Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Improving serotonin fast-scan cyclic voltammetry detection: New waveforms to reduce electrode fouling

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I. Supplemental Figures

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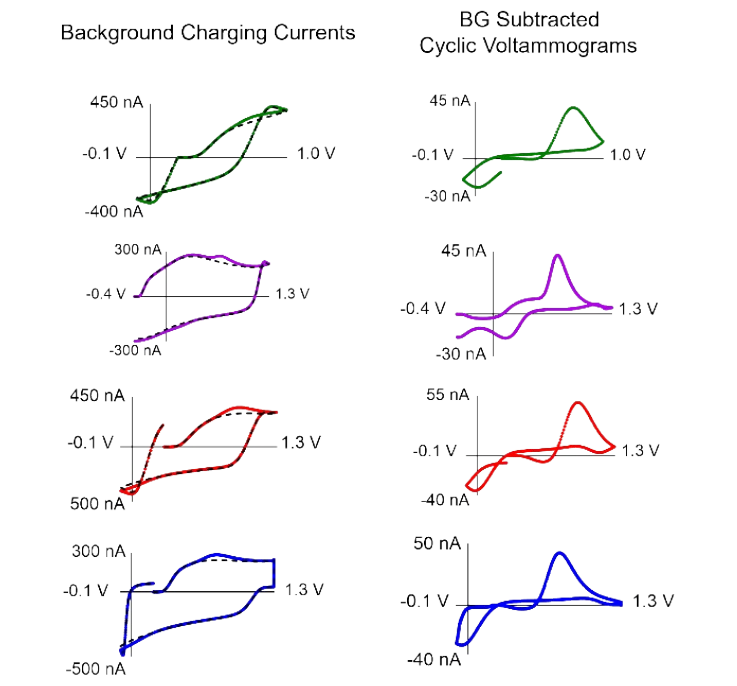
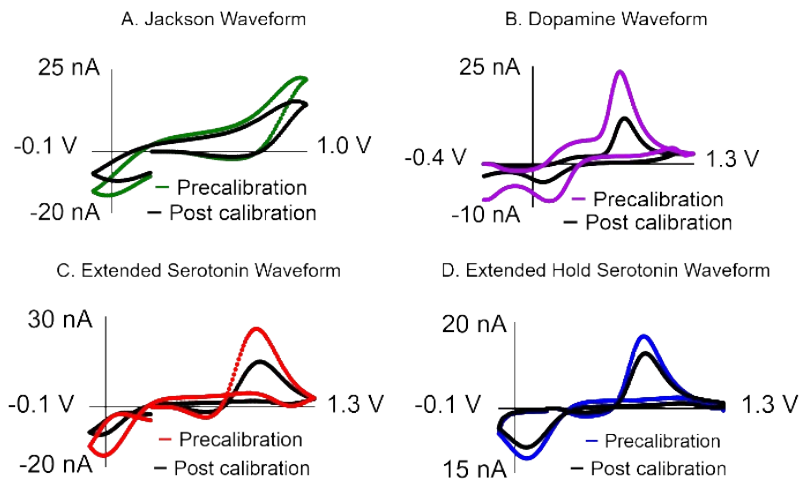
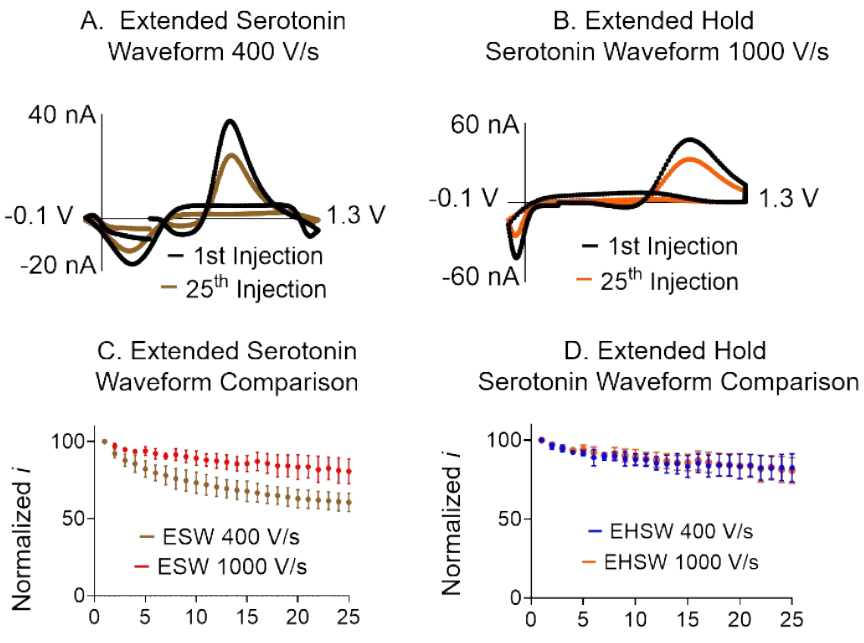


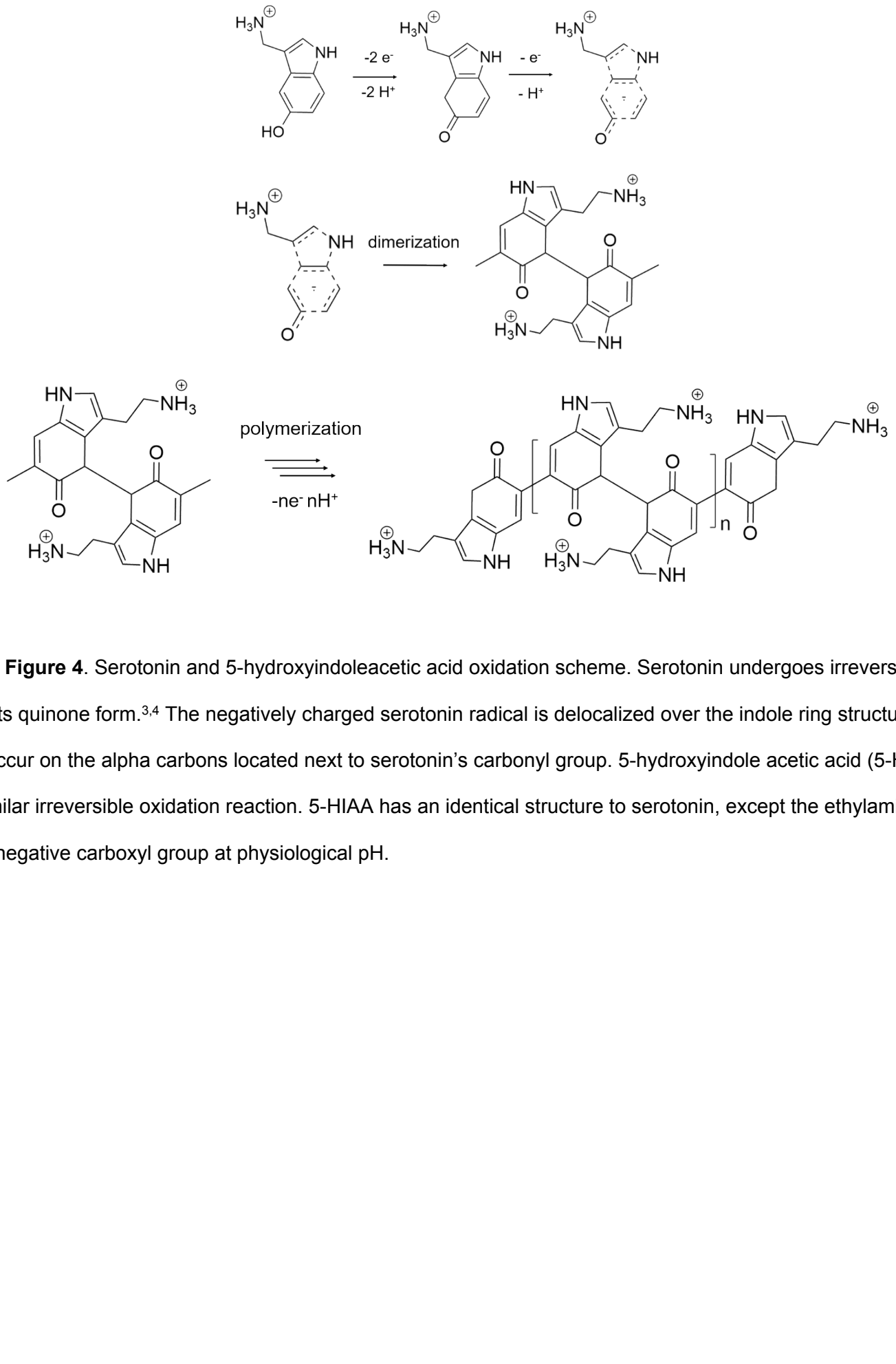
Supplementary Figure 1. Fast-scan cyclic voltammetry (FSCV) background (BG) charging currents. FSCV cyclic voltammograms on the left column show BG charging currents (dotted line) for each waveform (no analyte) along with BG charging current with 1 μM serotonin (colored). The right column shows BG subtracted cyclic voltammograms for 1 μM serotonin with each waveform. When a potential is applied to the CFME, the double layer charges like a capacitor and produces a background (BG) charging current.[1] The charging currents are proportional to the size (surface area) of the CFME and scan rate applied. CFMEs that are 25-75 μm in length usually produce BG currents between 200-800 nA. Larger background currents are observed for the Jackson waveform and ESW with 1000 V/s scan rates. The potential is applied for 10 minutes to allow the BG current to equilibrate before the experiment is run. Ten scans before the serotonin injection were averaged for background currents to subtract, and 5 CVs from when serotonin were injected were averaged for the analyte.



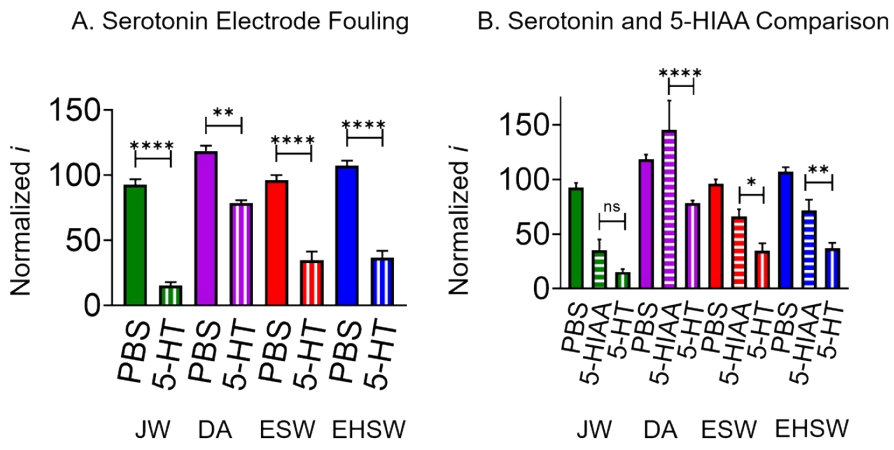
Supplementary Figure 2. Precalibration and post calibration responses of electrode after *Drosophila* tissue implantation. Example precalibration and post calibration cyclic voltammogram responses with to 1 µM serotonin flow cell injection for **A.** Jackson Waveform, **B.** Dopamine Waveform, **C.** Extended Serotonin Waveform, and **D.** Extended Hold Serotonin Waveform. CFMEs foul 20-50% after inserting and removing the electrodes into larval ventral nerve cord tissue. Electrode fouling can be caused by entropy-driven unfolding and adsorption of proteins to the charged electrode surface.[2] Although CFMEs foul from tissue exposure, serotonin detection was stable for each waveform (Fig 7A-D, Fig. S5). On average, CFMEs fouled 40 ± 6% with the Jackson waveform, 43 ± 5% with the Dopamine waveform, 38 ± 4% with the ESW, and 36 ± 3% with the EHSW.



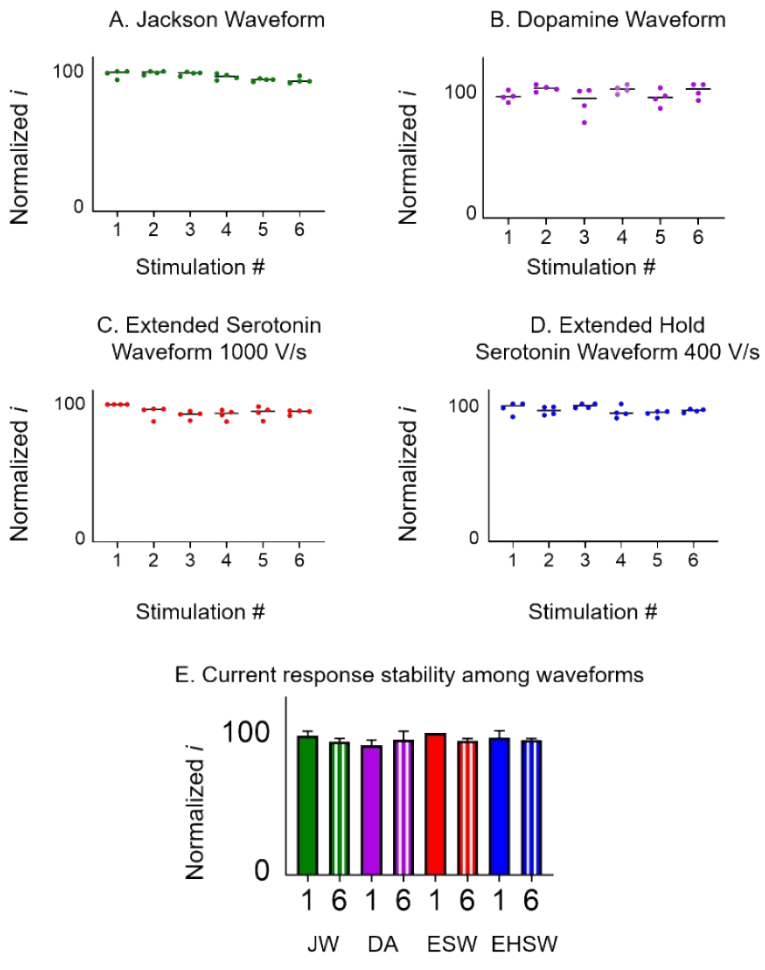
Supplementary Figure 3. Extended serotonin waveforms scan rate comparison. Repeated serotonin measurement electrode fouling was determined by injecting 1 µM serotonin for 5 seconds every 30 seconds repeated 25 times in a flow cell. Cyclic voltammograms show initial (1st, black) and final (25th, color) injections for each waveform. **A**. The CFME current using the extended serotonin waveform (ESW) at 400 V/s is reduced about 30% on the 25th injection. **B**. The current for the extended hold serotonin waveform at 1000 V/s is about 20% lower on the 25th injection. **C**. Comparison of repeated serotonin injection CFME responses for the ESW at 400 and 1000 V/s show the currents are lower using the ESW at 400 V/s than at 1000 V/s (Fig. 3C). ESW electrode fouling is significantly different at 400 V/s compared to 1000 V/s with a 39 ± 2% decrease and 19 ± 2%, respectively (Tukey's post-hoc, $p = 0.0001$). **D**. Comparison of repeated serotonin injection CFME responses for the EHSW at 400 V/s (Fig. 3D) and 1000 V/s show current decreases similarly with both scan rates. The ESW at 400 V/s exhibited similar cyclic voltammograms to the dopamine waveform and EHSW at 400 V/s with oxidation peaks at 0.6 V and reduction peaks at 0.0 to 0.2 V (Fig. 2B and D). There was a significant effect of injection number versus normalized current detected comparing the four waveforms (One-way ANOVA, $p = 0.0001$, $n = 6$). No significant differences in electrode fouling were observed between the ESW 1000 V/s, EHSW 400 V/s, and the EHSW 1000 V/s. The EHSW at 400 and 1000 V/s displayed similar electrode fouling behaviors and suggest extending the switching potential for prolonged periods of time (≥1 ms) compensates for using a slower scan rate.



Supplementary Figure 4. Serotonin and 5-hydroxyindoleacetic acid oxidation scheme. Serotonin undergoes irreversible oxidation after it reaches its quinone form.[3,4] The negatively charged serotonin radical is delocalized over the indole ring structure. Dimerization and extension occur on the alpha carbons located next to serotonin's carbonyl group. 5-hydroxyindole acetic acid (5-HIAA) undergoes a similar irreversible oxidation reaction. 5-HIAA has an identical structure to serotonin, except the ethylamine group is replaced with a negative carboxyl group at physiological pH.



Supplementary Figure 5. Fouling after long exposure to serotonin. To determine electrode fouling effects with long serotonin exposure, the current response to a 1 μM serotonin injection was recorded, the CFME was soaked in serotonin for 1 hour with a waveform applied, and then the current response to serotonin was analyzed again and compared to the PBS control (Fig. 4E). **A**. Long exposure to serotonin causes CFMEs to foul severely with all waveforms. There were significant effects of waveform (Two-Way ANOVA, $F_{(3,36)} = 60.50$, $p = 0.0001$, $n = 6$ for 5-HIAA, $n = 3$ for PBS, $n = 3$ 5-HT) and soaking in either 5-HT or PBS ($F_{(2,36)} = 84.36$, $p = 0.0001$) on current response with significant interaction between the groups ($F_{(6,36)} = 9.77$, $p = 0.0001$). CFMEs using the Jackson waveform (green) fouled the most with 85 ± 1% current decrease, and responses were significantly different from the PBS control (Tukey's post-hoc, $p = 0.0001$). Electrodes using the ESW (red, 65 ± 4%) and EHSW (blue, 63 ± 3%) fouled similarly and less than the Jackson waveform. Current responses were significantly different in serotonin compared to the control (both $p = 0.0001$). Using the dopamine waveform (purple), electrodes also fouled with a 22 ± 1% decrease in current, and serotonin responses were significantly different than the control ($p = 0.001$). However, serotonin electrode fouling with the dopamine waveform was remarkably less and significantly different compared to the Jackson waveform ($p = 0.00001$), ESW ($p = 0.001$), and EHSW ($p = 0.001$). **B.** Comparing electrode fouling responses to serotonin with 5-HIAA, using the Jackson waveform, CFME fouling is not significantly different ($p = 0.48$). However, the ESW ($p = 0.01$), EHSW ($p = 0.001$), and dopamine waveform ($p = 0.00001$) show significantly different CFME fouling responses with serotonin versus 5-HIAA. The 1 hour exposure to 1 μM serotonin purposefully fouls electrodes, and may not replicate responses *in vivo* where the electrode is only subjected to high concentrations over a short period of time, like in our optogenetic experiments. In Abdalla et al. 2017, they estimated the basal concentration of serotonin in mice brain tissue *in vivo* was approximately 60 nM,[5] so this concentration is substantially higher than that.



Supplementary Figure 6. Optogenetic stimulation waveform stability. A 5 minute wait period was applied between stimulations and six stimulations were applied. **A**. The Jackson waveform (green) had an average stimulation stability of 95 ± 1% (*n* = 4) and all trials were similar. **B**. The dopamine waveform (purple) had an average current of 104 ± 3% of the first stimulation, indicating stability. C. The ESW (red) had an average of 94 ± 2% normalized current and **D.** the EHSW (blue) had an average of 98.1 ± 1.9%. All waveforms remained above 90% stable after six repeated stimulations. **E**. Electrode response stability was determined by comparing normalized currents for the 1st (solid) and 6th stimulations (striped). There are no significant differences.

References

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(2) Norde, W. Driving Forces for Protein Adsorption at Solid Surfaces. *Macromol. Symp.* **1996**, *103*, 5–18. https://doi.org/10.1002/masy.19961030104.

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(5) Abdalla, A.; Atcherley, C. W.; Pathirathna, P.; Samaranayake, S.; Qiang, B.; Peña, E. A.; Morgan, S. L.; Heien, M. L.; Hashemi, P. In Vivo Ambient Serotonin Measurements at Carbon-Fiber Microelectrodes. *Anal. Chem.* **2017**, *89* (18), 9703–9711. https://doi.org/10.1021/acs.analchem.7b01257.