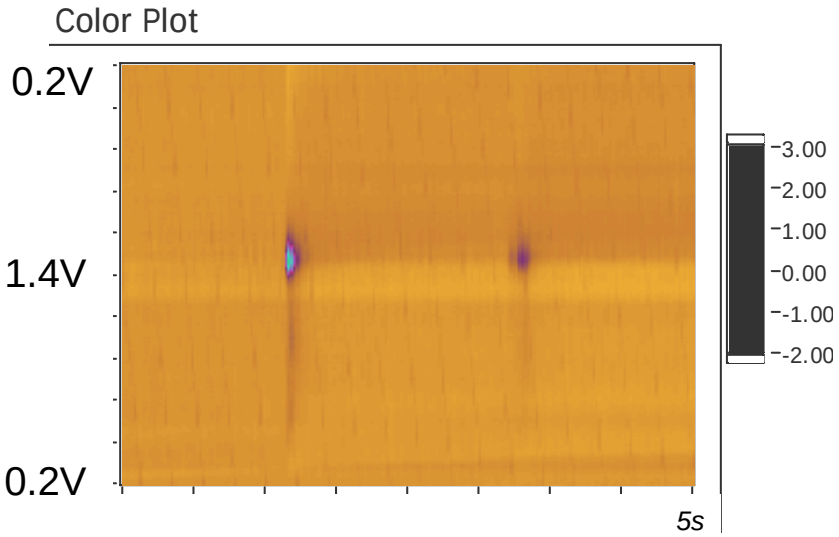


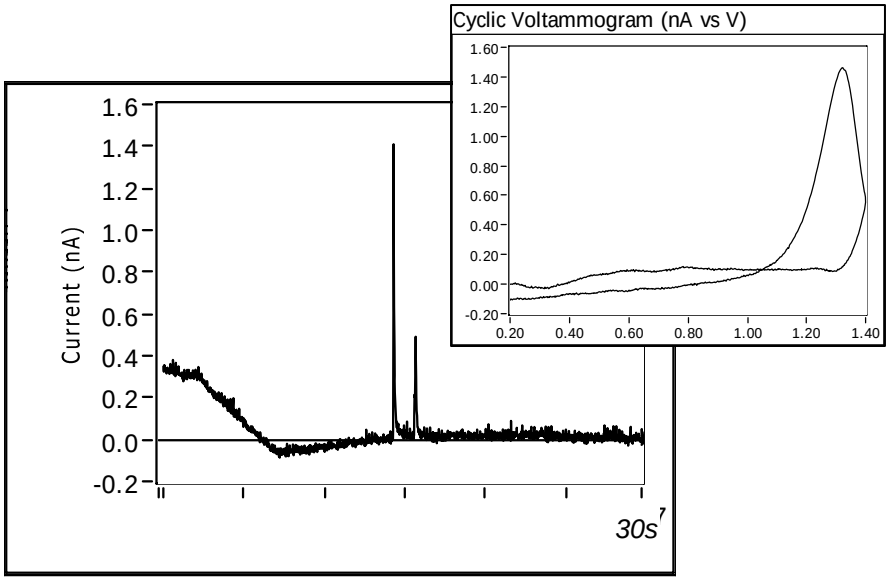
Exocytosis in Single cells



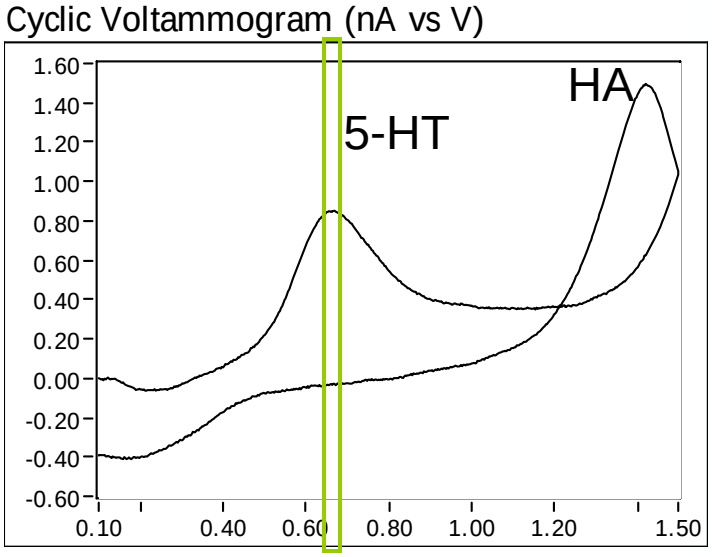


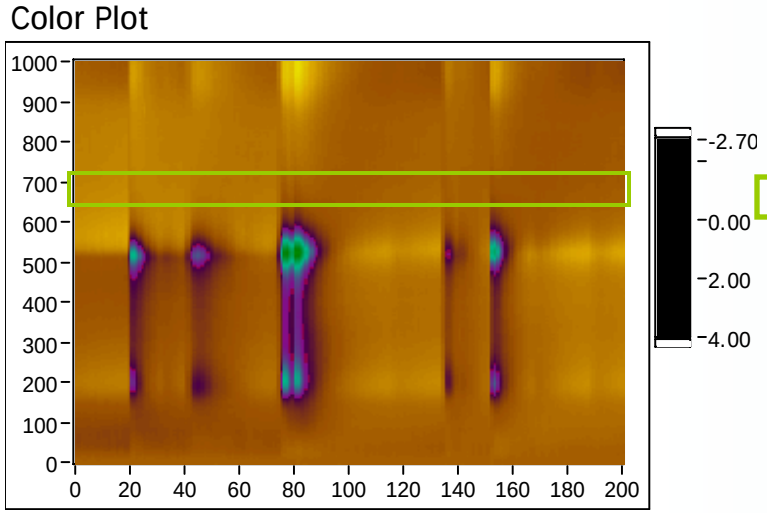
Following single granular release of histamine from a human basophil





Cyclic Voltammetry



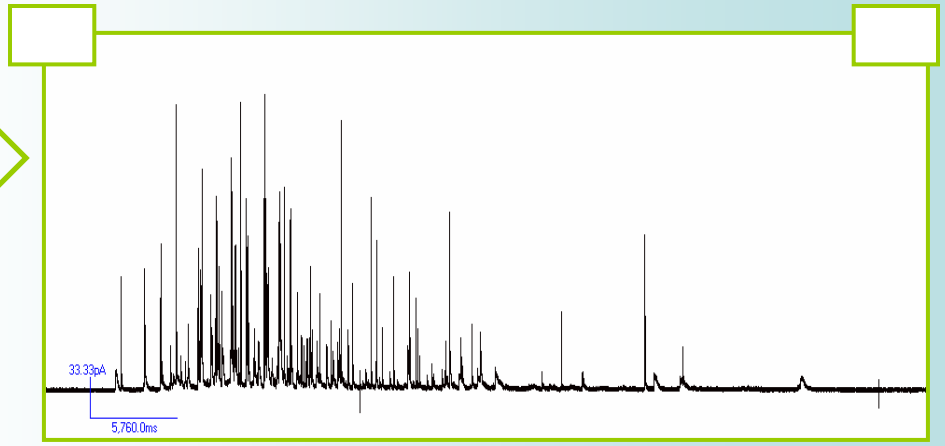


Amperometry

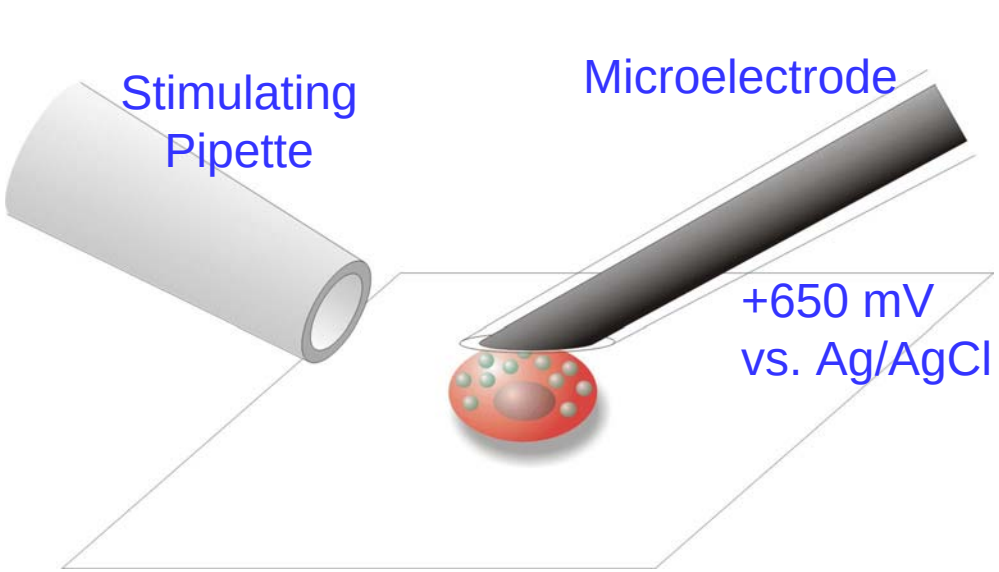
Electrode held at constant potential

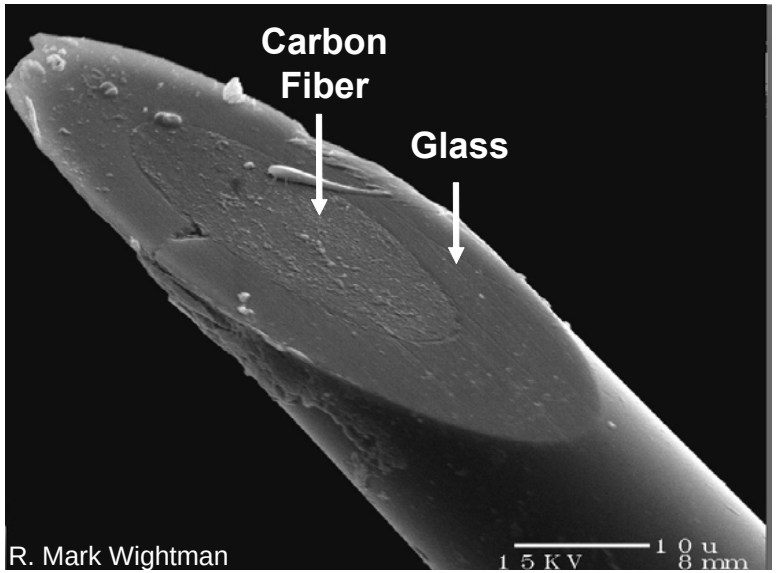
Current corresponds to oxidation or reduction of analyte

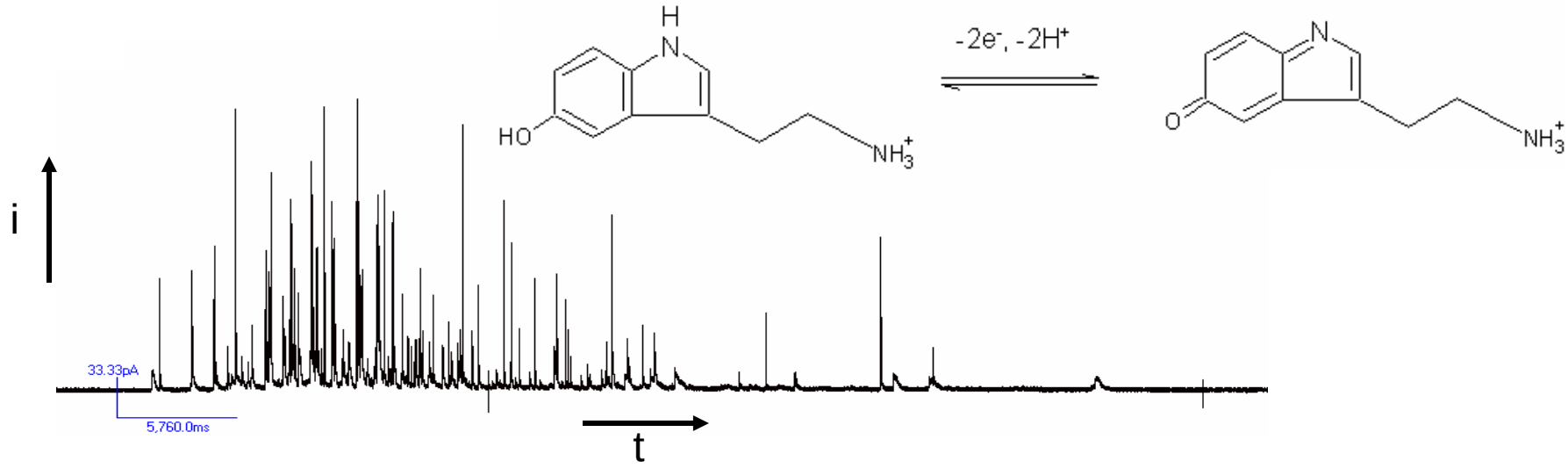
- **Improved time resolution**
- No need for calibration
- Loss of chemical information



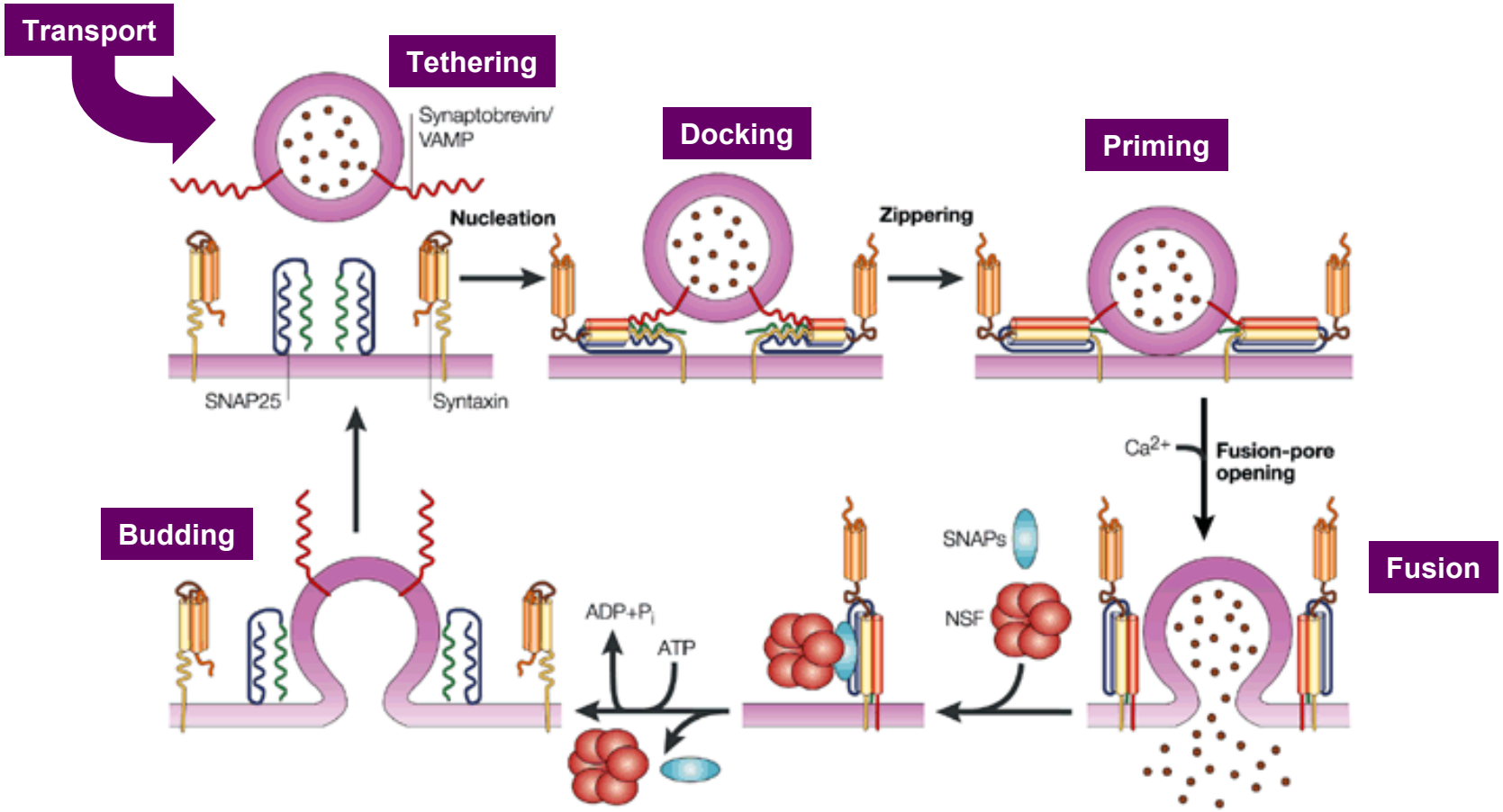
Single Cell Amperometry







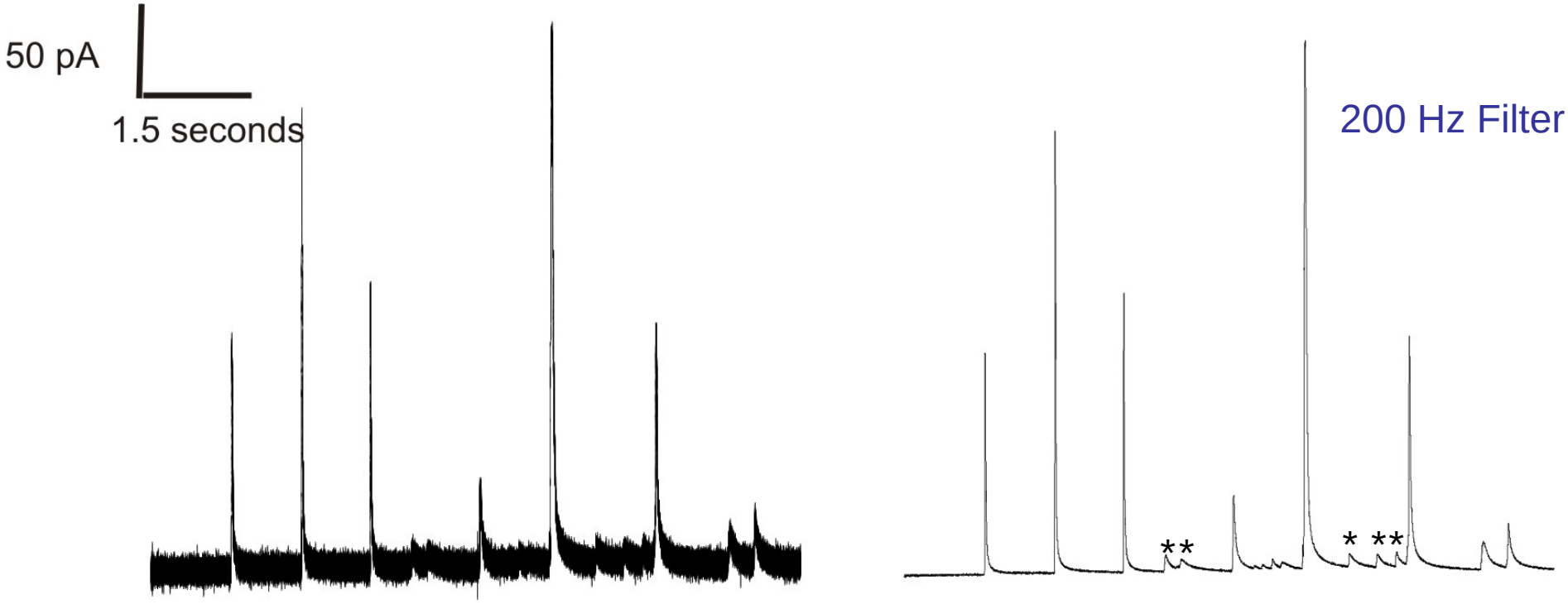
Mechanism of Exocytosis



Rizo and Sudhof. *Nature Reviews Neuroscience*. 3:641-653, 2002.

Digital Filtering

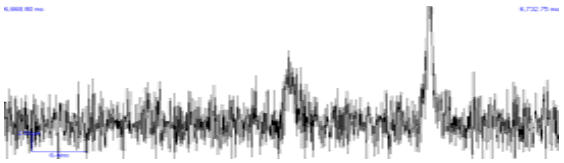
From Mast Cells:



Filter cut-off frequencies must be chosen carefully and vary by cell type.

Over filtering

5 kHz



From dopaminergic neurons

1 kHz

400 Hz

250 Hz

over shoot distortion

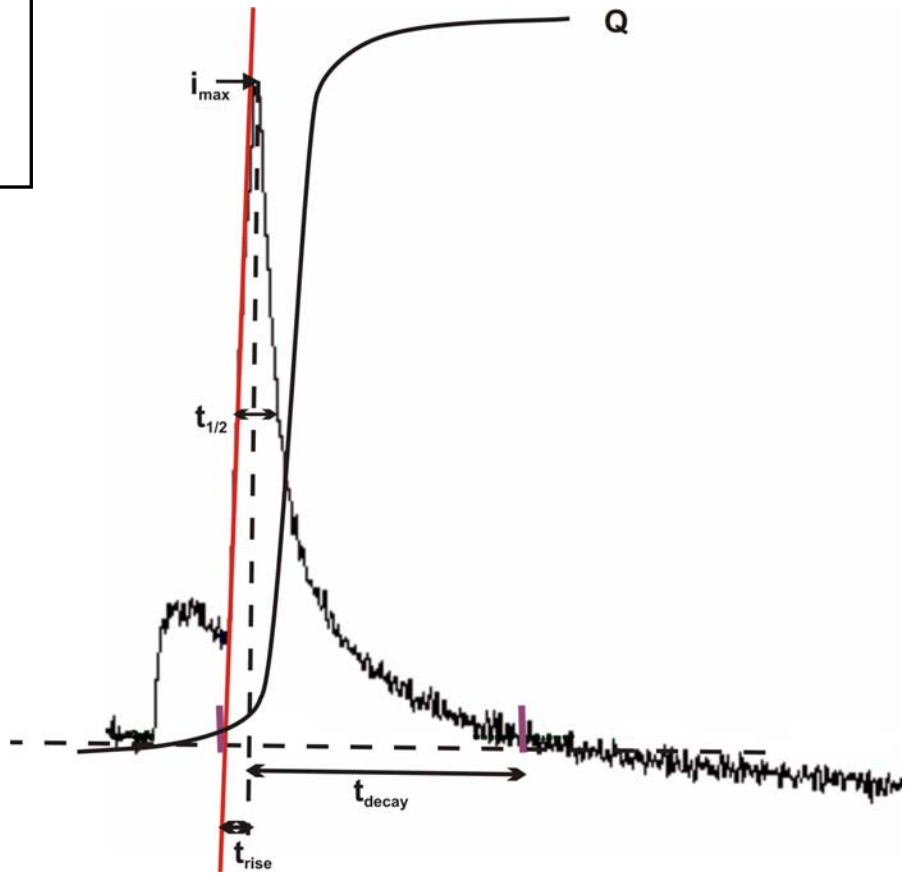
Data Analysis

Spike detection parameters:

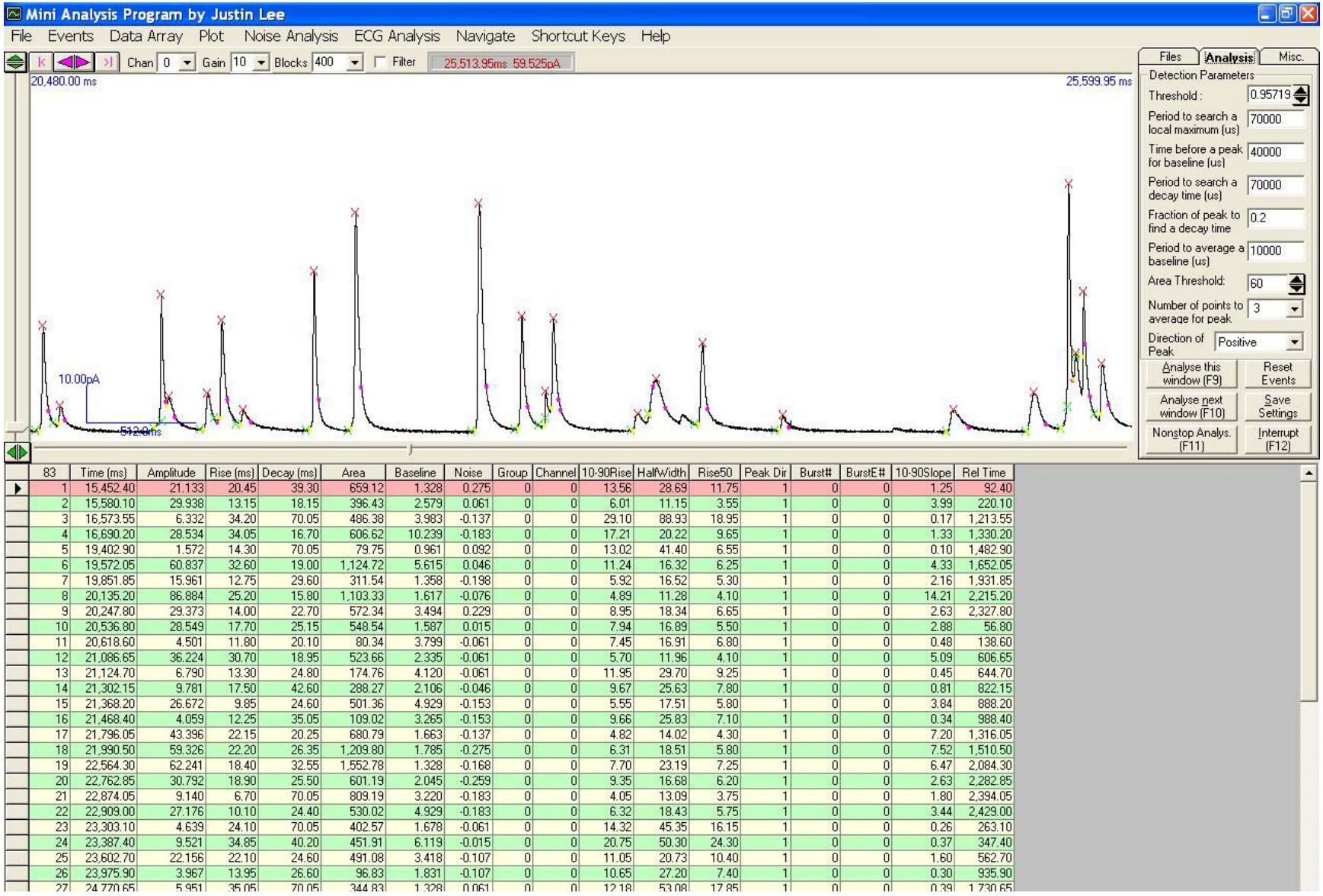
- **Amplitude threshold (5xRMS)**
- **Area threshold**
- **Period to find local maximum**
- **Period to set base line**
- **Fraction to find decay**

Data output parameters:

Peak maximum
Spike Area
Half-width
Rise time
Decay time
Spike number
Spike frequency
% of Spikes with feet
Foot area / Spike Area



Spike Analysis using Mini Analysis



83	Time (ms)	Amplitude	Rise (ms)	Decay (ms)	Area	Baseline	Noise	Group	Channel	10-90Rise	HalfWidth	Rise50	Peak Dir	Burst#	BurstE#	10-90Slope	Rel Time
1	15,452.40	21.133	20.45	39.30	659.12	1.328	0.275	0	0	13.56	28.69	11.75	1	0	0	1.25	92.40
2	15,580.10	29.938	13.15	18.15	396.43	2.579	0.061	0	0	6.01	11.15	3.55	1	0	0	3.99	220.10
3	16,573.55	6.332	34.20	70.05	486.38	3.983	-0.137	0	0	29.10	88.93	18.95	1	0	0	0.17	1,213.55
4	16,690.20	28.534	34.05	16.70	606.62	10.239	-0.183	0	0	17.21	20.22	9.65	1	0	0	1.33	1,330.20
5	19,402.90	1.572	14.30	70.05	79.75	0.961	0.092	0	0	13.02	41.40	6.55	1	0	0	0.10	1,482.90
6	19,572.05	60.837	32.60	19.00	1,124.72	5.615	0.046	0	0	11.24	16.32	6.25	1	0	0	4.33	1,652.05
7	19,851.85	15.961	12.75	29.60	311.54	1.358	-0.198	0	0	5.92	16.52	5.30	1	0	0	2.16	1,931.85
8	20,135.20	86.884	25.20	15.80	1,103.33	1.617	-0.076	0	0	4.89	11.28	4.10	1	0	0	14.21	2,215.20
9	20,247.80	29.373	14.00	22.70	572.34	3.494	0.229	0	0	8.95	18.34	6.65	1	0	0	2.63	2,327.80
10	20,536.80	28.549	17.70	25.15	548.54	1.587	0.015	0	0	7.94	16.89	5.50	1	0	0	2.88	56.80
11	20,618.60	4.501	11.80	20.10	80.34	3.799	-0.061	0	0	7.45	16.91	6.80	1	0	0	0.48	138.60
12	21,086.65	36.224	30.70	18.95	523.66	2.335	-0.061	0	0	5.70	11.96	4.10	1	0	0	5.09	606.65
13	21,124.70	6.790	13.30	24.80	174.76	4.120	-0.061	0	0	11.95	29.70	9.25	1	0	0	0.45	644.70
14	21,302.15	9.781	17.50	42.60	288.27	2.106	-0.046	0	0	9.67	25.63	7.80	1	0	0	0.81	822.15
15	21,368.20	26.672	9.85	24.60	501.36	4.929	-0.153	0	0	5.55	17.51	5.80	1	0	0	3.84	888.20
16	21,468.40	4.059	12.25	35.05	109.02	3.265	-0.153	0	0	9.66	25.83	7.10	1	0	0	0.34	988.40
17	21,796.05	43.396	22.15	20.25	680.79	1.663	-0.137	0	0	4.82	14.02	4.30	1	0	0	7.20	1,316.05
18	21,990.50	59.326	22.20	26.35	1,209.80	1.785	-0.275	0	0	6.31	18.51	5.80	1	0	0	7.52	1,510.50
19	22,564.30	62.241	18.40	32.55	1,552.78	1.328	-0.168	0	0	7.70	23.19	7.25	1	0	0	6.47	2,084.30
20	22,762.85	30.792	18.90	25.50	601.19	2.045	-0.259	0	0	9.35	16.68	6.20	1	0	0	2.63	2,282.85
21	22,874.05	9.140	6.70	70.05	809.19	3.220	-0.183	0	0	4.05	13.09	3.75	1	0	0	1.80	2,394.05
22	22,909.00	27.176	10.10	24.40	530.02	4.929	-0.183	0	0	6.32	18.43	5.75	1	0	0	3.44	2,429.00
23	23,303.10	4.639	24.10	70.05	402.57	1.678	-0.061	0	0	14.32	45.35	16.15	1	0	0	0.26	263.10
24	23,387.40	9.521	34.85	40.20	451.91	6.119	-0.015	0	0	20.75	50.30	24.30	1	0	0	0.37	347.40
25	23,602.70	22.156	22.10	24.60	491.08	3.418	-0.107	0	0	11.05	20.73	10.40	1	0	0	1.60	562.70
26	23,975.90	3.967	13.95	26.60	96.83	1.831	-0.107	0	0	10.65	27.20	7.40	1	0	0	0.30	935.90
27	24,770.65	5.951	35.05	70.05	344.83	1.328	0.061	0	0	12.18	53.08	17.85	1	0	0	0.39	1,730.65

Spike detection:

Parameters:

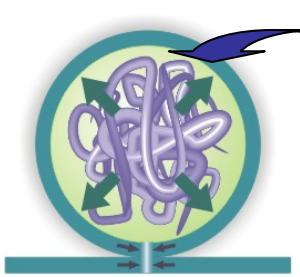
Amplitude threshold (5xRMS)

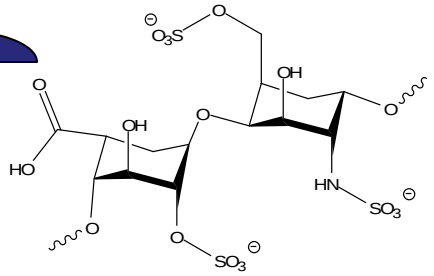
Area threshold

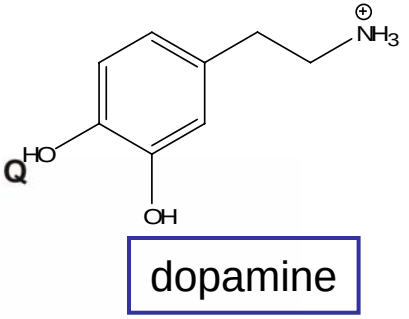
Period to find local maximum

Period to set base line

Fraction to find decay

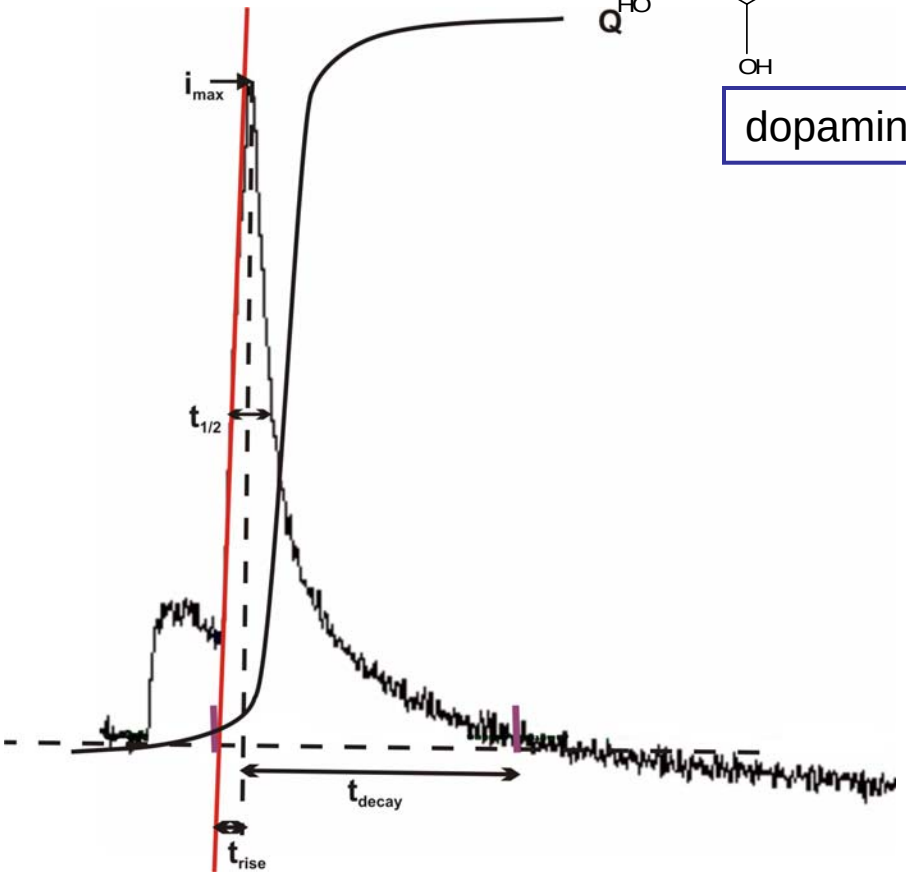






Data output parameters:

- **Peak maximum**
- **Spike Area (N = Q/nF)**
- **Half-width**
- **Rise time**
- **Decay time**
- Spike number
- Spike frequency
- % of Spikes with feet
- Foot area / Spike Area



Spike detection:

Parameters:

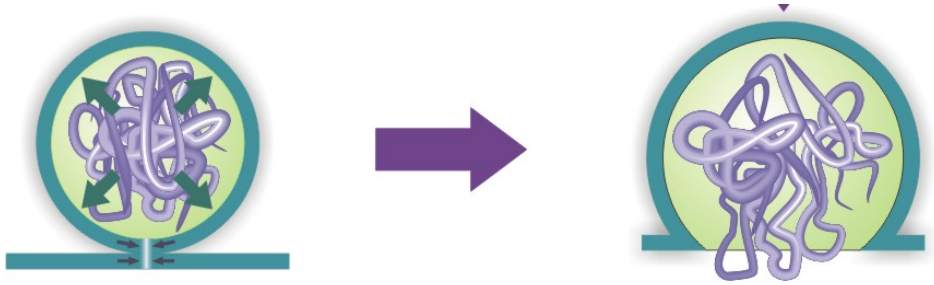
Amplitude threshold (5xRMS)

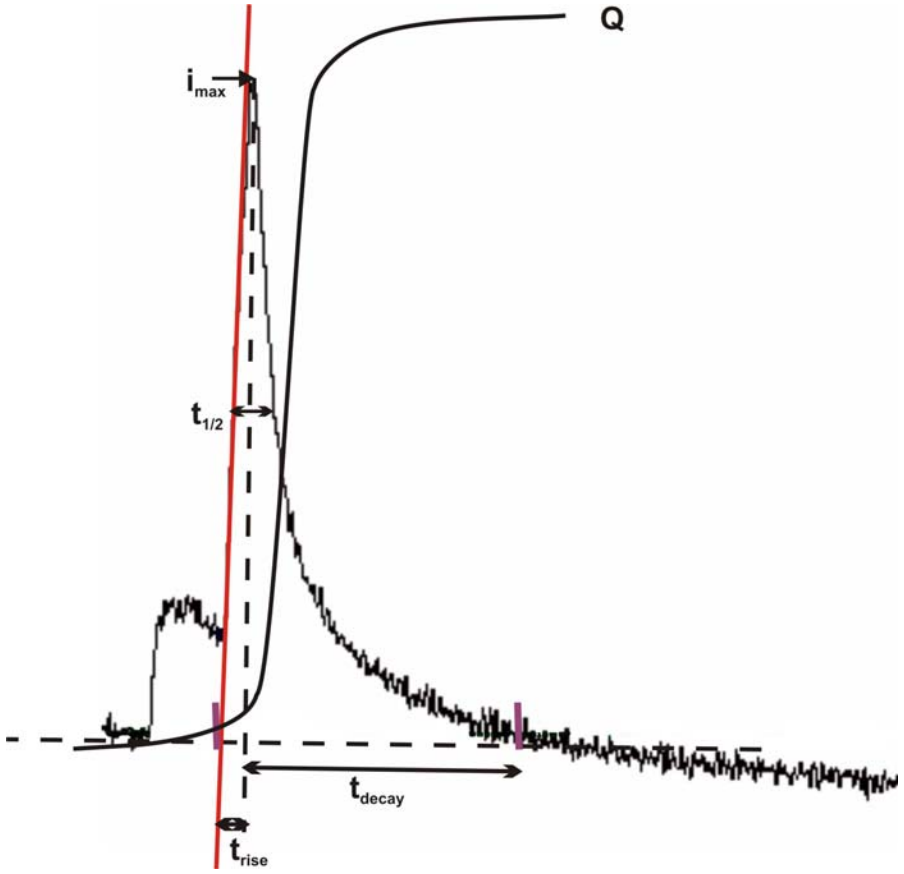
Area threshold

Period to find local maximum

Period to set base line

Fraction to find decay





Data output parameters:

- **Peak maximum**
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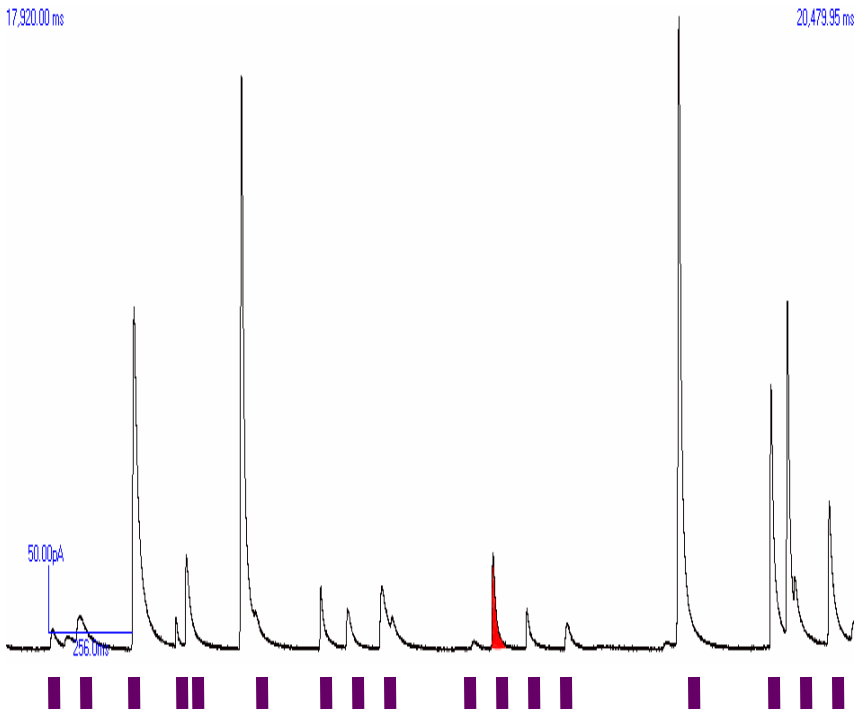
- **Half-width**
- **Rise time**
- **Decay time**
- Spike number
- Spike frequency
- % of Spikes with feet
- Foot area / Spike Area

Spike detection:

Parameters:

- Amplitude threshold (5xRMS)
- Area threshold
- Period to find local maximum
- Period to set base line
- Fraction to find decay



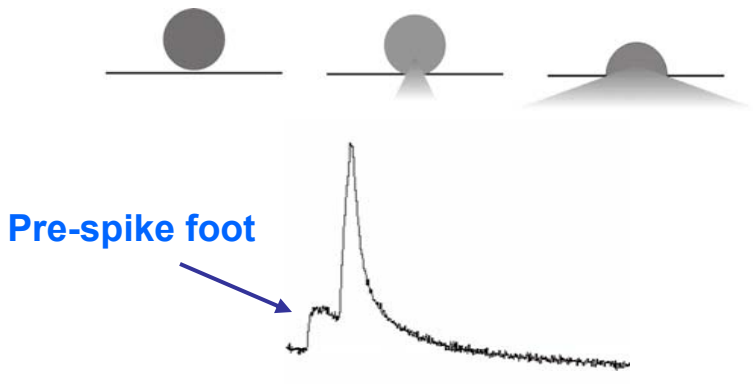
Data output parameters:

- Peak maximum
- Spike Area
- Half-width
- Rise time
- Decay time
- **Spike number**
- **Spike frequency**
- % of Spikes with feet
- Foot area / Spike Area

Spike detection:

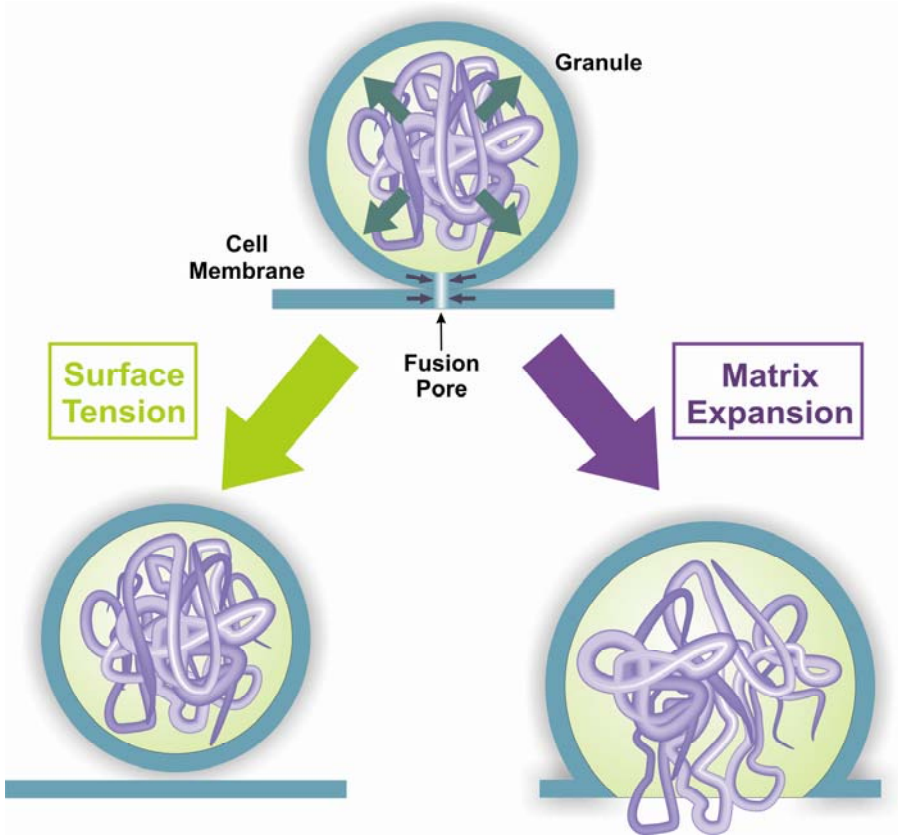
Parameters:

- Amplitude threshold (5xRMS)
- Area threshold
- Period to find local maximum
- Period to set base line
- Fraction to find decay



Data output parameters:

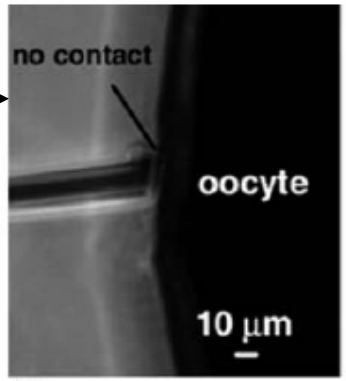
- Peak maximum
- Spike Area
- Half-width
- Rise time
- Decay time
- Spike number
- Spike frequency
- % of Spikes with feet
- Foot area / Spike Area

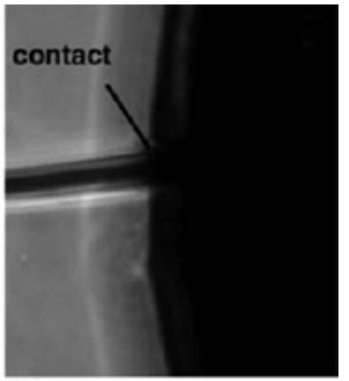


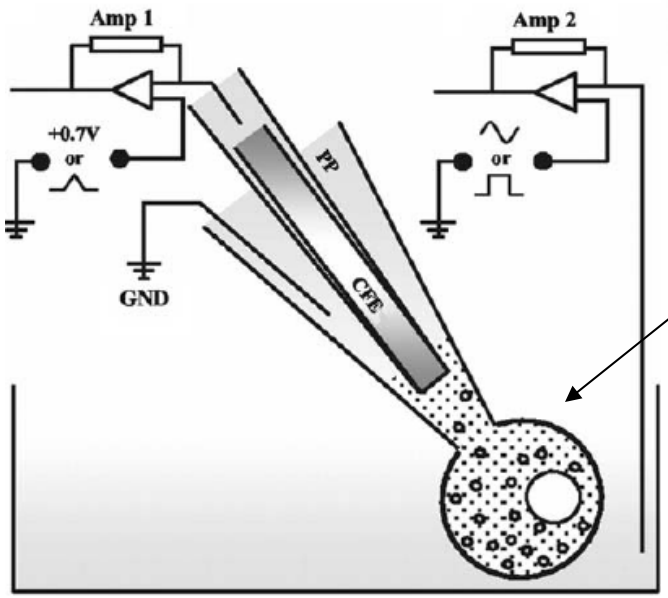
Biological Applications of Microelectrodes

Extracellular Measurements

Patch Amperometry
SECM / Amperometry
Cholesterol Enzyme Electrode







Intracellular Measurements

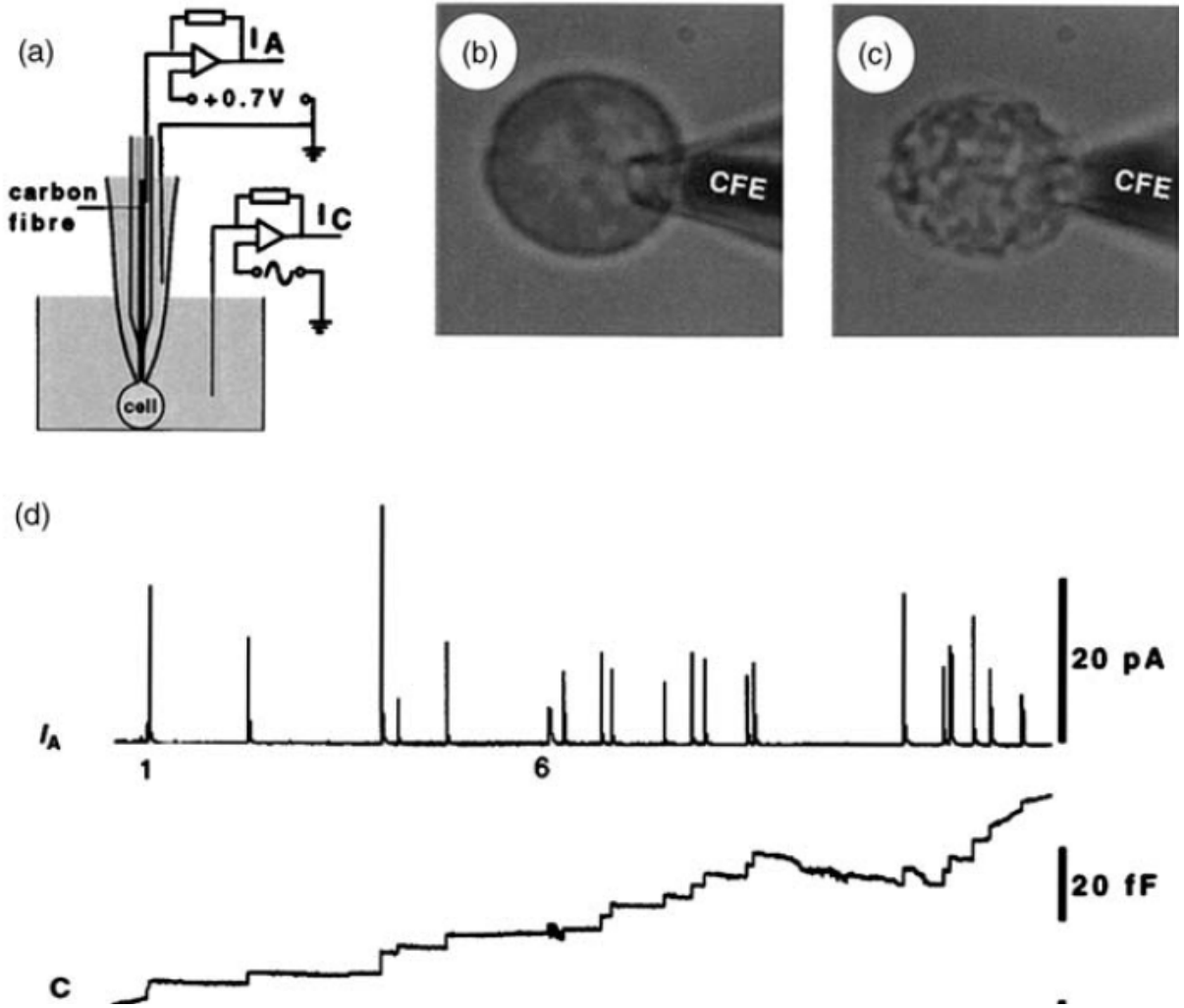
Electrodes and Analytes
Intracellular Patch Electrochemistry

In Vivo Measurements

Release of NT's in the brain correlates with behavior

Extracellular Measurements: Patch Amperometry

Combination of Amperometry and Electrophysiology



M. Lindau and coworkers Nature **1997** *389* 509-12.

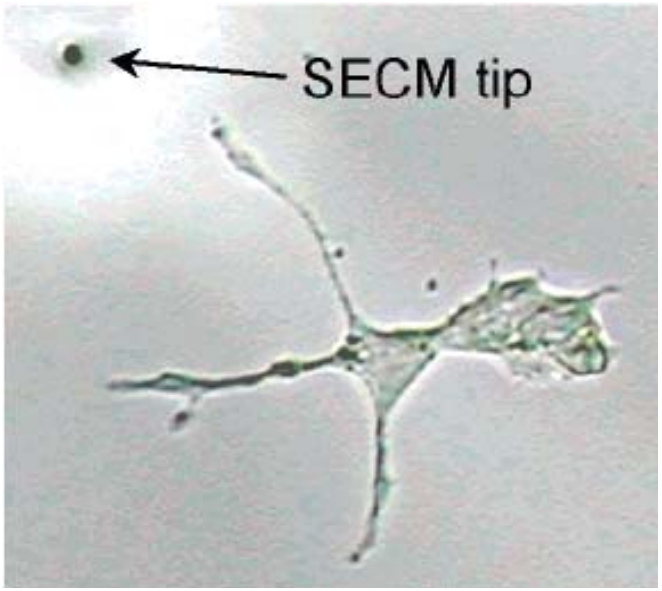
Extracellular Measurements – Patch Amperometry

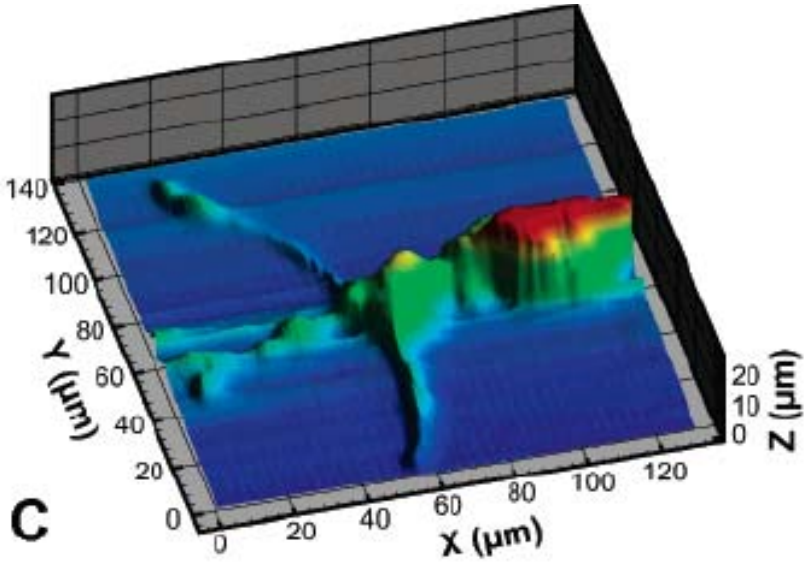
Pros: Best of both worlds

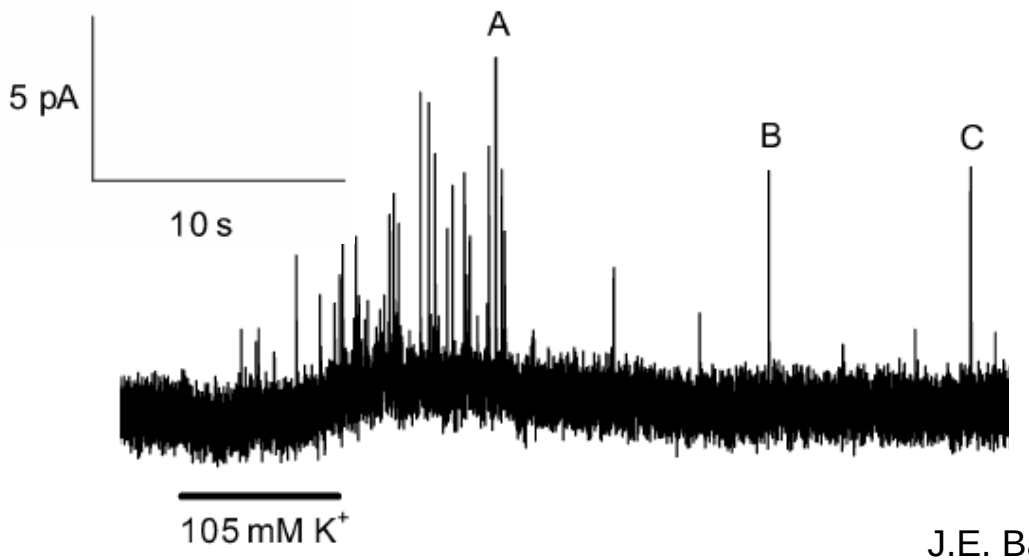
- Simultaneous Determination of:
  - Neurotransmitter flux (Faradaic current transient)
  - Membrane fusion (capacitance step)
  - Fusion pore opening (conductance change)
- Investigation of:
  - Nature of the fusion pore (evolution, size, lifetime)
  - Kiss-and-Run events
  - Coupling of vesicle size and content
  - Ionic exchange through fusion pore

Cons: Difficult implementation

Extracellular Measurements: Electrochemical Imaging and Amperometry







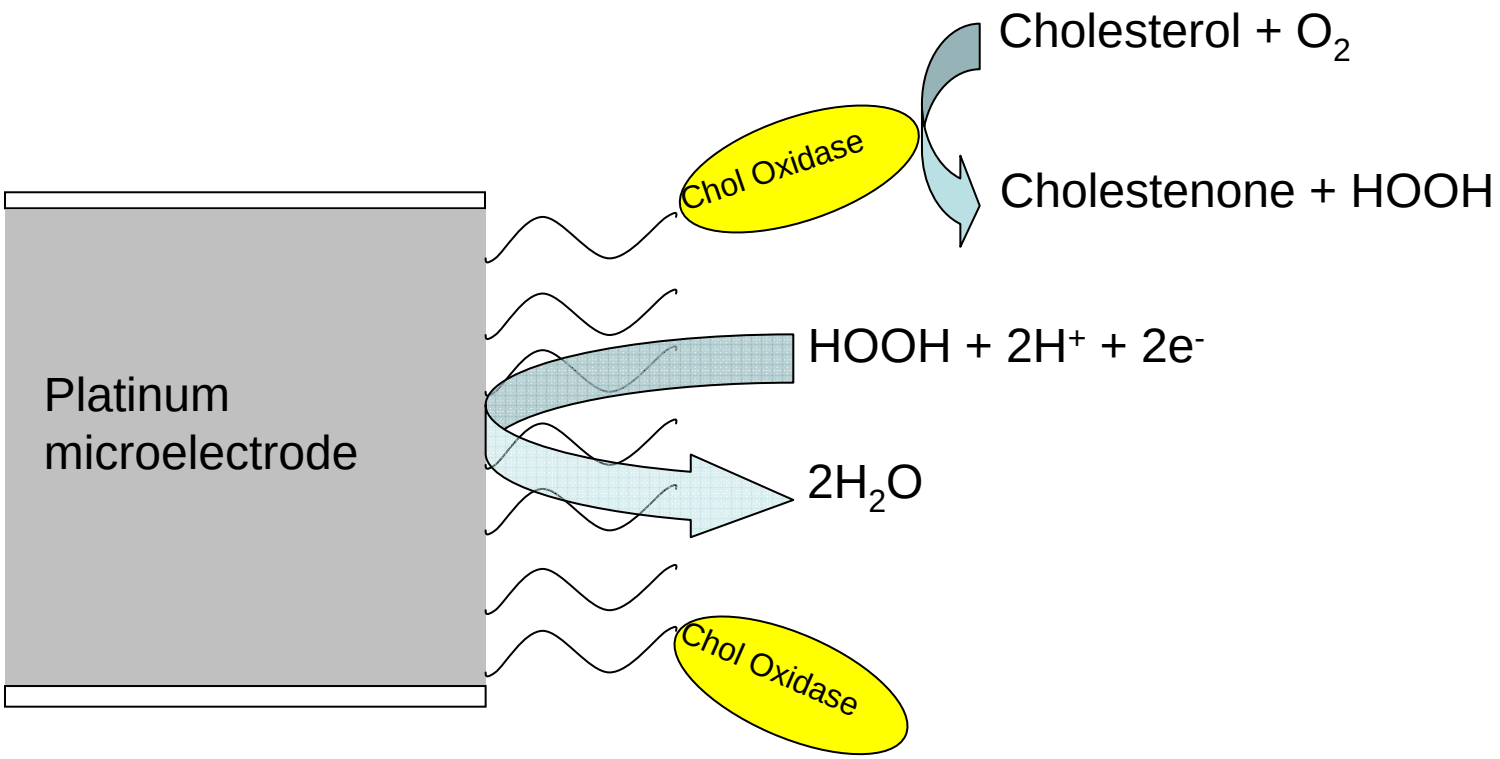
3-D imaging of cellular morphology

Morphological changes
concurrent with exocytosis

J.E. Baur and coworkers *Anal. Chem*. **2005,** *77*, 1111-1117

Extracellular Measurements: Electrochemical Determination of Membrane Cholesterol

Platinum electrode with covalently attached cholesterol oxidase

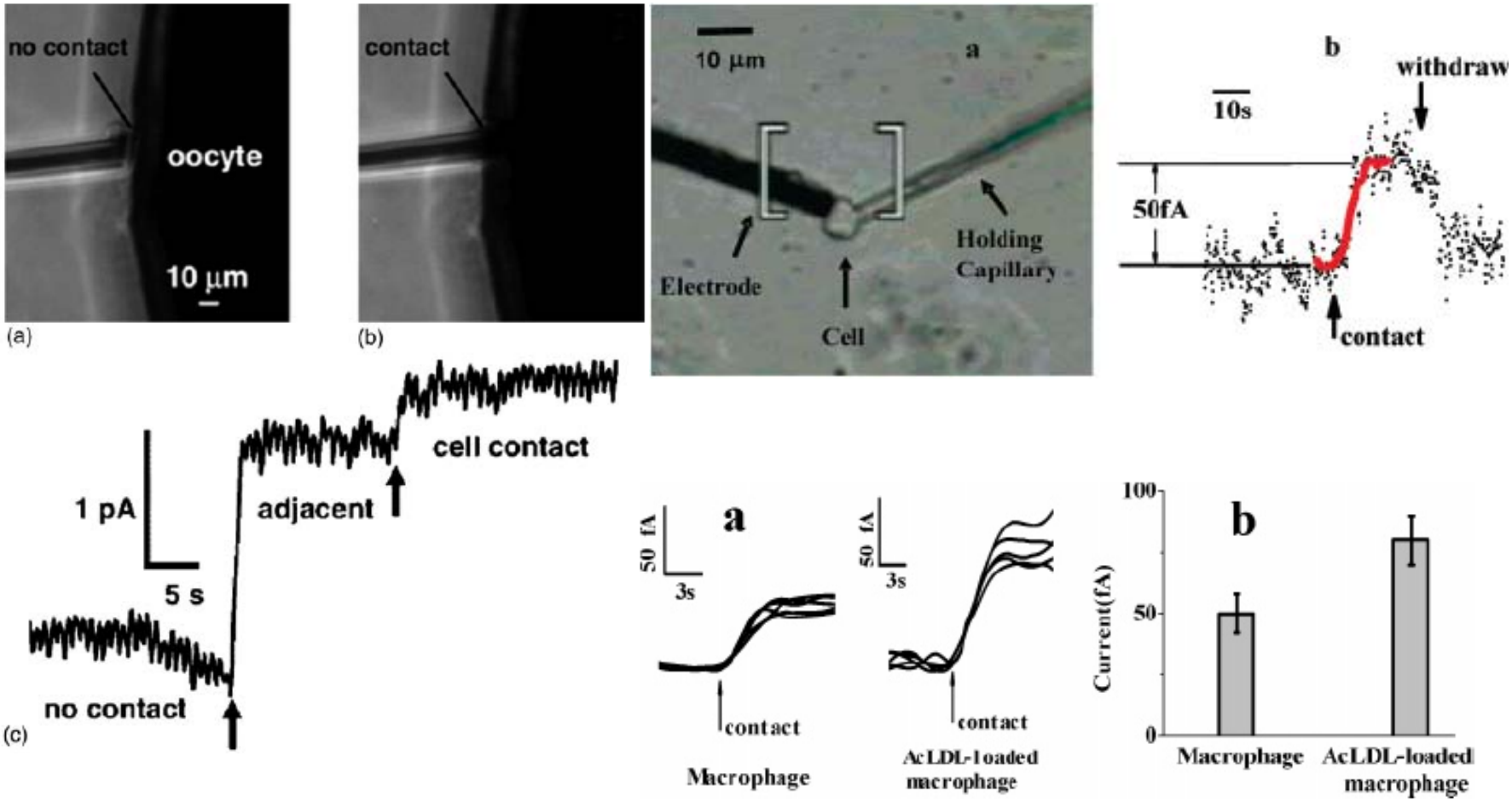


James D. Burgess and coworkers *JACS* **2007**, *129*, 11352

Extracellular Measurements: Electrochemical Determination of Membrane Cholesterol

Xenopus Oocytes

Mammalian Macrophages



James D. Burgess and coworkers *JACS* **2007**, *129*, 11352

Intracellular Electrochemical Measurements

Almost exclusively inside invertebrate cells

Aplysia californica

Cholinergic neurons
Metacerebral serotonergic neurons

Planorbis corneus

Giant dopamine neuron

Intracellular Electrochemical Measurements

Electrode Materials

Carbon fiber (disk)
Carbon ring
Platinum
Platinized carbon
Immobilized enzymes on carbon or Pt

Intracellular Analytes

Oxygen
Dopamine
Serotonin
Metronidazole (drug)
Antipyrine (drug)
Glucose

Intracellular Electrochemical Measurements

Carbon ring microelectrodes

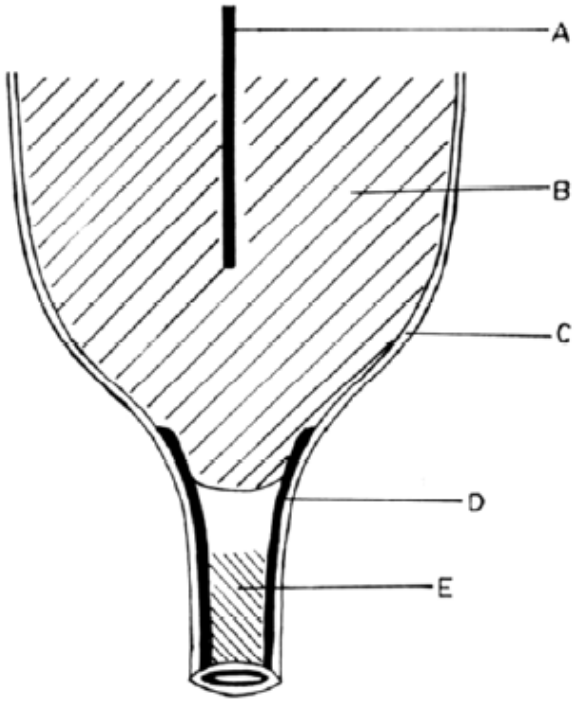


Figure 1. Quartz capillary, ring-shaped, ultrasmall electrode: A, nickel–chromium wire; B, Hg; C, quartz; D, carbon deposit; E, epoxy.

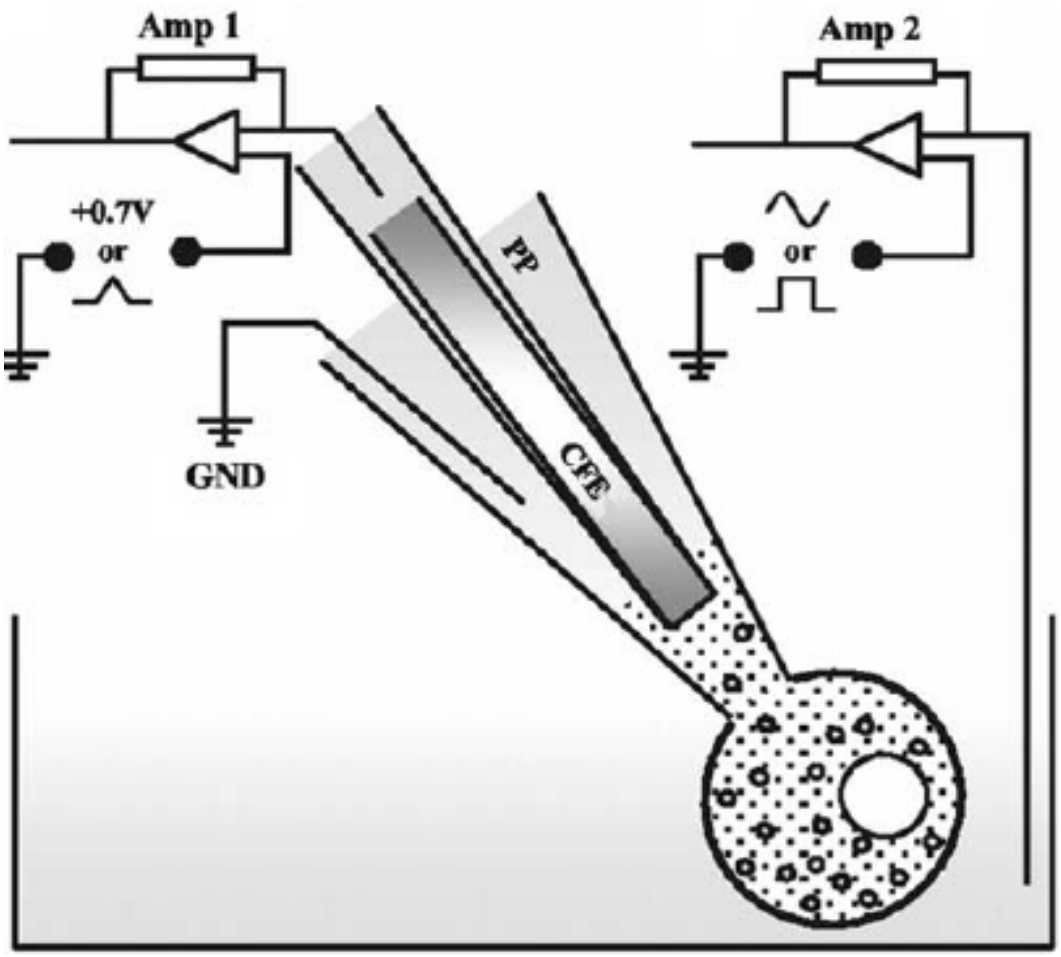
Carbon deposition by pyrolysis of methane

~1 μm tip diameter
12 – 120 nm ring thickness

Ewing and coworkers *Anal Chem* **1986,** *58*, 1782

Intracellular Electrochemical Measurements

Intracellular Patch Electrochemistry

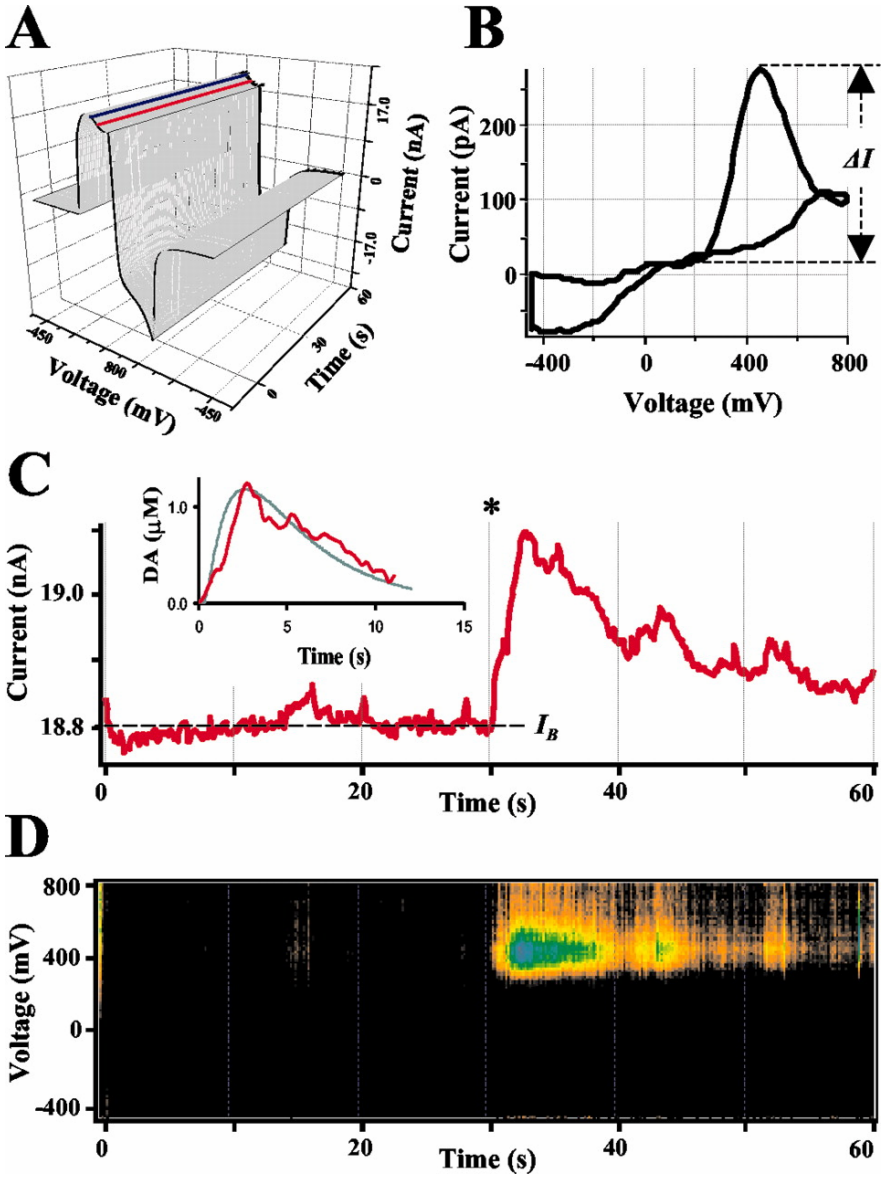


Applicable to mammalian cells

D. Sulzer, M. Lindau and coworkers *J Neurosci* **2003**, *23*, 5835

Intracellular Electrochemical Measurements

Intracellular Patch Electrochemistry



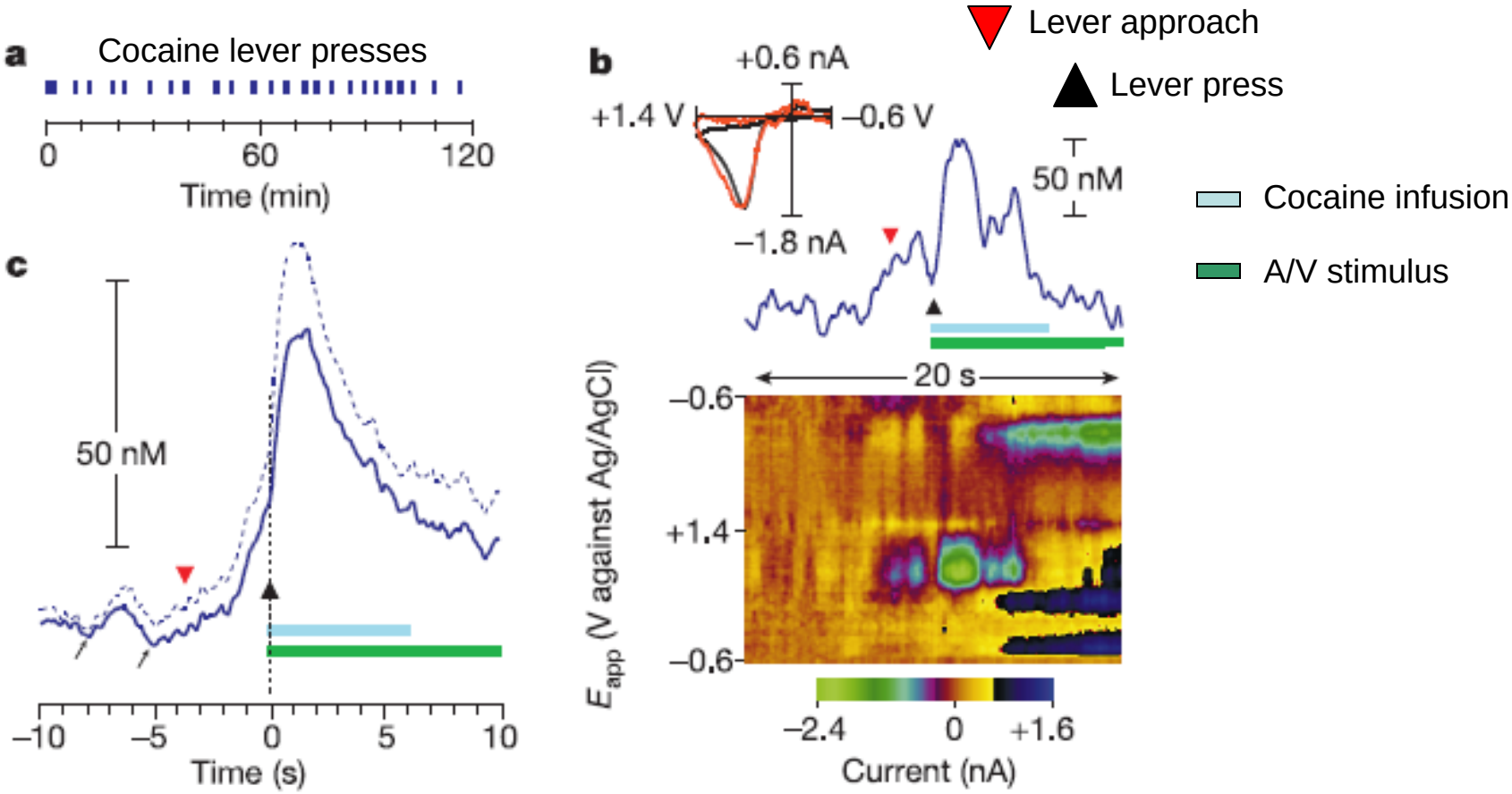
Voltammetry AND Amperometry

Catecholamines AND metabolites

D. Sulzer, M. Lindau and coworkers *J Neurosci* **2003**, *23*, 5835

In Vivo Electrochemical Measurements

Correlation with behavior

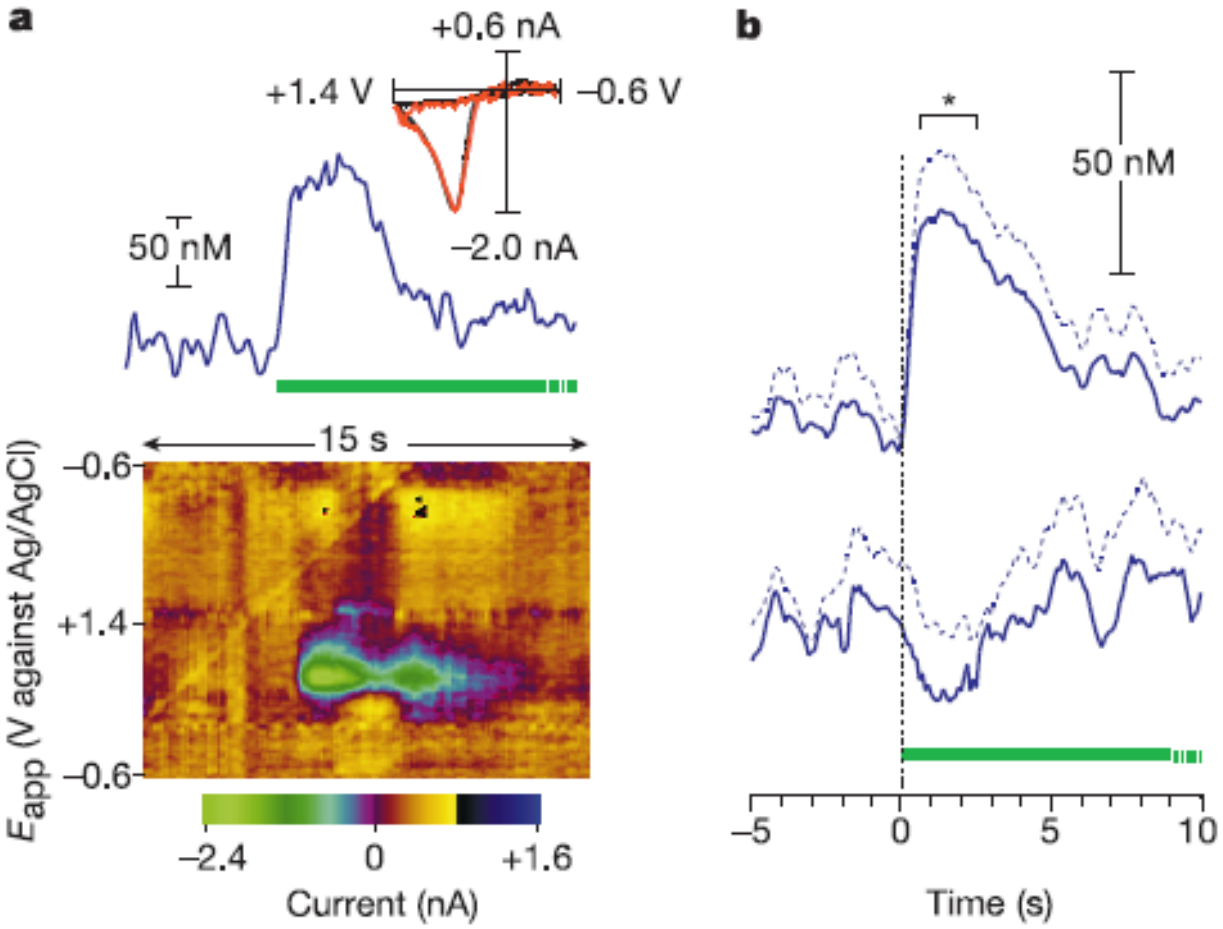


Wightman, Carelli and coworkers *Nature* **2003**, *422*, 614.

In Vivo Electrochemical Measurements

Correlation with behavior

Dopamine is released after cocaine-associated stimuli only

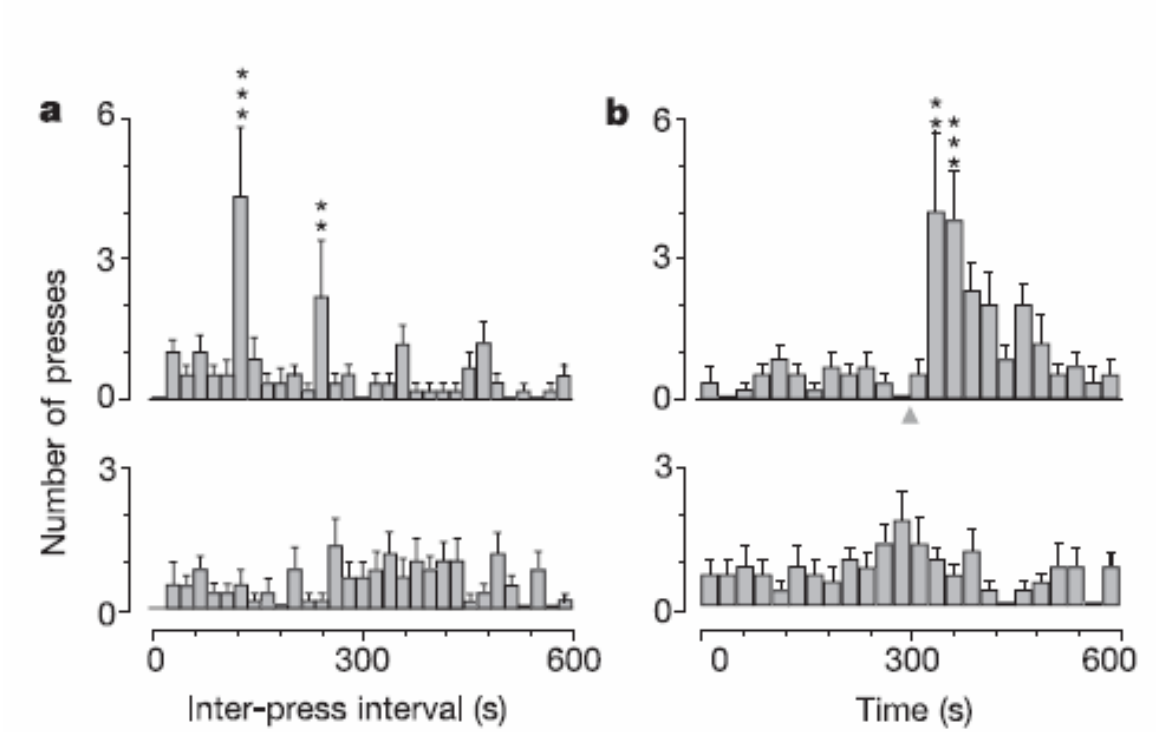


Wightman, Carelli and coworkers *Nature* **2003**, *422*, 614.

In Vivo Electrochemical Measurements

Correlation with behavior

Electrically evoked dopamine release promotes cocaine seeking behavior



Wightman, Carelli and coworkers *Nature* **2003**, *422*, 614.