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Biogenic Amines in Microdissected Brain Regions of *Drosophila melanogaster* Measured with Micellar Electrokinetic Capillary Chromatography – Electrochemical Detection

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Abstract

Micellar electrokinetic chromatography with electrochemical detection has been used to quantify biogenic amines in microdissected *Drosophila melanogaster* brains and brain regions. The effects of pigment from the relatively large fly eyes on the separation have been examined to find that the red pigment from the compound eye masks much of the signal from biogenic amines. The brains of *white* mutant flies, which have characteristically low pigment in the eyes, have a significantly simplified separation profile in comparison to the red-eyed, wild-type, Canton S fly. Yet, the *white* mutant flies were found to have significantly less amounts of dopamine, *l*-3,4-dihydroxyphenylalanine (L-DOPA), salsolinol, and N-acetyltyramine in their dissected brains when compared to dissected brains of Canton S flies. In addition, significant variation has been observed in the dissected brains between individual flies that might be related to changes in neurotransmitter turnover. The transgenic GFP fly line (TH-GFP), for which the overall profile of biogenic amines is not found to be significantly different from Canton S, can be used to visualize the location of dopamine neurons. Biogenic amines were then quantified in three brain regions observed to have dopamine levels, the central brain, optic lobes, and posterior superior medial protocerebrum (PPM1) region.

INTRODUCTION

Drosophila melanogaster has been a broadly used model organism due to many of its inherent features. *Drosophila*'s small size (<0.5 cm) and short reproduction life cycle (12–14 days from conception to sexual maturity) have made it feasible to keep large quantities of genetically homogenous samples available to study. Fewer genes and fewer functional redundancies have facilitated the identification of individual genes^{1, 2} and ultimately produced a vast number of genetic mutants available to help investigate the physiological mechanisms underlying behavior.³ Pigmentation mutations result in easily identifiable phenotypes and are the oldest identified genetic mutation to be isolated in *Drosophila*, specifically the *white* mutant.⁴ In *white* mutants, the ATP-binding cassette (ABC) transporter, which transports guanine and tryptophan precursors to the red (drospterin) and brown (ommochrome) screening pigments into fly larvae eyes, is not properly expressed and thus prevents the formation of eye pigments.^{5, 6} As such they suffer from defective vision stemming from their inability to screen bright lights, difficulties in movement,^{7, 8} and

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deficiencies in learning.⁹ Care must be taken to compare them to their wild-type counterparts.

Although *Drosophila* has a less complex nature, due to its smaller genome and simpler nervous system of approximately 200 000 neurons, it can exhibit many of the same high-ordered behavioral and molecular functions found in vertebrates.^{2, 10–14} Biogenic amines such as dopamine, serotonin, tyramine, and octopamine are known mediators of diverse physiological and behavioral functions within the fly.¹⁵ Despite the important role these molecules play within the nervous system, the underlying neuronal mechanisms are still not completely understood.

Traditionally, detection of biogenic amines within the *Drosophila* brain has been studied using immunohistochemical,^{16–18} histofluorescence,¹⁹ and pharmacological²⁰ methods. These methods are invaluable tools for visualization of neurons containing biogenic amines;^{21, 22} however, they are ineffective in quantifying them. More recently, small-volume methods have been developed for analysis in *Drosophila*. These include dynamic methods, especially voltammetry in *Drosophila* larvae^{23–25} and adult flies,^{26, 27} and several small-volume separations-based techniques. Early separation experiments to quantify biogenic amines in *Drosophila* heads utilized gas chromatography-mass spectrometry²⁸ and high performance liquid chromatography (HPLC) with electrochemical (EC)^{29–32} detection, but these studies utilized complex extraction and derivatization protocols or could only detect a few biogenic amines, mainly dopamine. Recent HPLC-EC methods have improved upon the separation methodology to increase the number of quantifiable biogenic amines and have used dissected fly brains to reduce the number of interfering electroactive compounds.^{33–37} Micellar electrokinetic chromatography (MEKC) is a mode of capillary electrophoresis (CE) where surfactant exceeding the critical micelle concentration is present in the running buffer. The high-resolution capabilities and low mass detection limits of MEKC have been used to detect biogenic amines and their modulators in both head homogenates^{38–40} and individual heads of the fly.⁴¹

In this paper, we report the use of MEKC to investigate the levels of biogenic amines and their metabolites present in dissected whole brains and specific *Drosophila* brain regions. The analytical challenge here has been to develop the protocols to look at even smaller tissues from a complex brain substrate. Furthermore, different fly strains, wild-type (Canton S), *white* mutant, and GFP-TH mutant, have been used to investigate the effects fly-eye pigment has on these separations and analyze specific regions within the fly brain. Significant differences are observable between these different fly types and some brain regions.

EXPERIMENTAL SECTION

Chemicals

All chemicals were used as received and purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. Perchloric acid was obtained from Honeywell Riedel-de Haën (Seelze, Germany), while N-acetyldopamine, N-acetyloctopamine, and N-acetyltyramine were obtained from The National Institutes of Mental Health (NIMH) chemical synthesis and drug supply program (Research Triangle Park, NC, USA). Separations were performed in 25 mM borate buffer containing 50 mM SDS and 2% 1-propanol at pH = 9.66. All standards were prepared as 10 mM stock solutions in 0.1 M perchloric acid and were diluted to the desired concentration with additional 0.1 M perchloric acid.

Drosophila Strains and Preparation

Three strains of *Drosophila melanogaster*, wild-type Canton S, *white* mutant, and TH-GFP mutant, were used in this study. Canton S flies have red-pigmented eyes, while the *white* mutant strain contains a mutated copy of the *w* gene affording unpigmented eyes. TH-GFP flies are a transgenic strain that carry tyrosine hydroxylase (TH)-GAL4 and UAS-mCD::GFP (membrane tethered green fluorescent protein) which is used to visualize dopamine neurons.^{3, 18} Images were acquired using a stereomicroscope (Olympus, SZX10, Hamburg, Germany) and an Olympus DP71 camera. All flies were cultured on standard potato meal/agar medium at 25 °C and collected 4–5 days after emerging from pupal cages.

Two types of fly samples were prepared, head homogenates and dissected brains (see below). Collection of all fly samples began by briefly immobilizing male flies on ice for 10 s, dipping them in a bath of cold 95% ethanol for 30 s to remove their waxy coating, and placing them in a petri dish containing 0.1 M perchloric acid on ice. Fly heads were removed from their bodies using small forceps (Fine Science Tools, Heidelberg, Germany) under an Olympus SZ40 stereoscope.

Homogenates were prepared by collecting three heads and homogenizing in 1.0 µL of 0.1 M perchloric acid/100 µM catecholamine (internal standard). The homogenized heads were centrifuged using an Eppendorf centrifuge with a fixed angle rotor (Eppendorf, Hamburg, Germany) and spun at 14,000 rpm for 5 min at 0 °C. The supernatant was collected and centrifuged (2 h, 14,000 rpm, 0 °C) through a 3-kDa centrifuge filter (VWR International, LLC, West Chester, PA, USA). The filtrates were then immediately injected into the capillary.

Fly brains were dissected from their heads using small forceps under an Olympus SZ40 stereoscope. To dissect a fly, it was held at the belly facing up with one pair of forceps, while a second pair pinched at the neck to separate the head from the body. The proboscis was removed by pulling it down and away from the head to create a cavity in the lower region of the head. Holding onto each side of the cavity, forceps were gently pulled away from one another until the cuticle was broken into two pieces making sure the brain stayed intact. The brain was removed from the pieces of cuticle, eliminating any remnants of the air sacs, compound eye, or cuticle debris. Brain region samples were collected by further dissecting a TH-GFP brain under fluorescent light to visualize the central brain, optic lobes, and PPM1 regions. All individual dissections were performed in less than 5 min to prevent the fly from being dead for too long as analyte levels may change due to the stress placed upon the fly. A discussion of fly dissections has been described in Nature Protocols.⁴²

Dissected brains or brain regions were collected in 200-µL PCR tubes (VWR International, West Chester, PA, USA) with 1.0 µL of 0.1 M perchloric acid/25–100 µM catecholamine (internal standard) and centrifuged (VWR International, Galaxy Mini, West Chester, PA, USA) at 6000 RPM for 30 s. The heads were then ultrasonicated (Sonics & Materials, Inc, Satigny, Switzerland) with a 2 mm stepped microtip at 100% amplitude for 30 s in a cold-water bath. The sample was centrifuged for 30 s for collection and promptly injected onto the capillary.

Separation Instrumentation and Analysis

The CE system with end-column amperometric detection utilized in this study was built in-house and has been described previously.^{43, 44} A 45-cm length of fused-silica capillary with an outer diameter of 151 µm and inner diameter of 14 µm (Polymicro Technologies, Phoenix, AZ, USA) was used for the separations. Amperometric EC detection was carried out in a two-electrode format in which a 5-µm carbon fiber microelectrode was held +0.75 V versus a Ag/AgCl reference electrode (Harvard Apparatus, Holliston, MA, USA). Carbon-

fiber microelectrodes were fabricated by aspirating a 5 μm diameter carbon fiber (Amoco, Greenville, SC, USA) into a borosilicate glass capillary (B120-69-10, Sutter Instruments, Novato, CA, USA). The glass capillary was then pulled to a narrow tip with a glass-capillary puller (Narishige, PE-21, Tokyo, Japan) and epoxy (Epo-Tek, Epoxy Technology, Billerica, MA, USA) was placed at the carbon-fiber/glass junction and allowed to cure. The carbon fiber was cut to a length of 400–500 μm from the glass junction to form a cylindrical electrode. Electrical contact was made by back-filling the capillary with 3.0 M KCl and inserting a Ni:Cr wire. Current was measured using a Keithly model 428 current amplifier (Cleveland, OH, USA) and recorded at 10 Hz using a LabView 8.0 (National Instruments, Austin, TX, USA) interface, written in-house. Microsoft Excel (Redmond, WA, USA) was used to generate the electropherograms and statistics. Data analysis was performed using Peakfit Separation and Analysis Software v4.11 (SPSS Inc., Chicago, IL, USA).

Buffer solutions were filtered through a 0.2- μm nylon filter (Alltech, Deerfield, IL, USA) prior to use. Capillaries were filled with separation buffer using a stainless steel reservoir with applied N_2 pressure (3.5 MPa) and washed between changes in buffer with deionized water (18.0 M Ω) at 15 kV for ~15 min. Injections were then performed electrokinetically at 5 kV for 5 s to extract ~1 nL of homogenate from the homogenate. To enhance microelectrode placement, the capillary inner diameter was enlarged via HF etching.⁴⁴ Approximately 2 mm of the polyimide coating was burned off from the capillary to expose the fused silica. The exposed portion of the capillary then was placed in HF for 15 min with a pressure of 3.5 MPa (N_2) through the capillary. Subsequently, the same segment of capillary was placed in a sodium bicarbonate solution to neutralize the acid and then washed with water to remove any excess salt.

Safety Considerations

An in-house-built safety interlock box was utilized to protect the user from high voltage. Since HF could cause severe burns, it was used with extreme care in a fume hood with proper precautions taken.

RESULTS AND DISCUSSION

MEKC Separations of Canton S and *white* Mutant *Drosophila* Heads

Electrochemical detection methods offer a powerful tool for the determination of biogenic amines within biological samples owing to their high specificity and sensitivity. However when attempting to analyze biogenic amines within the heads of *Drosophila melanogaster*, an abundance of electroactive species from the eyes, particularly the pigment within the eye (Figure 1A), can overwhelm analyses. When homogenized wild-type Canton S fly heads are separated using MEKC, a large separation profile of variable intensities is produced (Figure 1B). As neuromodulators are cellularly regulated and are present in low amounts, they produce low (few picoampere) signals. Other signals can be over three orders of magnitude larger, with the peaks at 550 and 1150 s having heights over 10 000 pA, and can end up hindering quantification of less abundant signals with their wide elution times. Under these conditions, the internal standard catechol must be used at a higher concentration (100 μM) otherwise; it can become obscured by other peaks in the separation. To help reduce the effects of the high-concentration species and to investigate the effect eye pigment has upon the separation, homogenized *white* mutant fly heads have been examined (Figure 1C) resulting in a vastly different separation. Approximately 90% of the highly expressed and concentrated peaks observed from the Canton S flies are not observed in the *white* mutant flies producing mostly baseline-resolved peaks of the lower concentration species. This indicates that these abundant electroactive species result from species related to the red eye

pigment of the fly. With the removal of these peaks, the internal standard is now easily identified in the separation and can be reduced to a more appropriate level (25 μ M).

MEKC Separations of Three Types of *Drosophila* Brains

Homogenization of fly brains can be extremely challenging. In contrast to the hard exterior cuticle of the fly head, the fly brain is small and pliant. Additionally, commercially available homogenizers or home-made, miniature, tissue homogenizers⁴¹ are too large and bulky to break apart fly brains making it difficult to obtain a homogeneous sample. Thus, tissue disruption and homogenization is achieved by use of ultrasonication in the work presented here. The 2-mm ultrasonication probe is small enough to reach the solution at the bottom of a 200- μ L centrifuge tube. Care is taken to avoid contact with the bottom of the tube with the tip of the sonicator and to carry out sonication in a cold-water bath, to avoid burning a hole in the sample tube. Sonication appears to yield an efficient and systematic method to obtain a uniform sample with no solid cell material visible.

To investigate the biogenic amine differences between *Drosophila* mutants and Canton S, three types of fly brains, wild-type, *white mutant*, and TH-GFP mutant, have been dissected, homogenized, and separated (Figure 2). Figure 2 shows the separations of typical *Drosophila* homogenates of three brains for each type of fly. In previous works, homogenates of 50 heads or more were commonly used to analyze the entire fly head.^{38–41} Dissection of that many fly brains is too time consuming creating reproducibility and tissue degradation issues from air oxidation and diffusion. It was possible to reproducibly dissect three brains, which was strategically used here to minimize variation between individual flies.

The separation profiles of all three types of the fly brains shown in Figure 2 most closely resemble the separation profile for the *white mutant* homogenized whole heads (Figure 1C inset). The large, overloading peaks, apparently from the eyes, have been eliminated in the wild-type samples, while preserving peaks associated with neuromodulators. A similar effect was also observed with the TH-GFP samples (data not shown). Two immediate differences can be seen between all three dissected samples and the *white mutant* heads. The large peak at elution time of 800 s seems to be reduced in the dissected brain samples. More significantly, many of the peaks corresponding to the neuromodulators in the dissected samples have larger peak areas. This is likely a consequence of the faster preparation time and lack of use of a centrifugal filter when preparing the dissected samples. Shorter preparation times help to prevent air oxidation, and analytes might adsorb to the filter excluding them from quantification.

While *white mutant* samples help to show that the eye pigment from Canton S flies produces much of the electroactive species seen in these separations, they result in some biological ramifications. As discussed earlier due to their inability to produce screening pigments in their eyes, *white* mutants experience defective vision when in bright lights. It has also been reported that they suffer from difficulties in movement,^{7, 8} deficiencies in learning, and reduced amounts of histamine, dopamine, and serotonin.³⁷ As such, it is important to quantify and compare these and perhaps other neuromodulators in this mutant species relative to the wild-type species (Canton S).

Quantitative information has been acquired from three independent preparations of the wild-type, *white mutant*, and TH-GFP mutant dissected brains. Neuromodulators dopamine, salsoinol, N-acetyldopamine, L-DOPA, octopamine, N-acetyltyramine, N-acetylserotonin have been identified by correlating migration times to a 25- μ M standard separation run directly after the brain homogenate sample. The average value and standard error of the mean (SEM) for each of these analytes is reported by comparison to the same 25- μ M

standard separation run directly after the brain homogenate and to a 25- μ M internal standard of catechol (Table 1).

The small (femtomole) amounts of dopamine, salsolinol, N-acetyltyramine, N-acetylserotonin, and L-DOPA detectable in the *white* mutant all appear to be reduced when compared to wild-type and TH-GFP. Statistical analysis with a two-tailed T-test with equal variances revealed that the *white* mutant is significantly lower in dopamine, salsolinol, and N-acetyltyramine ($p < 0.05$) and L-DOPA ($p < 0.10$) relative to Canton S flies. When compared to the TH-GFP flies, the *white* mutant was significantly lower in salsolinol, N-acetyltyramine, and N-acetylserotonin ($p < 0.05$) and L-DOPA ($p < 0.10$). It is also interesting to note the differences present in the three fly types between dopamine, L-DOPA, salsolinol, and N-acetyldopamine. The level of dopamine in the *white* mutant was found to be significantly lower than in the Canton S and TH-GFP flies. As L-DOPA is a precursor and salsolinol is a metabolite to dopamine, it is not surprising that they too are lower in the *white* mutant than in the other two flies. Also in contrast to previous reports,³⁷ we find that octopamine is not different between wild-type and *white* mutant flies, although dopamine is lower in the *white* mutant fly as previously reported.³⁷ This could be due to differences in the separation methodology used or possibly the use of dissected fly brains here versus homogenized whole heads.

Variability of Biogenic Amines Among Single *Drosophila* Brains

As previously reported, small sample volumes can be used with capillary electrophoresis making it possible to analyze and study individual fly heads.⁴¹ Analysis of the dissected brain adds a new facet to such analyses, as it provides sample clean up prior to separation by removing many unidentified species that interfere with the separation and detection of biogenic amines and their metabolites. To determine the sensitivity of the MEKC-EC system for single dissected drosophila brains, the biogenic amine and metabolite levels have been calculated for five individual wild-type dissected brains. Individual brains were dissected and ultrasonicated in 1.0 μ L of solution containing internal standard, instead of the previously reported 250 nL.⁴¹ The sample volume was increased to the minimum volume that could consistently be used to homogenize fly brains using the 2-mm ultrasonication probe.

Figure 3 shows the electropherograms and calculated numeric values for the average amount of analyte detected for each of the five single-fly brain homogenates. The overall separation profile of a single fly-brain homogenate remains consistent between samples with well-defined, baseline-resolved peaks. Migration times correlate well with little variation between runs, particularly in the beginning of the separations. However, some variation in peak intensities of the more intense peaks is observed between the flies, highlighting the potential patterns that may be discerned from analyzing individual animals. Of note are the peaks at 550 s, 700 s, and 1050 s. The peak at 500 s is considerably larger for the first two flies examined vs. the last three, whereas the peak at 700 s is considerably smaller for the first two flies examined vs. the last three. While these two peaks might suggest some co-variation between the five flies, the intensity of the third peak at 1050 s follows a completely different pattern from that of the previous two peaks. Other peaks are also present and show similar features with no covariance between flies. We are working to identify these substances; however, the pattern is the important feature in the data here, not the absolute chemical identity.

Sample degradation or column changes might be an issue in quantification. To examine this, samples for flies 1–3 were run in duplicate (not shown), and absolute areas for every peak in each of the second runs were found to significantly decrease from the first runs despite storage of samples at -70°C between runs. This decrease might be attributed to the

combination of small sample size (1 μ L) and loss due to air oxidation or from transfer during the first injection. Some peaks (~15–20% per sample) were decreased enough that they were no longer discernable from baseline, but relative areas between peaks and the internal standard catechol were found to not significantly vary.

The biogenic amines quantified in Table 1 were also measured for these single brain analyses (Figure 3B). The average analyte values measured for the five dissected single brains were found not to vary significantly from the Canton-S three-brain homogenates reported in Table 1. However, variation still exists among the individual flies for the different analytes and helps to demonstrate the ability of this technique to study individual fly-to-fly variability. For example, the values of L-DOPA, the dopamine precursor, are observed to be present in higher quantities in fly 5 than in fly 2, but the amounts of dopamine and two of its metabolites, N-acetyldopamine and salsolinol, are higher in fly 2 than in fly 5. This is in contrast to L-DOPA levels and might indicate different levels of metabolism in the two flies. A similar example also occurs between flies 3 and 4, where L-DOPA and dopamine levels are relatively similar between the two flies, but the values of the two metabolites, N-acetyldopamine and salsolinol, are much lower in fly 3 than fly 4. In fact, this trend extends to the two other metabolites, N-acetyltyramine and N-acetylserotonin; for both metabolites, the value measured for fly 3 is lower than in fly 4.

MEKC Separations of *Drosophila* Brain Regions

As this methodology is easily sensitive enough to detect and measure biogenic amines and their metabolites in whole fly brains, we decided to examine individual regions of the brain to push the limits of the technology to levels not achieved before for sampled chemical analysis in this system. In addition, dissection of the *Drosophila* brain into segments offers the ability to obtain quantitative chemical anatomy within the brain. *Drosophila* brains are well categorized physically and physiologically and have many substructures from the overall structure of larger tissues to small clusters of individual neurons, providing the background to match out and map the quantitative chemistry of its regions.

To demonstrate the feasibility of MEKC-EC to detect and assess biogenic amines in these smaller regions, separations of three independent cultures of three brain regions, the two optic lobes, the central brain, and the PPM1 region were examined (Figure 4) and quantified (Table 2). Figure 4A shows a representative optical image of the anterior of a dissected brain. The optic lobes exist on each side of the central brain and connect the central brain to the compound eyes. The optic lobe processes and integrates visual information from the retina and transmits it to the central brain. Together these two units make up the largest two structures in the fly brain. Conversely, the outer layer of the cortex contains thirteen small clusters of dopamine neuronal cells distributed throughout the *Drosophila* brain. One of these clusters, the PPM1 brain area, contains about five cell bodies, which are not split between the two hemispheres of the brain due to its location along the midline of the brain.²² To visualize the PPM1 region of the brain, fluorescence microscopy of TH-GFP flies was utilized. Figure 4B shows a representative false-color image of the posterior of a dissected brain with GFP-labeled dopamine neurons. The white box outlines the exposed brain region where PPM1 neurons were dissected, while the fluorescent cells outside of the box represent other dopamine neuronal clusters. PPM1 regions were dissected to a depth of about one-quarter of the total depth of the brain (~12 μ m) as attempts to dissect smaller lengths were found to be too variable.

Dopamine was examined first. The amount of this transmitter detected in the central brain is close to that reported for the dissected brain homogenates and single dissected brains, whereas very little was observed in the optic lobes. Although there are dopamine neuronal cell bodies present in the optic lobes, they are very few, occur close to the boundary between

the optic lobe and central brain, and project into the central brain.²² As transmitter is typically expected to be higher in the nerve endings, the amount of dopamine present in the optic lobes is likely below the limit of detection for the system. The amount of octopamine measured is almost equal in both the central brain and optic lobes. The optic lobes contain 6 of the 100 octopamine neurons in the fly; however, elaborate octopamine arborizations project into the optic lobes,⁴⁵ leading to the localization of many nerve endings and likely account for the increase in measured octopamine. Octopamine is not detected in the PPM1 region of the fly brain. If any is present in this region of the brain, it must be at trace levels, below the limit of detection. The total measured amount of dopamine in the central brain was almost five times greater than that found in the PPM1 region, yet when the concentration of dopamine was compared for these two regions, the PPM1 region is twenty-five times as concentrated as the central brain (this is because the PPM1 region is considerably smaller than the central brain).

CONCLUSIONS

We present a method to analyze dissected whole brains and brain regions for the analysis of biogenic amines and metabolites present in *Drosophila melanogaster*. We also compare these results to earlier work using homogenized whole heads (including the eyes and cuticle). Electroactive species from whole heads produce intense signals in separations that can overlap neurochemical species of interest. Analysis of these isolated dissected regions serves as a form of sample clean up that helps to alleviate this and create a more reproducible and sensitive separation. Utilizing microdissection, the biogenic amines in homogenates of three types of flies, wild-type, *white* mutant, and TH-GFP, were compared. The *white* mutant flies were confirmed to have reduced amounts of dopamine, salsolinol, N-acetyltyramine, N-acetylserotonin, and L-DOPA in the isolated brains. We also demonstrate that this method can be used to obtain information about spatial differences in biogenic amine levels within different brain regions. These data clearly demonstrate that MEKC-EC has the capability to investigate small brain regions of *Drosophila* brains.

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Figure 1.

(A) Image of two *Drosophila* heads that are analyzed in B and C. On the left is a wild-type Canton S, and the right is a *white* mutant. (B) Electropherogram of three homogenized Canton S *Drosophila* heads with 25 μM catechol internal standard, peak identified with arrow. Many peaks are observed with some being extremely large. At such a low concentration, the internal standard peak can be difficult to find and quantify. (C) Electropherogram of three homogenized *white* mutant *Drosophila* heads with 25 μM catechol as internal-standard; peak identified with arrow. The electropherogram is on the same scale as in B and demonstrates that the intense electroactive peaks observed in B are from the pigment in the eye. (Inset) A magnified view of the electropherogram. The absence of eye-pigment in this sample generates a simpler electropherogram with higher detail within the peaks.

Figure 2.

Electropherograms of dissected *Drosophila* brain homogenates for (A) wild-type, (B) *white* mutant and, (C) TH-GFP mutant flies. Highlighted are peaks corresponding to dopamine (1), salsolinol (2), octopamine (3), N-acetyltyramine (4), N-acetylserotonin (5), N-acetyldopamine (6), L-DOPA (7), and the internal standard catechol (8). Some differences in peak intensities are present between these electropherograms, but intensities can be misleading due to changes in electrode efficiency from fouling which is compensated by the internal standard.

Figure 3.

Separation (A) and quantification (B) of neurochemicals from five single fly brains. (A) Highlighted peaks are unidentified but correspond to different changing peak intensities between the five single-fly brain samples. (B) Bars represent the femtomole amounts of each analyte (dopamine, salsolinol, octopamine, N-acetyltyramine, N-acetylserotonin, N-acetyldopamine, and L-DOPA) measured in brains of these five flies. Flies 1–5 have different shades from left to right, and the average of all five is reported as the solid gray bar to the right of each group. Numeric averages and SEM are reported above the bar corresponding to the average amount.

Figure 4.

(A) Anterior image of an intact, dissected *Drosophila* brain. Brackets show the two optic lobes at the sides and the central brain in the middle. (B) False-color fluorescent image of the posterior of a TH-GFP fly. Green areas indicate cells containing tyrosine hydroxylase, the enzyme responsible for catalyzing the conversion of L-tyrosine to L-DOPA and the rate limiting step in the creation of dopamine. White areas are autofluorescence from larger structures within the brain. The white box denotes the location of PPM1 region, which lies ventrally along midline of the central brain that separates it into its two halves. The 500- μm scale bar is applicable for both panels.

Quantification of Biogenic Amines, Precursor, and Metabolites in Canton S, *white* Mutant, and TH-GFP *Drosophila* Brains^a

Table 1

Analyte	Canton S			<i>white</i> mutant			TH-GFP		
	Mean (fmol)	SEM		Mean (fmol)	SEM		Mean (fmol)	SEM	
Dopamine	91**	4.8		63	7.7		71	5.4	
Salsolinol	140**	14		94	9.5		140**	13	
Octopamine	780	77		700	140		700	130	
N-Acetyltyramine	1600**	300		400	220		1500**	250	
N-Acetylserotonin	500	190		120	50		450**	99	
N-Acetyldopamine	800	190		570	52		900	140	
L-DOPA	1600*	230		900	260		1700*	290	

^aSeparation of three separate fly culture homogenizations showing quantification of analytes in Canton S, *white* mutant, and TH-GFP *Drosophila* brains. Each value corresponds to an amount contained in one brain.

* indicates significantly different from *white* mutant brain samples with p value less than 0.10

** indicates significantly different from *white* mutant brain samples with p value less than 0.05

Table 2

Quantification of Biogenic Amines in the Central Brain, Optic Lobes, and PPM1 Region in Single TH-GFP *Drosophila* Brains^a

Analyte	Location Dissected	Measured Amount (fmol)		Calculated Volume (nL)		Concentration (μM)	
		Mean	SEM	Mean	SEM	Mean	SEM
Dopamine	Central Brain	99	8.7	6.2		16*	1.4
Dopamine	Optic Lobes	0.3	0.20	4.8		0.6*	0.41
Dopamine	PPM1	21	5.0	0.051		400**	98
Octopamine	Central Brain	370	73	6.2		60	12
Octopamine	Optic Lobes	310	65	4.8		60	13
Octopamine	PPM1	-	-	0.051		-	-

^a Averages of three single, TH-GFP, fly-brain region samples ± standard error of the mean (SEM). Mass amounts are reported in femtomoles per fly head. Brain region volumes were calculated by measuring areas in Figure 4 and using 12 μm deep for the PPM1 region and 50 μm deep for the central brain and optic lobe regions.

- values indicate they are below the limit of detection for this technique.

* indicates significantly different from the other marked values with p value of 0.985

** indicates significantly different from the other marked values with p value of 0.999