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Analysis of biogenic amines in a single *Drosophila* larva brain by capillary electrophoresis with fast-scan cyclic voltammetry detection

Huaifang Fang, **Trisha L. Vickrey**, and **B. Jill Venton***
Dept. of Chemistry, University of Virginia, Charlottesville, VA

Abstract

Drosophila, the fruit fly, is a common model organism in biology, however quantifying neurotransmitters in *Drosophila* is challenging because of the small size of the central nervous system (CNS). Here, we develop neurotransmitter quantification by capillary electrophoresis with fast-scan cyclic voltammetry detection, which allows peak identification by both migration time and the cyclic voltammogram, in contrast to traditional amperometric detection which provides no chemical identification. Tissue content of biogenic amine neurotransmitters was determined in a single CNS dissected from a *Drosophila* larva. Low detection limits, 1 nM for dopamine and serotonin, 2.5 nM for tyramine, and 4 nM for octopamine, were achieved using field-amplified sample stacking by diluting the homogenized tissue with percholoric acid and acetonitrile. Two different strains of wild-type flies, Oregon R and Canton S, have similar dopamine and serotonin levels but different octopamine content. When flies are fed NSD-1015, which inhibits dopamine decarboxylase (Ddc) a synthesis enzyme in the dopamine and serotonin pathways, dopamine significantly decreases by 52%. A genetically altered driver line, *Ddc-GAL*4, had lower serotonin and dopamine content as did w^{118} flies. When the Ddc-GAL4 line was used to produce flies overexpressing the serotonin synthesis enzyme tryptophan hydroxylase (*Ddc*-GAL4;UAS-*Trh*), serotonin tissue content was greater than for *Ddc-GAL*4, but not significantly different than wild-type. These results show that CE-FSCV is useful for monitoring the impact of genetic and pharmacological manipulations on the content of multiple neurotransmitters in a CNS from a *Drosophila* larva.

Drosophila melanogaster, the fruit fly, has been used for nearly 100 years as a model organism for understanding complex biological processes. Their small size and short life cycle (12–14 days from conception to sexual maturity) make *Drosophila* easy to rapidly breed in large quantities.[1] The *Drosophila* genome is fully mapped[2] and many genetic mutants are available. Adult flies are often used for behavioral assays while larvae are used in many fundamental neuroscience studies because the entire CNS can be removed and cultured.[3] *Drosophila* neurotransmitter signaling is largely homologous to mammals. Biogenic amine neurotransmitters such as dopamine and serotonin are present in *Drosophila* [4,5] and regulate similar functions as in mammals, including circadian rhythms, reward, learning and memory.[6 – 9] Differences do exist; for example, the phenolamines octopamine and tyramine in *Drosophila* play analogous roles to the adrenergic neurotransmitters norepinephrine and epinephrine in mammals.[10] Key molecules involved in synaptic transmission such as synthesis enzymes, receptors, and transporters are highly conserved between *Drosophila* and mammals.[5,11 – 13] Thus, *Drosophila* are a useful tool for probing common mechanisms of neurotransmission.

*corresponding author: PO Box 400319, Charlottesville, VA 22904 (434) 243-2132 jventon@virginia.edu.

Traditionally, biogenic amines in *Drosophila* brains have been studied using immunohistochemical methods, which facilitate visualization of neurons but are not highly quantitative.[3,14] Our lab has pioneered a method to implant a microelectrode in an intact *Drosophila* larval CNS to track dynamic changes in endogenous neurotransmitters using fast-scan cyclic voltammetry.[15 – 17] Separations-based methods can be used to determine *Drosophila* tissue content. Gas chromatography-mass spectrometry (GC-MS)[18] and high performance liquid chromatography with electrochemical detection (HPLC–EC)[19,20] are used to quantify biogenic amines in tissue from multiple samples. HPLC-EC has been used predominantly with adult flies[19,21] and whole bodies of larvae,[22 – 24] while only a single study has examined biogenic amine content in isolated larval brains.[20] More recently, capillary electrophoresis (CE) has been applied to separate the contents of individual fly samples.[25,26] With CE, highly efficient, rapid separations can be achieved with excellent mass detection limits and low sample volume requirements. Micellar electrokinetic chromatography, a separation mode of CE, with electrochemical detection has been employed to detect electroactive neurotransmitters, neuromodulators, and metabolites in head homogenates,[25,27,28] individual heads,[29] and individual brains[30] of the adult fly, but has not been used for larvae.

Amperometry is the predominant electrochemical method used for detection after separations because of its high sensitivity. Amperometry has very little chemical selectivity and many compounds can be detected at potentials sufficient to oxidize biogenic amines.[31] Therefore, to identify analyte peaks, the real sample must be compared to separations of standards run before and after because the migration may vary between runs as protein from the sample adsorb to the capillary.[25,27,29] Peaks co-eluting with unidentified analytes are also difficult to discern. Fast-scan cyclic voltammetry (FSCV) would be advantageous as a detector for CE because measurements are made every 100 ms and the cyclic voltammogram (CV) provides a chemical signature of the compound detected. [31] CE with FSCV detection has been used to detect metal ions[32,33] and antibiotics[34] in standard samples. However, CE-FSCV has not been used for biological samples, likely because detection limits are 100-fold less than with amperometry.[35] To circumvent higher limits of detection, sample enrichment can be used. Field-amplified sample injection (FASI) can provide up to 1000-fold sensitivity enhancement.[36]

This paper describes the first application of CE-FSCV with FASI for analyzing biogenic amine neurotransmitters in a real biological sample. Femtomole quantities of dopamine, serotonin, and octopamine are quantified in a CNS from a single *Drosophila* larva. The variation in neurotransmitter content in wild-type flies is determined as well as the effects of pharmacological and genetic manipulations of neurotransmitter synthesis. CE-FSCV is a useful tool for measuring and verifying the identity of biogenic amine neurotransmitters in *Drosophila* larva and could be applied in the future to other biological samples.

EXPERIMENTAL SECTION

Chemicals

Tyramine, octopamine, serotonin, dopamine were purchased from Sigma (St. Louis, MO) and 10 mM stock solutions prepared in 0.1 M perchloric acid. A modified Schneider's insect medium was used in *Drosophila* dissections: 15.2 mM $MgSO_4$, 21 mM KCl, 3.3 mM KH_2PO_4, 36 mM NaCl, 5.8 mM NaH_2PO_4, 5.4 mM $CaCl_2$, 11.1 mM glucose, and 5.3 mM trehalose, pH 6.2 (all from Fisher, Suwanee, GA).[15] The separation buffer was 200 mM NaH_2PO_4 with 1 mM tetraborate (pH 4.5). The detection cell buffer was 100 mM NaH_2PO_4 (pH 6.5). Buffer solutions for CE were filtered with a 0.2-μm nylon filter (Alltech, Deerfield, IL).

Fly Homogenate Preparation

Sample vials were made by rotating the end of a gel-loading pipette tip (i.d. 0.4 mm, o.d. 0.57 mm, Fisherbrand Premium Plus Gel-Loading Tips, Fisher) in the flame of a butane torch to seal the end of the tube. A new vial was used for each sample.

Ddc-GAL4, Canton-S, w^{118}, and Oregon-R fly stocks were supplied through the Bloomington, IN *Drosophila* Stock Center. *UAS-Trh* was provided by Amita Sehgal (UPenn).[37] To prepare the sample, the entire central nervous system (CNS) was removed from a 5-day old, 3rd instar *Drosophila* larva and placed in a plastic Petri dish containing 4 mL of modified Schneider's buffer, which can keep the CNS alive for about 2 hours.[15] The CNS was transferred to the sample vial by pipette, the buffer removed, and 2 μL of 5 mM perchloric acid with acetonitrile added. The sample vial was placed in an Eppendorf centrifuge (Brinkman Instruments, Westbury, NY), and spun at 14,760 g (11,000 rpm) for 5 min at room temperature. A metal wire (28 gauge, o.d. 0.325 mm) was used as a pestle to break up the CNS. The sample was centrifuged again at 14,760 g for 5 min. Electrokinetic injections were made by placing the end of the separation capillary into this vial.

For pharmacological experiments, NSD-1015 (Tocris, Ellisville, MO) was mixed with water and the *Drosophila* yeast food for a final concentration of 20 μM. Larvae were fed the drug-yeast mixture for two days prior to experimentation.[38]

Instrumentation and Data Analysis

The CE with end column fast-scan cyclic voltammetry was built in house. The separation capillary was 39–42 cm long (11 μm i.d., 148 μm o.d., Polymicro Technologies, Phoenix, AZ). The detection end of the capillary was etched by removing 1.5 cm of the polyimide coating to expose the fused silica, sealing the end in super glue, and placing the exposed portion of the capillary in 25% hydrogen fluoride (HF) for 3 hours. Sodium bicarbonate solution was used to neutralize the acid, the capillary washed with water, and the tip cut off to remove the super glue. The etched region was 20–30 μm o.d., while the inner diameter remained unchanged. The etched capillary was polished at a right angle on a polishing wheel (Sutter, Novoto, CA) and filled with separation buffer before use using a syringe. Between fly samples, the capillary was rinsed with separation buffer for 5 min.

Positive high voltage (15 kV) was applied at the injection end of the capillary through a platinum wire in the buffer reservoir using a DC power supply (Spellman, Plainview, NY). The detection end was grounded through stainless steel tubing attached to the detection cell, a Lucite block which was adapted from a flow gate injector (Supplemental Fig. 1).[39] The block was placed under a Stereomaster microscope (Fisher) and the end of the capillary positioned across from the microelectrode (distance of 15 μm±2). When the distance between the capillary and electrode was less than 10 μm, background noise from the electrophoretic voltage was high while the signal decreased for the distances larger than 20 μm. The position was adjusted according to the response of 50 nM standard sample every day. The working electrode was a 34.5 ± 2.5 μm diameter carbon-fiber disk microelectrode (Specialty Materials, Lowell, MA). A 250 μm diameter chloridized silver wire reference electrode was placed about 50 μm from the working electrode. A cross flow detection buffer flowed slowly (0.5 mL/min) between the other arms to flush the area around the electrode and prevent analytes from accumulating.

Detection was performed with fast-scan cyclic voltammetry with a two-electrode configuration using a Dagan ChemClamp potentiostat (Dagan, Minneapolis, MN with a custom-modified headstage). The data acquisition software and hardware were the same as described by Heien et al.[40] The electrode was scanned from −0.4 to 1.3 V and back at 400 V/s every 100 ms. The instrument was housed in a Faraday cage in order to minimize the

effects of external noise sources. Estimates of the concentration in fly samples were made from standard samples run before and after the fly sample and from internal standards used in some samples.

Statistics

Error bars are standard error of the mean (SEM). Statistics were performed in GraphPad Prism (La Jolla, CA). T-tests were used to compare means from 2 groups. One-way ANOVA was used to compare 3 or more groups.

Safety Considerations

HF is corrosive and can cause severe burns and depletion of calcium in the body if exposed to the skin. HF should only be handled in a hood and the experimenter should wear a face shield, lab coat, and double gloves as personal protective equipment. HF was immediately neutralized with sodium bicarbonate after use.

RESULTS AND DISCUSSION

Capillary electrophoresis with fast-scan cyclic voltammetry detection

Capillary electrophoresis (CE) allows rapid separations of small amounts and volumes and is therefore advantageous for use with biological samples such as *Drosophila*. In this study, dopamine, serotonin, octopamine and tyramine were targeted because they are the major biogenic amine neurotransmitters in flies.[5] Fast-scan cyclic voltammetry (FSCV) was chosen as the electrochemical detection method because it provides rapid temporal resolution and a CV to help identify the peaks. The main challenge of CE with electrochemical detection is decoupling the high separation voltage from the electrode response. End column detection was used and the end of the separation capillary was etched to permit ions to pass though small holes, thus decoupling the voltage.[41,42]

We chose to scan the electrode from −0.4 to 1.3 V at 400 V/s (10 Hz repetition rate) because this waveform allows high sensitivity detection with FSCV.[40] While other waveforms have been developed to combat fouling by oxidation products of serotonin,[43] octopamine, and tyramine,[44] the trade-off with all of the modified waveforms is reduced dopamine sensitivity. Fouling is expected to be minimal because the amount of biogenic amines in a single fly CNS is small, the 1.3 V switching potential renews the carbon electrode surface,[45] and the cross flow buffer in the detection cell flushes analytes away from the electrode. When concentrations of serotonin, octopamine, and tyramine were less than 200 nM, the sensitivity of the electrode was maintained.

To optimize separation conditions, the pH and borate concentration of the buffer were varied. Dopamine and octopamine are the most difficult to separate, as they have the same molecular weight. Figure 1A shows resolution improved when the buffer pH decreased from 6.5 to 4.5, where baseline separation for octopamine and dopamine was achieved in the electropherogram trace taken at 0.5 V. The inset cyclic voltammograms (CVs) help identify the analytes. While the CVs for octopamine and tyramine are similar, CE can clearly separate these compounds. The combination of migration time and CV allows identification of the analytes, an advantage over amperometry (Supplemental Fig. 2). To further improve the resolution, tetraborate was added to the separation buffer. Borate forms a complex with diols, such as dopamine, and slows the migration time by changing the mass and charge.[46] In Figure 1B, the migration time of dopamine increased with increasing borate concentration, while the migration time for the other analytes remained constant. Thus, 200 mM phosphate buffer, pH 4.5, with 1 mM tetraborate was chosen for the separations.

Field amplified sample stacking

Field amplified sample injection (FASI) is employed in electrophoresis to increase analyte loading onto a capillary.[36] The sample is diluted in a low conductivity matrix so the high electric field in the sample during electrokinetic injections causes analytes to rapidly migrate toward and stack in a narrow zone at the beginning of the capillary. FASI results in low detection limits and narrow peaks. Some dilution is necessary when working with small volume samples, such as the 8 nL *Drosophila* CNS. By employing FASI, the dilution media can be adjusted to stabilize the analytes as well as optimize detection limits.

Biogenic amines quickly air oxidize, so samples are usually kept in acid to prevent degradation.[47] We found that 5 mM perchloric acid stabilized the sample so that it could be stored for at least one day at room temperature. Samples were also diluted with mixtures of perchloric acid and acetonitrile, a good solvent for sample stacking.[36,48] Figure 2 shows that detection was most sensitive when the sample matrix was 30% acetonitrile:70% 5 mM $HClO_4$. Higher concentrations of acetonitrile occasionally caused air bubble formation and separation failure. For comparison, analytes were also diluted in the separation buffer (Fig. 2) but the limits of detection (LOD) were only 50 nM for dopamine and serotonin and 100 nM for octopamine and tyramine (S/N=3), insufficient for detection of analytes in a single larva. Diluting with perchloric acid and acetonitrile results in LODs of about 1 nM for dopamine and serotonin, 2.5 nM for tyramine, and 4 nM for octopamine (S/N=3).

Quantification of biogenic amines in a single *Drosophila* larval CNS

Drosophila go through three larval stages (1^{st}, 2^{nd} and 3^{rd} instar) before they undergo pupation and become adults. Third instar larva have fully developed neurotransmitter systems [3,49] and whole brain culture techniques for these larvae facilitate studies on live tissue, including electrochemical measurements of endogenous neurotransmitter release.[15,17] Detection of tissue content in a single fly larval CNS has not been previously reported and is challenging because the sample volume is less than 8 nL and the amount of each neurotransmitter is less than a pmol. Samples were prepared in a sample vial made from a sealed, gel-loading pipette tip (Supplemental Fig. 3). A single CNS was placed in the vial, diluted with perchloric acid:acetonitrile matrix, homogenized, and then centrifuged. Since the sample preparation and injection were performed in the same vial, sample loss was avoided due to transfer. To ensure that the tissue was properly homogenized, some samples were also sonicated in a bath sonicator for 30 min. Tissue content was similar with and without sonication, so this step was not used for future samples. The overall sample preparation is simple, requires only one vial, and takes only 12 min.

Figure 3 compares the separation and identification of neurotransmitters in a standard sample and in a single Canton S larval CNS, a common wild-type fly strain. The analyses were run sequentially on the same capillary under the same conditions. The top panels show the entire electropherogram trace at 0.5 V. The 4 neurotransmitters of interest migrate fastest because they are positively charged. As expected, the *Drosophila* sample is more complex and contains more peaks, including several unidentified compounds, but the targeted neurotransmitters were clearly separated. Future studies could focus on identifying and separating more peaks, perhaps employing micellar electrokinetic chromatography.

The bottom panels of Figure 3 show enlargements of the area of interest for the electropherogram, along with the color plot and cyclic voltammograms for each peak. The color plots are more useful for visualizing the separation than the electropherogram trace at one voltage because the compounds have different peak potentials. The different shapes and potentials of the compounds also aid in their identification. The CVs and color plots for the larva sample (Fig. 3B) look nearly identical to those for the standard sample (Fig. 3A) and

the migration times are also consistent. The voltammetry in conjunction with the separation confirms the identity of the compounds detected. Peak heights from the standards analyzed before the *Drosophila* sample were used to quantify the amount of neurotransmitter present. For the sample in Figure 3B, there was 30 fmol of tyramine, 100 fmol of serotonin, 220 fmol of octopamine and 130 fmol of dopamine.

To study variability between animals, tissue content was determined for individual larva from two wild-type strains, Oregon R and Canton S. Dopamine, serotonin, and octopamine were detected in all samples. Tyramine was detected only in some samples, likely because the concentration is small and near our limit of quantitation, which is 8 nM (or 17 fmol/CNS, S/N=10) for tyramine. The scatter plots in Figure 4 show the variability in neurotransmitter content. Octopamine content was significantly different between Oregon R and Canton S flies (t-test, $p<0.05$), demonstrating that different strains can have different levels of neurotransmitters. The relative standard deviation (RSD) for each neurotransmitter in a single strain ranges from 24 to 34 %. In comparison, the RSD is around 3% when the same concentration standard sample is analyzed multiple times, so the variance is due to differences in the samples and not the method. While the amount of amines in a single CNS is variable, the average content for dopamine and serotonin are the same order of magnitude as a previous study in which 25 pooled Canton S CNSs were analyzed (Table 1).[20] Table 1 also compares the values determined in adult brains, which are similar to the larva results. We expect our method could be extended to detect monoamine content in single adult brains as well.

Effects of manipulating dopamine and serotonin synthesis on tissue content

Drosophila are useful for pharmacology and genetic experiments to probe neuronal function. First, we investigated pharmacological manipulation of neurotransmitter synthesis. Dopamine decarboxylase (Ddc, also known as amino acid decarboxylase) catalyzes the last step of dopamine synthesis, L-dopa to dopamine, and the last step of serotonin synthesis, L-tryptophan to serotonin. Figure 5 shows that serotonin content decreased 25% after chronic NSD-1015 administration ($p=0.11$) while dopamine decreased significantly by 52% ($p<0.01$). The percentage decrease of dopamine is similar to the decrease in stimulated dopamine release (47%) after chronic NSD-1015 administration.[17] Because chronic administration of a drug can lead to compensations, such as changes in autoreceptor or transporter expression, stimulated release might not always follow tissue content. The CE studies measure both intracellular and extracellular dopamine, because the tissue is homogenized, while electrochemistry measures releasable dopamine. The agreement between the two measurements shows that stimulated release was proportional to the total amount of dopamine. Serotonin was not significantly decreased, even though Ddc is involved in serotonin synthesis, which could indicate that Ddc inhibition has a greater effect on dopamine synthesis. The ability to detect changes in neurotransmitters after pharmacological agents allows a better understanding of how chronic drug administration affects neurotransmission.

As a second biological test, flies with genetic mutations were investigated. The UAS (upstream activating sequence)-GAL4 expression system is commonly used in *Drosophila* for expression of genes in specific cells.[50] When GAL4 is produced in a cell, it binds to the UAS and activates transcription of the inserted gene. Because the GAL4-UAS system is derived from yeast, expression of GAL4 in *Drosophila* is thought not to affect cells if UAS is not present. We studied *Ddc-GAL4* flies that produce GAL4 in cells expressing dopamine decarboxylase.[51] *Ddc-GAL4* flies were then crossed to *UAS-Trh* flies, where Trh is the serotonin synthesis enzyme tryptophan hydroxylase, to create *Ddc-GAL4;UAS-Trh* flies (written as *Ddc-Trh* in shorthand). The *Ddc-Trh* flies were expected to express more tryptophan hydroxylase and thus have higher serotonin contents. As a control, w^{118} flies

were also examined, as they are the background strain for the Ddc-GAL4 flies. These flies lack a transporter that normally transports pigment to the eyes, so their eyes are white. Lack of this transporter has also been reported to affect histamine, dopamine, and other neurotransmitter levels, although not all studies see a definitive effect.[8,19,30,52]

Dopamine and serotonin levels were significantly different in w^{118} flies than Canton S wild type flies (Fig. 5, t-test, $p<0.05$). This decrease in synthesis could be due to the lack of transporter and reduced expression of dopamine has previously been observed in w^{118} flies.[30] Ddc-GAL4 flies had slightly lower serotonin and dopamine contents than w^{118}, although the effect was not significant. Therefore, the driver line (Ddc-GAL4) and background strain (w^{118}) have lower serotonin and dopamine contents than Canton S wild-type. Homozygotes with 2 copies of the *Ddc-GAL*4 reportedly have lower lifespans, an effect postulated to be due to the death of dopamine neurons.[50] Our *Ddc-GAL*4 flies are smaller and darker than wild-type flies, another indication of adverse effects. Understanding the neurochemistry of *Ddc-GAL*4 is critical because these flies have been used to create Parkinson disease models by crossing to other lines with genes linked to a UAS.[53,54] Because deficits in the dopamine system are already present, the effects of the mutations would need to be judged with the *Ddc-GAL*4 fly as a baseline, not wild-types.

The *Ddc-Trh* flies should express more tryptophan hydroxylase and therefore have increased serotonin content. However, serotonin content is not different than Canton S wild-type flies, but is significantly greater in *Ddc-Trh* than *Ddc-GAL*4 flies (1-way ANOVA with Bonferonni post-test, Fig. 5). Because *Ddc-GAL*4 flies have lower levels of serotonin, the extra expression of Trh only brings levels back to normal. Dopamine levels in *Ddc-Trh* were not significantly different than *Ddc-GAL*4 flies, which serves as a control to demonstrate that extra Trh expression did not change all neurotransmitters. These studies provide important information to biologists that *Ddc-Trh* flies might not exhibit phenotypes consistent with higher serotonin levels because serotonin is not different than wild-type flies. The w^{118} flies with the white eyes and the *Ddc-GAL4* driver lines had impaired monoamine content and effects of additional synthesis in *Ddc-Trh* flies must be compared to these flies and not Canton S. Thus, understanding the effect of the genetic mutations on tissue content provides valuable knowledge for predicting biological effects.

CONCLUSIONS

Capillary electrophoresis with fast-scan cyclic voltammetry detection can be used to determine the content of biogenic amines in a single larval *Drosophila* CNS. The advantage of FSCV is that peaks can be identified using the cyclic voltammogram in addition to migration times. Field-amplified sample stacking leads to higher amounts of analyte injected and low limits of detection. CE-FSCV was used to show that pharmacological and genetic munipulations of synthesis affect tissue content and that w^{118} and the drive line *Ddc-GAL*4 have decreased neurotransmitter levels. Therefore, this tool should be useful for studying changes in neurotransmitter levels and interactions between neurotransmitters in the *Drosophila* larval nervous system. CE-FSCV could also be used to detect low amounts of monoamine neurotransmitters in other tissue or microdialysis samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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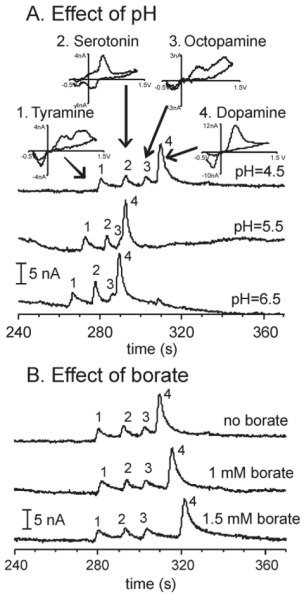

Figure 1.

Optimization of separation. A. Effect of varying the pH from 4.5 to 6.5 (no borate). B. Effect of adding tetraborate to the buffer (pH 4.5). The labeled peaks are 1. 50 nM tyramine, 2. 25 nM serotonin, 3. 50 nM octopamine, and 4. 50 nM dopamine. The inset cyclic voltammograms verify the peaks being detected. Separation conditions: 39 cm capillary (i.d. 11 μm); electrokinetic injection 5 kV for 15 s; detection waveform: −0.4 to 1.3 V, 400 V/s, 10 Hz.

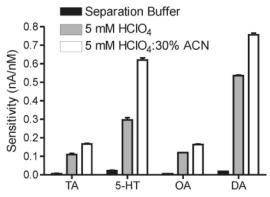

Figure 2.

Effect of sample stacking. The sensitivity (nA/nM) for detection is shown for three conditions: analytes dissolved in separation buffer, 5 mM $HClO_4$, and a 30% v:v solution of acetonitrile: 5 mM $HClO_4$.

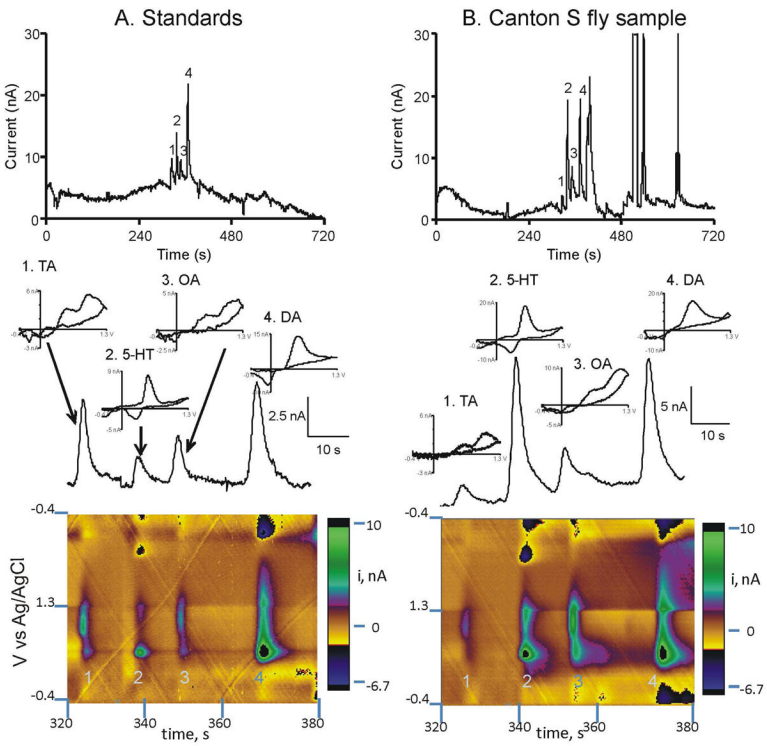

Figure 3.

Example separations. A. A separation of a standard sample (50 nM tyramine, octopamine, dopamine and 25 nM serotonin). B. A separation of a single Canton S fly larval CNS in 2 μL 5 mM $HClO_4$ with 25% acetontrile, performed sequentially to the standard sample on the same capillary. Separation conditions are the same as Fig. 1 except capillary length: 42 cm, separation buffer: 200 mM phosphate, 1 mM borate, pH 4.5. The top shows the entire electropherogram (taken at 0.5 V). The bottom shows a close-up of the region of interest. The color plot allows shows all the data collected, with voltage on the y-axis, time on the x-axis, and current in pseudocolor. Using the peak heights from the standard sample, the calculated concentration of the analytes in the fly sample is 16 nM tyramine, 51 nM serotonin, 111 nM octopamine, and 64 nM dopamine.

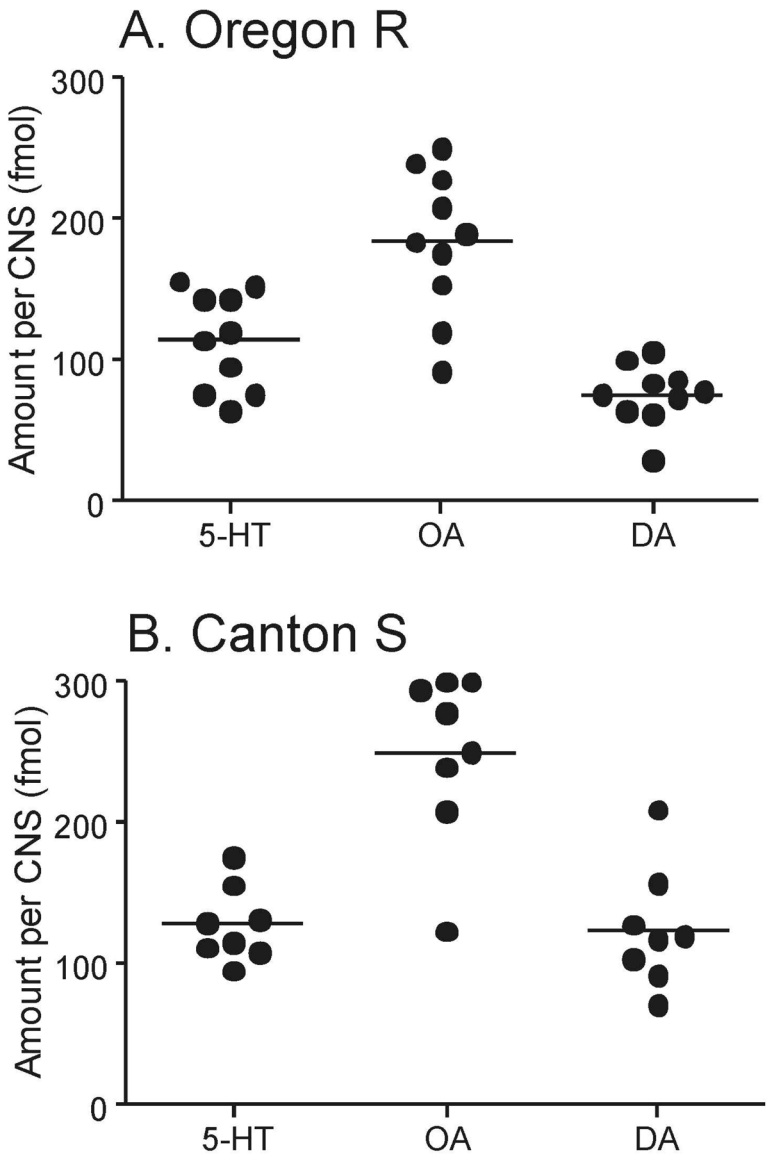

Figure 4.

Variation of biogenic amines among individual flies. A. Oregon R and B. Canton S. Each point represents one sample and the line indicates the mean. Values are shown for serotonin (5-HT), octopamine (OA), and dopamine (DA). t-tests show a difference in octopamine content between strains ($p<0.05$) but no differences for dopamine or serotonin.

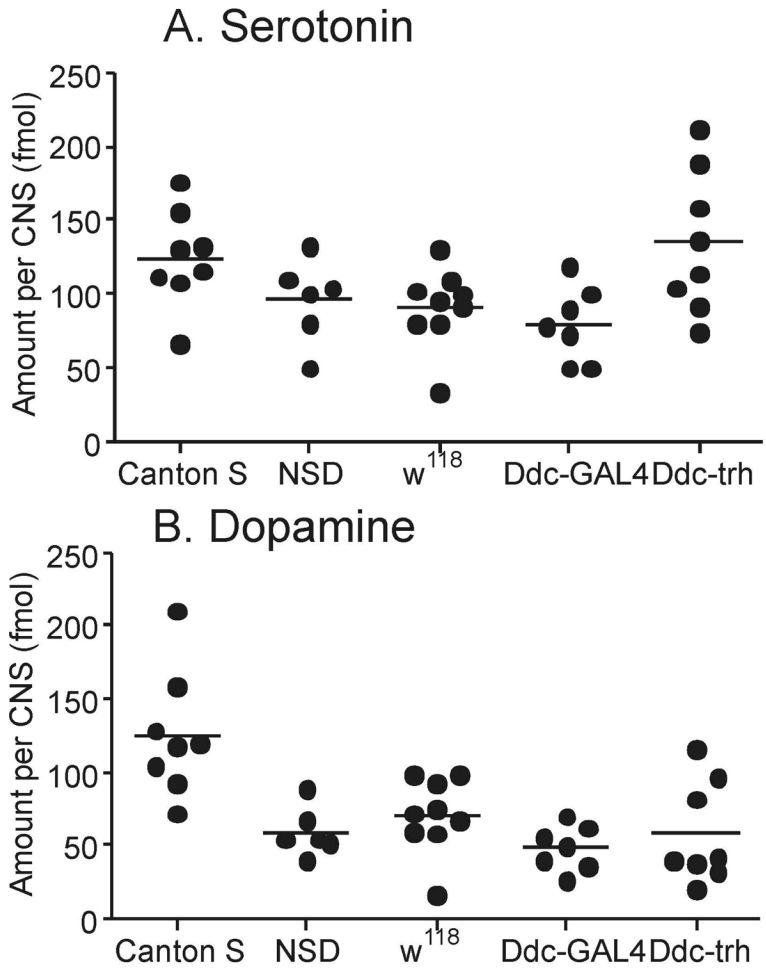

Figure 5.

Effect of pharmacological and genetic manipulations on neurotransmitter levels. Differences are compared for A. serotonin and B. dopamine. The groups are Canton S wild-type, Canton S fed for 2 days with NSD-1015 (a synthesis inhibitor), w^{118} white-eyed background strain, *Ddc-GAL4*, and *Ddc-GAL4;UAS-Trh* (abbreviated *Ddc-Trh*), which overexpress the serotonin synthesis enzyme tryptophan hydroxylase. T-tests to compare the Canton S and NSD 1015 groups show that dopamine is significantly decreased ($p<0.05$) but not serotonin. A one-way ANOVA of the Canton S, w^{118}, *Ddc-GAL4*, and *Ddc-Trh* flies shows a significant main effect of genotype ($p=0.011$ for serotonin, $p=0.0002$ for dopamine). Bonferonni post-tests indicate that *Ddc-GAL4* and w^{118} levels are lower than Canton S for dopamine ($p<0.01$) and *Ddc-GAL4* is lower than Canton S for dopamine ($p<0.05$). For serotonin, *Ddc-Trh* flies are significantly larger than *Ddc-GAL4* ($p<0.05$) but not different than Canton S. For dopamine, *Ddc-Trh* levels are significantly different from Canton S ($p<0.05$).

Table 1

Reported biogenic amine levels (fmol/brain) in larval or adult Drosophila brains

	Fly strain	Octopamine	Dopamine	Serotonin	Samples pooled	Ref.
Larval brain	Canton S	250±20	125±15	132±9	1	this work
	Canton S		74±2	70±1	25	20
	Oregon R	180±16	76±7	114±11	1	this work
	w^{118}	165±24	70±9	91±9	1	this work
Adult brain	Canton S	450±27	118±13	159±6	10	8
	Canton S	1720	527	376		55
	Canton S	780±77	91±5		3	30
	Canton S	500±94	70±13		1	30
	Oregon R		485±12		10	21
	w^{118}	460±98	137±30	160±35	10	8
	w^{118}	540±18	340±15	223±7	10	19
	w^{118}	700±140	63±8		3	30

Errors are SEM.

Blanks indicate that the data was not reported in that paper.