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ORIGINAL ARTICLE

Overexpression of the *Drosophila* vesicular monoamine transporter increases motor activity and courtship but decreases the behavioral response to cocaine

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Aminergic signaling pathways have been implicated in a variety of neuropsychiatric illnesses, but the mechanisms by which these pathways influence complex behavior remain obscure. Vesicular monoamine transporters (VMATs) have been shown to regulate the amount of monoamine neurotransmitter that is stored and released from synaptic vesicles in mammalian systems, and an increase in their expression has been observed in bipolar patients. The model organism *Drosophila melanogaster* provides a powerful, but underutilized genetic system for studying how dopamine (DA) and serotonin (5HT) may influence behavior. We show that a *Drosophila* isoform of VMAT (DVMAT-A) is expressed in both dopaminergic and serotonergic neurons in the adult *Drosophila* brain. Overexpression of DVMAT-A in these cells potentiates stereotypic grooming behaviors and locomotion and can be reversed by reserpine, which blocks DVMAT activity, and haloperidol, a DA receptor antagonist. We also observe a prolongation of courtship behavior, a decrease in successful mating and a decrease in fertility, suggesting a role for aminergic circuits in the modulation of sexual behaviors. Finally, we find that DMVAT-A overexpression decreases the fly's sensitivity to cocaine, suggesting that the synaptic machinery responsible for this behavior may be downregulated. DVMAT transgenes may be targeted to additional neuronal pathways using standard *Drosophila* techniques, and our results provide a novel paradigm to study the mechanisms by which monoamines regulate complex behaviors relevant to neuropsychiatric illness.
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Introduction

Although *Drosophila* and mammalian neuroanatomy differ considerably, many molecular elements of aminergic neurotransmission are conserved.[1–3] In addition, *Drosophila* exhibit a variety of complex behaviors that are regulated by dopamine (DA) and serotonin (5HT).[4–12] Thus, *Drosophila* provides a powerful system to explore the mechanisms by which aminergic neurotransmission is regulated, and how changes in the presynaptic release of monoamines may influence behavior.

Both pharmacologic and genetic methods may be used to increase transmitter release. Psychostimulants such as cocaine and amphetamines dramatically alter a variety of behaviors that may be similar to some aspects of psychosis as well as bipolar, obsessive–compulsive and tic disorders.[13–15] However, these drugs bypass normal mechanisms of vesicular neurotransmitter release, and the effects of increasing aminergic signaling through normal mechanisms of exocytosis are not clear. Furthermore, it is not feasible to direct psychostimulants or other drugs to specific cells, and it is therefore not possible to map the location of the aminergic neurons responsible for their behavioral effects. In contrast, molecular genetic manipulations of neurotransmitter transporters may provide a method for increasing neurotransmission using more physiologically relevant mechanisms of neurotransmitter release, and in specific subpopulations of aminergic neurons.

Monoaminergic neurons express two distinct classes of neurotransmitter transporters, including: (1) plasma membrane transporters that are responsible for terminating synaptic transmission and (2) vesicular transporters that transport classical transmitters into secretory vesicles for their release from

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presynaptic nerve terminals.[16,17] Two vesicular monoamine transporters (VMAT1 and 2) have been identified in mammals.[18] Both recognize DA and 5HT as substrates, and VMAT2 is expressed in all dopaminergic and serotonergic neurons in the CNS.[19]

VMAT2 knockout mice show a dramatic reduction in the storage and release of monoamine transmitters,[20–23] while overexpression of VMAT2 *in vitro* increases DA release.[24,25] The potential clinical relevance of increasing VMAT expression has been suggested based on studies showing that VMAT2 levels are increased in bipolar patients.[26,27] Conversely, inhibition of VMAT by the drug reserpine causes a state that resembles depression.[28] Surprisingly, although the behavioral effects of decreasing VMAT expression *in vivo* are well documented,[20–23] complementary models to study the effects of increasing VMAT activity and monoamine release *in vivo* have not been developed.

The molecular-genetic tools available in *Drosophila* allow temporally and spatially restricted patterns of expression unavailable in other systems.[29] To investigate how increasing the vesicular release of DA and 5HT may affect behavior, we have overexpressed in dopaminergic and serotonergic cells an isoform of *Drosophila* VMAT that is structurally similar to mammalian homologs (DVMAT-A[30]). We find that overexpression of DVMAT-A results in an increase in stereotypic grooming behavior, locomotion and courtship. We also find that an increase in DVMAT expression alters the fly's behavioral response to cocaine and haloperidol. These results establish a genetic system to investigate the cellular mechanisms that regulate complex behaviors involving aminergic neurotransmission.

Materials and methods

Fly stocks

Flies were raised on standard corn meal-agar media at room temperature. Oregon R was used as wild type. Fly cultures and crosses were performed according to standard procedures.[31] To create transgenic flies, we used a previously constructed expression vector containing a full-length DVMAT-A cDNA in pEX-UAS (pEX-UAS-DVMAT-A).[30] pEX-UAS-DVMAT-A was coinjected with the Δ2–3 plasmid, a source of transposase, into approximately 500 *yw* embryos.[32] pEX-UAS-DVMAT-A includes an HA tag to facilitate the detection of the transgene and does not affect transport activity or protein trafficking.[30] We obtained a series of several *UAS-DVMAT-A* transformants including those that we mapped to the X (*UAS-DVMAT.X*), second (*UAS-DVMAT.II*) and third (*UAS-DVMAT.III*) chromosomes using standard genetic techniques. GAL4 expression lines used in our studies included *GMR-GAL4*,[33] *Ddc-GAL4* on the second chromosome and X chromosomes (*Ddc-GAL4.II*) and (*Ddc-GAL4.X*), gifts of Dr J Hirsh, and *elav-GAL4*, a gift of Dr C Goodman. These were mated to *UAS-DVMAT-A* lines to generate following lines homozygous for both the driver and UAS constructs: (1) *Ddc-GAL4.II;UAS-DVMAT.III*; (2) *Ddc-GAL4.II, UAS-DVMAT.II*; (3) *Ddc-GAL4.X,UAS-DVMAT.X*; (4) *UAS-DVMAT.II;elav-GAL4.III*; (5) *GMR-GAL4.X;UAS-DVMAT.II.* In addition, for some experiments we used flies heterozygous for the *GAL4* driver or *UAS-DVMAT* as indicated in the text and figure legends.

Westerns

To quantitate DVMAT-A expression, homozygous flies of the following genotypes were aged in parallel for 4 days: (1) *Ddc-GAL4.II* (driver alone), (2) *UAS-DVMAT.III* (target alone), and (3) *Ddc-GAL4.II;UAS-DVMAT.III* (DVMAT-A overexpressing flies). Individual fly heads were manually collected and homogenized in SDS-PAGE sample buffer (5 μl/head, 6 heads/sample) using a glass on glass micro-tissue grinder (Kontes), then briefly microfuged to remove insoluble debris. Samples of 10 μl (2 head equivalents) from each genotype were subjected to SDS-PAGE and blotted to nitrocellulose as previously described.[30,34] The samples were probed using (1) a MAb to HA (Covance) and (2) the affinity-purified antiserum to DVMAT-A, followed by the appropriate HRP-conjugated secondary antibody (Amersham) and a chemiluminescent substrate (Pierce SuperSignal West Pico). To quantitate band intensity, films were scanned (Epson Perfection 2450), and the total pixels/band in digitized images (Adobe Photoshop) measured using the NIH Image 1.63 Gel Macro. We confirmed uniform transfer of protein to the membranes by staining with Ponceau S (0.1% solution, Sigma).

Immunofluorescence

Commercially available primary antibodies used for immunofluorescence included mouse MAb HA.11 (BabCo/Covance), rat anti-serotonin (Chemicon International, Inc.), mouse anti-tyrosine hydroxylase (ImmunoStar) and mouse MAb 24B10 (Developmental Studies Hybridoma Bank, University of Iowa).[35] Additional noncommercial antisera included mouse anti-FasII, a gift of Dr Corey Goodman, and a rabbit antibody to DVMAT-A that we have described previously.[30] Secondary antibodies were coupled to Cy3, fluorescein, or Cy5 (Jackson ImmunoResearch). Whole adult and pupal fly heads were dissected and fixed for 1 h in 2.5% paraformaldehyde (PFA) in PLP (10 mM $NaIO_4$, 75 mM lysine in 37 mM phosphate buffer, pH 7.4) then washed 3 × in PBST (0.03% Triton in PBS, pH 7.4) as previously described[36] and incubated in primary antibodies in PBST with 5% normal goat serum (NGS) overnight at 4°C. After washing 3 × in PBST, adult and pupal brains were incubated with secondary antibodies for 4 h in PBST with 5% NGS, washed 3 × in PBST and then mounted in Antifade (Molecular Probes). For serotonin immunolocalization in pupal brain, the tissues were incubated for 30 min in Ringer solution containing 0.1% collagenase/dispase (Boehringer Mannheim) to partially dissociate cells prior to fixation. Third

instar larval brains were dissected in Ringer solution, fixed for 30 min in 4% PFA in PLP and similarly stained. Stained tissue was visualized using Zeiss AxioskopII microscope or a Leica TCS SP laser scanning confocal microscope housed in the Carol Moss Spivak Cell Imaging Facility in the UCLA Gonda Center, and images were processed using Adobe Photoshop 6.0.

For quantitation of 5HT staining, digital images from larval brains were obtained using a Zeiss $\times 100$ Plan-Neofluar objective and a constant exposure time of 1 s. The pixel intensity within each 5HT-stained bouton was quantitated using AxioVision 4.0 software and an adjacent area of background fluorescence subtracted from each value.

Gradient fractionation

Glycerol gradient fractionation of synaptic vesicles was performed as described[37,38] with minor modifications, including the use of gradients generated with a BioComp Gradient Master (Fredericton, Canada) without a sucrose cushion to capture heavier membranes. Fractions were recovered from the bottom of the gradient using a Beckman Fraction Recovery System. Samples were probed on Western blots using a MAb to the HA epitope in DVMAT (HA.11, 1/500) and *Drosophila* synaptobrevin (1/500), generously provided by Dr Regis Kelly.

Neurochemical analysis

Fly heads (3/sample) were manually collected and homogenized in 0.1 M perchloric acid containing 0.1% EDTA using a glass on glass micro-tissue grinder (Kontes). Insoluble debris were sedimented by centrifugation and the supernatant filtered through a Millipore MC cartridge. The filtrate was diluted 10-fold prior to analysis, and 5 μl of the diluted sample was analyzed using high-performance liquid chromatography (HPLC) with electrochemical detection (Antec Leyden, Leiden, Netherlands) employing a mobile phase consisting of sodium acetate (75 mM), sodium dodecane sulfonate (0.75 mM), EDTA (10 mM), triethylamine (0.01%), acetonitrile (12%), methanol (12%), tetrahydrofuran (1%), pH 5.5, pumped at a rate of 200 μl/min (Shimadzu model LC-10AD, Columbia, MD, USA) through a 100×2 mm column (3 μm, Hypersil C18, Keystone Scientific, Bellefonte, PA, USA). The system was calibrated at regular intervals and provided a limit of detection for 5HT of 0.5 fmol for a 5 μl injection of sample. Data were collected and analyzed using ChromPerfect software (Justice Innovations, Mountain View, CA, USA).

Behavioral assays

For manual locomotion assays, flies were habituated for 1 h in an $80 \times 55 \times 2\,mm^2$ chamber containing a $6\,mm^2$ grid and the number of grid lines that were crossed over periods of 1–2 min scored in real time or from video-recordings. For automated assays, activity was monitored for 1–3 days using a *Drosophila* Activity Monitoring System (Trikinetics, Waltham, MA, USA) fitted with individual or population monitors for assays of either single flies or groups of flies, respectively. For grooming assays, flies aged for 1–10 days after eclosion were observed in individual chambers or standard culture vials, using a stereomicroscope fitted with a digital video camera. A single grooming event was scored each time a fly rubbed its legs over its head or abdomen, or rubbed two legs together. For each fly, grooming behavior was observed over three separate 2 min periods and tallied as an average number of grooming events per minute. Scoring periods were initiated by the observation of a single grooming event, and at least 20 flies were scored for each genotype. An increase in grooming behavior was confirmed by two independent observers blind to genotype. For negative geotaxis assays, groups of 30 flies were placed in glass, 100 ml graduated cylinders. After gently tapping the cylinder, the flies were allowed to climb for 15 s. Then each group was scored for the number of flies that climbed above the 50 ml mark versus those remaining below the 50 ml mark. To quantitate tracking behavior during courtship, groups of males and females were observed for periods of 20 min in chambers used for manually scoring locomotion. An individual tracking event was scored each time two flies moved as pair for at least 30 s. The duration of each event also was recorded. To quantitate additional courtship behaviors and the frequency of copulation following courtship, pairs of flies were placed in individual courtship chambers, videotaped and observed until copulation occurred or for up to 30 min. The copulation index was calculated as the percentage of four simultaneously tested mating pairs that copulated within 30 min. For feeding drugs, stock solutions were rapidly mixed with molten fly food. For acute administration of volatilized cocaine, groups of 10 male flies were exposed to a fixed dose (200 μg) of free-base cocaine by volatilization and then subjected to a negative-geotaxis climbing assay that is blinded to the experimenter as described.[9] Data are presented as the percentage of flies that *fail* to climb (climbing index).

Results

Dopaminergic and serotonergic cells in the adult CNS express DVMAT

To use DVMAT-A to investigate the effects of DA and 5HT release on adult fly behavior, we first tested whether it is expressed in neurons that store and release these transmitters in the adult CNS. Since flies do not appear to synthesize norepinephrine or epinephrine, and the related transmitter octopamine is synthesized via a distinct biochemical pathway,[39] tyrosine hydroxylase (TH) serves as a marker for dopaminergic neurons in these experiments. We have previously identified two splice variants of DVMAT (A and B), one of which, DVMAT-A, is similar to mammalian orthologs.[30] We have generated a specific

antibody that recognizes the carboxy-terminus of DVMAT-A, and we have used this antibody to demonstrate DVMAT-A expression in dopaminergic, serotonergic and octopaminergic neurons in the developing larval CNS and neuromuscular junction.[30] As shown previously, the affinity-purified antiserum recognizes a single band in cultured cells transfected with DVMAT-A cDNA, but not untransfected cells, and also recognizes a single band on Westerns of head homogenates.[30] Using this antibody to stain whole-mounts of adult brain, we detect colocalization of DVMAT-A and TH in dopaminergic neurons throughout the adult CNS (Figure 1). These include two distinct clusters of neurons (DM and DL_1) in the protocerebrum of the central brain that have been used recently to study neurodegenerative processes in dopaminergic cells.[40,41] We observe six cells in the DM cluster and 12–14 cells in the DL_1 cluster (Figure 1a, d, e, f) similar to previous reports using specific probes for TH and Ddc or chemical methods to detect the intrinsic fluorescence of DA.[42–46]

Similar to these earlier reports, we also detect several additional clusters of interneurons in the central brain (AM and PM groups, data not shown) and the DL_2 cluster (Figure 1a, e, f). DVMAT-A also localizes to a relatively large number of small dopaminergic cells in the innermost optic ganglion (the medulla, Figure 1a, b) that arise late during pupal development.[44] These results indicate that most if not all dopaminergic cells in the adult CNS express DVMAT-A.

To determine whether DVMAT-A is expressed in serotonergic neurons in the adult fly, we costained adult brains with antibodies to 5HT and DVMAT-A (Figure 2). To facilitate permeabilization of the antibodies, we also stained pharate (late pupal) flies before the complete scelerotization of the cuticle (Figure 2). We find that DVMAT colocalizes with 5HT in the 12–14 cells of the LP_2 cluster (Figure 2a, b). These cells send a prominent fiber pathway through the medulla and arborize extensively in the outermost optic ganglion, the lamina (Figure 2b–c). Additional branches from this fiber tract appear as three bands in defined layers of the second optic ganglion, the medulla, including the inner medulla (iM), and layers M1, 2 and 4 (Figure 2d). These projections are nearby but distinct from photoreceptor cell 7 and 8 axons that project into the medulla (Figure 2e, arrowheads). They also are distinct from projections of the monopolar L1 and L2 cells (Figure 2f) that are innervated by photoreceptor cells. The close apposition of serotonergic processes to both photoreceptor and monopolar cells are consistent with previous studies indicating that 5HT may modulate visual function in *Drosophila*.[47] We also find that serotonergic cells innervating the central complex (Figure 2g) express DVMAT-A (Figure 2h). The central complex includes the ellipsoid and fan-shaped bodies, the protocerebral bridge and the noduli; mutants that affect the morphology of these structures indicate that they are involved in modulating locomotor behavior.[48]

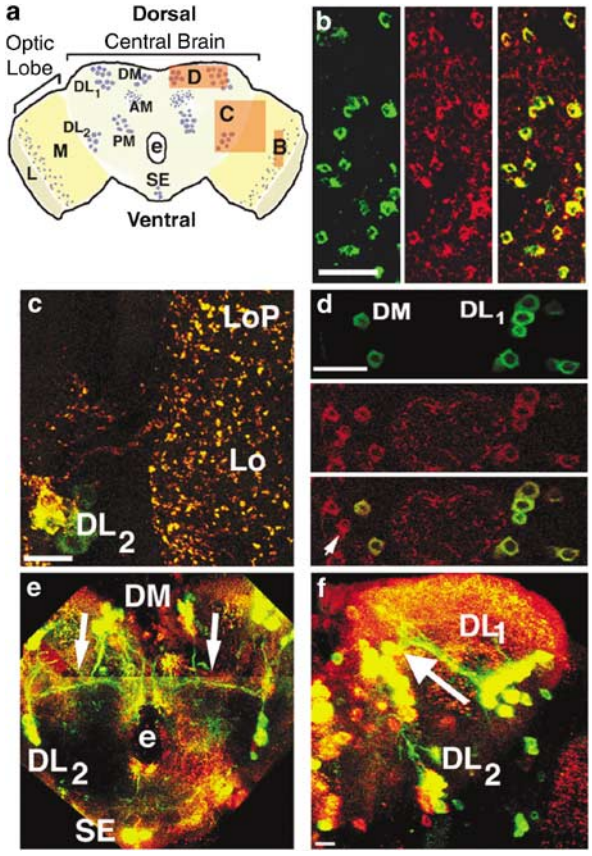
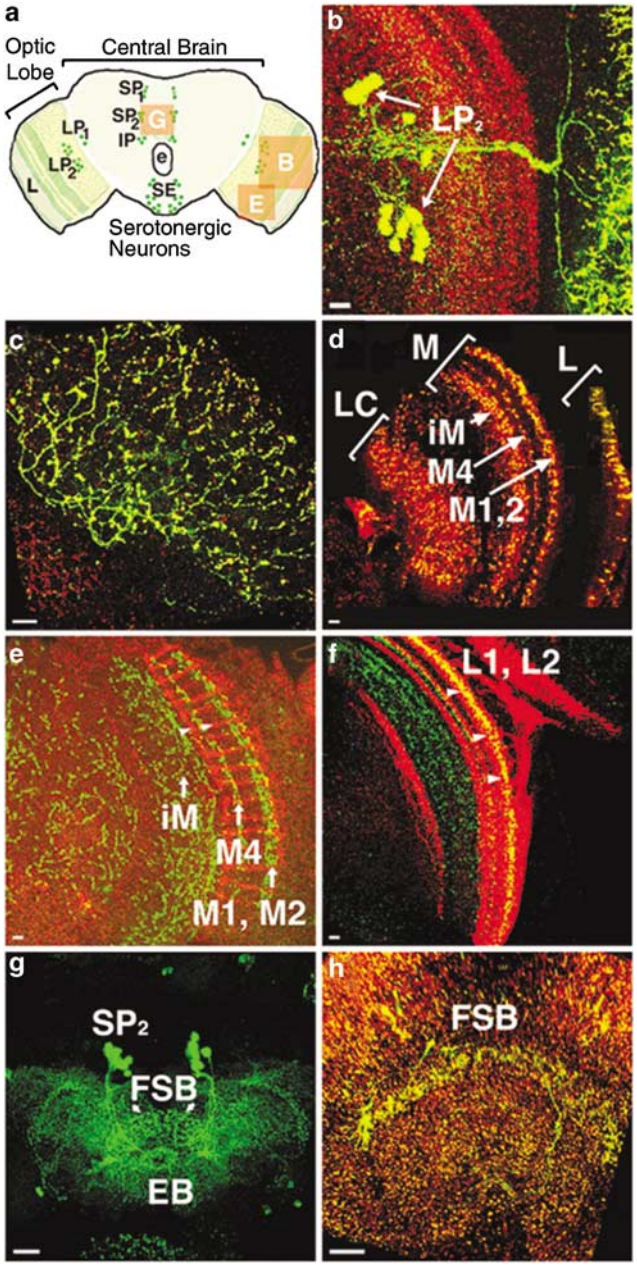


Figure 1 DVMAT is expressed in adult dopaminergic neurons. (**a**) The adult CNS includes the central brain surrounding the esophagus (e) and the bilaterally symmetric optic lobes, which include the lamina (L) and the medulla (M). Blue circles represent dopaminergic cell bodies in the previously identified[44] dorsomedial (DM), dorsolateral (DL_1, DL_2), anterior medial (AM), posterior medial (PM), subesophogeal (s.e.) cell clusters, and a large number of smaller cell bodies in the medulla. The red rectangles indicate areas shown in adjacent panels. (**b–d**) Adult brains were costained with antisera to TH and DVMAT-A, and secondary antibodies conjugated to FITC or Cy3, respectively. (**b**) Confocal, optical sections of the medulla show staining for TH (green, left panel), DVMAT (red, center panel) and merged images (right panel), and indicate coexpression of TH and DVMAT in dopaminergic cells. (**c**) TH and DVMAT colocalize in the DL_2 cluster that projects to the lobula and lobular plate posterior to the medulla. (**d**) Optical sections of cells in the DM and DL_1 clusters show expression of TH (green, top panel), DVMAT-A (red, middle) and colocalization of TH and DVMAT-A in the merged image (bottom panel). Note that several cells medial to the DM cluster stain for DVMAT but not TH (e.g. arrow in bottom panel of **d**), and may be serotonergic. (**e**) Merged images of several optical sections show cells in the DM cluster projecting ventrally, and DL_2 processes projecting dorsally then toward the midline (arrows). (**f**) Processes from DL_1 project medially (along the arrow) toward the DM cell cluster. Scale bars: (**d**), 25 μm, others 10 μm.

Mammalian VMAT2 is responsible for histamine storage in mast cells and is expressed in the histaminergic nucleus in the CNS.[19] We therefore expected that DVMAT would be expressed in fly

photoreceptors that store and release histamine as the primary neurotransmitter.[49] However, we do not detect expression of DVMAT-A in photoreceptor cell processes in the medulla (Figure 2e) or the lamina (data not shown). We also have stained adult and larval brains with an additional antiserum to the amino-terminus of DVMAT, as well as an antiserum to a structurally related vesicular transporter that we have recently identified (data not shown), but neither appear to stain photoreceptor cells. Thus, the vesicular transporter responsible for histamine storage in adult photoreceptor cells remains unclear.



Behavioral effects of inhibiting DVMAT

To determine whether inhibition of DVMAT activity would affect motor behavior in flies, we tested the effects of the drug reserpine, which we have shown inhibits the transport activity of DVMAT-A *in vitro*,[30] and has been used to investigate the function of VMAT orthologs in mammals[50] and *Drosophila*.[51] We observe a similar decrease in locomotor activity (Figure 3a) and grooming (Figure 3b) in flies treated with reserpine, confirming that the storage of monoamines by DVMAT is required for motor behaviors in the adult fly. We also find that treatment of adult flies with reserpine decreases the number of adult progeny produced by wild-type flies at concentrations as low as 1 μM (Figure 3c), consistent with other reports similarly investigating the effects of this and other aminergic agents on fertility.[52,53] In addition, we directly tested the effects of reserpine on larval development by transferring 30 freshly laid embryos to food containing increasing concentrations of drug. Although 1000 μM reserpine had a significant effect

Figure 2 DVMAT expression in serotonergic neurons. (**a**) Green circles in the cartoon indicate serotonergic cells that localize to previously identified clusters[5] in the superior protocerebrum (SP_1, SP_2), the lateral protocerebrum (LP_1, LP_2), the inferior medial protocerebrum (IP) and the subesophageal ganglion (s.e.) ventral to the esophagus (e). Selected serotonergic nerve terminals in the optic ganglia, including the lamina (L), and in the central brain are indicated as green stippling. The shaded rectangles correspond to adjoining panels (**b**, **e** and **g**) showing areas visualized in pupal brains (75% developed) costained with antisera to 5HT and DVMAT-A followed by appropriate fluorescently labeled secondary antibodies and visualized using confocal microscopy. (**b**) A merged image of several optical sections shows the LP_2 cell cluster (arrows) sending axonal projections from the lobula that express both 5HT (green) and DVMAT (red) and colocalize (yellow) in the medulla and the lamina. (**c**) Rotation of a similar image stained for 5HT (green) and DVMAT (red) shows the extensive arborization of the LP_2 projections in the lamina. (**d**) A low-power image of the optic lobe shows colocalization of 5HT (green) and DVMAT (red) in the lamina (L), in the inner medulla (iM), layers M1, 2 and 4 of the outer medulla (M), and more diffusely in the lobular complex (LC). (**e**) Costaining with antisera to DVMAT (green) and the photoreceptor-cell-specific antibody MAb24B10 (red), shows that DVMAT expression in the medulla (arrows) does not localize to photoreceptor R7 or R8 axon terminals (arrowheads). (**f**) A low-power image of the optic ganglia stained with DVMAT (green) and fasciclin II (red), a marker for processes projecting from monopolar neurons (L1, L2, asterisk), shows a broad yellow crescent (arrowheads) due to the close apposition of DVMAT and fasciclin II expressing processes, although DVMAT is not expressed in monopolar cells. (**g**) Serotonergic projections that appear to emanate from the SP_2 cluster innervate the ellipsoid body (EB) and fan-shaped body (FSB) of the central complex and may modulate limb coordination. (**h**) Serotonergic processes in the central body coexpress 5HT (green) and DVMAT (red) with colocalization seen as yellow in this image highlighting the fan-shaped body (FSB). Scale bars, 10 μm.

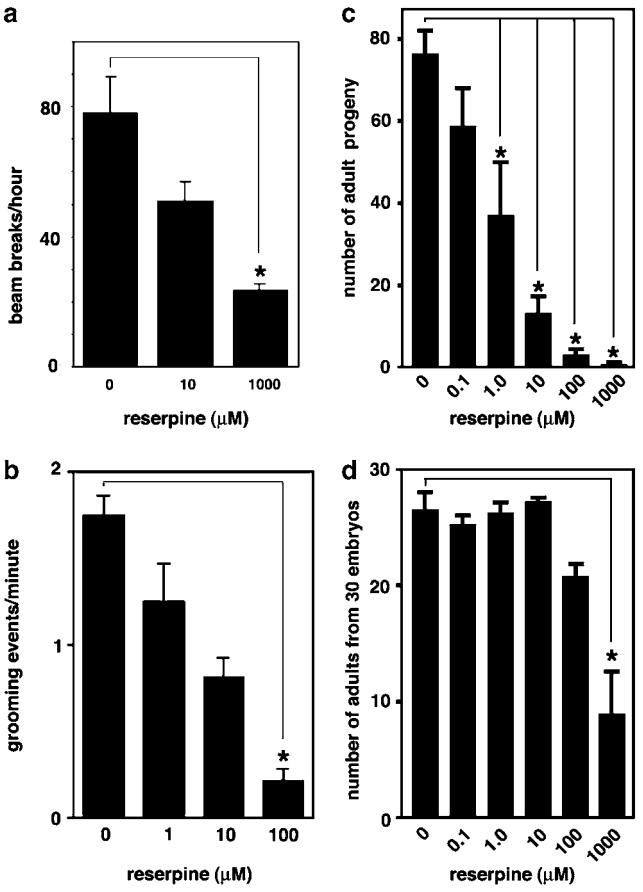


Figure 3 Reserpine decreases motor activity and fertility. (**a**) Flies were fed reserpine (10, 1000 μM) or vehicle alone, and an automated activity monitoring system (Trikinetics) was used to measure locomotion of single flies. The data are plotted as the number of times per hour that flies crossed the photoelectric beam over a 4-hour period of peak activity, mean $\pm$ s.e.m., $n=4$ flies per dose, ANOVA $P=0.0019$, with Dunnett's multiple comparison test $P<0.01$ for the effect of 1000 μM reserpine versus control (*). (**b**) Similar to the effects on locomotion, treatment with reserpine decreased grooming behavior, mean $\pm$ s.e.m., $n=20$ flies per dose, ANOVA $P=0.018$, with Dunnett's multiple comparison test $P<0.01$ for the effect of 1000 μM reserpine versus control (*). (**c**) The number of adult progeny produced by six pairs of parents fed the indicated dose of reserpine or vehicle are shown, mean $\pm$ s.e.m., $n=3$ experiments, with ANOVA $P<0.0001$ and Tukey's multiple comparison test $P<0.001$ for the effects of 1 through 1000 μM reserpine (*). (**d**) In contrast, the number of adults that eclosed from 30 larva raised on food containing reserpine (mean $\pm$ s.e.m., $n=3$ experiments, ANOVA $P=0.0001$) differed from vehicle only at 1000 μM reserpine (*Tukey's multiple comparison test $P<0.001$).

on the number of adult progeny that eclosed ($P<0.001$), lower concentrations did not. This suggests that the change in fertility that we observe at reserpine concentrations of 1–100 μM (Figure 3c) are due to the effects on the adult rather than on larval development. Although these results are surprising in the face of the extensive literature on the requirement for DA in cuticle formation during development,[54] they are consistent with our observation that DVMAT is expressed in embryos only in the nervous system and not in cuticle-forming tissue.[30]

Overexpression of DVMAT and monoamine storage in vivo

To investigate the effects of *increasing* DVMAT function and monoamine release *in vivo*, we generated a series of *UAS-DVMAT-A* transgenic lines capable of overexpressing DVMAT-A protein in a tissue-specific fashion using the GAL4/UAS system.[29] In addition, to facilitate the detection of the DVMAT-A transgene, we have previously inserted an HA epitope-tag into the lumenal loop between the first and second transmembrane domains,[30] a site which does not alter transport activity or trafficking.[30,55,56]

Since our goal in overexpressing DVMAT-A was to increase the vesicular storage and release of DA and 5HT, we first asked whether the transgenic protein targeted to the subcellular organelles that store and release these neurotransmitters. To determine whether the overexpressed DVAMT-A localized to synaptic vesicles, we used glycerol gradient fractionation, an established method for studying the localization of synaptic vesicle proteins in neuroendocrine cells[57] and *Drosophila*.[37] To enhance our ability to detect the HA-tagged transgene, we drove expression in all neurons by crossing *UAS-DVMAT-A* lines with flies containing *elav-GAL4*. Glycerol gradient fractionation of homogenenates from these flies shows that HA-tagged DVMAT cosediments with the synaptic vesicle marker synaptobrevin, thereby indicating that exogenously expressed DVMAT localizes to synaptic vesicles (Figure 4).

We next expressed the DVMAT-A transgene in dopaminergic and serotonergic neurons by crossing *UAS-DVMAT-A* flies to those expressing the dopa decarboxylase (Ddc) promoter linked to GAL4 (*Ddc-GAL4).*[8] *Ddc-GAL4* has been previously shown to drive transgene expression in both dopaminergic and serotonergic cells.[8] Costaining with antibodies to HA and DVMAT-A confirmed these results and shows that HA-tagged DVMAT is expressed in both serotonergic and dopaminergic neurons, consistent with their use of Ddc for transmitter biosynthesis; in contrast, biosynthesis of octopamine does not require Ddc,[58] and we do not detect expression of the HA-tagged transgene in the octopaminergic cells that localize to the midline of the ventral nerve cord (data not shown).

Using the anti-HA antibody to probe Western blots, we detect expression of a $\sim$60 kDa band representing exogenous DVMAT in homogenates from flies overexpressing HA-tagged DVMAT-A, but not in control flies expressing *Ddc-GAL4* or *UAS-DVMAT* alone (Figure 5a). Identical samples probed with the antibody to DVMAT-A show a two-fold increase in the expression in DVMAT overexpressing flies as compared to age-matched controls (Figure 5a, b). To determine whether overexpression of DVMAT simi-

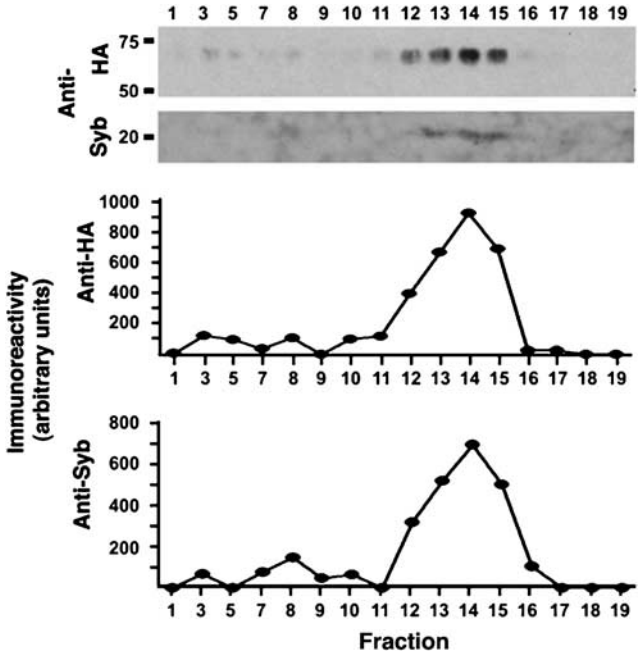


Figure 4 Localization of transgenic DVMAT to synaptic vesicles. Heads isolated from flies homozygous for the *elav-GAL4* driver and *UAS-DVMAT-A* were homogenized and subjected to glycerol gradient fractionation. Fractions were probed on Western blots for HA-tagged DVMAT, or synaptobrevin as indicated, and the resultant bands quantified and shown in the lower panels. HA-tagged-DVMAT cosediments with synaptobrevin, indicating that it localizes to synaptic vesicles.

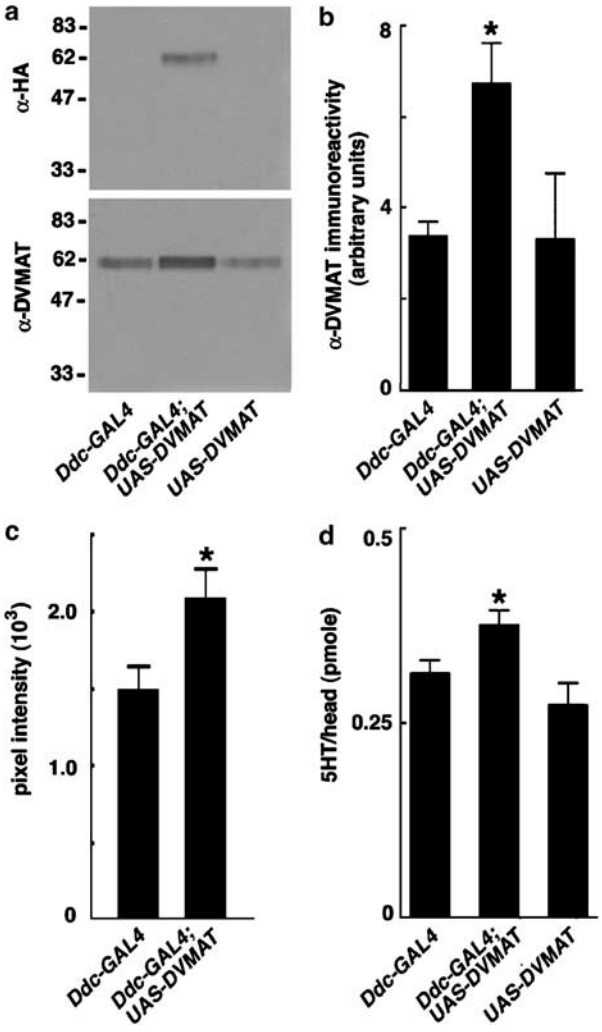


Figure 5 Overexpression of DVMAT increases 5HT storage. (**a**) Western blots using flies homozygous for both *Ddc-GAL4.II* and *UAS-DVMAT.III* (*Ddc-GAL4;UAS-VMAT* in **a–d**) show robust expression of a band immunoreactive to the HA epitope tag encoded in the DVMAT-A transgene (**a**, α-HA, top panel); homozygotes of *Ddc-GAL4.II* or *UAS-DVMAT.III* alone do not. *Ddc-GAL4;UAS-VMAT* flies also show a two-fold increase in immunoreactivity to DVMAT-A relative to control flies (α-DVMAT-A, bottom panel), shown quantified in (**b**), (mean ± s.d., Student's *t*-test, $P<0.05$ relative to *Ddc-GAL4**). (**c**) Immunofluorescent quantitation of 5HT levels using a MAb to 5HT shows a 40% increase in the labeling of serotonergic puncta in the brains of larva homozygous for both *Ddc-GAL4.II* and *UAS-DVMAT.III* (mean ± s.e.m.), $n=21$ larva, 205 individual puncta, relative to *Ddc-GAL4.II* homozygotes, $n=15$ larva, 120 individual puncta, Student's *t*-test, $P=0.037$, (*). (**d**) HPLC analysis indicates that flies homozygous for both *Ddc-GAL4.II* and *UAS-DVMAT.III* (*Ddc-GAL4;UAS-VMAT*) also show a higher level of 5HT/head (mean ± s.e.m., $n=4$ samples, 3 heads/sample) relative to *Ddc-GAL4* ($P=0.049$, Student's *t*-test, two-tailed*) or *UAS-DVMAT.III* homozygotes.

larly increases monoamine storage, we stained larval brains with an antiserum to 5HT, and determined the pixel intensity of stained, serotonergic nerve terminals. DVMAT overexpressing flies show a 40% increase in staining at serotonergic nerve terminals as compared to control flies expressing *Ddc-GAL4* alone (Figure 5c). We were unable to perform similar experiments using an antibody to DA because of high background (data not shown). We also measured serotonin content in fly heads using HPLC and observe a 20% increase in the 5HT content of tissue overexpressing DVMAT (Figure 5d). Similar experiments did not reveal an increase in total tissue DA, but data on tissue content on DA may be difficult to interpret in insects because of the presence of DA in the cuticle.[9,54] Nonetheless, our data indicate that an increase in the expression of DVMAT increases the storage of serotonin. Moreover, they are consistent with previous studies in which overexpression of mammalian VMAT homologs *in vitro* were shown to increase the storage and release of catecholamines.[24,25]

Similar to cocaine, overexpression of DVMAT increases motor behaviors

Pharmacologic studies using cocaine and other aminergic drugs indicate that enhancing aminergic signaling increases both locomotion and grooming in flies, presumably due to an increase in extracellular amine concentrations.[4,6,59] Furthermore, inhibition of DVMAT activity using reserpine decreases locomotor

activity (see Figure 3). These results suggest that an *increase* in DVMAT-A activity might potentiate motor behaviors. Therefore, we tested whether DVMAT-A overexpressing flies would show an increase in locomotion. Indeed, similar to the effects of feeding wild-type flies a low dose of cocaine-HCl for 7 days (1 μg/ml added to standard fly food, Figure 6a), we observe a marked increase in basal locomotor behavior in flies overexpressing DVMAT using *UAS-DVMAT* transgenes on either the second or third chromosomes (*UAS-DVMAT.II* and *UAS-DVMAT.III*, respectively) relative to wild-type flies (wt), and to flies homozygous for either *Ddc-GAL4.II* or *UAS-DVMAT.III* (Figure 6b) or for *UAS-DVMAT.II* (data not shown). The increase in locomotion was most apparent during daylight periods when flies are normally active (data not shown).

In addition to increased locomotor activity, wild-type flies administered volatilized, freebase cocaine show an increase in grooming behavior in which they rub their legs together or over their head and body in a stereotypic manner.[6] We therefore tested whether increasing the vesicular release of DA and 5HT by overexpressing DVMAT would potentiate grooming activity. Indeed, similar to the increase in grooming seen in wild-type flies fed a low dose of cocaine (Figure 6c), DVMAT-A overexpressing flies show a marked increase in the number of grooming episodes compared to controls (Figure 6d) and spend 5- to 10-fold more total time grooming (data not shown). Additional measurements performed with flies homozygous for *Ddc-GAL4* and heterozygous for *UAS-DVMAT* (*Ddc-GAL4.II/Ddc-GAL4.II;UAS-DVMAT.III/+*) show 8 ± 3 grooming events/min, and suggest that grooming behavior is relatively proportional to DVMAT gene-dosage. Flies containing insertions of DVMAT on the second and third chromosomes (*UAS-DVMAT.II* and *UAS-DVMAT.III*, respectively, Figure 6d) and the X chromosome (data not shown) all show an increase in grooming behavior, indicating that this phenotype is not the result of a particular insertional event. We also note that *Ddc-GAL4;UAS-DVMAT* flies do not show defects in cuticle formation or color (data not shown). It is therefore unlikely that the increased grooming that we observe is a result of changes in the

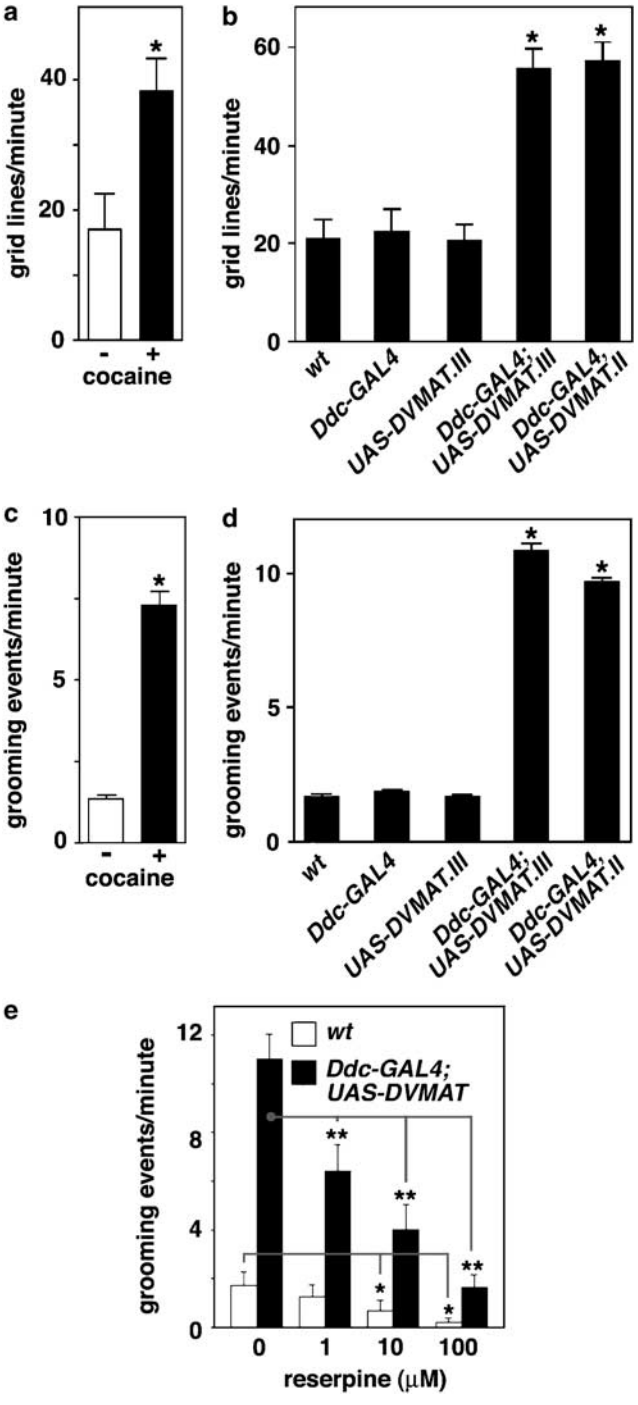


Figure 6 Similar to cocaine, overexpression of DVMAT increases locomotion and grooming. (**a**) Feeding cocaine ('+' cocaine) to wild-type flies increases baseline locomotion as measured by manually counting the number of grid lines crossed per minute (grid lines/min), mean $\pm$ s.e.m., $n = 20$, Student's *t*-test, two-tailed, $P < 0.01$(*). (**b**) Overexpression of DVMAT also causes an increase in locomotion (grid lines/min), ANOVA $P < 0.0001$, with Bonferonni's post-test $P < 0.001$ (*), comparing *Ddc-GAL4.II; UAS-DVMAT.III* or *Ddc-GAL4.II,UAS-DVMAT.II* to either *Ddc-GAL4.II* or *UAS-DVMAT.III* homozygotes or to wild-type flies. (**c**) Cocaine causes a three-fold increase in grooming behavior in wild-type flies, Student's *t*-test, $P < 0.0001$ (*), $n = 36$. (**d**) DVMAT overexpressing flies showed a similar increase in grooming behavior (grooming events/min) mean $\pm$ s.e.m., $n = 20$/genotype, ANOVA $P < 0.0001$, with Bonferonni's post-test $P < 0.001$ (*) for *Ddc-GAL4.II;UAS-DVMAT.III* or *Ddc-GAL4.II,UAS-DVMAT.II* versus either *Ddc-GAL4.II* or *UAS-DVMAT.III* homozygotes, or wild-type flies. (**e**) Treatment with increasing concentrations of reserpine (0–100 μm) showed concentration-dependent inhibition of grooming indicating a requirement for DVMAT transport activity, two way ANOVA $P < 0.0001$ for both genotype and drug treatment, and their interaction, with Bonferenni's post-test $P < 0.001$ for the effects of 10, and 100 μM reserpine versus vehicle alone (0) in wild-type flies (*), and $P < 0.001$ for the effects of 1, 10, and 100 μM reserpine versus vehicle alone (0) in DVMAT overexpressing flies (**).

function of the hypoderm. Indeed, flies homozygous for *elav-GAL4* and *UAS-DVMAT* also show an increase in grooming, indicating that this phenotype is a result of DVMAT overexpression in neurons rather than in the periphery (data not shown).

To confirm that the behavioral effects we observed were due to an increase in DVMAT activity and transmitter storage, we treated wild-type and DVMAT overexpressing flies with reserpine (Figure 6e), which blocks DVMAT transport activity *in vitro*.[30] We find that treatment with reserpine causes a dose-dependent decrease in grooming behavior in *Ddc-GAL4.II; UAS-DVMAT.III* flies overexpressing DVMAT (*Ddc-GAL4;UAS-DVMAT*, Figure 6e). These data indicate that grooming behavior is directly dependent on DVMAT activity and confirm that the behavioral effects we observe are the result of an increase in the vesicular storage and, presumably, release of DA and 5HT. Taken together, our data therefore indicate that an increase in DVMAT-A expression causes an increase in vesicular monoamine release which in turn potentiates motor behaviors, similar to the effects of cocaine and aminergic agonists.[4,6,59]

Overexpression of DVMAT alters additional amine-linked behaviors

In addition to increased grooming and locomotion, the treatment of flies with cocaine impairs negative geotaxis, the instinctive drive of adult flies to climb upwards against gravity.[6] Similar to this behavioral effect of cocaine, we observe that flies expressing two copies of *UAS-DVMAT.III*, and two copies of the *Ddc-GAL4.II* driver show reduced climbing in negative geotaxis assays (*Ddc-GAL4;UAS-DVMAT*, Figure 7a). However, decreasing the dosage of either the driver or the *UAS-DVMAT* transgene eliminates this effect, for example, *Ddc-GAL4.II/+,UAS-DVMAT.III/UAS-DVMAT.III* flies do not show a defect in negative geotaxis (data not shown), allowing negative geotaxis to be used in additional assays that tested the effects of cocaine (see below). We note that *Ddc-GAL4.II;UAS-DVMAT.III* flies appear to be engaged in grooming during negative geotaxis assays (data not shown), which may contribute to this phenotype.

A limited number of previous studies in *Drosophila* support the possibility that adult sexual behavior may be regulated by DA and 5HT release.[10–12] We therefore reasoned that DVMAT-A overexpression might provide a means to further test whether DA and 5HT can modulate sexual behavior. Indeed, we observe an increase in courtship activity including for example, wing song, in which males hold their wings at a stereotypic right angle and vibrate the extended wing, and the pursuit of female flies by the males (tracking behavior); the increase in courtship is shown quantified using male–female tracking behavior (Figure 7b). Interestingly, despite the increase in courtship behavior, male–female pairs of DVMAT overexpressing flies show a relatively low rate of sustained copulation (Figure 7c). Although we observed occasional attempts, these lasted only a few seconds, were terminated by the males dismounting, and often followed by the resumption of courtship behavior. We also observe a decrease in fertility relative to wild-type flies (see Figure 9).

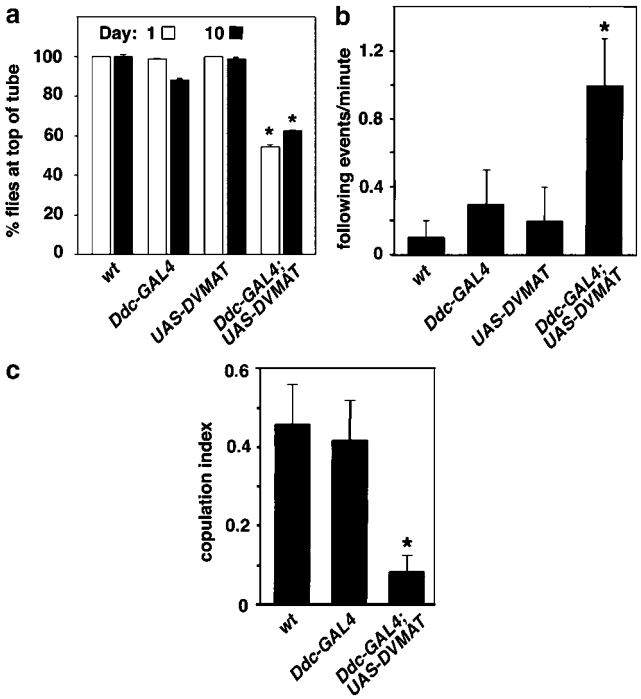


Figure 7 DVMAT overexpression alters additional amine-linked behaviors including courtship. (**a**) Flies homozygous for both *Ddc-GAL4.II* and *UAS-DVMAT.III* (*Ddc-GAL4;UAS-DVMAT)* show a defect in negative geotaxis, ANOVA, $P<0.0001$ for the effects of genotype, with Bonferonni's post-test $P<0.001$ (*) for *Ddc-GAL4.II;UAS-DVMAT.III* versus other indicated genotypes at 1 and 10 days. Each bar represents the mean ± s.e.m. for three experiments, and with thirty flies tested in each experiment. The data are plotted to show the percentage of flies aged for 1 or 10 days after eclosion that climbed to the top of a graduated cylinder in fifteen seconds. (**b**) The frequency of male-female tracking behavior (mean ± s.e.m.) and other elements of courtship (data not shown) are increased in DVMAT overexpressing flies relative to *Ddc-GAL4* or other indicated controls, one way ANOVA, $P<0.001$, with Dunnett's multiple comparison test showing a difference between *Ddc-GAL4.II* versus *Ddc-GAL4.II;UAS-DVMAT.III*, $P<0.05$, but not wild-type or *UAS-DVMAT.III*. (**c**) Individual male-female pairs of DVMAT overexpressing flies (*Ddc-GAL4.II;UAS-DVMAT.III)* are less likely to copulate when placed together in mating chambers than either wild-type (odds ratio 9.3) or *Ddc-GAL4.II* homozygote flies (odds ratio, 7.8, χ^2 using logistic regression, $P<0.005$ for effect of genotype), showing the copulation index (mean ± s.e.m.) as calculated in Materials and methods.

Overexpression of DVMAT decreases the behavioral sensitivity to cocaine

Psychostimulants such as cocaine and amphetamines function by increasing extracellular DA and cause changes in the synaptic signaling machinery.[60]

Furthermore, these changes have been proposed to underlie some of the behavioral effects of chronic cocaine exposure such as sensitization in mammals.[60] Treatment with cocaine also appears to induce sensitization in *Drosophila*, suggesting that an increase in extracellular DA or 5HT in flies might generate similar adaptive changes in aminergic signaling pathways.[6] Since overexpression of DVMAT-A appeared to mimic some of the behavioral effects of cocaine, we speculated that it, too, might also induce adaptive changes at the synapse.

Similar to previous reports using volatilized free-base cocaine,[6] or application of cocaine–HCl to decapitated flies,[59] we find that feeding cocaine–HCl to wild-type flies increases locomotion (Figure 8a, see also Figure 6). In contrast, the motor behavior of DVMAT overexpressing flies appears to be affected much less by this treatment (Figure 8a). Similarly, DVMAT overexpression appears to blunt the increase in grooming induced by cocaine (Figure 8b). Whereas wild-type flies show a dose-dependent increase in grooming, flies overexpressing DVMAT show the same, relatively high rates of grooming despite increasing dosages of drug (Figure 8b).

These results suggest the possibility that DVMAT overexpression causes some form of adaptive change that modifies the fly's response to cocaine. However, since both treatment with cocaine and overexpression of DVMAT cause an increase in motor activity, it is difficult to rule out the possibility that a ceiling effect mitigates the results of these experiments, that is, that flies are unable to locomote or groom at a higher rate than that induced by either cocaine administration or DVMAT overexpression alone, thus making it appear that DVMAT overexpression blocks the effects of cocaine. Therefore, to further explore the effect of DVMAT overexpression on the fly's response to cocaine, we tested the effects of acute, volatilized drug using a previously described assay in which the climbing ability of wild-type flies is *reduced* by administration of cocaine.[6,9] Importantly, in this assay, we measured whether DVMAT-overexpression could *reverse* the inhibition of climbing caused by cocaine and thereby *increase* the relative number of flies observed to climb the side of the testing chamber in these assays. Since the measured behavioral response is opposite to the defect caused by cocaine, the potential for a ceiling effect is less problematic. We find that flies overexpressing DVMAT and containing one copy of *Ddc-GAL4.II* and two copies of *UAS-DVMAT.III* (*Ddc-GAL4/+;UAS-DVMAT*, Figure 8c, bar 4) show significantly less impairment due to cocaine in their ability to climb than control flies expressing *Ddc-GAL4* alone (Figure 8c, bar 1). Flies containing one copy each of *Ddc-GAL4.II* and *UAS-DVMAT.III* (*Ddc-GAL4/+;UAS-DVMAT/+*, bar 3) and overexpressing DVMAT to a lesser extent show a similar trend toward reversing the effects of cocaine, but did not reach statistical significance. All lines showed essentially identical levels of baseline climbing ability in the absence of cocaine (data not shown).

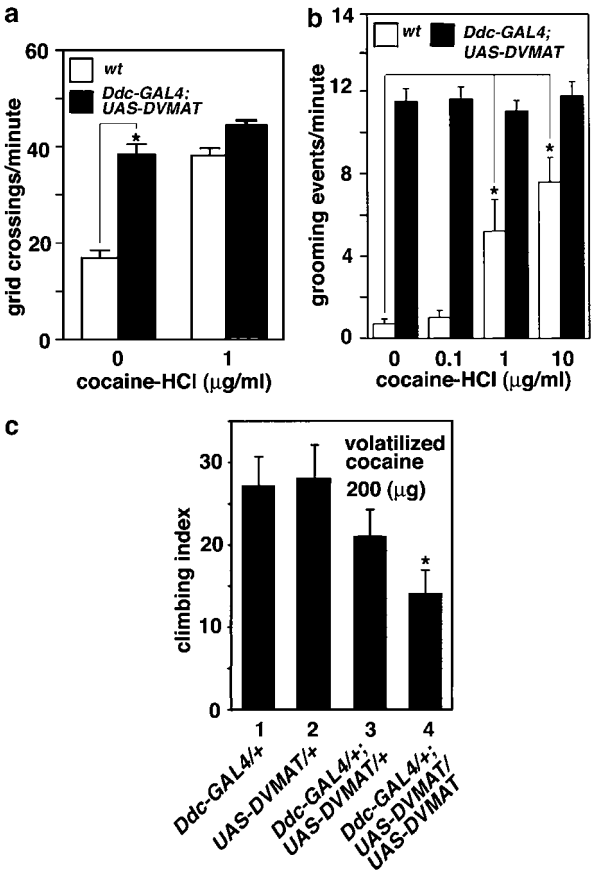


Figure 8 DVMAT overexpression decreases the fly's behavioral sensitivity to cocaine. (**a**) Wild-type flies fed cocaine (1 μg/ml) for 5 days show an increase in motor activity but DVMAT overexpressing flies do not, two-way ANOVA, $P=0.002$ and 0.036 for the effects of drug and genotype, respectively, with Bonferroni's post-test $P<0.05$ for the effects of cocaine on wild-type flies (*), $n=36$ for each condition. (**b**) Wild-type flies fed cocaine show a dose-dependent increase in grooming (mean $\pm$ s.e.m. events per minute), whereas grooming in DVMAT overexpressing flies (*Ddc-GAL4.II;UAS-DVMAT.III*) appears to be unchanged, two-way ANOVA, $P<0.0001$ for the effects of drug, genotype and their interaction, with Bonferroni's post-test $P<0.001$ for the effects of 0.1 and 1 mg/ml cocaine on wild-type flies (*). (**c**) In climbing assays following an acute dose of free-base cocaine (200 μg), flies containing one copy of *Ddc-GAL4.II* and two copies of *UAS–DVMAT.III* (*Ddc-GAL4/+;UAS-DVMAT/UAS-DVMAT*, bar 4) are significantly less sensitive to cocaine, mean $\pm$ s.e.m., $n=12$ flies/genotype, ANOVA $P<0.05$, with Tukey's multiple comparison test $P<0.05$, (*) than flies containing either the *Ddc-GAL4* driver alone (*Ddc-GAL4/+*, bar 1) or *UAS-DVMAT.III* alone, (*UAS-DVMAT/+*, bar 2), that is, DVMAT overexpressing flies show less drug-induced impairment in climbing. Flies containing one copy each of *Ddc-GAL4.II* and *UAS-DVMAT.III* (*Ddc-GAL4/+;UAS-DVMAT/+*, bar 3) show a similar trend toward reversing the effects of cocaine but did not reach statistical significance.

Changes in the behavioral response to DA receptor blockade

In mammalian systems, changes in aminergic signaling can profoundly alter the synaptic response of DA

receptors.[61–63] For example, both chronic and acute administration of DA receptor antagonists can induce changes in the circuitry responsible for motor control; in clinical settings this can result in tardive dyskinesias that are thought to involve adaptive changes in the function of either DA receptors themselves or the downstream signaling machinery.[62,64] To investigate the possibility that DVMAT overexpression might cause adaptive changes that affect the response of DA receptors, we tested the effects of haloperidol, which is widely used as a DA receptor antagonist in mammals and also binds to DA receptors in insects.[2] We find that treatment with haloperidol rescued the increase in grooming seen in DVMAT overexpressing flies (Figure 9a). These data suggest that dopaminergic pathways are involved in grooming behavior, consistent with previous pharmacologic studies using decapitated flies.[4,59] In contrast, grooming in wild-type flies appears to be relatively insensitive to treatment with haloperidol (Figure 9a). The difference between the response of wild-type and DVMAT overexpressing flies to haloperidol supports the idea that DA receptors in DVMAT overexpressing flies may be more sensitive to antagonist and that a chronic increase in vesicular DA release induced by DVMAT overexpression may cause changes in the sensitivity of either the DA receptors themselves or the downstream signaling machinery (see Discussion).

Since previous pharmacologic experiments suggest that sexual behavior and fertility also may involve DA,[11,12,52,53,65] we tested the effects of haloperidol on fertility. Haloperidol administration appeared to increase fertility in DVMAT overexpressing flies. In contrast, haloperidol reduced the fertility of wild-type animals (Figure 9b). Similarly, haloperidol increased the number of eggs laid by DVMAT overexpressing flies, but decreased the number of eggs laid by wild-type flies (data not shown). These differences suggest that a relatively narrow window of dopaminergic neurotransmission is compatible with optimal fertility. Furthermore, similar to the effects of cocaine, these data suggest that DVMAT overexpression and the consequent increase in monoamine release changes the synaptic machinery responsible for aminergic neurotransmission.

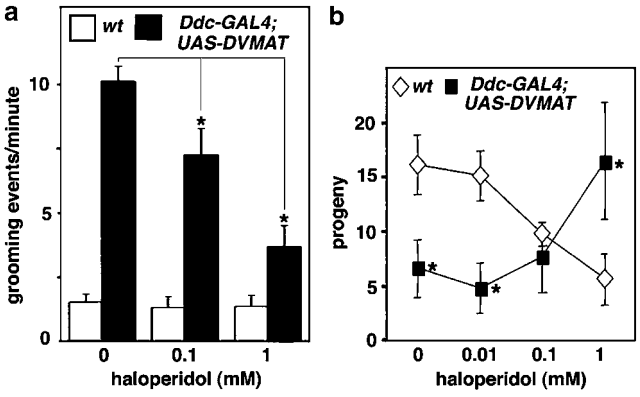


Figure 9 Alterations in the response to a DA receptor antagonist. (**a**) DVMAT overexpressing flies show a dose-dependent decrease in grooming in response to treatment with haloperidol, with two-way ANOVA $P<0.0001$ for the effects of drug, genotype and their interaction, and Bonferroni's post-test $P<0.001$ (*) for the effects of 0.1 and 1 mM haloperidol versus no drug for DVMAT overexpressing flies, but no significant effect of haloperidol on wild-type flies. (**b**) Treatment with haloperidol (0–1 mM) increased the number of adult progeny (black squares, mean ± s.e.m.) produced by DVMAT overexpressing flies (*Ddc-GAL4.II;UAS-DVMAT.III)* but decreased the number produced by wild-type flies (white diamonds), with two-way ANOVA showing a significant interaction between genotype and dosage ($P=0.0086$, $n=3$ experiments) and significant differences between strains ($P<0.05$) at dosages of 0, 0.01, and 1 mM (*).

Discussion

A growing body of literature indicates that the amount of transmitter stored in individual vesicles can vary and that vesicular neurotransmitter transporters regulate the quantity and mode of transmitter storage and release.[24,38,66,67] To exploit this phenomenon to study the behavioral effects of increasing vesicular monoamine release, we have identified a *Drosophila* isoform of VMAT similar to mammalian orthologs (DVMAT-A[30]). We now show that DVMAT-A localizes to most if not all dopaminergic and serotonergic cells in the adult CNS and that pharmacologic inhibition of DVMAT with reserpine inhibits motor activity and fertility. We also find that the behavior of flies overexpressing DVMAT-A is markedly altered, suggesting that upregulation of VMAT expression or activity could have important neurophysiologic consequences *in vivo*.[26,27,68–70] Furthermore, since a variety of GAL4 driver lines are available that target small subsets of neurons, our data suggest that overexpression of DVMAT may be used to map the neuronal circuitry responsible for the response to cocaine and other behaviors in *Drosophila* that are regulated by monoamines. Finally, these results provide the basis for genetic and pharmacologic screens for genes that regulate VMAT and aminergic neurotransmission.

In addition to DVMAT, other genes involved in aminergic neurotransmission are conserved in the fly, including tyrosine hydroxylase and Ddc, and these, too, have proven useful for exploring the effects of monoamines on behavior.[11,12,53,71] However, while the enzymes required for DA synthesis are expressed both in the nervous system and in cuticle-forming tissue, DVMAT is more specifically expressed in neurons.[30] Furthermore, consistent with the notion that DVMAT is not required for cuticle formation, we show here that larval development is relatively insensitive to the inhibition with reserpine, and we suggest that DVMAT may be particularly useful for studying the neuronal functions of monoamines.

Clinical studies indicate that inhibition of VMAT by reserpine can cause a state resembling depres-

sion,[28] and more recently, several labs have created VMAT2 knockout or knock-down mice.[20–23,72] Although VMAT2 knockout homozygotes die soon after birth, heterozygotes are viable as adults, and show an increased locomotor response when challenged with psychostimulants and an increased sensitivity to dopaminergic neurotoxicity.[20–22,72] Mutants in the *Caenorhabditis elegans* homolog of VMAT also show changes in amine-linked behaviors, including a decrease in the animal's ability to slow its movements in response to a food source.[73] Our results complement these studies and demonstrate for the first time in any system that an *increase* in the function of VMAT can generate robust behavioral changes. We emphasize that the behavioral effects we observe are the result of a relatively modest increase in neurotransmitter storage and co-opt normal, physiological mechanisms of neurotransmitter release. This is in marked contrast to psychostimulants such as amphetamines and cocaine, which circumvent normal mechanisms of exocytosis and essentially flood the synapse with neurotransmitter. Thus, our results more specifically indicate that upregulation in the storage and vesicular release of DA and 5HT may have important behavioral consequences.

Our findings also underscore the potential importance of previously described mechanisms for regulating VMAT expression and activity.[68–70,74,75] Furthermore, these results suggest that relatively modest changes in the expression of mammalian VMAT orthologs could have behaviorally significant effects relevant to psychiatric illness. Although no change in VMAT2 expression was detected in patients suffering from Tourette's syndrome or OCD,[76] children with ADHD show a decrease in VMAT2 levels on platelets.[77] In addition, VMAT2 binding in the thalamus and ventral brainstem of bipolar patients is elevated by 31 and 28%, respectively as compared to control subjects.[27] Furthermore, VMAT2 concentrations in these regions correlated with performance on measures of frontal, executive function.[27] In another study, VMAT2 expression in the thalamus was shown to be higher in bipolar disorder patients than in either controls or schizophrenic patients.[26] These results suggest that higher than normal VMAT2 expression may represent a trait-related abnormality in patients with bipolar disorder, and perhaps other psychiatric disorders linked to aminergic systems.

The behavioral effects that we observe in flies in response to increased DVMAT expression include an altered response to cocaine. A number of previous studies have established *Drosophila* as a model system to study the behavioral effects of cocaine.[6–9,59,78] To study how aminergic cells contribute to this response, the *Ddc-GAL4* driver used here was initially developed to ectopically express the $G\alpha_i$ or $G\alpha_s$ subunits in serotonergic and dopaminergic neurons, and thereby decrease or increase DA synthesis, respectively.[8] Expression of $G\alpha_i$ caused hypersensitivity to a challenge dose of cocaine, whereas $G\alpha_s$ resulted in a decreased behavioral response.[8] The effects of $G\alpha_s$ were suggested to be the result of a compensatory downregulation in aminergic signaling, presumably in response to an increase in DA synthesis and release.[8] Similarly, the altered response to cocaine shown by DVMAT overexpressing flies suggests that increasing the vesicular release of transmitter causes adaptive changes at aminergic synapses. One possible mechanism to explain the blunted response to cocaine that we observe would be a decrease in the sensitivity of aminergic receptors.[79] A complementary increase in receptor sensitivity has been previously proposed to explain the increased behavioral response of VMAT2 knockout mice to aminergic drugs.[20–22,72] We find that DVMAT overexpressing flies show an altered response to the DA receptor antagonist haloperidol; however, comparing the effects of haloperidol on grooming in wild-type and DVMAT overexpressing flies suggests that DA receptors in DVMAT overexpressing flies may be more, rather than less sensitive than wild-type flies to DA receptor blockade. We therefore speculate that both the results of previous experiments in VMAT2 knockout mice and our own observations in VMAT overexpressing flies could involve adaptive changes in the function of other synaptic elements, and may include the plasma membrane DA or serotonin transporters.[80]

It remains possible that some of the behavioral effects we observe are due to ectopic expression of DVMAT, and may not be completely attributable to changes in DA or 5HT pathways. Since VMATs do not transport non-aminergic transmitters, it is unlikely that overexpression of DVMAT in glutamatergic, cholinergic or GABAergic cells would have an appreciable effect. However, in addition to DA and 5HT, flies use octopamine, histamine and possibly tyramine as neurotransmitters.[39] Similar to DA and 5HT, overexpression of DVMAT in neurons synthesizing these transmitters is likely to increase their storage and release, and ectopic expression in these cells could conceivably contribute to some of the behaviors we observe. Recently, a GAL4 driver line for expression in octopaminergic neurons has been developed,[81] and a TH-GAL4 line also is available.[82] Future experiments using these flies will be useful to more precisely determine the contribution of DA and 5HT to particular behaviors potentiated by DVMAT overexpression.

Indeed, our results suggest a novel strategy to map the neuronal circuitry in the fly involved in the behavioral response to cocaine and other amine-linked behaviors. The broad range of available GAL4 promoters as well as more complex genetic methods to drive expression in small clonal populations of cells[83] will allow the overexpression of DVMAT-A in restricted subsets of aminergic neurons (see Figures 1 and 2). This in turn may allow the relevant modulatory circuits for particular amine-linked behaviors to be identified in *Drosophila* for the first time. Importantly, most other paradigms used to map

neuronal circuitry in the fly use probes that affect neuronal function more broadly, for example, a temperature-sensitive allele of *shibire*, and a mutant form of a potassium channel *eag* alter synaptic transmission in all neurons in which they are expressed.[84] In contrast, overexpression of DVMAT is likely to only affect neurons that synthesize aminergic neurotransmitters, greatly increasing the relative specificity of each driver. For example, to determine which aminergic neurons cause the increase in courtship that we observe, any number of GAL4 drivers that include, but are not absolutely specific for aminergic cells in the CNS may be employed.[11,12,85,86]

Finally, we suggest that flies overexpressing DVMAT-A may provide a genetic model for the study of amine-linked changes in motor activity and other complex behavior. Importantly, dopaminergic and serotonergic pathways also play a critical role in regulating motor activity and other stereotypic behavioral patterns of mammals,[87] and in humans, aminergic agents are used in treatment of depression, bipolar disorder, obsessive–compulsive disorder and tic-related disorders such as Tourette's syndrome. It is possible that the increase in overall activity and sexual behavior seen in bipolar patients may be related to the increase in VMAT2 expression.[26,27] However, genetic models to explore the molecular mechanisms by which VMAT is upregulated, or may be suppressed, have not been available. We speculate that genetic and pharmacologic screens of flies overexpressing DVMAT may be used to identify novel regulatory pathways for both VMAT and more generally, aminergic neurotransmission. Although flies and vertebrates are neuroanatomically distinct, the molecular pathways involved in dopaminergic and serotonergic signaling are remarkably conserved. We therefore speculate that the use of *Drosophila* genetics and flies overexpressing DVMAT could help improve the range of molecular targets for the pharmacologic treatment of amine-linked, pathologic behaviors in humans.

Acknowledgments

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