Dynamic In Vitro Biosensing with Flexible Microporous Multimodal Cell-Interfacial Sensors

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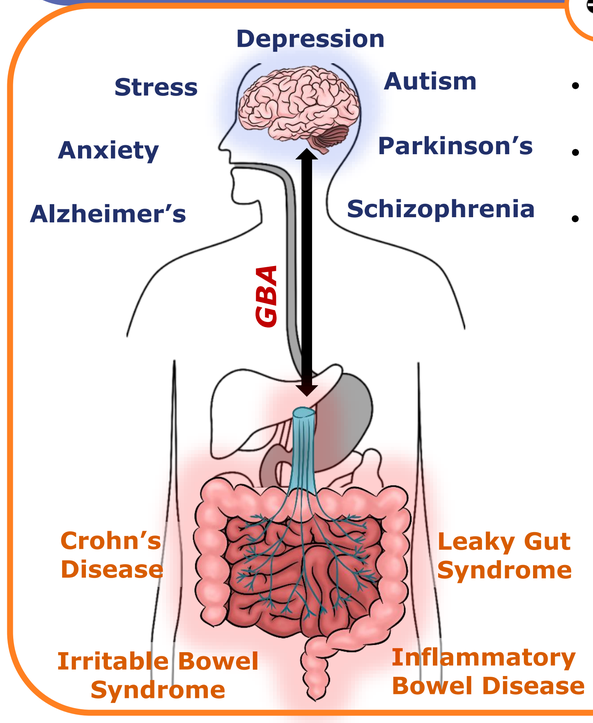
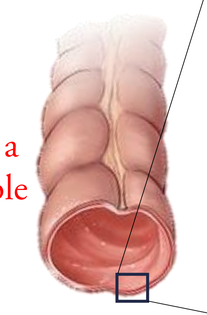
Electrodes fabricated on cell culture substrates have direct, real-time access to molecular dynamics

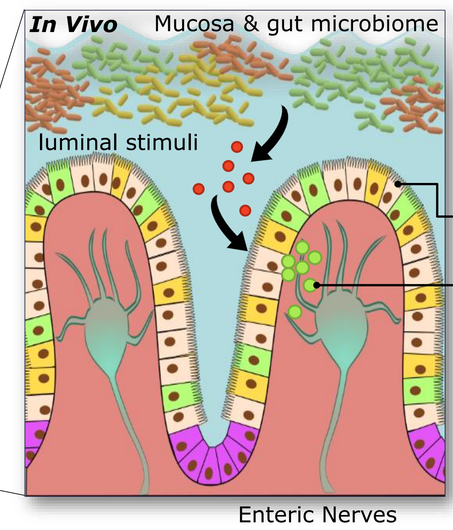
Motivation and Significance

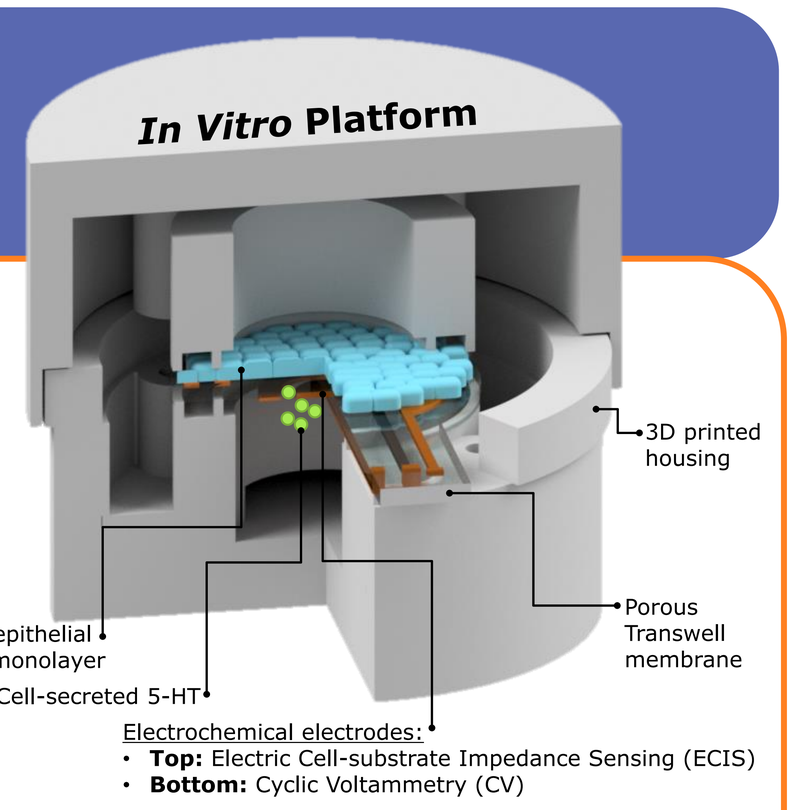


- The **Gut-Brain-Axis (GBA)** connects the Gastrointestinal (GI) tract and the brain via Enteric Nervous System (ENS) and the vagus nerve.[1]
- Neural disease development is highly correlated with GI tract infections/diseases.[2]
- **Proposed pathway:** Luminal stimuli induce serotonin (5-HT) release from enterochromaffin cells,[3] which activates enteric nerves and propagates signals to the brain.

We have a limited understanding of this dynamic pathway due to a lack of technology capable of real-time *in situ* serotonin monitoring from relevant tissue.







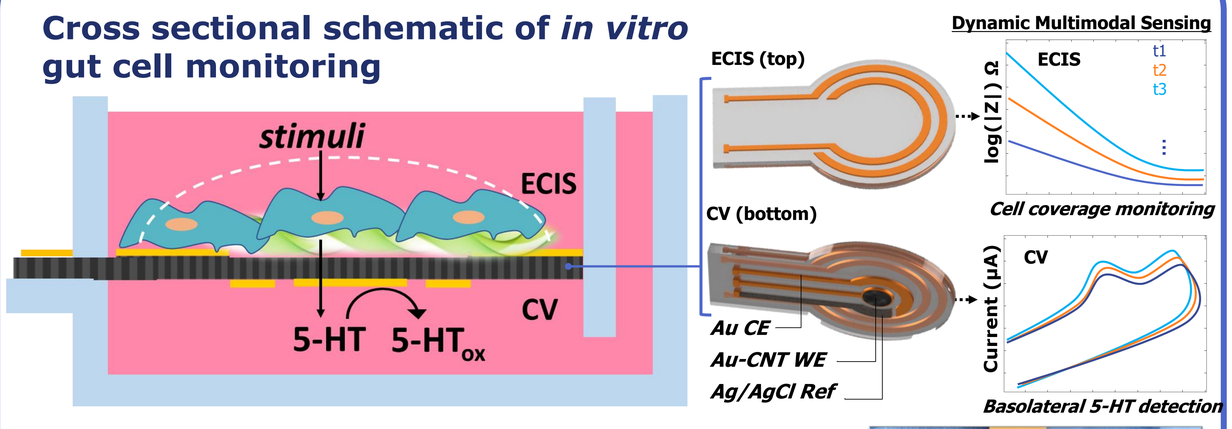
Electrochemical electrodes:
- **Top:** Electric Cell-substrate Impedance Sensing (ECIS)
- **Bottom:** Cyclic Voltammetry (CV)

Our Technological Innovation

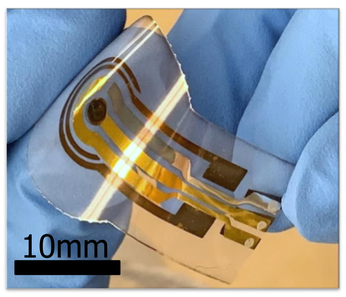
Electrodes fabricated directly on a cell culture substrate can **monitor gut cells** and their **5-HT release dynamics**, to correlate specific stimuli with their influence on the GBA pathway.

Electrodes for 5-HT Detection

Cross sectional schematic of *in vitro* gut cell monitoring

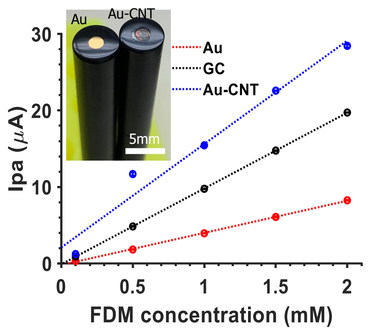


- 3D printed housing with **cell-interfacial electronics**
- Electrodes are integrated on a flexible, porous Transwell membrane (Polyester Track Etched, PETE, ≤1μm pores)
- Cultured Enterochromaffin RIN14B cells
- **ECIS:** non-invasively monitor cell growth and coverage
- **CV:** detect chemically-stimulated RIN14B basolateral release of 5-HT and diffusion through membrane

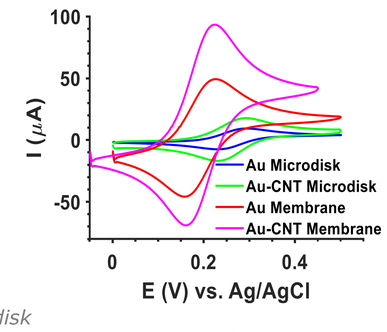


Carbon Nanotube (CNT) electrode characterization

CNTs have been used to increase electrode nanostructured surface area[4] and sensitivity to 5-HT[5].



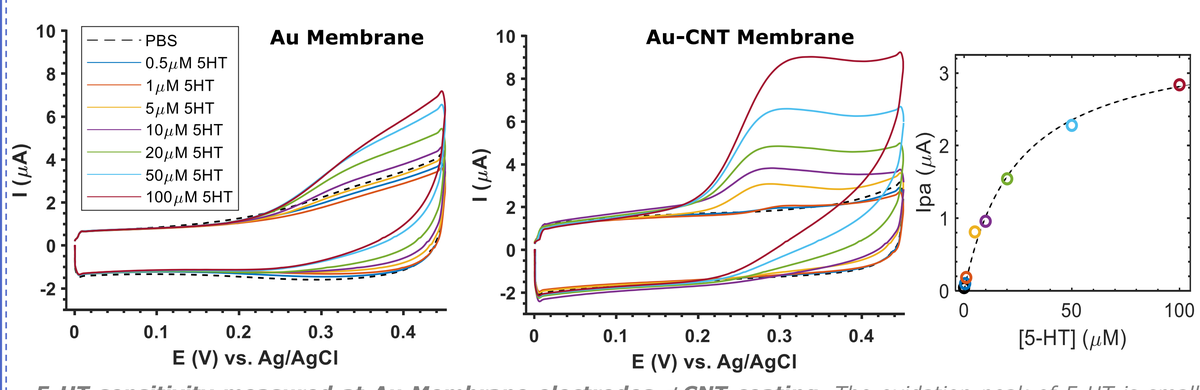
CNT coating *evaluated on Au microdisk electrode, vs. bare Au and glassy carbon, by measuring ferrocene dimethanol (FDM).*



	Epa (V)	Ipa / SA (μA/mm²)
Au Microdisk	0.287 ± 0.0002	2.28 ± 0.0023
Au-CNT Microdisk	0.287 ± 0.0002	4.023 ± 0.0400
Au Membrane	0.220 ± 0.0001	2.5 ± 0.1000
Au-CNT Membrane	0.218 ± 0.0003	4.28 ± 0.0050

Epa – Anodic peak voltage
Ipa – Anodic peak current
(n=5)

Microdisk and Membrane electrodes ±CNT coating *compared at 2mM FDM. Assessed Ipa normalized to footprint surface area (SA).*



5-HT sensitivity measured at Au Membrane electrodes ±CNT coating*. The oxidation peak of 5-HT is small and wide at the bare Au electrode, whereas CNT coating increases peak height and definition, presumably due to increased 5-HT binding strength and electroactive surface area. (Right) Langmuir curve fit to Ipa values measured at the Au-CNT membrane electrode, slope of linear region denotes sensitivity: ~100nA/μM (n=5).*

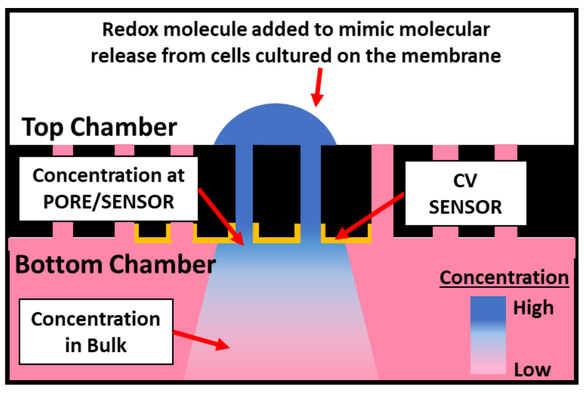
Significance:

- Electrodes fabricated on **thin, porous, flexible membranes** perform near identically to standard microdisk electrodes
- **CNT electrode modification** increases:
  - Electroactive surface area
  - 5-HT sensitivity

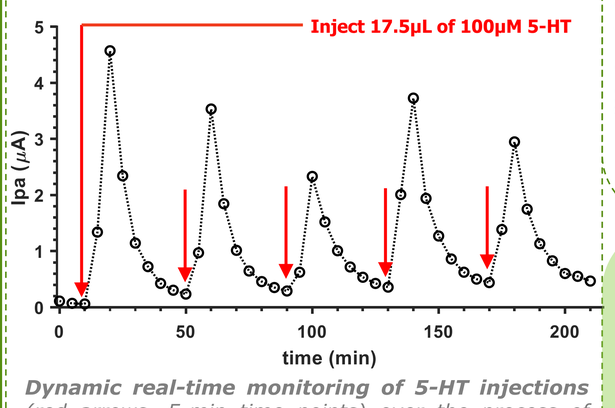
Functional In Vitro Sensing

Dynamic 5-HT detection

- **Interfacial electrodes** have close local proximity to the site of molecular release – **real-time detection**
- 5-HT released into into nanoliter pore volumes – **high local concentrations**

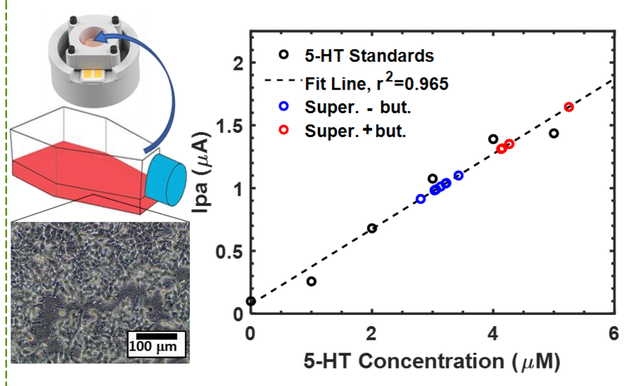


Schematic of 5-HT injection *to mimic detection of molecular release by cells cultured atop the membrane.*



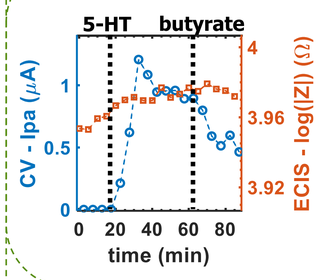
Dynamic real-time monitoring of 5-HT injections *(red arrows, 5-min time points) over the process of diffusion through the porous membrane.*

RIN14B cell-released 5-HT



Detection of 5-HT released from T75-cultured RIN14B cells. *(Left) Schematic (Right) 5-HT measurement from cell supernatant before (blue) and after (red) stimulation with 100μM butyrate. Concentration calculated against standard curve: 1μM 5-HT released over 1 hour.*

Multimodal measurements



Monitoring of RIN14B cell culture with ECIS and CV. *Measurements staggered by 2.5min:*
ECIS … 2.5min … CV … 2.5min … ECIS …
Detection of exogenous 1μM 5-HT via CV. ECIS of cell layer shows stability at 10Hz over duration of test.

Significance:

Interfacial porous electrodes benefit from:
- Close proximity to molecular events **– high time resolution of molecular dynamics**
- Low pore volumes **– high concentrations**
- Multiple on-site electrode integration

Future Work

- Optimization of RIN14B cell culture and molecular detection on electrode-integrated membrane
- *In vitro gut model:* incorporate other epithelial cell types and gut bacteria
- Use EIS + CV monitoring to correlate bacterial colonization/degradation of the mucosa with effects on 5-HT release patterns → **Uncover real-time GMBA signaling**

References

1. Carabotti, et. al., "The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems." Ann Gastroenterol. 2015; 2. Foster, et. al. "Gut–brain axis: how the microbiome influences anxiety and depression." Trends Neurosci. 2013; 3. O'Mahony, et. al. "Serotonin, tryptophan metabolism and the brain-gut-microbiome axis." Behav Brain Res. 2015; 4. McCreery. "Advanced carbon electrode materials for molecular electrochemistry." Chem Rev. 2008. 5. Swamy, et. al. "Carbon nanotube-modified microelectrodes for simultaneous detection of dopamine and serotonin in vivo." R Soc Chem. 2007.

Acknowledgements

This work was funded by the Brain and Behavior Initiative – BBI Seed grant. Travel funding was provided by the International Conference Student Support Award – ICSSA. The authors would like to thank the staff at the Maryland Nanocenter for support in fabrication and imaging.

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