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In Vivo* Electrochemical Measurements of Exogenously Applied Dopamine in *Drosophila melanogaster

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Abstract

Carbon-fiber microelectrodes coupled with electrochemical detection have been used extensively for the analysis of biogenic amines. In order to determine the functional role of these amines, *in vivo* studies have primarily used rats and mice as model organisms. Here, we report on the development of these microanalytical techniques for *in vivo* electrochemical detection of dopamine in the adult *Drosophila melanogaster* central nervous system (CNS). A triple-barrel micropipette injector was used to exogenously apply three different concentrations of dopamine, and a cylindrical carbon-fiber microelectrode was placed in the protocerebral anterior medial brain area where dopamine neurons are densely populated. Background-subtracted fast-scan cyclic voltammetry was used to measure dopamine concentration in the fly CNS. Distinct differences are shown for the clearance of exogenously applied dopamine in the brains of wild type flies versus fumin (*fmn*) mutants lacking a functional dopamine transporter. The current response due to oxidation of dopamine increased significantly from baseline for wild type flies following cocaine incubation. Interestingly, the current remained unchanged for mutant flies under the same conditions. These data confirm the accepted theory that cocaine blocks dopamine transporter function and validates the use of *in vivo* electrochemical methods to monitor dopamine uptake in *Drosophila*. Furthermore, after incubation with tetrodotoxin (TTX), a sodium channel blocker, there was a significant increase in peak oxidation current in the wild type flies; however, the current did not significantly change in the *fmn* mutant. These data suggest that factors that affect neuronal activity via ion channels such as TTX also influence the function of the dopamine transporter in *Drosophila*.

Introduction

The field of *in vivo* electrochemistry in the brain began in the 1970's with Ralph Adams pioneering the detection of electroactive species. His group measured neurochemicals in the brains of anesthetized rats with carbon electrodes using cyclic voltammetry and chronoamperometry.^{1, 2} Subsequently, background-subtracted fast-scan cyclic voltammetry (FSCV) coupled with carbon-fiber microelectrodes has been developed and extensively used as an analytical technique for *in vivo* measurements of electroactive neurotransmitters.³⁻⁷ *In vivo* electrochemistry has mainly focused on the rat as the primary model system to address fundamental questions regarding neurotransmission mechanisms.⁸⁻¹¹ While similar studies have been conducted in other model systems such as mice and primates, microanalytical

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methods for *in vivo* studies in a model organism as small as *Drosophila melanogaster* have remained undeveloped.¹²⁻¹⁵

Drosophila has been extensively used as a model organism because its genetic manipulation is relatively straightforward, and the genome contains fewer genetic redundancies compared to the mammalian genome, facilitating identification of functions of individual genes or molecules.^{16, 17} *Drosophila* has a short life cycle (12–14 days) and thus it is quite feasible to generate mutants that are genetically homogeneous in comparison to other model organisms used for *in vivo* electrochemistry including rats and mice. Although *Drosophila* has a relatively simple nervous system containing approximately 200,000 neurons, it exhibits many of the same higher-order brain functions as vertebrates at the molecular, cellular, and behavioral levels.¹⁶⁻¹⁸ Flies are capable of learning from prior experiences and storing learned information.^{16, 17} Many monoamines including dopamine, serotonin, tyramine, and histamine that regulate human physiological processes are also found in the *Drosophila* central nervous system (CNS). In addition, octopamine, specific to invertebrates, has similar roles to mammalian norepinephrine.¹⁹

The neurotransmitter dopamine has been implicated in physiological human processes including attention, motivation, emotion, sleep, and addiction.²⁰⁻²² In particular, the reinforcing properties of psychostimulants such as cocaine and amphetamine that block the dopamine transporter or other addictive substances such as ethanol and nicotine involve an elevated level of extracellular dopamine.^{20, 23-25} However, the underlying neuronal mechanisms concerning how dopamine affects vulnerability and addiction remain as yet poorly understood.

Constant-potential amperometry, chronoamperometry, and FSCV are the common electrochemical techniques that have been used to detect dopamine *in vivo* using model systems.²⁶⁻²⁸ While constant-potential amperometry has the advantage of excellent temporal resolution over most other electrochemical techniques, its lack of chemical specificity makes it useful only in a system where the identity of the analyte is known or when combined with a more chemically selective technique.^{11, 29, 30} Voltammetry is one of the most widely accepted techniques used to identify single electrochemical substances. Specifically, background-subtracted FSCV is a dominant technique used for neurotransmitter detection *in vivo* because of its chemical selectivity, relatively high sensitivity, and sub-second temporal resolution.³⁰⁻³²

The current study reports on the development of these microanalytical techniques for *in vivo* electrochemical detection in the *Drosophila* CNS. Voltammetry has been carried out to monitor dopamine in the adult brain of the wild type fly versus the mutant fly lacking functional dopamine transporter, and significant differences are detectable for the clearance of exogenously applied dopamine by the transporter.

Experimental Section

Chemicals

All chemicals were used as received and purchased from Sigma (St. Louis, MO) unless otherwise stated. Adult-hemolymph like (AHL) saline (108 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 8.2 mM MgCl₂, 4 mM NaHCO₃, 1 mM NaH₂PO₄, 5 mM trehalose (Fluka BioChemika, Buchs, Switzerland), 10 mM sucrose, 5 mM Tris, pH 7.5) was made using ultrapure (18 MΩ·cm) water and filtered through a 0.2 μm filter.³³ All collagenase, dopamine, cocaine, and tetrodotoxin (TTX) solutions were prepared using AHL saline.

***In vivo Drosophila* preparation**

The Canton-S strain of *D. melanogaster* was used as the wild type fly in this study. The transgenic flies carrying tyrosine hydroxylase (TH)-GAL4 and UAS-mCD:GFP (membrane tethered green fluorescent protein) were used to visualize the dopamine neurons.^{34, 35} The genetic background in the *w¹¹¹⁸* mutant with a genetic lesion in the dopamine transporter gene was replaced with the Canton-S background.³⁶ All flies were maintained at 25 °C on a standard cornmeal-agar medium and 4–7 day-old male flies were used in all experiments. For *in vivo* imaging and voltammetry, the flies were immobilized on ice and mounted in a homemade collar (a 38.1 mm diameter concave plexiglass disk with a 1.0 mm hole in the center) with low melt agarose (Fisher Scientific, Pittsburgh, PA). Microsurgery was performed on a stereoscope (Olympus SZ60, Melville, NY) using small dissection scissors and forceps (World Precision Instruments, Sarasota, FL). After the cuticle was removed from the top portion of the head to expose the brain, the head was covered with 0.1% collagenase solution for 30 min to relax extracellular matrix in the brain and then rinsed and covered with AHL saline. The images were acquired using an Olympus SZX10 stereomicroscope and an Olympus DP71 digital camera (Figure 1A) or a Leica MZ16 stereomicroscope and a Leica DFC290 digital camera (Figure 1B and 1C; Leica, Mannheim, Germany).

Electrochemical measurements

Carbon-fiber microelectrodes were fabricated as previously described.⁶ Briefly, a single 5 μ m diameter carbon fiber (Amoco, Greenville, SC) was aspirated into a borosilicate glass capillary (B120–69–10, Sutter Instruments, Novato, CA), and the capillary was pulled using a regular glass capillary puller (P-97, Sutter Instruments). Electrical contact was made by back-filling the capillary with silver paint (4922N DuPont, Delta Technologies Ltd., Stillwater, MN) and inserting a tungsten wire. To form a cylindrical electrode, the carbon fiber was cut to a length of 40–50 μ m, as measured from the glass junction. Electrode tips were dipped into epoxy (Epo-Tek, Epoxy Technology, Billerica, MA) for 30 s to ensure a good seal between the fiber and the glass and then dipped into acetone for 15 s to remove epoxy from exposed carbon fiber. A Ag/AgCl electrode served as the reference electrode in all experiments. A silver wire (0.25 mm diameter, 99.999% purity, Alfa Aesar, Ward Hill, MA) was chloridized in bleach overnight. Micropipette injectors were fabricated by pulling glass capillaries in a glass capillary puller to an opening of approximately 5 μ m.

Electrochemical data were collected using an Axopatch 200B Amplifier (Axon Instruments, Foster City, CA) and two data acquisition boards (PCI-6221, National Instruments, Austin, TX) run by the TH 1.0 CV program (ESA, Chelmsford, MA).³⁷ For amperometric experiments, a constant potential (+750 mV) was first applied to the working electrode with respect to the reference for at least 15 min to stabilize background current. All cyclic voltammograms were obtained using a triangular waveform (scanned –0.6 V to +1.0 V versus Ag/AgCl at 200 V/s) repeated every 100 ms. Prior to voltammetric experiments, all electrodes were cycled (–0.6 V to +1.0 V at 200 V/s) for at least 15 min to stabilize the background current. Electrochemical responses were plotted and statistical analysis performed using Prism 3.0 (GraphPad Software, La Jolla, CA).

All electrodes were positioned using micromanipulators (421 series, Newport, Irvine, CA). Either a single-barrel glass micropipette or a three-barrel glass micropipette (3B120F-6, World Precision Instruments) was used to exogenously apply dopamine solutions. Each barrel was individually coupled to the micro-injection system (Picospritzer II, General Valve Corporation, Fairfield, NJ) using a PolyFil apparatus (World Precision Instruments).

Results and Discussion

Drosophila preparation for *in vivo* electrochemical measurements

Electrochemical methods provide a new tool for studying electroactive neurotransmitters in *Drosophila*. We are particularly interested in studying dopamine neurotransmission since it plays crucial roles in numerous CNS functions in *Drosophila* as in mammals.¹⁹ In the *Drosophila* brain, multiple clusters of dopamine neuronal cell bodies are spread throughout the outer layer of the brain cortex and innervate many brain regions. In particular, the dopamine neuronal cluster in the protocerebral anterior medial (PAM) brain area project to the nearby mushroom body (MB) structure that is crucial for many higher-order neuronal functions including learning and memory.³⁸⁻⁴⁰ Thus, we focused on the PAM neurons for *in vivo* analysis of dopamine neurotransmission. To place microelectrodes in the area where the PAM neurons are located, we implemented a microsurgery procedure. A single adult fly was immobilized in a homemade fly collar using agarose applied to the body and the bottom portion of the head (Figure 1A), leaving the upper portion of the head uncovered and positioned for dissection. The cuticle was then removed, and the brain was kept bathed in AHL saline (Figure 1B). The salts in the AHL solution were at physiological concentrations, keeping the immobilized fly viable for 1.5 – 2.5 hours providing sufficient time to perform electrochemical measurements.³³ A micromanipulator was used to guide the cylindrical working electrode into the PAM region. The micropipettes used for dopamine application throughout these experiments were positioned above the PAM area, approximately 10 μm from the working electrode (Figure 1B inset). The reference electrode was submerged in the AHL saline. Fluorescence microscopy was used to visualize the location of the PAM dopamine neurons in the brain of the transgenic TH-GAL4/UAS-GFP fly expressing GFP in dopamine neurons. The PAM area represents the largest cluster of dopamine neurons and is easily identifiable.³⁸ Figure 1C shows a representative fluorescence image of the dissected brain with GFP-labeled dopamine neurons. The white box outlines the exposed brain regions where PAM neurons are clearly visible, while the fluorescent cells below the box represent other dopamine neuronal clusters. Experiments to investigate dopamine uptake were performed at the PAM dopamine neuronal area.

Measuring Exogenously Applied Dopamine in *Drosophila*

In previous studies, electrochemical detection using FSCV has been used to monitor *in vivo* dopamine concentrations in rats.³ Exogenously applied dopamine can be measured at the surface of a carbon-fiber microelectrode inserted into the PAM area of the *Drosophila* system. To further characterize dopamine detection in the PAM area, color plots were used to display FSCV data. In these experiments, we ejected small amounts of a dopamine solution in the area near the electrode and used voltammetry to quantify the dopamine changes in the brain and to track its temporal characteristics. Here, 1.0 mM dopamine was exogenously applied to the adult wild type brain using a single micropipette injector, and a microelectrode was used for dopamine detection in the PAM area. The potential was scanned from -0.6 V to $+1.0\text{ V}$ versus Ag/AgCl (200 V/s, repetition frequency = 10 Hz). A false-color representation of current (Figure 2A) allows one to visualize cyclic voltammograms over time. The oxidation of dopamine is represented in green while blue corresponds to the reduction of the orthoquinone, allowing discrimination of a particular analyte from other species that may be present in the same brain region. Cyclic voltammetry can be used to identify electroactive species based on the potential at which oxidation occurs and the overall shape of the wave.^{11, 30, 31} For example, the cyclic voltammogram in Figure 2B is a background-subtracted average of ten successive cyclic voltammograms taken at the peak current from the color plot (Figure 2A). By inspection, the shape of the voltammogram and peak potentials leads us to conclude that the increase in current in Figure 2A corresponds to the measurement of dopamine. Finally, the current can be converted to dopamine concentration using *in vitro* calibration (Figure 2C), and the time required for the concentration to decrease to half of its maximum value, $t_{1/2}$, determined. The

difference in dopamine concentration applied versus that detected (millimolar versus micromolar) at the electrode is attributed to reuptake and diffusion of the analyte into the surrounding solution and tissue. Importantly, the time course of the uptake monitored in the fly brain following application of exogenous dopamine solution ($t_{1/2} \sim 50$ s) is consistent with measurements of clearance from tissue in other model systems like the rat following exogenous application of dopamine solution.⁴¹ Thus, this method is a viable approach to measure changes in exogenously applied dopamine concentration occurring *in vivo* in the adult fly brain.

Voltammetric versus amperometric detection of dopamine *in vivo*

Oxidation of dopamine produces a current which is dependent on the concentration of applied dopamine and its diffusion, uptake, and metabolism as it traverses through tissue. However, the local geometry and position of the micropipette injector also influence the signal. Specifically, the relative distance of the micropipette to the electrode in the PAM area (Figure 1B) affects the amplitude of the current measured. Because a single micropipette is difficult to position the same distance from the electrode multiple times, a pulled triple-barrel capillary was used to exogenously apply three different concentrations of dopamine to the PAM area in series. The current response from 1.0 mM dopamine, approximately 150 pmol (Supporting Information), applied to the PAM region was measured over time, and repeated with 2.0 mM and 5.0 mM dopamine solutions, with each solution loaded into a separate barrel of a triple-barrel micropipette injector. Results obtained using amperometry to measure the dopamine concentration *in vivo* proved to be variable. Indeed, the measured concentration at the electrode does not increase linearly with the applied concentration ($r^2 = 0.36$, $n = 4$). Hence, we used FSCV for analysis. Representative data collected using FSCV are shown in Figure 3. The measured peak currents were converted to dopamine concentration by calibration of the electrode *in vitro* with standard solutions (Supporting Information). The plot of normalized measured dopamine concentration versus injected concentration constructed using FSCV measurements has a slope of 0.73 ± 0.08 ($r^2 = 0.84$, $n = 6$), close to the expected value of 1. Thus, controlled concentrations of dopamine solutions can be applied locally to the fly CNS and measured voltammetrically. The differences observed between amperometry and FSCV are not surprising when one takes into account the limited sample volume of the *Drosophila* PAM region. During amperometric measurements, we hypothesize that local dopamine is “consumed” by oxidization to the orthoquinone, and the local dopamine concentration is altered, making the dopamine unavailable for repeated measurements. The orthoquinone might also be involved in mechanisms of oxidative stress that could affect surrounding tissue in the local environment. In contrast, voltammetric measurements regenerate the measured analyte, minimizing the effect on surrounding tissue. Additionally, the diffusion layer, and thus the volume sampled, with FSCV is smaller than that sampled using amperometry (~ 3 pL versus ~ 50 pL based on the parameters used in these experiments, Supporting Information). Amperometry effectively measures dopamine changes that are averaged over a larger tissue volume, whereas FSCV measures the dopamine concentration locally around the electrode. This apparently leads to a more accurate measurement of dopamine concentration in this system.

Comparison of dopamine uptake in wild type versus *fmn* mutant flies and the effect of cocaine

The *fmn* mutants are a *Drosophila* line where the dopamine transporter function has been eliminated through genetic mutation. Thus, the cells that normally remove dopamine from the extracellular fluid after it is released cannot do so, or at least not by the normal mechanism, in *fmn* mutants. We used *in vivo* voltammetry to investigate the relative magnitude of uptake of dopamine in the fly brain by comparing the *fmn* mutants to wild type flies.

Using the same FSCV parameters described in a previous section, differences in uptake between the wild type and *fmn* mutant brains were first investigated. Dopamine was exogenously applied to the PAM area (1.0 mM) with a single micropipette injector, and the current response recorded. Two measurements were taken for each fly, and the maximum currents averaged together. The current was then converted to dopamine concentration using *in vitro* calibration. Interestingly, comparison of the black traces in Figure 4A and 4B shows that the peak dopamine concentration observed after injection, $[DA]_{\max}$, is considerably smaller in the wild type compared to the *fmn* mutant. When the average baselines for signals in multiple flies are considered (Figure 4C), the $[DA]_{\max}$ was significantly higher in *fmn* flies compared to wild type flies ($9.5 \pm 2.4 \mu\text{M}$ versus $3.1 \pm 0.8 \mu\text{M}$, $p = 0.02$ (*), Student's *t*-test). This indicates that less dopamine is detected at the electrode after exogenous application in the wild type flies and is likely due to a high rate of dopamine uptake via the functional transporter in the PAM neurons in these flies versus the nonfunctional transporter in the *fmn* flies. Thus, $[DA]_{\max}$ can be used to measure changes in dopamine uptake. It is important to point out that the measurements reported here are highly dependent on electrode and injector placement, resulting in some variation in the values in different flies of the same genotype. However, experiments comparing the relative amount of dopamine in different flies can be carried out by normalization to baseline signals following initial dopamine application, and temporal changes of uptake in the same fly with different conditions can be carried out.

The validity of this theory is demonstrated by using a known dopamine uptake inhibitor, cocaine, to block reuptake of exogenously applied dopamine. To account for differences in the injector positioning and fly-to-fly variability, the maximum currents of two baseline measurements were averaged for each fly and used to normalize all measurements for that particular fly. After the baseline measurements, the fly brain was bathed with 1.0 mM cocaine in AHL saline, and a voltammogram was obtained for exogenously applied dopamine after five minutes. Representative traces for wild type and *fmn* mutant flies are shown in Figure 4A and 4B. After the cocaine application, higher dopamine concentrations were detected at the electrode compared to baseline in wild type flies (Figure 4A). *Fmn* mutants lacking functional dopamine transporters showed no change from baseline following the cocaine incubation (Figure 4B). When multiple cocaine-treated flies were considered (Figure 4D), the wild type flies had significantly increased normalized $[DA]_{\max}$ and $t_{1/2}$ compared to the cocaine-treated *fmn* mutant flies (Student's *t*-test, $p = 0.01$ (*) for $[DA]_{\max}$; two way ANOVA, $p = 0.05$ (*) and $F = 4.1$ for genotype for $t_{1/2}$). This data supports existing evidence that cocaine blocks dopamine transporter function in *Drosophila*.²⁵

The effect of tetrodotoxin (TTX) on uptake

We also investigated the effect of neuronal activities on dopamine uptake by treating the brains of the two fly genotypes with TTX. TTX is a neurotoxin that blocks action potentials through the blockade of voltage-sensitive sodium channels.⁴²⁻⁴⁴

To examine the effects of TTX, the fly brain was bathed with 1.0 μM TTX in AHL saline after the baseline measurements, and voltammograms were obtained with injections of dopamine every five minutes. Representative traces for wild type and *fmn* mutant flies are shown in Figure 5A and 5B. The *fmn* mutant clearly exhibited a different response compared to the wild type flies following incubation with TTX. After TTX treatments in wild type flies, higher dopamine concentrations were detected at the electrode compared to baseline (Figure 5A). This could be due to several factors. For example, dopamine uptake in the fly brain may depend on neuronal activity in which case inhibition of the action potential by TTX would abolish the uptake. Alternatively, TTX might directly inhibit the uptake process. Both possibilities are supported by the result that *fmn* mutants lacking functional dopamine transporters showed no significant change from baseline following TTX incubation (Figure 5B). Interestingly, the TTX-treated

wild type flies contained significantly increased normalized $[DA]_{\max}$ and $t_{1/2}$ compared to the TTX-treated *fmn* mutants (Figure 5C; two way ANOVA, $p = 0.0001$ (***) and $F = 32.3$ for genotype for $[DA]_{\max}$, $p = 0.04$ (*) and $F = 4.9$ for genotype for $t_{1/2}$). It is possible that the *fmn* mutant may have a compensatory increase in the transporter-independent process (i.e. an increased N-methylation) for inactivating endogenously released as well as exogenously applied dopamine, leading to decreased dopamine concentrations detected at the electrode. Previous studies have reported the activity of the dopamine transporter to be dependent on membrane potential.⁴⁵ TTX blocks voltage-gated sodium channels, thereby reducing the activity of neurons via action potentials. Our data thus suggest that the dopamine transporter is activity-dependent, as uptake is reduced in the wild type flies with TTX.

Conclusions

Microanalytical tools have been developed for *in vivo* electrochemical measurements in the adult *Drosophila* CNS. Exogenously applied dopamine is detected using a cylindrical carbon-fiber microelectrode inserted into the dopamine neuronal cluster projecting to the mushroom bodies. The signal has been characterized using FSCV. A known dopamine uptake blocker, cocaine, was used to validate this method for *in vivo* measurement of *Drosophila* dopamine transporter function. Electrochemical detection with FSCV was used to investigate the effect of TTX on the dopamine transporter, and the peak dopamine concentration measured which is dependent on uptake. This work presents a new method for studying electroactive neurotransmitters *in vivo* in *Drosophila* which can be used to measure changes in dopamine uptake.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Series of images taken of *Drosophila* during microsurgery. (A) Fly immobilized in a homemade fly collar (Scale bar = 500 μm). (B) Fly after cuticle has been removed. The exposed brain area with the PAM dopamine neurons is outlined by the black box (Scale bar = 100 μm, electrode and injector not to scale). Inset: Schematic showing relative electrode and micropipette injector placement for experiments. (C) Fluorescence image highlighting GFP-labeled dopaminergic neurons. White box outlines the PAM region (Scale bar = 100 μm).

Figure 2. Exogenously applied 1.0 mM dopamine detected *in vivo* in an adult wild type fly. (A) Successive voltammograms plotted as applied potential versus time with false color representation showing current. (B) Background-subtracted fast-scan cyclic voltammogram of dopamine application. (C) Changes in dopamine concentration over time. Dopamine concentration was determined as described in Figure 2. Black arrow corresponds to a 1.0 s dopamine application beginning at 5.0 s.

Figure 3.

Voltammetric detection of exogenously applied dopamine solutions in the PAM area of the adult wild type *Drosophila* brain. A triple-barrel micropipette was used to apply 1.0 mM (black), 2.0 mM (red), and 5.0 mM (blue) dopamine solutions in series for 1.0 s beginning at 5.0 s (black arrow). Dopamine (DA) concentrations were determined by converting the maximum current from the sampled amperometry plot using the *in vitro* calibration average of three electrodes.

Figure 4. Effect of cocaine on dopamine uptake

(A) Representative concentration trace of exogenously applied dopamine in wild type *Drosophila* before (black line) and after (red line) cocaine application. An increase in dopamine concentration in the adult wild type fly was observed following a 5 min exposure to 1 mM cocaine. Black arrow corresponds to a 1.0 s dopamine application beginning at 5.0 s. (B) Representative concentration trace of exogenously applied dopamine in the *fmn* mutant before (black line) and after (red line) cocaine application. No significant change was observed in the adult *fmn* mutant fly. (C) Baseline comparison of [DA]_{max} for the wild type and *fmn* mutant (mean ± SEM; Student's *t*-test, $p = 0.02$ (*), $n = 9$). (D) Comparison of adult wild type versus *fmn* mutant flies when 1.0 mM dopamine is exogenously applied before and after application of 1.0 mM cocaine. The increases in [DA]_{max} are significantly higher in wild type flies compared to *fmn* flies when treated with 1.0 mM cocaine (mean ± SEM; Student's *t*-test, $p = 0.01$ (*), $n = 6$.)

Figure 5.

Effect of TTX on dopamine uptake. (A) Representative concentration trace of exogenously applied dopamine in wild type *Drosophila* before and after TTX application. An increase in dopamine concentration in the adult wild type fly was observed following exposure to TTX. Black arrow corresponds to a 1.0 s dopamine application beginning at 5.0 s. Baseline 2, 10 min, and 20 min traces were omitted for clarity. (B) Representative concentration trace of exogenously applied dopamine in the *fmn* mutant before and after TTX application. No significant change was observed in the adult *fmn* mutant fly. (C) Comparison of adult wild type versus *fmn* mutant flies when 1.0 mM dopamine is exogenously applied before and after application of 1.0 μM TTX. The increases in $[\text{DA}]_{\text{max}}$ are significantly higher in wild type flies compared to *fmn* flies when treated with 1.0 μM TTX (mean $\pm$ SEM; two way ANOVA, $p = 0.0001$ (***) and $F = 32.3$ for genotype, $n = 3$; SEM in the baseline bars are too small to see).