IEEE Sensors Letters Volume 2(3) (2017)
Biomarker Sensing Technologies

Serotonin Sensing Technologies to Promote Understanding of the Gut-Brain Axis

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Received 1 Nov 2016, revised 25 Nov 2016, accepted 30 Nov 2016, published 5 Dec 2016, current version 15 Dec 2016.

Abstract— The gut-brain axis (GBA) is the bidirectional biochemical signaling pathway between the gastrointestinal (GI) tract and the central nervous system (CNS). Although the GBA is recognized as an important signaling network, its specific mechanics are not fully understood. The monoamine neurotransmitter serotonin (5-HT) is identified as an important signaling molecule in the CNS and GI tract. 5-HT's concurrent and widespread role in both neurological and GI physiology implicates it as a biomarker in the GBA, motivating the development of sensing technologies to study its dynamics, and subsequently lending to a fuller understanding of the GBA. To simultaneously understand the contribution of 5-HT to underlying signaling pathways and its downstream physiological effects, we have concurrently developed nano-micro-bio devices and systems to measure 5-HT dynamics *in vitro*, *ex vivo*, and *in vivo*. Our *in vitro* platforms measure 5-HT release events in response to stimuli in a simplified gut model, our *ex vivo* sensing technology measures 5-HT concentrations in the CNS of dissected crayfish, and our *in vivo* platforms measure 5-HT dynamics and aim to help elucidate the physiological role of 5-HT. Together, these sensing technologies provide engineering approaches for monitoring 5-HT dynamics and promote understanding of GBA pathways.

Index Terms— Gut-brain axis, Biomarker sensing technologies, Electrochemical sensing, Micro-electromechanical systems, Serotonin

I. INTRODUCTION

A. The Need for Studying 5-HT Dynamics in the Gut-Brain Axis

The gut and the brain are strongly connected through a physicochemical network known as the gut-brain axis (GBA) where conditions of the gastrointestinal (GI) tract are strongly linked to neurological conditions [1]. The cooccurrence of GI disorders with neurological disorders has motivated interest in the GBA and into identifying relevant biomarkers for both diagnostics and understanding the underlying pathways connecting the GI tract and central nervous system (CNS). Serotonin (5-hydroxytryptamine, 5-HT) has been demonstrated as a major signaling molecule in the GBA, with more than 90% produced in the GI tract in response to various conditions in the microbiome and gut epithelium [2]. 5-HT dynamics in the GI tract contribute to GI motility, secretory reflexes, and visceral sensations [3]. Additionally, 5-HT is an important neurotransmitter, responsible for regulating vital functions such as sleep, cognition, memory, thermoregulation, appetite, and general brain health [4]. The coincident role of 5-HT in the GI tract and CNS makes 5-HT an excellent candidate for studying the GBA and motivates the development of sensing technology for real-time 5-HT measurements, which will further enable insight into specific mechanisms contributing to GBA pathways.

B. Electrochemical 5-HT Sensing

Current understanding of the role of 5-HT within the GBA and its downstream physiological effects are limited due to the lack of appropriate quantitative tools for real-time detection. To detect 5-HT release in response to stimuli, sensors must achieve both high sensitivity and precise spatiotemporal resolution. Electrochemical sensing provides a real-time method for measuring 5-HT molecular events, enabling detailed studies of specific mechanisms [5-7]. Moreover, cyclic voltammetry allows for selective analysis of molecules based on their oxidation potential. Unlike standard analytical techniques [8], electrochemical sensing is low power, suitable for small sample sizes, and miniaturizable – making is well suited for implementing into study tools aiming to understand the role of 5-HT in the GBA.

We have developed micro-nano-bio devices for *in vitro*, *ex vivo*, and *in vivo* measurements of 5-HT to detect release events and elucidate downstream physiological effects under different conditions. Implementing our sensing technologies in different environments potentiates the ability to understand how molecular 5-HT events in the body regulate physiological changes. *In vitro* platforms are utilized to study specific 5-HT release events in response to stimuli while *in vivo* systems aim to study the downstream effects of 5-HT in animal models and humans. Furthermore, 5-HT is measured *ex vivo* in biologically realistic models to validate our sensing technologies. An overview of our 5-HT sensing devices and systems for multiple conditions is depicted in Fig. 1 [9, Fig. 1, 10, Fig. 1, 11, Fig. 1].

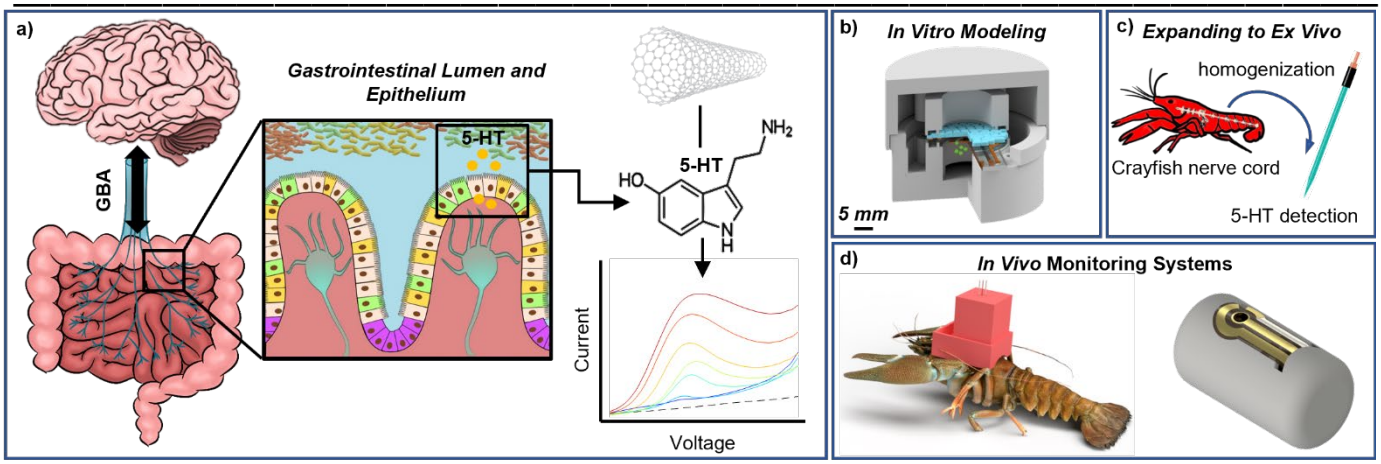

Fig. 1. (a) Illustration of the gut-brain axis and representative electrochemical detection as illustrated in [6, Fig. 1]. (b) Conceptual CAD diagram of the arrangement of the *in vivo* transwell device, showing cultured cells (blue) and 5-HT secretion (green) on a sandwiched porous membrane electrode [9, Fig. 1]. (c) *Ex vivo* experiments measure 5-HT in homogenized crayfish nervous tissue to study sensing technologies in biologically relevant environments with interferents. (d) *In vivo* systems aim to monitor 5-HT dynamics in animal and human models, these systems include: (left) an implantable biosensing system on an adult crayfish [10, Fig. 2] and (right) the SeroPill shown in a conceptual CAD diagram, with a flexible Au-CNT sensor fixed to the capsule [11, Fig. 1].

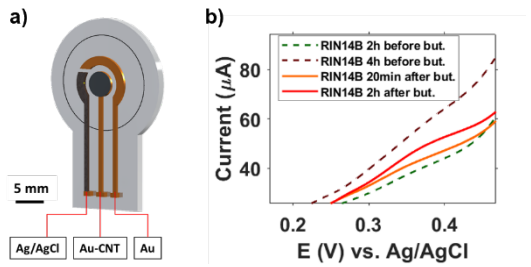

Fig. 2. (a) CAD diagram of planar Au-CNT three-electrode sensor with working, counter, and reference electrodes labeled, all deposited on a permeable membrane [9, Fig. 1]. (b) The cyclic voltammograms of RIN14B cells grown on membrane before and after butyrate stimulation and select accumulation times [9, Fig. 8].

II. SENSING TECHNOLOGIES FOR UNDERSTANDING THE GBA

A. In Vitro Platform to Detect 5-HT Stimulation Response

In vitro sensing platforms are useful tools for studying cultured cells, but typically employ techniques with poor spatiotemporal resolutions which are not appropriate for resolving release events. We have developed an *in vitro* sensor platform for studying 5-HT release concentrations through a permeable membrane [9]. RIN14B cells were directly cultured on a permeable biosensor and stimulated to detect the presence of 5-HT. The RIN14B cell line was chosen as a surrogate for enterochromaffin cells (ECC) due to its ability to secrete 5-HT in response to stimuli. The three-electrode planar electrode (Fig. 2a [9, Fig. 1]) is composed of a gold (Au)-carbon nanotubes (CNTs) working electrode (WE), an Au counter electrode (CE), and a silver/silver chloride (Ag/AgCl) reference electrode (RE). The electrodes were deposited using electron beam deposition on a porous PETE membrane (~1 μm pore diameter) and CNT films were subsequently drop-casted on the WE to create a permeable, electrochemical sensor.

To stimulate 5-HT release, RIN14B cells were incubated in 100 μM sodium butyrate for 1 hr. Following a 2 hr accumulation period, an appreciable peak was observed (Fig. 2b, [9, Fig. 8]), indicating an estimated concentration of 7.2 μM 5-HT. However, no detectable peaks were observed during shorter accumulation conditions, supporting accumulation-dependent sensitivity. This may be due to the ECC-like immortalized cell lines (including RIN14B) secreting much lower 5-HT concentrations than primary *in vivo* ECCs. This is a major challenge for *in vitro* 5-HT platforms and future work is focused on methods to produce higher concentrations of 5-HT from release events to accurately model ECCs.

The electrochemical 5-HT detection by our *in vitro* setup demonstrates the system can perform sensitive 5-HT detection on a porous polymeric membrane and with future improvements may allow for access to molecular information via real-time measurements that have previously been inaccessible.

B. Carbon Fiber Microelectrode (CFME) to Investigate 5-HT Dynamics

A Nafion-CNT surface-modified CFME [12] was developed to address the limitations of the planar electrode and to expand our sensing capabilities to realistic biological models. The CFME demonstrated the following improvements from the planar electrode: (1) small sensor size; (2) small sample volume; (3) high spatial sensing resolution; and (4) minimally invasive sensing capability. The CFME was characterized in a RIN14B cell culture: 5-HT release was stimulated by allyl isothiocyanate and resulted in a peak current (Ipa) of 0.018 μA. A calibration curve was performed with 100 nM to 200 nM 5-HT, yielding a limit of detection (LOD) of 120 nM and a sensitivity of 254.0 nA/μM (R^2=0.9853) (Fig. 3).

C. Ex Vivo 5-HT Sensing Using the CFME

Building upon the *in vitro* characterization, we have successfully expanded our sensing capabilities to *ex vivo* measurements of 5-HT in realistic biological settings. Homogenized crayfish nervous tissue was used to evaluate the ability of the CFME to measure neurohormonal 5-HT in the CNS. *Ex vivo* measurements focused on crayfish because it is an established model for investigating neural

mechanisms underlying the GBA and 5-HT signaling [13].

Cerebrospinal fluid 5-HT concentrations have been reported between 120-3870 pg/mL [14]. A volume of 0.5 mL of 3 mM, corresponding to approximately 1780 pg/mL, 5-HT was injected into crayfish hemolymph (a fluid equivalent to blood in invertebrates). Subsequently, the nerve cord was excised, the hemolymph was removed, and the dissected nervous tissue was homogenized. Fig. 4a depicts the voltammetric responses of the sensor for tissue with and without injection of 5-HT into the hemolymph. A clear oxidation peak at 0.35 V was observed, which is attributed to the oxidation of 5-HT on the sensor and supports the hypothesis that our sensor can measure biologically relevant concentrations of 5-HT in the CNS. The Ipa with 5-HT is 0.02 µA versus 0.005 µA without 5-HT injection (Fig. 4b). While we have successfully measured 5-HT in sub-micromolar concentrations after injection in real biological samples, we are limited in measurements of naturally occurring 5-HT. Future *ex vivo* work will focus on improvements to sensitivity to enable the quantification of 5-HT in non-treated samples.

D. Towards In Vivo Sensing Platforms

By leveraging miniaturization, electrochemical sensors and front-end electronics have been integrated into *in vivo* sensing systems to elucidate the downstream physiological role of 5-HT in the GBA for animal models and humans. We have successfully demonstrated the measurement of 5-HT in treated crayfish with an integrated backpack system and a proof-of-concept for an ingestible capsule device for 5-HT monitoring *in situ*.

1. CrayPack: Wearable 5-HT sensing system for crayfish

We have translated our sensing technologies to a standalone system for *in vivo* 5-HT monitoring in a freely behaving crayfish which could be used to study the relationship between neurohormonal and natural behavior. Literature suggests that stressed crayfish exhibit elevated levels of 5-HT in their nervous system, and real-time measurement could yield insight into its role in aggression and anxiety [15]. System miniaturization allows for 5-HT measurements underwater and in freely behaving crayfish to quantify the correlation between 5-HT and behavior in this well-known animal model. We successfully developed CrayPack, illustrated in Fig. 5a [10, Fig. 1], which is a wired, integrated system, to monitor 5-HT using a CFME sensor integrated with our flexible printed circuit board (PCB) [16], alleviating the need for a wired benchtop potentiostat to take electrochemical measurements [10].

The integrated backpack system is capable of monitoring 5-HT dynamics in response to saline and 5-HT injections. Injection events contained either 0.5 mL of crayfish saline or 0.5 mL of 3 mM of 5-HT. 5-HT concentrations in the hemolymph were monitored by CrayPack. Fig. 5b [10, Fig. 7] depicts a significant change in Ipa following 5-HT injection events (maximum current 82.9 nA), whereas no appreciable 5-HT was observed after saline (control) injections. Our results demonstrate the successful monitoring of *in vivo* 5-HT dynamics in crayfish hemolymph using CrayPack. Future work will include expanding our current system to a completely wireless, integrated system capable of measuring and wireless reporting endogenous 5-HT concentrations in freely-moving crayfish.

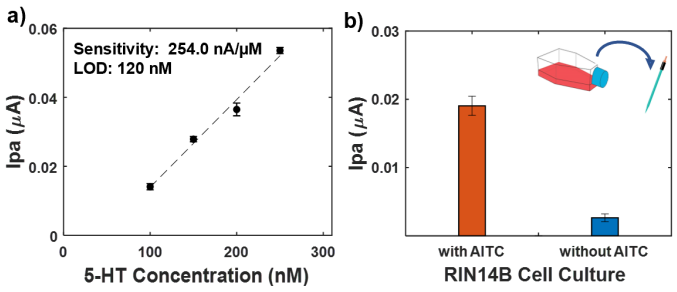

Fig. 3. (a) *In vitro* calibration curve for 5-HT concentrations from 100-250 nM. (b) Changes in Ipas for RIN14B with (orange) and without (blue) stimulation by AITC to simulate 5-HT release.

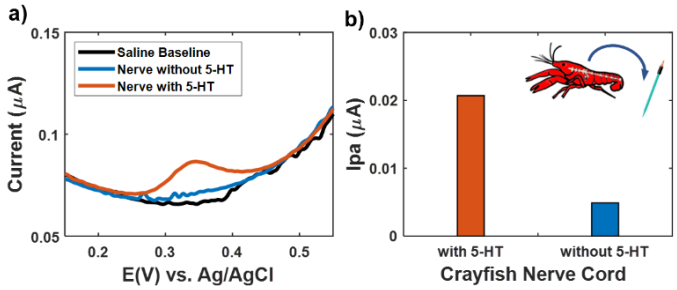

Fig. 4. (a) The cyclic voltammograms of homogenized crayfish nervous tissue (blue) and after 0.5mL of 3mM 5-HT injection (orange), and saline baseline (black). (b) Changes in Ipas for *ex vivo* homogenized crayfish CNS tissue with (orange) and without (blue) 5-HT injection.

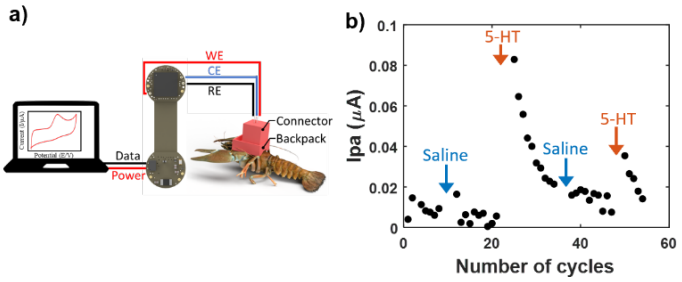

Fig. 5. (a) Schematic illustration of the wearable 5-HT sensing system on an adult crayfish from [10, Fig. 1]. (b) Changes in oxidation peak currents vs. time of multiple injection events: a 0.5 mL saline injection (blue) @9 min and @35 min and a 5-HT injection (orange) @22 min and @48 min [10, Fig. 7].

2. SeroPill: 5-HT Sensing Ingestible Capsule

To better understand the role of 5-HT in the GBA, we have developed the first wireless ingestible device for real-time quantification of 5-HT. We have previously investigated ingestible capsules for GI tract monitoring, diagnostics, and drug delivery [17-19]. We have expanded our capabilities to monitoring of 5-HT in the GI tract which will be instrumental in clarifying its role in GI diseases in regions that standard endpoint techniques cannot access. The SeroPill (Fig. 6a) [11, Fig. 3] is a fully integrated system featuring a flexible three-electrode Au-CNT sensor and our previously demonstrated PCB electronics, which are packaged in a 3D printed shell for *in situ* 5-HT monitoring [11]. We have adapted the previously demonstrated three-electrode planar sensor design [9] onto a flexible film to conform to the cylindrical enclosure of the ingestible capsule. By applying system miniaturization, we have created this proof-of-concept design for an ingestible sensing platform capable of measuring luminally released 5-HT in the GI tract.

In this work, biologically relevant concentrations of 5-HT were measured in a beaker using wireless communication. Fig. 6b [11, Fig. 5] summarizes the response of the sensor to 5-HT concentrations from 1 µM to 10 µM. A calibration curve was performed with 1 µM to 10 µM 5-HT, yielding a limit of detection (LOD) of 140 nM and a sensitivity of 470.0 nA/µM (R^2=0.99). The goal of the SeroPill is to monitor 5-HT in the GI tract with wireless electrochemical sensing. Future work will focus on validating the sensor for selectivity against interferents and operation in a simulated GI environment.

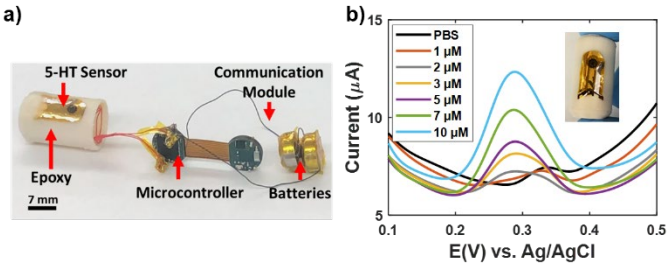

Fig. 6. (a) An image of the system depicting the connections between the internal components [11, Fig. 3]. (b) The cyclic voltammograms response of 5-HT concentrations in a beaker from 0-10 µM, depicting the peak current response from 0.2-0.5 V [11, Fig. 5].

III. DISCUSSION AND OUTLOOK ON FUTURE SENSING PLATFORMS

We have successfully demonstrated our 5-HT sensing technologies for a variety of *in vitro*, *ex vivo*, and *in vivo* settings. Our *in vitro* sensing platform has detected 5-HT released by ECC-like cells. We have concurrently developed a CFME for detecting 5-HT in *ex vivo* tissues and created an *in vivo* system capable of monitoring 5-HT in an animal model and a fully integrated, ingestible sensing capsule.

Future work will, in part, address the remaining challenges of electrochemical sensing 5-HT, including fouling, sensitivity, and selectivity of the sensor [8]. We aim to expand our sensing capabilities – addressing diminished sensitivity due to biofouling in long-term monitoring, improving sensitivity to detect low concentrations of endogenous 5-HT, and further miniaturizing the demonstrated systems. In future work, it will also be critical to maximize sensor selectivity against interfering molecules. Additionally, modifying electrode surfaces and system designs to improve resilience against biofouling is crucial to achieving long-term 5-HT measurements. Moreover, the miniaturization of sensor and PCB electronics, as well as optimization of device configurations, will enable further size reduction of our ingestible devices for facile GI translation. We aim to fully characterize these systems *in vivo* to validate their capabilities.

By exploring the dynamics of 5-HT both *in vitro* and *in vivo*, we can obtain a clearer understanding of how molecular 5-HT events regulate critical physiological processes and play a role in the GBA pathways. Our *in vitro* platform is an impactful tool for modeling simplistic release events and improving our sensing technologies before attempting *in situ* monitoring. Complementary *in vivo* sensing systems improve the ability to study 5-HT's role in animal behavior and its coincident role in CNS and GI tract physiology. The combination of *in vitro* and *in vivo* methodologies will symbiotically pave the way for future developments in 5-HT monitoring systems and help to clarify GBA physiochemistry.

ACKNOWLEDGMENT

This work was supported in part by the National Science Foundation under Integrative Strategies for Understanding Neural and Cognitive Systems (NCS) Grant #1926793. The authors acknowledge support from TerrapinWorks and the University of Maryland NanoCenter and its FabLab. Special thanks to Dr. Ashely Chapin for her helpful discussions and contributions.

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