Indirect Voltammetry Detection of Non-Electroactive Neurotransmitters using Glassy Carbon Microelectrodes: The Case of Glutamate

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Section S1

Agarose hydrogels of varying concentrations were prepared and loaded by a rectangular weight cell of 50 kg load in a direct compression test using a TA.XT plusC Texture Analyzer (TA) (Stable Micro Systems Products) shown in Figure 1. This test, similar to a method reported for testing of microneedles [53], helped to determine the concentration of agarose resulting in a gel with a Young's modulus comparable to the one of brain tissue [54]. Five different compression tests were performed with a speed of loading of 2 mm/sec.

Section S2

Figure S1 summarizes the outcome of the compression tests that allowed the determination of concentration of agarose in the hydrogel for it to serve as *in vitro* brain model. The recorded force-time curves (Figure S1a) were converted into strain-stress plots (Figure S1b), where the slope during the initial compression phase was used to extract the modulus of the gel. An agarose hydrogel concentration of 0.6% was found to offer a mean modulus of 5.04 kPa corresponding the modulus of brain tissue [55-56]. In an earlier study, agarose gel concentration of 1.5% has been shown to be a good model for skin [53,56].

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[1] Address correspondences to Sam Kassegne • Professor of Mechanical Engineering, NanoFAB.SDSU Research Lab, Department of Mechanical Engineering, College of Engineering, San Diego State University, 5500 Campanile Drive, CA 92182-1323. E-mail: kassegne@sdsu.edu • Tel: (760) 402-7162.
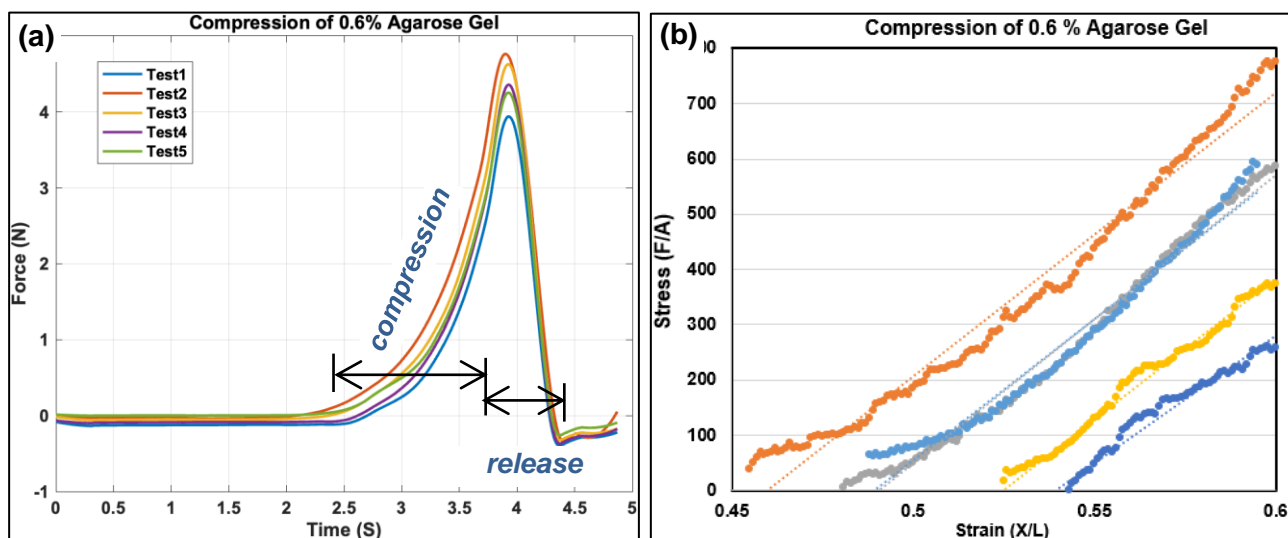
Figure S1. Compression test plot for (a) force-time and (b) close-up stress-strain curve for 0.6% agarose hydrogel model.
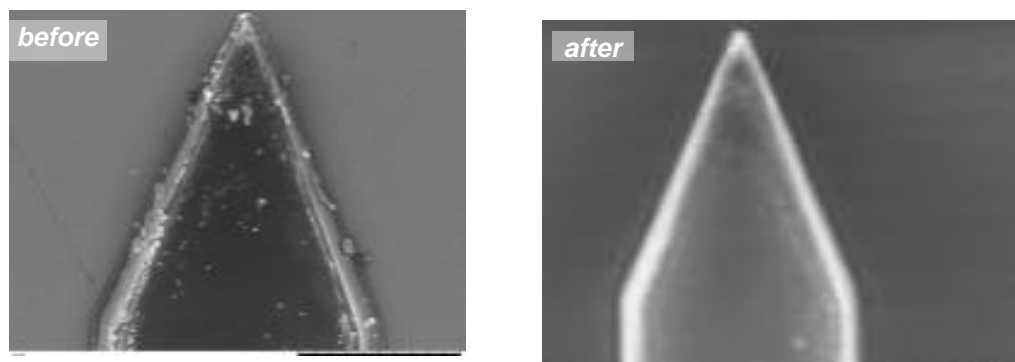

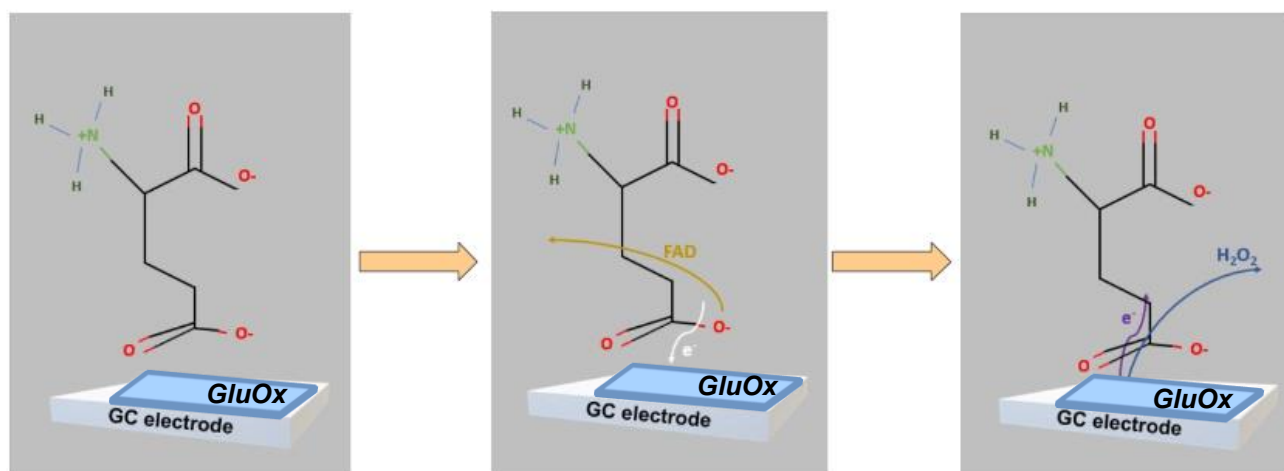**Figure S2.** Tips before and after penetration tests.

$$\textit{L-glutamate} + O_2 + H_2O \rightarrow \textit{oxoglutarate} + NH_3 + H_2O_2$$

Figure S3. Reactions driving FSCV detection on *GluOx* functionalized GC microelectrode.

Table S1. Active Site Amino Acids of Glutamate Oxidase (GluOX).

Active Site Amino Acids of Glutamate Oxidase (GluOx)				
Amino Acid	**Chemical Formula**	**Number of Atoms**	**Functional Group**	**Property**
Tyr	$C_9H_{11}NO_3$	24	Carbon Ring	Hydrophobic
Arg	$C_6H_{14}N_4O_2$	26	NH_4	Positive Charge
Trp	$C_{11}H_{12}N_2O_2$	27	Carbon Ring	Hydrophobic
Ala	$C_3H_7NO_2$	13	Carbon Strand (CH_3)	Flexible Linker
Glu	**$C_5H_9NO_4$**	19	Carboxylic Acid	Negative Charge
Asp	$C_4H_7NO_4$	16	Carboxylic Acid	Negative Charge
His	$C_6H_9N_3O_2$	20	Amino-Ring	Positive Charge

Table S2. Injection of *glutamate* and H_2O_2 as a function of time with sufficient time provided in-between each step to allow for stabilization of FSCV data collection.

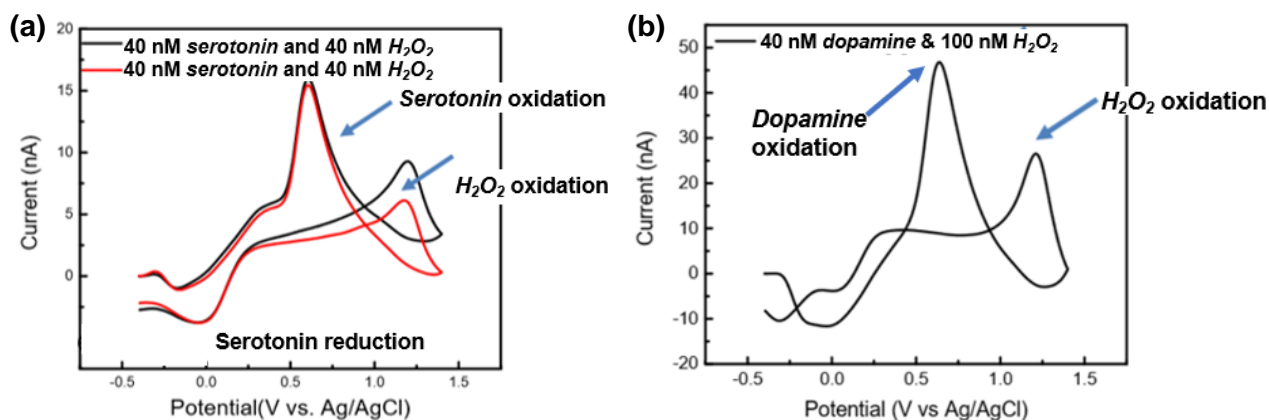| Time	Concentration
10 sec	10 nM
1 min	30 nM
2 mins	90 nM
3 mins	200 nM
5 mins	400 nM
6 mins	800 nM (1000 μM for H_2O_2)
8 mins	1.6 μM (2 μM for H_2O_2)

(a)

(b)

Figure S4. Experimenting for demonstrating selectivity of analytes using GC microelectrodes. In this experiment, we injected a mix of 40 nM of *serotonin* and 40 nM of H_2O_2 and using GC microelectrode to selectively detect the distinct oxidation and reduction of *serotonin* along with the distinct H_2O_2 peaks. We repeated the same experiment for a mix of 40 nM of *dopamine* and 100 nM of H_2O_2 where again, distinct peaks of the two analytes were determined as shown below.
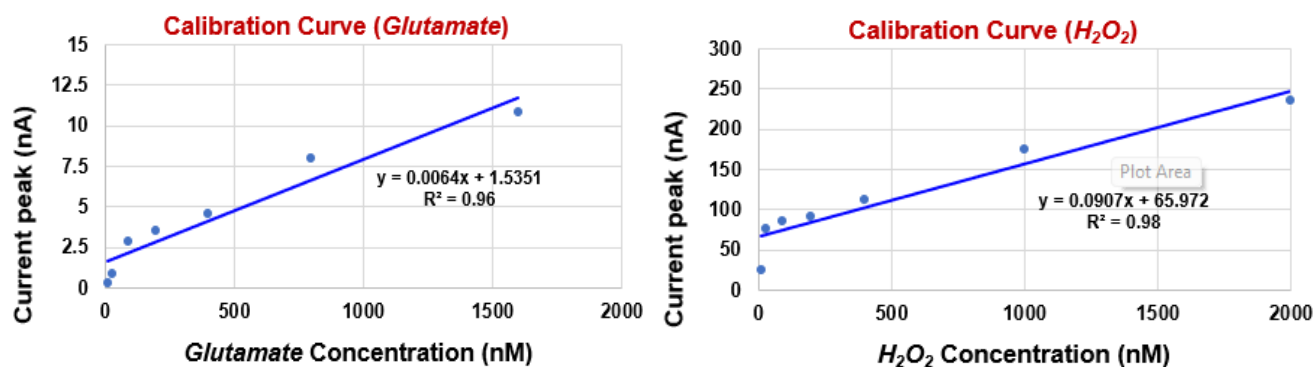

Figure S5. Lower limit of detection (LOD) of the microelectrodes was estimated to be 1.51 and 1.71 nM for *glutamate* and H_2O_2, respectively.