A WEARABLE SYSTEM FOR ELECTROCHEMICAL SENSING OF SEROTONIN IN CRAYFISH

*Jinjing Han, Justin M. Stine, Ashley A. Chapin, Tawen Ho, Norma Pena-Flores, Jens Herberholz, and Reza Ghodssi**

University of Maryland, College Park, Maryland, USA

ABSTRACT

We present a wearable system for *in vivo* serotonin (5-hydroxytryptamine, 5-HT) detection in crayfish hemolymph (i.e., blood). Surface-modified electrodes, with 4.9x improved 5-HT sensitivity, were integrated with a 3D-printed package to perform cyclic voltammetry (CV) by connecting to potentiostat electronics, enabling real-time data readout. The system was mounted on the crayfish dorsal carapace allowing electrode implantation into the heart for continuous *in vivo* 5-HT measurements (over 75 min) with a benchtop potentiostat. This system successfully monitored 5-HT dynamics in the targeted region with a high temporal resolution, providing a reliable platform for real-time neurochemical sensing.

KEYWORDS

Biosensing, electrochemical devices, microelectrodes.

INTRODUCTION

5-HT is a key neurotransmitter in the brain, underlying mental illnesses such as anxiety and depression [1]. 5-HT modulates the behavior and physiology of vertebrate and invertebrate animals via neurohormonal routes. For example, stressed crayfish show increased levels of 5-HT in hemolymph, and injection of 5-HT into hemolymph induces anxiety-like behavior. HPLC is commonly used to determine 5-HT levels in hemolymph to study its physiological pathways [2]. However, such techniques are costly, time-consuming, and have a low temporal resolution.

Electrochemical methods have emerged as a simple and inexpensive alternative, capable of rapid detection of 5-HT in biological samples with high sensitivity and selectivity [3]. CFMEs are commonly used for detection of neurotransmitters, especially *in vivo* in rodents. However, these electrochemical systems require bulky benchtop equipment and wires to connect with the implanted microelectrodes, limiting animal movement [4]. Animal behavioral studies would benefit from real-time *in vivo* quantitative 5-HT measurements using a fully miniaturized, wireless, wearable system. This work presents a wearable system towards this goal, showing: (i) successful measurements of *in vivo* 5-HT in crayfish by wiring to a benchtop potentiostat, and (ii) a prototype of a fully miniaturized electrochemical sensing system integrated with a PCB potentiostat. This system has the potential to facilitate real-time 5-HT measurements in freely behaving animals.

SYSTEM DESIGN AND ASSEMBLY

Figure 1a shows a schematic of the wearable system comprised of a **3D-printed package** with **customized implantable electrodes**, which can perform electrochemical measurements driven by either a benchtop or miniaturized PCB potentiostat.

The three-electrode configuration includes a surface-modified CFME as a working electrode (WE), an Ag/AgCl wire as a reference electrode (RE), and a Pt wire as a counter electrode (CE) (Figure 1b). The CFME is modified by dip-coating in 0.5 mg/mL carbon nanotube dispersed 2.5% Nafion solution (Nafion-CNT), followed by electrochemical activation (Nafion-CNT/EC). Successful coating of the Nafion-CNT film and surface etching by electrochemical activation were observed using SEM. Ag is electrochemically converted to Ag/AgCl by oxidation in 1 M KCl.

The 3D-printed package consists of two components: (i) an alignment spacer printed with clear resin using digital light processing (DLP) (M50, CADWorks3D) to connect customized electrodes with potentiostat electronics, and (ii) a PLA enclosure printed with fused filament fabrication (FFF) (MK3S+, Prusa) to provide an interface with the crayfish. A small cutout (4 × 3 mm) on the bottom of the enclosure allows electrode access to the hemolymph in the crayfish heart.

A hole was drilled through the crayfish carapace using a 26G needle and small drill bits. The enclosure base was fixed onto the carapace using super glue. The alignment spacer was lowered onto the enclosure, through which the electrodes were implanted under the dorsal carapace directly into the heart. The device showed minimal impact on crayfish movement. A week after electrodes implantation and removal, the crayfish remained alive, indicating the potential for repeated measurements in the same animal.

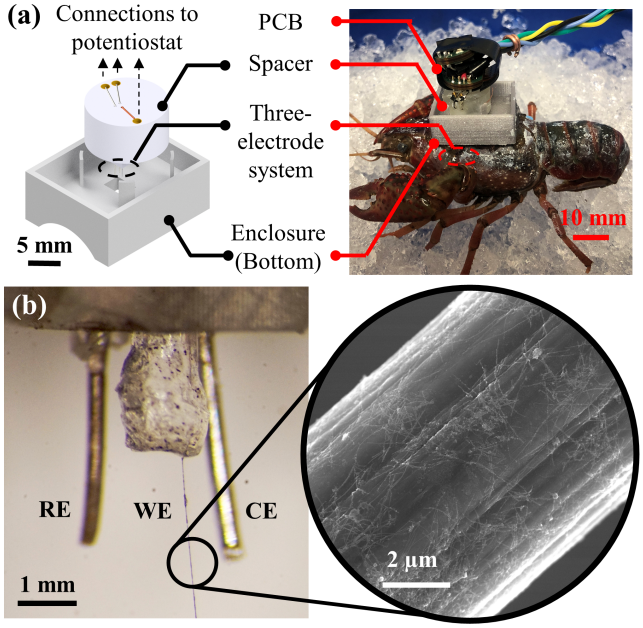

Figure 1: (a) Schematic of the wearable system and image of this system on an adult crayfish. Electrodes are implanted in the heart for in vivo hemolymph CV measurements. (b) Three-electrode electrochemical system with an SEM of the Nafion-CNT/EC WE.

RESULTS

The surface-modified WEs were characterized and compared with unmodified and partially modified CFMEs using CV. Figure 2 shows the anodic peak currents (Ipas) resulting from 5-HT detection at the WEs. While the bare CFMEs showed no response to 5-HT, the Nafion-CNT/EC CFMEs showed a 4.9x higher signal response than the Nafion-CNT CFMEs.

A PCB potentiostat, containing an analog-front-end (AFE) AD5941 and a microcontroller (MCU) BGM13S, was integrated with the customized electrode system and compared to a benchtop potentiostat for 5-HT detection using CV. The oxidation peak potentials (Epas) were observed to be 0.31 V for both instruments, while Ipas of 0.21 µA and 0.11 µA were recorded from the benchtop and PCB potentiostats, respectively (Figure 3). The cyclic

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voltammogram recorded by the PCB was subject to system noise, which required data processing by a low pass filter to properly analyze the current peaks.

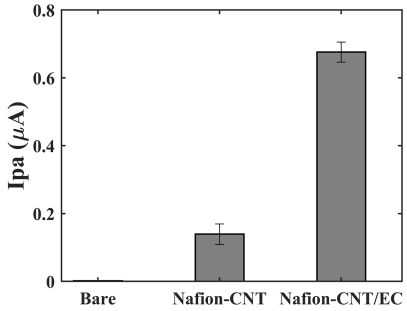

Figure 2: Ipas from CV signals of 10 μM 5-HT measured at bare, Nafion-CNT, and Nafion-CNT/EC surface-modified CFMEs (n=6). Average signals are 0 μA, 0.14 μA, and 0.68 μA, respectively, using a benchtop potentiostat (VSP-300, Biologic).

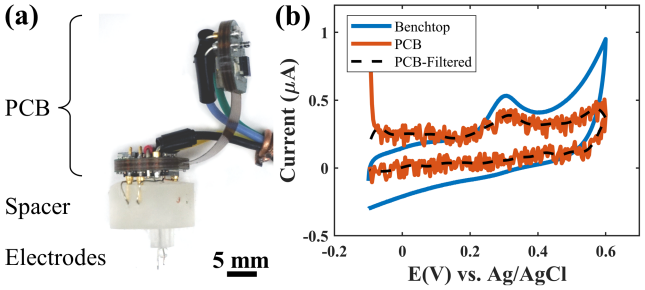

Figure 3: (a) Image of PCB-integrated electrode system used for 5-HT measurements. (b) Cyclic voltammograms of 10 μM 5-HT in PBS, comparing benchtop (CompactStat.h, Ivium) and PCB potentiostats using customized electrodes (Nafion-CNT/EC WE).

The PCB-integrated wearable system was then implanted into a crayfish heart to perform *in vivo* electrochemical measurements in hemolymph (Figure 4). The experimental setup is shown in Figure 1(a). The wearable system interfaced well with the crayfish carapace, facilitating minimally invasive measurements. The cyclic voltammograms for the *in vivo* hemolymph measurement had a similar shape compared to PBS, indicating successful *in vivo* electrochemical measurements (Figure 4b). 5-HT was undetectable in hemolymph, indicating low basal 5-HT concentrations. Two major challenges arose from this experiment. The Nafion-CNT/EC CFMEs have improved sensitivity but are fragile due to surface etching, which limits their lifetime in *in vivo* experiments. Future work will attempt to harden the electrodes with additional surface coatings. Furthermore, challenges in fabrication of the miniaturized electrode system resulted in low yield due to poor electrical connection between electrodes and PCB.

Given these challenges, *in vivo* measurements of 5-HT were performed using Nafion-CNT WEs and a benchtop potentiostat, which were more reliable. 5-HT was injected into the crayfish ventral sinus cavity, where it was expected to circulate through the animal to increase 5-HT concentration in the heart. CV was used to continuously monitor Ipas during this 5-HT circulation. The Ipa reached a maximum at 45 min after the 1st 5-HT injection (1 mL of 3 mM) and then started decreasing. The 2nd 5-HT injection (0.2 mL of 3 mM) was performed 65 min after the 1st injection, resulting in an increase in Ipa (Figure 5). These results indicate selectivity and successful continuous measurements of 5-HT *in vivo*, which follows the expected trend of changing 5-HT concentrations due to the repeated injections. For example, the 5-HT concentration may rise and fall as it circulates through the heart.

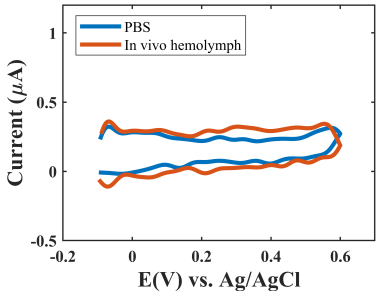

Figure 4: Filtered cyclic voltammograms of a PCB-integrated system using a Nafion-CNT/EC WE in crayfish hemolymph, showing a similar shape as PBS.

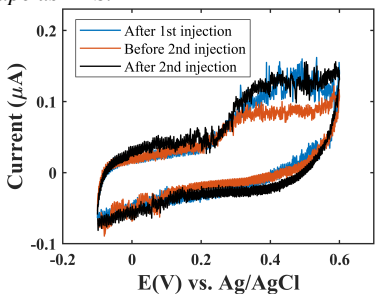

Figure 5: Cyclic voltammograms of in vivo 5-HT-spiked hemolymph using a benchtop potentiostat (CompactStat.h, Ivium) with a Nafion-CNT WE. The Ipas were 50.7 nA, 26.2 nA and 40.9 nA after the 1st injection (blue), before the 2nd injection (orange), and 10 min after the 2nd injection (black), respectively.

CONCLUSION

This work demonstrates a wearable system that integrates modified implantable electrodes with a 3D-printed package for electrochemical measurements in crayfish. The developed system has been integrated with a PCB potentiostat for miniaturization, as well as connected to a benchtop potentiostat for more sensitive electrochemical measurements. The PCB-integrated system achieved electrochemical measurements in hemolymph without 5-HT injection. When connected to a benchtop potentiostat, successful continuous monitoring of *in vivo* 5-HT was achieved in an anesthetized animal outside water. A fully realized PCB-integrated, miniaturized wearable system could potentially provide real-time measurements of 5-HT concentrations in freely moving animals, and identify the role of 5-HT in regulating various behaviors.

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CONTACT

*R. Ghodssi, tel: +1-301-405-8158; ghodssi@umd.edu