Probing Brain Chemistry
From Micro to Nano

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“Understanding the reaction muck that is a cell, while of some considerable significance, remains nonetheless a daunting target for a chemistry community accustomed to the luxury of physically isolating variables. Nevertheless, life is chemistry (plus a little electricity), and biological functions result from the ebb and flow of molecule concentrations within a leaky membrane beaker. By using analytical methods to determine cell chemistry, we will come to understand the dynamic mechanisms of cell biology.”

--- Anthony W. Cznarnik
The human brain contains ~100 billion neurons.

**Synapse** - 20 nm gap between neurons where *chemical signaling* takes place.

Over **100 different substances** have already been identified as *neurotransmitters*.
Neurons Have Thousands of Connections with Each Other

Each neuron receives signals from 1,000 to 10,000 different neurons simultaneously

http://www.people.fas.harvard.edu/~wtyler/Imagingneurons.html
Serotonin Transmitter System

PSYCHIATRIC & DEGENERATIVE DISORDERS

Anxiety Disorders
Major Depressive Disorder
Alzheimer's Disease
Addiction
Autism
Bulimia
Alcoholism
Obesity
Migraine

Serotonin

Current Methods

- **Microdialysis**
  - Chemically selective (coupled to HPLC, CE)
  - Higher limits of detection (pM)
  - Long sampling times - (1-20 min)
  - Large probe diameters (240 μm)

- **Microelectrode voltammetry**
  - Short sampling times (0.1-1 s)
  - Small probe diameters (5-30 μm)
  - Low chemical selectivity (Nafion)
  - Lower limits of detection (nM)

Effects of Ecstasy (MDMA)

- Emotional openness
- Euphoria
- Stimulation
- Reduction of critical and cynical thoughts
- Decrease inhibitions
Current Hypothesis: MDMA Inhibits SERT and Causes Reverse Transport

Serotonin Transporter Deficient Mice

$[^{125}\text{I}] \text{RTI-55}$ binding to SERT

Same sections stained with Alcian Blue

The Locomotor Stimulating Effects of MDMA but Not AMPH Are Attenuated in SERT Deficient Mice

Working Hypotheses

- MDMA-induced serotonin release in striatum is reduced in SERT+/- mice and absent in SERT-/- mice.
- Decreased SERT-mediated 5-HT efflux results in decreased striatal dopamine release.
- Together these account for the gene dose-dependent insensitivity of SERT deficient mice to the locomotor stimulating effects of MDMA.
Microdialysis

CMA/7 or CMA/11 Probes
Membrane Diameter: 0.24 mm
Membrane Length: 2 mm
MW Cutoff: 6000 kDa

Brain section demonstrating dialysis membrane placement in striatum
Baseline 5-HT Levels Are Increased in Mice Lacking SERT

Zero Net Flux in Striatum of SERT Deficient Mice

Effects of MDMA on 5-HT Efflux in Striatum

Effects of MDMA on DA Efflux in Striatum

Conclusions

Serotonin release, while necessary, is not sufficient to produce MDMA stimulated motor activity in animals (and possibly humans) with decreased serotonin transporter expression.

MDMA may release serotonin by other mechanisms, in addition to reverse transport at serotonin transporters.
Measuring Uptake Rates by Chronoamperometry

A. 5-Hydroxytryptamine

\[
H^+ + 2e^- + 2H^+ \rightarrow \text{NH}_3 \]

B. Synaptosome

Reference Electrode

Working Electrode

Potentiostat

Model: IVEC10

Workstation

Headstage

Data Analysis

C. Change in Concentration

\[
\mu M \text{ Change in Concentration}
\]

D. 100 μm

Serotonin Uptake is Reduced in a Gene Dose-Dependent Manner

Boron-Doped Diamond Electrodes to Determine Serotonin Uptake Kinetics

- Wide working potential window
- Low background current
- Relatively rapid electron transfer kinetics
- Weak adsorption of polar molecules
  - Low fouling due to adsorption of serotonin or proteins

Collaboration with Bhavik Patel, Dept. of Bioengineering, Imperial College London
Electrodes from Professor Greg Swain, Chemistry Department, Michigan State University

http://www.chemistry.msu.edu/faculty/swain/index.html
Measuring Serotonin Uptake Rates Using Boron-Doped Diamond Electrodes

BDD electrodes show higher and more stable current responses to serotonin.

BDD electrodes show less fouling in response to high concentrations of serotonin or biological medium compared to carbon fiber electrodes.

**Serotonin Neurotransmission in Frontal Cortex in SERT+/- Mice**

<table>
<thead>
<tr>
<th>MFB Stimulation</th>
<th>Dorsal Raphe Stimulation</th>
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**Dorsal Raphe Stimulation**

**Stimulation Protocol**
- Stim Frequency = 60 Hz
- Stim Pulse = 120
- Polarity = Monophasic (+)
- Width of Pulse (ms) = 4
- Current = 300-400 µA

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Singh YS and Andrews AM, unpublished
Fig. 1. Photograph of the improved ceramic-based Pt multisite microelectrode. The Pt recording sites are $50 \times 150 \mu m^2$ with $50 \mu m$ edge-to-edge spacing. Sites are numbered 1–4 starting from the microelectrode tip.

Previous work by others has demonstrated tethering and recognition of proteins and peptides


Our goal is to tether small neurotransmitter probes (<200 MW) so as to permit selective recognition by large biomolecules!
DNA Aptamer for AMP

Biosensors using nucleic acid aptamers as selective molecular recognition elements (chemically sensitive/selective, small & fast)
Identify sequences of DNA or RNA that bind specifically to the tethered small molecules – **aptamers**

**Aptamers:**
- chemically synthesized (vs. biologically for antibodies)
- can be made more specific than antibodies, especially to specific interferents

![Chemical structures of Theophylline and Caffeine](image)

Theophylline and caffeine differ by one methyl group and aptamers exist to select for theophylline over caffeine by 10,000× in binding affinity.
Images of the biochip containing 26 arrayed SWCNT-FET devices

Aptamers for IgE linked to CNT to detect IgE in solution

Demonstrate molecular recognition of neurotransmitters tethered to SAM surfaces

Use surfaces to capture native binding proteins

Use surfaces to screen for high affinity aptamers

Construct multiplexed in vivo nanosensors

Identify novel neurotransmitter binding proteins

Investigate regulation of neurotransmitter signaling pathways

Study dynamic neurotransmitter signaling

Mass spectrometry proteomics

Patterned Si nanowire sensors
Traditional SELEX: Sepharose functionalized with serotonin failed to produce capture materials with high selectivity.

Exploit advanced mixed monolayers with minimal defects and optimized probe spacing to maximize specific recognition & minimize nonspecific binding.

Alkanethiols can be functionalized with many different head groups:
- OH
- COOH

Goal: To fabricate neurotransmitter-functionalized chips to be used for protein and nucleic acid capture that resist nonspecific binding.

http://www.ifm.liu.se/Applphys/ftir/sams.html
Oligoethylene glycol monolayers terminated in -OH or -COOH

Elimination of non-specific absorption
Recognition of polyclonal 5-HT antibodies (N = 13)

Szapacs ME and Andrews AM, unpublished
Is recognition biospecific at 50% surface coverage ???

• Optimal density for specific binding interactions (reduce steric hindrance)

• Surfaces with <5% coverage are not amenable to interrogation by ellipsometry
  - Quartz crystal microbalance
  - Fluorescence spectroscopy
  - Scanning probe & fluorescence microscopies
  - Surface plasmon resonance spectroscopy
Defects play key roles in patterning and pattern dissolution

Dodecanethiol on Au\{111\}

\[ V_{sample} = -1 \text{ V, } I = 1 \text{ pA} \]
Figure 2: Insertion Self-Assembly and 5-HT Functionalization. (a) Schematic of the insertion process. A sample with a monolayer of 1 is incubated in a solution of 2. Single molecules of 2 insert themselves into the existing monolayer matrix at defect sites, leading to dilute, non-phase separated coverage of the inserted molecule. (b) Using standard NHS/EDC chemistry as an intermediary, the carboxyl terminus of 2 is used to tether a serotonin molecule via its primary amine group.

Antibodies covalently coupled directly to an alkanethiol monolayer assembled on a QCM chip can be detected by a decrease in the oscillation frequency.

\[ \Delta F = -2(F_0^2) / A(\mu_q^* \rho_q)^{1/2} * \Delta m \]

- \( \Delta F \): change in frequency
- \( F_0 \): resonant frequency of the fundamental mode of the crystal (Hz)
- \( A \): active measurement area (cm²)
- \( \mu_q^* \): density of the crystal (2.648 g/cm³)
- \( \rho_q \): shear modulus (2.947 x 10¹¹ g/cm-s²)
- \( \Delta m \): change in mass (g)
Primary (1°) antibodies bind to surface immobilized 5-HT

Secondary (2°) antibodies bind to 1° antibodies and are tagged with fluorescent molecules
Fluorescence Spectroscopy

1. Lamp housing
2. Adjustable slits
3. Excitation Monochromator
4. Sample compartment
5. Baffle
6. Filter holders
7. Excitation/emission optics
8. Cuvette holder
9. Emission port shutter
10. Excitation Correction
11. Emission Monochromator
12. PMT detector

Source

Detector

2° Antibody

1° Antibody

5-HT functionalized SAM

Glass 10 nm Au
Antibody Saturation Binding

-Δ Frequency (Hz)

Immobilized 5-HT pAb \(10^{-4} \mu g/\mu l\)

Sample 1
Sample 2
Sample 3

Vaish A and Andrews AM, unpublished
Native serotonin receptors bind to free solution serotonin with high affinity.

Vaish A and Andrews AM, unpublished
Couple 5-hydroxytryptophan (5-HTP) to the surface through its carboxyl group, instead of coupling serotonin through its amine group to provide assess that more closely mimics free serotonin.
Selective Capture Using Functional Surfaces

Create dilute, non-phase-separated coverage of tether molecules using insertion self-assembly.

neurotransmitter functionalization chemistry

neurotransmitter precursor functionalization chemistry

neurotransmitter antibody incubation

neurotransmitter receptor incubation

Vaish A and Andrews AM, unpublished
(ii) XPS spectra of the N1s peak in (A) mixed SAMs (95:5), (B) SAMs with tethered ethylenediamine linkers, and (C) the SAMs in (B) derivatized with 5-HTP. The area under the curves (inset) indicates twice the amount of nitrogen is present in (C) as compare to (B), consistent with the stoichiometry of the surface chemistry.

Vaish A and Andrews AM, unpublished
L-5-HTP-functionalized surfaces (N = 4) show high affinity for native human serotonin-7 subtype receptor. Decrease in QCM frequency is double that observed for DL-5-HTP-functionalized surfaces (N = 4). 5-HT-functionalized surfaces (N = 3) show limited affinity to bind this receptor.

Vaish, Shuster, Weiss and Andrews, unpublished
Selective Capture of Native Membrane-Associated Serotonin Receptors

Vaish, Shuster, Weiss and Andrews, unpublished
Microcontact Insertion Patterning

Student Researchers

- Trevor Brown, Chemistry
- Rob Bridges, Chemistry
- Eric Horowitz, Chemistry
- Matt Szapacs, Chemistry
- Beth Anderson, Chemistry
- Mitchell Shuster, Physics
- Amit Vaish, Bioengineering
- TJ Mullen, Chemistry
Awesome Young Scientists

Stefanie Altieri
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Amanda Bressler
Tara Chrzanowski
Chubi Ihunah
Alex Lewis
Brooke Osbourn
Stefan Pajtek
Mitchel Shuster
Yogesh Singh
Laura Taylor
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