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***Drosophila melanogaster* as a genetic model system to study neurotransmitter transporters**

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Abstract

The model genetic organism *Drosophila melanogaster*, commonly known as the fruit fly, uses many of the same neurotransmitters as mammals and very similar mechanisms of neurotransmitter storage, release and recycling. This system offers a variety of powerful molecular-genetic methods for the study of transporters, many of which would be difficult in mammalian models. We review here progress made using *Drosophila* to understand the function and regulation of neurotransmitter transporters and discuss future directions for its use.

Keywords

Drosophila; neurotransmitter transporter; vesicular transporter; VMAT; VGAT; VGLUT; VACht; EAAT; DAT; SERT; GAT; inebriated; SLC6; SLC1; SLC18; SLC17; dopamine; serotonin; GABA; octopamine; glutamate; portabella; acetylcholine; ChT1; VNUT

Introduction

Several features of the model genetic system *Drosophila melanogaster* make it attractive for the study of neurotransmitter transporters. These include a powerful molecular-genetic toolset, a short lifespan, low cost and the availability of an essentially limitless supply of “test subjects”. Here we provide an overview of current studies on neurotransmitter transporters in *Drosophila* and discuss potential uses for this system in the future. By including additional background, we hope to provide a general introduction to the field for both *Drosophilists* and non-*Drosophilists* alike. Conversely, for “fly people” with other

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primary interests, we also provide an overview of neurotransmitter transporters in general and some of the outstanding questions that remain unanswered.

Neurotransmitter Transporters

Neurotransmitter transporters are responsible for the movement across biological membranes of classical neurotransmitters-- the biogenic amines and acetylcholine-- and the amino acid neurotransmitters-- GABA, glutamate and glycine. Plasma membrane and vesicular neurotransmitter transporters represent two distinct activities (Figure 1). Plasma membrane neurotransmitter transporters are responsible for the termination of synaptic transmission and recycling neurotransmitters after they are released (Blakely and Edwards, 2012) (Figure 1).

Vesicular neurotransmitter transporters localize to the membranes of secretory vesicles and are responsible for transport and storage of neurotransmitters into the vesicle lumen (Blakely and Edwards, 2012) (Figure 1). Vesicular transporters are required for the storage of neurotransmitters in synaptic vesicles (SVs) as well as large dense core vesicles (LDCVs), which also store and release peptide neurotransmitters (Fei et al., 2008). However, peptides are not transported into LDCVs via vesicular transporters, but rather packaged into the lumen of the vesicle as it is being formed (Dikeakos and Reudelhuber, 2007). Peptides also do not undergo transport at the plasma membrane. Similarly, “novel” neurotransmitters such as nitrous oxide do not require specific transport proteins since they are synthesized on demand and pass relatively freely through lipid membrane barriers (Boehning and Snyder, 2003). It remains unclear whether lipid-based signaling molecules such as anandamide require specific transporters for movement across biological membranes (Fowler, 2013).

All known plasma membrane and vesicular transporters are members of the Solute Carrier (SLC) family of proteins and share several characteristic features (Blakely and Edwards, 2012). All contain multiple transmembrane domains and are responsible for moving relatively small (~75–200 Daltons) hydrophilic molecules across lipid membranes. As “active” transporters, all use energy to drive the movement of neurotransmitter against a concentration gradient, and as “secondary” active transporters, the energy is supplied by an ion gradient rather than by direct hydrolysis of ATP. By contrast, the activities of “facilitated” transporters allow solute movement down a concentration gradient and do not require additional energy (e.g. (Richter and Hargreaves, 2013)), while primary active transporters directly hydrolyze ATP (e.g. (Dermauw and Van Leeuwen, 2014)

At the plasma membrane, transporters exploit the steep sodium gradient experienced by most eukaryotic cells. The sodium gradient drives the movement of the transporter and is coupled to neurotransmitter flux in a stoichiometrically defined manner, and in some cases with additional ions facilitating transport (Greuer et al., 2014; Kanai et al., 2014; Pramod et al., 2013; Rudnick et al., 2014). Since both sodium and the neurotransmitter move in the same direction at the plasma membrane, this is by definition a “symport” mechanism. Vesicular transporters use a distinct but related “antiport” mechanism in which a proton gradient is used to drive the movement of neurotransmitter in the opposite direction and into the lumen of the secretory vesicle (Parsons, 2000) (Figure 1).

The manner in which the transporters couple the movement of ions and neurotransmitter is not yet fully understood, but biochemical experiments and more recent crystallographic and modeling studies have begun to unravel the underlying mechanisms (Penmatsa and Gouaux, 2014; Zhao et al., 2011). Plasma membrane transporters may also function in an uncoupled or loosely coupled mode characterized by relatively large ionic currents (Fairman et al., 1995; Galli et al., 1997; Schicker et al., 2013). The potential function of these currents in synaptic physiology is not yet clear. Efflux represents an additional functional mode in which neurotransmitter moves out of rather than into the cell (Fleckenstein et al., 2007; Sulzer, 2011). The mechanism by which this occurs is a very active area of investigation, and includes work in *Drosophila* described in more detail below (Pizzo et al., 2013; Pizzo et al., 2012).

Vesicular transporters generally localize to secretory vesicles in the presynaptic neurons that synthesize and release neurotransmitter (Blakely and Edwards, 2012). Plasma membrane transporters for biogenic amines also localize to presynaptic neurons (Blakely and Edwards, 2012). By contrast plasma membrane glutamate and GABA transporters may be expressed in either neurons or glia (Figure 1) depending on their subtype (Zhou and Danbolt, 2013). We discuss the localization of specific *Drosophila* transporters below.

A circumscribed set of phylogenetic families encodes the plasma membrane and vesicular transporters (see Tables 1 and 2). Glutamate or Excitatory Amino Acid Transporters (EAATs) are members of SLC1; this family also contains neutral amino acid transporters (Greuer et al., 2014; Kanai et al., 2014; Sheldon and Robinson, 2007; Yang et al., 2009). All other known plasma membrane transporters, including those for GABA and the biogenic amines, are members of SLC6 (Pramod et al., 2013; Rudnick et al., 2014). SLC6 also includes a number of amino acid transporters, some of which are specific for insects, as well as a few remaining “orphans”, whose substrate is not known (Boudko, 2012; Boudko et al., 2005; Caveney et al., 2006). Acetylcholine is hydrolyzed before reuptake and there is no plasma membrane acetylcholine transporter. Rather, following ACh hydrolysis, choline is transported into the presynaptic neuron by a high affinity choline transporter, a member of SLC5 (Ferguson et al., 2004; Ribeiro et al., 2006).

Vesicular transporter subfamilies include SCL32, of which VGAT is the lone member (Schioth et al., 2014). SLC18 includes vesicular transporters for the biogenic amines and acetylcholine (Lawal and Krantz, 2013) and SLC17 includes the vesicular glutamate transporters as well as other transporters with divergent functions such as organic anion uptake at the plasma membrane (Reimer, 2013). Another member of SLC17 was recently identified in mammals as a vesicular ATP or nucleotide transporter (VNUT) (Sawada et al., 2008); a possible VNUT ortholog is present in the fly genome but has not yet been characterized (see Table 1).

Current Research Topics

Current research on neurotransmitter transporters includes investigations into the precise mechanisms by which they move solutes across membranes. Although some of the binding sites for ions and the neurotransmitters have been mapped, much remains unclear. At

present, we primarily have a static structural view of the binding sites, with only modeling able to capture the moment-to-moment movement of substrates (Penmatsa and Gouaux, 2014; Zhao et al., 2011). A surprisingly large number of commonly used drugs are known to bind to plasma membrane neurotransmitter transporters, yet their mechanism of action also remains incompletely understood. Many currently used antidepressants are Strongly Serotonergic Reuptake Inhibitors (SSRIs; e.g. fluoxetine/Prozac) and primarily inhibit the serotonin transporter (SERT), while others target the noradrenaline transporter (NET) or both NET and SERT (Hamon and Blier, 2013). Most psychostimulants, including cocaine, amphetamines and MDMA/ecstasy also interact with neurotransmitter transporters acting either as inhibitors or, in the case of amphetamines, to promote efflux (Fleckenstein et al., 2007; Sulzer, 2011). Importantly, drugs that act to block transport use a mechanism different from those that cause efflux and the nature of these differences remains a very important area of investigation (Fleckenstein et al., 2007; Foster et al., 2013; Kahlig et al., 2005; Sulzer, 2011). The downstream effects of both antidepressants and psychostimulants and the way they exert their therapeutic and/or addictive effects remains obscure. The developmental regulation of transport function and the potential effects of pharmacologically disrupting this activity is another fascinating topic (Daubert and Condron, 2010). The rapid development of the fly and the relative simplicity of its nervous system may be advantageous for these studies (Daubert and Condron, 2010).

A number of current investigations focus on the interplay between transporters and regulatory pathways. Transporters are essential elements of a network of interacting genes required for neurotransmitter homeostasis and behavior (see e.g. (Talkowski et al., 2008)), and a few elegant biochemical studies have highlighted direct protein-protein interactions and relatively direct regulatory processes (Bauer et al., 2012; Binda et al., 2008; Brunk et al., 2006; Sager and Torres, 2011; Winter et al., 2005; Yang et al., 2009). However, compared to our knowledge of the complex web of proteins implicated in receptor-mediated signaling, our understanding of transporter interactions and their regulation is limited.

Pathways critical for regulating the function of neurotransmitter transporters include those required for trafficking within the cell. Localization of vesicular transporters to different subsets of secretory vesicles has the potential to dramatically influence the site and amount of presynaptic neurotransmitter release. Some of the signals and machinery responsible for this process have been determined, but much remains unclear (Colgan et al., 2007; Foss et al., 2013; Grygoruk et al., 2010; Hua et al., 2011; Santos et al., 2013; Yao and Hersh, 2007). Current questions include the physiological function of vesicular transporter co-localization to the same vesicles in some cells. This phenomenon has important ramifications for both the possible co-release of neurotransmitter as well as the regulation of the electrochemical gradients that drive transport activity (Guzman et al., 2011; Hnasko and Edwards, 2011). *Drosophila* VMAT and VGLUT are co-expressed in at least some nerve terminals (Greer et al., 2005) but co-release has not been investigated in the fly. The trafficking of plasma membrane transporters through the secretory pathway and their regulated internalization is a particularly active and exciting area of study, and a number of the intrinsic signals required for internalization as well as the protein kinases that regulate these processes have been identified (Bauer et al., 2012; Chen et al., 2013b; Gabriel et al., 2013; Madsen et al., 2012;

Sakrikar et al., 2012; Vina-Vilaseca and Sorkin, 2010). The increasingly refined understanding of these events may help determine the mechanism of action of psychostimulants, as well as providing fundamental information about synaptic physiology.

Some research questions are particularly well suited to fly models. These include structure-function analyses in vivo and the relationship between transporter function and disease processes. Variation in transporter expression may function as markers for disease and in some cases may play a pathogenenic role (Hahn and Blakely, 2007; Lawal and Krantz, 2013; Yang et al., 2009). In addition, risk alleles for human disease have been associated with some neurotransmitter transporters (Adamczyk et al., 2011; Barwick et al., 2012; Hahn and Blakely, 2007; Hamilton et al., 2013; Jen et al., 2005; Mazei-Robison et al., 2008; Veenstra-VanderWeele et al., 2012), however the mechanisms by which these variants cause downstream phenotypic changes remains poorly understood. More generally, it remains unclear how variations in transport activity or regulatory processes may effect synaptic transmission and behavior. The powerful molecular genetic tools available in the fly coupled with their surprisingly complex array of behaviors provide an excellent platform to address these questions.

Although we focus here on *Drosophila*, many of the same principles apply to *C. elegans*, an equally important invertebrate model with a similarly large array of powerful molecular and genetic tools. As highlighted below, comparative studies in other insects including locusts, bees and moths represent another valuable source of information about the function and regulation of neurotransmitter transporters.

A Brief Fly Neurobiology Primer

To discuss the molecular genetics of fly transporters it is useful to first briefly describe a few characteristics of fly neurobiology and terms that may be obscure to those outside the *Drosophila* research community. After fertilization and egg-laying, embryos develop rapidly over the course of 24 hours, through 20 relatively distinct stages (Hartenstein and Wodarz, 2013; Menon et al., 2013). After hatching from the egg, larvae mature over ~5 days through three successive stages known as instars. Following a ~5 day pupal stage, the adult or “imago”, ecloses from the pupa. A dramatic reorganization of the nervous system and body plan occurs during pupation and includes the addition of adult-specific organs from discreet packets of stem cells known as imaginal discs. Both neurons and glia are present in both larval and adult nervous systems. These include cells that make up the glial sheath, which surrounds the nervous system at both stages and, acts as both a diffusion barrier and a site for active xenobiotic removal, analogous to the blood brain barrier (BBB) in mammals (DeSalvo et al., 2011; Mayer et al., 2009). The BBB and the glial sheath also express a similar complement of transporters for sugars, mono carboxylic acids and neutral amino acids, supporting the idea that they serve similar functions (R. Bainton, personal communication).

The structure of most neurons in flies and other insects differs somewhat from their canonical form in mammals. In flies, a single process emanates from the soma and gives rise to both dendrites and axons. This structure facilitates the organization of insect neurons into

glomeruli or ganglia in which both axons and dendrites project into a central neuropil, with the somata forming a surrounding cortical rind (Figure 2). Fly neurons support action potentials and calcium mediated release at the nerve terminal similar to mammals, but most terminals in the fly possess a characteristic presynaptic specialization known as a T-bar, around which synaptic vesicles (SVs) cluster. Large dense core vesicles (LDCVs), which release aminergic neurotransmitters and neuropeptides do not cluster near T-bars and few if any T-bars are found in terminals that contain primarily LDCVs either centrally (Karsai et al., 2013) or peripherally (Atwood et al., 1993; Jia et al., 1993).

Neuroanatomy

A complete description of fly neuroanatomy is beyond the scope of this review. However, a brief description of some particularly salient features may be useful for understanding the effects of mutations in neurotransmitter transporters. Although variation within individual cell clusters exist (Sykes et al., 2004), neuronal somata and processes are generally localized to stereotypic positions. The embryonic and larval central nervous systems include the segmented ventral nerve cord and the bulbous, anterior structure variously referred to as “the larval brain,” or the “fused subesophageal and supraesophageal ganglia”. Since the central nervous systems of both the larva and adult are small (on the order of 100 microns), most immunolabeling studies employ confocal imaging of the entire brain. Another common preparation for immunolabeling and electrophysiological studies is the larval fillet, which exposes the abdominal musculature and the neuromuscular junction (NMJ) (Jan and Jan, 1976).

Excellent resources for learning the neuroanatomy of the adult fly are available online http://www.virtualflybrain.org/site/vfb_site/home.htm. Here we describe a few well-characterized structures that may be encountered by the reader in studies of neurotransmitter transporters (Figure 3). These include the optic ganglia (lamina, medulla, lobula and lobula plate) (Melnattur and Lee, 2011; Menon et al., 2013) and the antennal lobes (a critical component of the olfactory system) (Masse et al., 2009; Mu et al., 2012; Wilson, 2013). The function and anatomy of two anatomically distinct structures in the “central brain” have been extensively studied: the mushroom bodies (MBs) shown to be critical for learning, memory and the integration of sensory information (Heisenberg, 2003), and the central complex (including the fan shaped body, ellipsoid body, protocerebral bridge and noduli), important for the coordination of movement (Strauss, 2002) and an increasing number of other behaviors (Lebestky et al., 2009; Liu et al., 2012a; Seelig and Jayaraman, 2013; Ueno et al., 2012).

A Brief Fly Genetics Primer

The growing number of molecular-genetic tools available in *Drosophila* are described in detail elsewhere (Bier, 2005; Frank et al., 2013). Here we provide a brief description of the Gal4/UAS expression system (Brand and Perrimon, 1993), a tool used in a number of studies on neurotransmitter transporters (see below). In the Gal4/UAS system, a promoter sequence of choice drives expression of the yeast-derived Gal4 transcriptional activator (Figure 4) (Brand and Perrimon, 1993). The Gal4 protein binds to its cognate Upstream

Activating Sequence (UAS) to express a cDNA of choice (e.g. for GFP, Figure 4). Expression of the Gal4 protein in a specific temporal and spatial pattern dictated by the chosen promoter will thus express the transgene in a highly specific manner. RNAi interference constructs can be similarly expressed in specific cell types using this system. The bipartite nature of the system allows any lines containing a Gal4 “driver” transgene to be paired with any other line containing a UAS transgene. Thousands of Gal4 and UAS lines are available including those representing specific neurotransmitter systems. These include *dVGLUT-Gal4* (Daniels et al., 2008), *dVGAT-Gal4* (Fei et al., 2010), *TH-Gal4* (Friggi-Grelin et al., 2003), *Tdc2-Gal4* (Cole et al., 2005), *TrH-Gal4* (Alekseyenko et al., 2010; Park et al., 2006) and *Ddc-Gal4* (Li et al., 2000) for glutamate, GABA, dopamine, serotonin, octopamine+tyramine, and dopamine+serotonin neurons respectively.

Additional, specialized UAS transgenes may be used to either activate or disrupt the function of specific populations of cells. For example, the fly ortholog of dynamin, *shibire* (*shi*), is required for endocytosis as well as other vesicle budding events (Kitamoto, 2001; Koenig and Ikeda, 1989; Poodry et al., 1973). The temperature sensitive (ts) mutant *shi^{ts}* can be shifted to a non-permissive temperature to block endocytosis and deplete the pool of SVs (Kitamoto, 2001). Another useful transgene encodes tetanus toxin (TNT), which cleaves SNARE proteins and thus blocks exocytosis (Frank et al., 2013). Optogenetic and temperature sensitive probes capable of activating or inactivating electrical activity in subpopulations of neurons are also available (Frank et al., 2013). Importantly, both optogenetic probes and TNT will affect exocytosis of both SVs as well as LDCVs, the latter containing both classical and peptide neurotransmitters. By contrast, transgenes or mutations that alter the function of neurotransmitter transporters or synthetic enzymes do not share this potentially confounding effect. Genetic reagents based on neurotransmitter transporters may become increasingly useful to specifically assess the affects of amines, GABA and glutamate in the fly “connectome.”

Thousands of mutations and useful chromosomal abnormalities are available in the fly (St Johnston, 2013). Remarkably, many of the first reported *Drosophila* mutants such as *white* are still in use. Point mutations have been traditionally generated using chemical mutagens such as the DNA alkylating agent Ethyl Methyl Sulfate (EMS) (St Johnston, 2013). Inversions, duplications and larger deletions have been traditionally generated using X-rays. More recently, mutations as well as small deletions have been generated using mobile, transposable elements. Several types of mobile elements have been used, including “P” elements, the first mobile elements to be identified in the fly (Bachmann and Knust, 2008; Engels, 1992). Mobile elements can cause mutations either by “hopping” into a gene, or by hopping out. Hopping out can be precise, leaving no trace of the insertion. Mutations are generated when transposons hop out via “imprecise excision”, either removing a portion of genomic DNA or leaving behind a portion of the transposable element. Of note, some types of mobile elements only generate precise excisions, and mutations can be generated only when they hop into a gene. Knock-out technology is also available in the fly (Rong and Golic, 2000), but the large number of existing mutations (and perhaps newer technologies such as CRISPR/Cas9 (Gratz et al., 2013)) may alleviate the need for de novo knockouts.

The nomenclature for *Drosophila* mutations is somewhat idiosyncratic. Alleles with no detectable function are labeled nulls whereas mutants that possess small or large amounts of residual activity are known as strong and weak “hypomorphs” respectively (Muller, 1932). The historic precedent of using the phenotype of the mutation to name the gene can also be confusing to those more familiar with other genetic systems. In the current molecular age, it may seem odd that a gene required for the transport of red and brown eye pigments is named *white*.

Drosophila Neurotransmitters and Their Metabolism

Drosophila use many of the same neurotransmitters as mammals including GABA, glutamate, acetylcholine and some of the same monoamines including dopamine, serotonin and histamine. Similar to mammals, GABA is synthesized via Glutamic Acid Decarboxylase (GAD) and GABA functions as an inhibitory transmitter (Lee et al., 2003). Ach is synthesized from choline and an activated acetyl moiety via Choline Acetyl Transferase (ChAT) (Kitamoto et al., 1998). The genomic organization of the cholinergic locus is phylogenetically conserved, with the vesicular acetylcholine transporter (VAcHT) contained within an intron of ChAT (Kitamoto et al., 1998). Both glutamate and acetylcholine act as excitatory transmitters, but unlike mammals, glutamate rather than ACh is released at the fly NMJ (Jan and Jan, 1976). In addition, glutamate in flies can also function as an inhibitory transmitter through activation of a glutamate-gated chloride channels (Liu and Wilson, 2013; Rohrbough and Broadie, 2002). Unlike mammals, flies and other insects do not appear to further process dopamine to noradrenalin or adrenalin, but rather use the structurally similar neurotransmitters tyramine and octopamine (OA) (Roeder, 2005), both classified as trace amines in mammals (Borowsky et al., 2001). OA and tyramine contain one rather than two hydroxyl (OH) groups on the phenyl ring and thus are classified as phenolamines rather than catecholamines. Unlike DA, synthesis of OA and tyramine does not employ tyrosine hydroxylase or Dopa decarboxylase. Rather, both are converted from tyrosine by Tyrosine decarboxylase (Tdc) (Cole et al., 2005) and in case of OA, an additional hydroxylation step catalyzed by Tyramine β Hydroxylase (T β H) (Monastirioti et al., 1996).

Neurotransmitter metabolism in the fly has not been documented as thoroughly as in mammals. In particular, the role of monoamine oxidation remains unclear. Although a monoamine oxidase (MAO) homolog is absent from the fly genome (Roelofs and Van Haastert, 2001), MAO-like activity has been reported (Chaudhuri et al., 2007; Wang et al., 2011) and some drugs active as MAO inhibitors in mammals also show activity in the fly (Yellman et al., 1997).

Unlike mammals, conjugation to β -alanine plays a role in histamine and dopamine metabolism in the fly. Interestingly, some of same biosynthetic and conjugation pathways used for cuticle formation and pigmentation are also used in neurotransmitter synthesis and degradation (Wright, 1987). One of the earliest identified fly mutants was *ebony*, which generates a darker cuticle than wild type (Wittkopp et al., 2003). *Ebony* has since been shown to encode a β -alanyl-dopamine synthetase and to include both histamine and DA as substrates (Borycz et al., 2002; Hovemann et al., 1998; Richardt et al., 2002). In addition to

metabolism of DA in the cuticle, *ebony* may also metabolize DA released as a neurotransmitter in the central nervous system (Suh and Jackson, 2007). In the visual system, *ebony* conjugates histamine to alanine to generate carcinine (Borycz et al., 2002). Since *ebony* is expressed in glia, histamine and its metabolites are presumably transported in and out of both glia and neurons, but the details of this process are poorly understood (Edwards and Meinertzhagen, 2010).

Based on the localization of the plasma membrane transporters, exocytotically released glutamate and GABA are likely to be taken up primarily into glia in flies, similar to the prominent role of glia in recycling glutamate and GABA in mammals (see below). Localization of DA and 5HT plasma membrane transporters on aminergic neurons suggests, that as is in mammals, these amines are recycled in part into presynaptic cells, although other, less specific transport pathways for amines are also possible in flies as in mammals (Daws, 2009).

***Drosophila* Vesicular Neurotransmitter Transporters**

dVAcHT

VAcHT was the first vesicular neurotransmitter transporter to be molecularly identified in the fly (Kitamoto et al., 2000). *dVAcHT* mutants include *dVAcHT¹* which is embryonic lethal, and the weaker allele *dVAcHT²* which survives through the second larval stage but locomotes slower than wild type animals (Kitamoto et al., 2000). Similar to VAcHT knock-down mice, heterozygous *dVAcHT* mutants survive to adulthood and show subtle phenotypic differences from controls. In particular, electrophysiological analysis of adult *dVAcHT* heterozygotes suggest that during periods of sustained vesicle release, at least one circuit in the adult CNS fails to maintain normal levels of ACh release (Kitamoto et al., 2000). This phenomenon may be related to changes in ACh release observed during periods of sustained release at the cholinergic NMJ of frogs (Naves and Van der Kloot, 1996; Van der Kloot, 2003) and in VAcHT knock-down mice (Lima et al., 2010; Rodrigues et al., 2013). The mechanisms underlying these changes may include the presence of variable numbers of VAcHT molecules per SV and a consequent reduction in the amount of transmitter stored in each vesicle (Prado et al., 2013). In addition, it is likely that under conditions of reduced VAcHT expression, some vesicles are essentially empty (Prado et al., 2013). The contribution of each mechanism may vary depending on the rate of SV recycling and remains an outstanding unanswered question (Prado et al., 2013).

dVGLUT

Prior to its molecular characterization, biochemical studies suggested vesicular glutamate transport activity was present in the fly (Tabb and Ueda, 1991). Unlike mammals, which have three distinct VGLUT genes, *Drosophila* contains only a single VGLUT ortholog, *dVGLUT* (Daniels et al., 2004). *dVGLUT* is expressed in all glutamatergic neurons in the larva and adult fly including the glutamatergic motoneurons innervating the larval NMJ (Daniels et al., 2004; Daniels et al., 2008). The membrane topology of *dVGLUT* has been examined using a series of epitope tags predicted to reside on the luminal and cytosolic loops between transmembrane domains as well as the cytosolic N- and C-termini (Fei et al.,

2007). Deletion of the cytosolic C-terminus abrogates lethality caused by dVGLUT over-expression, clearly implicating an important functional role for this domain (Grygoruk et al., 2010) and some critical trafficking motifs reside in the C-termini of mammalian VGLUTs (Foss et al., 2013; Voglmaier et al., 2006). However, the baseline localization of dVGLUT to SVs appears to be surprisingly unaffected by deletion of the C-terminus (Grygoruk et al., 2010) and recent studies of mammalian VGLUTs have identified trafficking motifs in both the N- and C-termini (Foss et al., 2013; Voglmaier et al., 2006). It is possible that the contribution of specific domains to VGLUT trafficking could vary between cell types or between species.

Post-synaptic excitatory potentials representing individual vesicles (minis) have been used to examine neurotransmitter content in those vesicles capable of glutamate storage and release in embryonic hypomorphic *dVGLUT* mutants (Daniels et al., 2006). Mini frequency was significantly lowered, suggesting that empty vesicles can fuse with the plasma membrane, consistent with other mammalian studies. More surprisingly, the size of the remaining minis-- an indication of SV filling-- was not altered in the mutant in comparison to controls, thus suggesting that a reduced number of VGLUT molecules, and perhaps only one, may suffice to fill a vesicle under these conditions (Daniels et al., 2006). Using other techniques, recent estimates of VGLUT copies per vesicle in wild type mammalian preparations have ranged from 4 to 14 (Mutch et al., 2011; Takamori et al., 2006). One electrophysiological study in VGLUT1 knock-out mice suggested a reduction in mini size (Wojcik et al., 2004), while another did not (Fremeau Jr et al., 2004). Unlike flies, the presence of multiple VGLUT isoforms in mammals complicates these analyses (Takamori et al., 2006). Although there is no evidence for oligomerization of vesicular transporters, it is possible that a single functional unit is composed of multiple molecules. It is also possible that multiple copies of vesicular transporters may be needed to increase the rate of filling during rapid recycling, with fewer copies sufficient for neurotransmitter release under some conditions (Blakely and Edwards, 2012). These questions remain an important topic in studies of vesicular transporters.

Over-expression of dVGLUT at high levels using relatively strong Gal4 drivers is larval lethal. Over-expression using a weaker driver allows survival through adulthood (Daniels et al., 2011). Interestingly, the surviving adults show large lacunae in their CNS, possibly the result of glutamate-mediated excitotoxicity (Daniels et al., 2011). Consistent with the observation that dVGLUT over-expression causes functional changes in SV homeostasis, electrophysiological studies of dVGLUT over-expression in larva show an increase in quantal size, as well as large spontaneous events at the larval NMJ (Daniels et al., 2004; Daniels et al., 2011), a phenotype similar to that seen with over-expression of vesicular transporters in mammals (Edwards, 2007; Wilson et al., 2005).

dVMAT

In contrast to the mammalian genome which contains two distinct VMAT genes (Liu et al., 1992), flies contains only one (Greer et al., 2005). The primary structures of dVMAT and mammalian VMATs are similar throughout the 12 predicted transmembrane domains, the regions likely to be responsible for substrate recognition and transport (Greer et al., 2005).

Indeed, neurotransmitter substrate specificity and relative affinity of dVMAT are generally similar to mammalian VMATs. For example, dVMAT is also inhibited by the drug reserpine at sub-micromolar concentrations (Greer et al., 2005).

dVMAT encodes at least two splice variants, including dVMAT-A and -B which differ at their C-termini (Greer et al., 2005). dVMAT-A is expressed in both larvae and adults in all dopaminergic, serotonergic and octopaminergic cells (Greer et al., 2005). Neither dVMAT-A nor B appear to be expressed in the otherwise well-characterized histaminergic photoreceptor cells of the adult eye (Chang et al., 2006), and the vesicular transporter responsible for the storage of histamine in the SVs of histaminergic photoreceptors is not known. It also is not known whether dVMAT is used for DA storage in other non-neuronal tissue, such as “crystal cells”, which are present in *Drosophila* hemolymph (blood) and release DA for melanotic scab formation (Galko and Krasnow, 2004; Rizki and Rizki, 1984).

The cytosolic C-terminus of dVMAT-A is similar to mammalian orthologs, whereas the C-terminus of dVMAT-B is divergent (Greer et al., 2005). The C-terminus of dVMAT-A, but not dVMAT-B, also contains endocytosis motifs active in vitro in S2 cells and in vivo ((Greer et al., 2005; Grygoruk et al., 2010) and manuscript under review). Both dVMAT-A and B contain a large amino terminal domain that is not conserved in mammals (Greer et al., 2005). Interestingly, this region contains a number of polyproline motifs that could conceivably play a role in protein-protein interactions, but this has not yet been tested.

In contrast to dVMAT-A and other VMAT orthologs, dVMAT-B is expressed in glia rather than neurons, and more specifically, in a small subset of glia in the adult optic lobes where its function appears to include the storage of histamine (Romero-Calderón et al., 2008). Although mammalian glia likely take up amines via low affinity mechanisms (Dahlin et al., 2007; Yoshikawa et al., 2013), the expression of a specific amine transporter in glia is clearly unusual. Further studies of dVMAT-B may help determine the role of glia in the recycling of histamine and perhaps other biogenic amines.

The *dVMAT* mutant shows a number of behavioral deficits consistent with the loss of exocytotic amine release in the nervous system (Chen et al., 2013a; Simon et al., 2009). Similar to mouse knockouts, mutant tissue levels of amines are dramatically reduced, presumably due to the degradation of amines that are not sequestered in secretory vesicles (Fon et al., 1997; Simon et al., 2009). Under standard fly culture conditions, loss of *dVMAT* is lethal, but reducing the density of the culture results in 100% viability, thus allowing behavioral assays. *dVMAT* mutants show a dramatic decrease in baseline larval locomotion (Simon et al., 2009), mirroring previously demonstrated effects of reserpine (Pendleton et al., 2000). *dVMAT* mutants also show a reduced startle response, reduced male courtship (Simon et al., 2009) and increased sleep (Nall and Sehgal, 2013). Treatment of adult mutant flies with reserpine also increases sleep (Nall and Sehgal, 2013). In contrast, amphetamines decrease sleep in flies (Andreatic et al., 2005), presumably via release of DA (Pizzo et al., 2013). In each case, changes in sleep were distinguished from changes in locomotion using previously established criteria (Hendricks et al., 2000; Shaw et al., 2000).

The Gal/UAS system was used to express dVMAT in subpopulations of aminergic neurons and thereby determine which aspects of the mutant phenotype result from loss of specific aminergic circuits (Chen et al., 2013a). Consistent with other reports on the function of OA signaling in flies and other insects (Adamo et al., 1995; Bacon et al., 1995; Fox et al., 2006; Koon et al., 2011; Lee et al., 2009; Monastirioti et al., 1996), expression of dVMAT in OA cells was required for rescue of baseline larval locomotion, female fertility, and the initial phase of the startle response (Chen et al., 2013a). In contrast, the later recovery phase of the startle response depends on serotonergic signaling since expression of dVMAT in 5HT cells rescued this deficit (Chen et al., 2013a). Male courtship could be rescued via expression in DA cells, consistent with recent studies using other genetic reagents to determine the function of DA circuits in the fly (Alekseyenko et al., 2010). The restoration of *dVMAT* function in smaller subsets of neurons in a null background may be used to map the identity of aminergic neurons responsible for other complex behaviors. Importantly, relatively broad Gal4 drivers can be used for these studies since, in general, *dVMAT* transgenes will only be active in cells in which amines are endogenously synthesized.

Current molecular-genetic techniques in *Drosophila* are particularly well-suited to in vivo over-expression studies, making it possible to determine how increased expression of a vesicular transporter may affect behavior. Flies over-expressing dVMAT in DA and 5HT cells (Li et al., 2000) survive through adulthood (Chang et al., 2006). The resulting changes in behavior, presumably caused by an increase in DA and 5HT release, include an increase in motor activity and a blunted behavioral response to cocaine (Chang et al., 2006). Similarly, administration of cocaine results in increased motor activity in the fly (Bainton et al., 2000; McClung and Hirsh, 1998; Torres and Horowitz, 1998). Similar to amphetamines (Andretic et al., 2005), over-expression of dVMAT in 5HT+DA cells also increases male courtship behavior (Chang et al., 2006).

Importantly, the effects of dVMAT and dVGLUT over-expression demonstrate that the electrophysiological changes previously seen with over expression of vesicular transporters in vitro (Pothos et al., 2000; Song et al., 1997; Wilson et al., 2005) can have significant downstream behavioral sequelae in an intact organism. This observation has important ramifications for drugs potentially able to increase vesicular transporter activity (see Future Directions below).

Over-expression of dVMAT as well as loss of function alleles have been used to explore the role of DA homeostasis in vivo in neurodegenerative processes relevant to Parkinson's disease (PD) (Lawal et al., 2010; Sang et al., 2007). DA has a high oxidative potential and may also conjugate to proteins implicated in genetic forms of PD (Conway et al., 2001; Hastings et al., 1996). Thus, it has been suggested that an increase in VMAT activity may have neuroprotective effects (Guillot and Miller, 2009). This has been tested in vitro in cell cultures using mammalian VMAT and dVMAT (Mosharov et al., 2009; Park et al., 2007), and in vivo in the fly (Inamdar et al., 2013; Lawal et al., 2010; Sang et al., 2007). Over-expression of dVMAT rescues the loss of DA neurons caused by either a genetic or chemical insult (Inamdar et al., 2013; Lawal et al., 2010; Sang et al., 2007). Conversely, loss of dVMAT activity increases the toxicity of both genetic and chemical insults to DA cells (Inamdar et al., 2013; Lawal et al., 2010; Sang et al., 2007). Parallel experiments have not

yet been reported in rodents. However, a mouse VMAT2 knockdown showed baseline loss of DA cells (Caudle et al., 2007) and some hVMAT2 alleles may decrease the risk of PD in patients (Glatt et al., 2006). It is therefore possible that over-expression of VMAT2, or drugs that increase VMAT2 activity, could show neuroprotective effects in mammalian models and perhaps patients at risk for PD.

dVMAT-A serves as a useful model for membrane trafficking to secretory vesicles. Mutation of signals in the C-terminus blocks endocytosis in cultured cells, and also disrupts trafficking to SVs in vivo (Grygoruk et al., 2010). Ongoing experiments will determine how these and other signals affect trafficking to SVs and LDCVs in neurons. Genetic rescue experiments in which these and other *dVMAT* mutant transgenes are expressed in a mutant *dVMAT* background provide a convenient method for analyzing the in vivo effects of these alleles without generating a large number of knock-in lines. Importantly, this type of in vivo structure-function analysis provides an opportunity to determine the effects of altered dVMAT trafficking on behavior. Similar studies on *Drosophila* dopamine transporter (*dDAT*) point mutants have been used to determine the behavioral effects of N-terminal phosphorylation and an hDAT mutant linked to autism (Hamilton et al., 2013; Pizzo et al., 2013; Pizzo et al., 2014). This method of analysis is likely to be useful for the study of a variety of other transporters (see Future Directions below).

dVGAT

Similar to mammals, the *Drosophila* genome contains a single vesicular GABA transporter gene (*dVGAT*) (Fei et al., 2010). *dVGAT* appears to be expressed in all GABAergic neurons in the larva, since it precisely co-localizes with GABA in the ventral nerve cord, and is also expressed in most, if not all adult GABAergic neurons (Enell et al., 2007; Fei et al., 2010). It is not clear whether *Drosophila*, like mammals, use glycine as a neurotransmitter and whether dVGAT could also serve to store glycine. It is also possible that dVGAT could play a role in β -alanine storage or metabolism in the fly although this has not been tested. The structure of β -alanine is intermediate between glycine and GABA and mammalian VGAT has been shown to transport β -alanine in vitro (Juge et al., 2013). As noted above, conjugation of β -alanine to histamine is required for histamine recycling in the fly (Borycz et al., 2002), and conjugation of β -alanine is also important for DA metabolism in both the cuticle and the central nervous system (Suh and Jackson, 2007; Wright, 1987).

Mutation of *dVGAT* causes developmental lethality (Fei et al., 2010). However, inducible expression of a *dVGAT* transgene has been used to restore dVGAT function during development and thereby allow adult behavioral studies. One adult phenotype demonstrated in the conditional rescue line was a surprisingly specific defect in the detection of small objects in the fly's visual field (Fei et al., 2010). Other aspects of reduced GABAergic signaling in this line remain to be explored.

portabella

Drosophila and some other insects express *portabella*, an additional vesicular transporter that appears to be absent from mammalian genomes (Brooks et al., 2011; Lawal and Krantz, 2013). The gene name was based on its robust expression in MBs (See Neuroanatomy

above) (Brooks et al., 2011). Prt is expressed in the intrinsic neurons of the MBs, the Kenyon cells (KCs) (Brooks et al., 2011). Surprisingly, the neurotransmitter stored and released by KCs is not known. The primary structure of the prt protein is most similar to DVMAT and it is possible that the substrate is also similar to known monoamines; however, the biosynthetic enzymes for DA, 5HT, OA and histamine are not expressed in KCs, suggesting that prt may transport a novel neurotransmitter (Brooks et al., 2011). In the absence of an identified substrate, a genetic approach was used to test the hypothesis that prt functions as a transporter. Mutation of residues conserved in prt and VMAT ablated the ability of a *prt* transgene to rescue the *prt* mutant phenotype (Brooks et al., 2011). These data support the idea that prt is likely to function as a bona fide transporter and that the substrate may be similar to known biogenic amines.

Mutation of *prt* results in a peculiar defect in sexual behavior, characterized primarily by an inability of males to maintain their position during copulation (Brooks et al., 2011). The mutants are nonetheless fertile (Brooks et al., 2011). Expression of prt using a Gal4 driver that is prominently expressed in MBs restores these behavioral defects (Brooks et al., 2011). MBs are best known for their role in learning and memory and the *prt* mutant shows a defect in olfactory learning, however the phenotype is relatively subtle (Brooks et al., 2011; Kahsai and Zars, 2011; Roman and Davis, 2001). It is possible that compensatory changes in the *prt* mutant mitigate its effect on learning. Alternatively, the release of a peptide, rather than a classical neurotransmitter, may be more important for some functions of KCs (Iliadi et al., 2008; Knapek et al., 2013).

Plasma membrane transporters

dSERT

The *Drosophila* serotonin transporter (*dSERT*) was the first plasma membrane neurotransmitter transporter to be identified in the fly (Corey et al., 1994; Demchyshyn et al., 1994). These studies revealed that the substrate specificity of dSERT diverged from mammalian orthologs, including a lower affinity for some antidepressants such as citalopram but a higher affinity for the mammalian DAT antagonist mazindol (Corey et al., 1994; Demchyshyn et al., 1994). Transport via dSERT also appeared to show a less stringent requirement for chloride than hSERT (Demchyshyn et al., 1994). Although one report suggested that the affinity of cocaine for dSERT was significantly higher than hSERT (Corey et al., 1994), another suggested similar affinities for both orthologs (Demchyshyn et al., 1994). Interestingly, the SERT ortholog that is expressed in the tobacco hornworm *Manduca sexta* is more similar to dSERT than hSERT but has an extremely low affinity for cocaine and most antidepressants (Sandhu et al., 2002).

Extensive structure-function comparisons of hSERT and dSERT have been performed using heterologous expression of hSERT/dSERT chimeras in cultured cells. These studies revealed the importance of a tyrosine residue (Y95 in hSERT, F90 in dSERT) in TM1 for some pharmacologic differences including the differential binding to citalopram (Adkins et al., 2001; Barker et al., 1998) Other sites in transmembrane domains 5–9 contribute to the apparent inability of dSERT to transport the neurotoxin MPP⁺ (Rodriguez et al., 2003).

Additional modeling studies have suggested an interaction between Y95 and E444 (Combs et al., 2011).

dSERT RNA is expressed late in embryonic developmental (stage 15) just before 5HT can be detected (stage 17) (Demchyshyn et al., 1994). In situ hybridization of larva shows a pattern consistent with the stereotyped localization of serotonergic neurons in larva (Giang et al., 2011) and adult brain (Thimman et al., 2006). The *dSERT* gene is predicted to be less than 4 kB and to encode two non-coding splice variants <http://flybase.org/cgi-bin/gbrowse/dmel/?Search=1;name=FBgn0010414>. Northern blots confirm the presence of at least two bands likely to represent mature mRNA species (Romero-Calderón et al., 2007).

A specific antiserum to dSERT has been used to map protein expression in embryo, larvae and adults (Giang et al., 2011). All cell bodies in the larva and adult that express dSERT also co-label with an antiserum to 5HT and vice versa (Giang et al., 2011). However, some processes in the adult brain, including those in the MB lobes, express dSERT but do not detectably label for 5HT. The function of dSERT at these sites is not clear but could conceivably involve uptake of a transmitter other than 5HT (Larsen et al., 2011), and perhaps the substrate of prt (see above).

Immunocytochemistry and fast scan cyclic voltametry have helped demonstrate the importance of reuptake mechanisms for 5HT clearance in *Drosophila* (Borue et al., 2010; Borue et al., 2009). Pharmacologic inhibition of amine uptake increased the time required for 5HT clearance (Borue et al., 2010; Borue et al., 2009) in larvae and reduced the pool available for release following repetitive stimulation (Borue et al., 2010; Borue et al., 2009).

A mutation in *dSERT* has been generated by Henrike Scholz's lab (University of Cologne, Germany). A phenotype characterization is now in progress and will determine how a potential increase in extracellular 5HT might affect fly behavior. Pharmacologic manipulation of 5HT release using amphetamine derivatives (fenfluramine and 3,4-methylenedioxymethamphetamine a.k.a. MDMA or 'ecstasy') has already demonstrated that increased extracellular 5HT causes enlarged varicosities in 5HT processes (Daubert et al., 2010). By contrast, direct application of exogenous 5HT decreases the number of varicosities in 5HT processes in the central neuropil (Sykes and Condrón, 2005), and loss of 5HT (and DA) synthesis in *Ddc* mutants results in increased branching of serotonergic processes (Budnik et al., 1989). The mechanisms underlying these phenomena remain unclear, but support the idea that both excessive and inadequate levels of extracellular 5HT, and possibly dSERT, activity could have significant developmental effects (Daubert and Condrón, 2010; Lesch and Waider, 2012). Further studies using *dSERT* transporter transgenes and mutants are likely to be useful to explore this issue. Importantly, the availability of a *dSERT* mutant will now allow in vivo structure-function studies in which specific mutations can be expressed in a loss of function background.

Further studies may also help to determine whether proteins other than neurotransmitter transporters are required for amine uptake and storage in 5HT cells. Mammalian thalamocortical projections appear to take up and release exogenous 5HT in the absence of cell-autonomous synthesis (Lebrand et al., 1996; Lebrand et al., 2006). In contrast, in the fly,

exogenously applied 5HT did not appear to be taken up or stored in non-aminergic cells ectopically expressing dSERT (and dVMAT) (Giang et al., 2011; Park, 2006). Although speculative, it is possible that additional factors beyond the transporters themselves are required for amine uptake and storage.

dDAT

The *Drosophila* dopamine transporter (*dDAT*) was identified via homology-based cloning and exhibits a kinetic profile similar to that of hDAT (Porzgen et al., 2001). In situ hybridization experiments showed that *dDAT* expression matched the pattern in larva previously established for DA neurons (Porzgen et al., 2001). Northern blots show a single mRNA species of 3–4 kB (Kume et al., 2005; Romero-Calderón et al., 2007) and in situ of in adult heads partially match the localization DA cells, although not all known DA cells were detectably labeled (Thimgan et al., 2006). As with all other DAT orthologs, dDAT-mediated transport is sodium dependent and able to support efflux as well as sodium-coupled transport (Porzgen et al., 2001). The affinity of dDAT for cocaine was noted to be relatively low compared to hDAT and dSERT (Porzgen et al., 2001). However, in vivo studies have shown that dDAT is likely to be required for at least some of the behavioral effects of cocaine (Bainton et al., 2000) and inhibition of dDAT by cocaine prolongs DA uptake in vivo (Makos et al., 2010; Vickrey et al., 2013).

Real time, in vivo measurements of DA have been made with carbon fiber electrodes using both fast scan cyclic voltametry in larvae (Vickrey et al., 2009) and amperometry in adults (Berglund et al., 2013; Makos et al., 2010). Both cocaine and nisoxetine block reuptake of endogenous DA in larvae (Vickrey et al., 2009). In the adult fly, clearance of exogenously applied dopamine was decreased by cocaine, amphetamine, methamphetamine, and methylphenidate (Ritalin). Additional experiments using a *dDAT* mutant demonstrated that these effects on DA uptake were mediated by dDAT, rather than another transporter such as dSERT (Makos et al., 2010).

The *dDAT* mutant was originally isolated in a screen for sleep defects and the *dDAT* gene was renamed *fumin* (*fmn*), meaning sleepless in Japanese (Kume et al., 2005). Whereas wild type flies follow a diurnal circadian rhythm, *fmn* mutants are active almost continuously over each 24-hour period. Moreover, after sleep deprivation they show no rebound need for rest (Kume et al., 2005; Ueno et al., 2012; Wu et al., 2008). *fmn* mutants also show an enhanced response to mechanical stimuli (Kume et al., 2005). The pathways mediating the sleep defect effect in *fmn* have been mapped using genetic interactions with the DA receptor, DopR (Ueno et al., 2012), which is similar to mammalian DA1 receptors (Kim et al., 2007; Liu et al., 2012b). These data indicate that that DA processes innervating the adult central complex help promote sleep (Ueno et al., 2012).

Mutations in *dDAT* have also been used to study the mechanism of action of amphetamines (Pizzo et al., 2013; Pizzo et al., 2014). By disrupting the function of membrane rafts in DA cells, the authors elegantly showed that localization of dDAT to these sites is required for efflux but not for transport in vivo (Pizzo et al., 2013) consistent with in vitro studies (Cremona et al., 2011). These studies also demonstrated that the behavioral effects of amphetamines in the fly are mediated through DA neurons rather than another aminergic

cell type (Pizzo et al., 2013; Pizzo et al., 2014). Further genetic rescue experiments used expression of a dDAT phosphoacceptor site mutant in the *dDAT* loss of function background to show that phosphorylation of the dDAT N-terminus is required for efflux in vivo (Fog et al., 2006; Khoshbouei et al., 2004; Pizzo et al., 2013). In addition, a dominant negative form of CamKII was used to demonstrate the specific requirement of this kinase for phosphorylation-mediated efflux through dDAT (Pizzo et al., 2014).

Another elegant study of *dDAT* used genetic rescue of the mutant to test the behavioral effects of a rare *de novo*, non-synonymous mutation (T356M) detected in a patient with autism (Neale et al., 2012). Importantly, the rarity of some *de novo* mutations in many genes can make it difficult to conclusively evaluate their phenotypic impact on the basis of human genetic studies alone. Thus, additional models to test the potential effect(s) of such mutants are quite useful. When expressed in cultured cells, the T356M mutation caused sustained constitutive efflux in the absence of amphetamines. When expressed in vivo in a wild type *dDAT* background, the mutant caused a genetically dominant increase in locomotion in adult flies similar to the effects of the *dDAT* loss of function *fmn* mutant (Hamilton et al., 2013). These data strongly suggest that physiologically significant effects can result from the T356M mutation and that this allele is unlikely to represent an innocuous polymorphism unrelated to disease.

Risk alleles associated with other transporters may also be investigated in this way. To our knowledge, only one mouse model containing a risk allele in hSERT for Attention Deficit Hyperactive Disorder (ADHD) has been generated (Veenstra-VanderWeele et al., 2012). The phenotypic effects of other possible risk alleles in SERT are not known (Ye and Blakely, 2011) and might be explored in the fly.

Octopamine uptake and metabolism in flies vs. other insects

OA is sometimes referred to as the invertebrate noradrenaline, since its structure differs by only a single ring oxygen and it serves some similar functions (Roeder, 2005). OA is a substrate of dVMAT (Greer et al., 2005) and the fly expresses an adrenergic-like OA receptor gene *OAMB* and three β -adrenergic-like receptors *Oct β 1R*, *Oct β 2R* and *Oct β 3R* (Evans and Maqueira, 2005). However, a plasma membrane OA transporter has not been identified in the fly. Surprisingly, a high affinity OA transporter has been identified in the cabbage looper *Trichoplusia ni* (*T. ni*) and several other insect species (Donly et al., 2007; Malutan et al., 2002), but is absent in *Drosophila* and other *Dipteran* species as well as bees (*Hymenoptera*) (Donly and Caveney, 2005). *T. ni* also express a high affinity DA transporter (Gallant et al., 2003). TriDAT and TriOAT show similar affinities for DA, but OA and tyramine inhibit TrnOAT to a greater extent than TrnDAT (Gallant et al., 2003). In the absence of an OAT ortholog, it remains unclear how OA is recycled in *Drosophila*. It is conceivable that dDAT functions as both an OA and DA transporter in the fly but this remains speculative.

dEAAT1

Drosophila EAATs were independently identified by three different groups (Besson et al., 1999; Kawano et al., 1999; Seal et al., 1998). *Drosophila* EAAT1 (CG3747) shares 41 and

35% amino acid identity, respectively, with human EAAT1 and EAAT2 (Besson et al., 1999). dEAAT2 is also similar, but current data suggest that it may not function as a glutamate transporter as we discuss below. We note that the naming of the dEAATs was based on their order of identification rather than correspondence to specific mammalian orthologs. dEAAT1 is sodium-dependent and shows high affinity for L-glutamate, as well as L- and D- aspartate (Seal et al., 1998). The sodium:glutamate stoichiometry of the homologous TriEAAT1 is 2: 1 (Donly et al., 1997), similar to mammalian EAATs (Wadiche et al., 1995). Also, similar to some mammalian EAATs (Fairman et al., 1995) dEAAT1 shows loosely coupled chloride channel-like activity (Seal et al., 1998).

Four non-coding *dEAAT1* mRNA splice variants are predicted, but their functional relevance is not known <http://flybase.org/cgi-bin/gbrowse/dmel/?Search=1;name=FBgn0026439>. In the embryo and larva, dEAAT1 mRNA appears to be confined to the nervous system and robustly expressed in glia, with little if any expression in neurons (Besson et al., 1999; Freeman et al., 2003; Soustelle et al., 2002; Stacey et al., 2010). *dEAAT1* is co-expressed with the well-characterized glial marker repo but some repo-positive cells do not express *dEAAT1* (Soustelle et al., 2002). dEAAT1 expression in adult glia has been confirmed using a dEAAT1 antiserum (Rival et al., 2006) in addition to a *dEAAT1-Gal4* driver (Rival et al., 2004; Stacey et al., 2010). *dEAAT1-Gal4* expressing cells co-label for the marker Prospero, indicating that a large majority of dEAAT1-expressing cells are of the anterior LG subtype of glia (Stacey et al., 2010). Surprisingly, dEAAT1 is not expressed in glia (or neurons) at the larval glutamatergic NMJ (Rival et al., 2006). Rather, uptake of glutamate at the larval NMJ is likely to be mediated by the cystine/glutamate exchanger encoded by the gene *genderblind* (Grosjean et al., 2008).

The reduction of *dEAAT1* expression in adult glia using RNA interference (RNAi) was reported to shorten life span, increase sensitivity to oxidative stress and cause neural degeneration (Rival et al., 2004), however, RNAi experiments in a later report showed less severe effects (Rival et al., 2006). In these later experiments, electrophysiological recordings from adult flight muscles showed broadening of peaks representing individual depolarization events, consistent with the idea that decreased glutamate uptake can have significant post-synaptic effects in flies as well as mammals (Rival et al., 2006).

Mutations in the *dEAAT1* gene were recently reported and are lethal at approximately 48 hours post-hatching (Stacey et al., 2010). Two mutant alleles were identified, including *dEAAT1^{SM1}* and *dEAAT1^{SM2}*, both likely to be null alleles since the start codon and the first two transmembrane domains were deleted and no expression of *dEAAT1* mRNA was detected. Additionally, the phenotypes of the homozygous mutants were equivalent to each other and to heterozygotes containing a mutant allele over a large chromosomal deletion (Stacey et al., 2010). We note that this type of genetic assay, rather than RNA or protein expression, is the gold standard for determining whether a *Drosophila* mutant is a true null allele.

The survival of the *dEAAT1* mutants as 1st instar larvae allowed a further phenotypic analysis (Stacey et al., 2010). Unlike wild type larvae that move almost continuously, *dEAAT1* homozygous mutants were nearly immobile with occasional slow contractions of

the body wall musculature (Stacey et al., 2010). In contrast to findings in RNAi studies (see above), no obvious morphologic or neurodegenerative defects were detected in the mutant (Stacey et al., 2010).

Additional functional studies of dEAAT1 may help determine how glutamatergic signaling is regulated and the potential effects of dysregulation. Mutations in human EAAT1 have been identified in a rare form of episodic ataxia (de Vries et al., 2009; Jen et al., 2005) and in collaboration with another lab (Joanna Jen, UCLA) we are currently testing the potential pathogenic effects of these mutations in *Drosophila*.

dEAAT2

Despite the similarity between dEAAT1 and dEAAT2, the latter may not function as a high affinity glutamate transporter (Besson et al., 2000; Besson et al., 2005; Besson et al., 2011). Similar to other EAAT orthologs, dEAAT2 transports aspartate, but its comparative affinity for glutamate is relatively low (Besson et al., 2000). Surprisingly, EAAT2 also transports taurine (aminoethane sulfonic acid), a β -amino acid whose physiological role in all species remains poorly understood (Besson et al., 2005; Besson et al., 2011). Some structures in the insect brain such as the MBs are labeled by antisera to taurine (Bicker, 1991; Strausfeld et al., 2003) and a role for taurine as a neurotransmitter has been suggested, but this remains incompletely defined (Wu and Prentice, 2010). Rather, taurine may function primarily as an osmolyte in the CNS and other tissue (Chesney et al., 2010; Schaffer et al., 2010). In mammals, taurine uptake is mediated by a distinct taurine transporter (Prasad et al., 2013), which appears to be absent in the fly and other insects (Besson et al., 2011). Consistent with an *in vivo* role for dEAAT2 in taurine uptake, mutations of *dEAAT2* exhibit dramatically decreased tissue content of taurine (Besson et al., 2011). The mutants also showed mild defects in the behavioral response to saline and propionic acid, but phototaxis and locomotion were similar to wild type controls; levels of aspartate and glutamate were also similar to controls (Besson et al., 2011).

In the embryo, *dEAAT2* mRNA is expressed in sensory organs in the peripheral nervous system and midline glia (Freeman et al., 2003; Soustelle et al., 2002). An antibody to dEAAT2 is not available but a *dEAAT2*-GAL4 construct has been used to localize expression in conjunction with a *UAS-GFP* marker. In contrast to *in situ* data, expression using the Gal4 driver was predominantly neuronal (Besson et al., 2011). However, the authors acknowledged that the 4.5-kb promoter fragment used to make the *dEAAT2*-GAL4 transgene could be incomplete and additional studies might uncover expression in some subsets of glia.

dGAT

A *Drosophila* GABA transporter (GAT) cDNA was identified prior to the sequencing of the fly genome (Neckameyer and Cooper, 1998) and corresponds to the single GAT ortholog present in the genome (CG1732). In parallel, similar GAT orthologs were identified in the tobacco hornworm *Manduca sexta* (Mbungu et al., 1995) (*MasGAT*) and the cabbage looper moth *Trichoplusia ni*. (*TrnGAT*) (Gao et al., 1999). All three insect orthologs are similar in primary structure to mammalian GAT-1. The pharmacology of cloned *MasGAT* and

TrnGAT have been investigated in vitro using heterologous expression systems and, similar to mammalian orthologs, both are sensitive to the mammalian GAT inhibitor DL-2,4-diaminobutyric acid (DABA) (Gao et al., 1999; Mbungu et al., 1995). In contrast to mammalian GATs, neither MasGAT nor TrnGAT were significantly inhibited by β -alanine. Nipecotic acid showed partial inhibition at high concentrations but significantly lower affinity for insect versus mammalian GATs (Gao et al., 1999; Mbungu et al., 1995).

The effects of both DABA and nipecotic acid have been tested in *Drosophila* membranes derived from homogenized embryos, larvae, pupae and adult flies (Leal et al., 2004; Leal and Neckameyer, 2002). The homogenates showed saturable uptake of tritiated GABA and inhibition by DABA and nipecotic acid; however, the required concentrations of inhibitors were higher than those used in assays of cloned transporters (Leal et al., 2004; Leal and Neckameyer, 2002). Feeding DABA to larva and adults or feeding nipecotic acid to adults disrupted several aspects of motor behavior (Leal et al., 2004; Leal and Neckameyer, 2002). At least some effects of DABA and nipecotic acid, such as disruption of negative geotaxis in the adult were reversible over the course of 2 days, which might prove useful for further behavioral studies. Although most insect GABA receptors (including RDL) are insensitive to the GABA_A receptor antagonist bicuculline (Buckingham et al., 2005) the behavioral effects of DABA could be reversed with this drug (Leal and Neckameyer, 2002). It was also surprising that the GABA analog gabapentin rescued the effects of DABA (Leal and Neckameyer, 2002), since the phenotype caused by blockade of GABA reuptake might be expected to be enhanced by a GABAergic agonist. A *dGAT* mutant would be useful to further explore these effects, but has not yet been identified.

dGAT expression is likely to be restricted to glia. In the adult fly, in situ hybridization showed an mRNA pattern similar to that of the glial marker repo (Thimman et al., 2006). Two possible splice variants have been predicted but both are the same predicted size <http://flybase.org/reports/FBgn0039915.html>. To our knowledge, a specific antiserum to *Drosophila* GAT is not available. Antisera to mammalian GATs 1–3 have been used in *Drosophila* as probes and showed three distinct patterns, but their relationship to the expression of *dGAT* is not clear. By contrast, a highly specific antiserum to MasGAT has been generated (Oland et al., 2010; Umesh and Gill, 2002) and used for both light microscopy and ultrastructural studies. A detailed study of the antennal lobe showed that MasGAT localizes to neuronal processes early in development (Oland et al., 2010) but labeling appeared to be confined to glia in the adult. Consistent with the idea that insect GATs in general may primarily localize to glia in adults, uptake of GABA into the optic ganglia of both *Drosophila* and larger flies (*Musca*) appeared to be primarily glial (Campos-Ortega, 1974) and in situ hybridization of *dGAT* also appeared to localize to glia rather than neurons (Thimman et al., 2006).

Choline Transport in other insects

Unlike most other neurotransmitters, acetylcholine is degraded extracellularly to choline prior to transport. Choline is then recycled by a specific high-affinity transporter that has been extensively studied in vertebrates (Parikh et al., 2013; Traiffort et al., 2013). A possible ortholog of the mammalian choline transporter (ChT1) is present in the *Drosophila* genome

(see Table 2) but has not been investigated. An ortholog from the cabbage looper (TrnChT) has been characterized and unlike mammalian orthologs, is weakly inhibited by Hemicholinium-3 (McLean et al., 2005). Interestingly, mammalian ChT resides primarily on synaptic vesicles at baseline (Blakely and Edwards, 2012). It is not known if this is also true in insects.

Additional SLC6 members

The *Drosophila* SLC6 family of sodium-dependent plasma membrane transporters contains several additional members whose function remains unclear. These include the gene *inebriated (ine)* (Soehnge et al., 1996; Stern and Ganetzky, 1992). A mutation at this locus was independently isolated in a screen for defects in the electroretinogram (ERG), the electrical field potential across the retina and downstream neurons in the optic ganglia (Pak, 1975; Wu and Wong, 1977). The characteristic oscillations in the mutant led to its designation as “receptor oscillation A”, or *rosA* (Burg et al., 1996; Pak, 1975; Wu and Wong, 1977). *rosA/ine* mutants also show reduced on and off transients in the ERG suggesting impaired signaling of photoreceptors to neurons immediately downstream in the optic ganglia (Burg et al., 1996; Pak, 1975; Wu and Wong, 1977). However, electrophysiological defects in *rosA/ine* are not confined to the eye and include potentiation of larval motoneuron depolarization (an increase in long term facilitation) (Huang and Stern, 2002). These observations are consistent with the idea that *rosA/ine* may generally function to regulate neuronal excitability.

A homolog of *ine* has been cloned from *Manduca sexta* (Chiu et al., 2000) and expression in oocytes was used to test a variety of potential substrates but to date, the substrates of *Manduca* and *Drosophila* *ine* remain unclear (Chiu et al., 2000) (however see below). *Ine* mRNA and protein appear to be broadly expressed in both *Drosophila* and *Manduca* and unlike some other members of SLC6, expression is not confined to the nervous system but also prominent in the gut and malpighian tubule, the “fly equivalent” of the mammalian kidney (Burg et al., 1996; Chiu et al., 2000). In these structures, *ine* has been proposed to function as an osmolyte transporter (Huang et al., 2002).

It has been suggested that *ine* could have a function in recycling histamine via transport of the histamine metabolite carcinine (Stuart et al., 2007). Carcinine is generated by *ebony*, which is expressed in a subset glia that surround photoreceptor terminals and functions to conjugate histamine to β -alanine (Stuart et al., 2007). Several tantalizing findings support the idea that *ine* could be involved in recycling histamine and perhaps function as a carcinine transporter (Gavin et al., 2007). For example, feeding flies carcinine phenocopies *ine* (Gavin et al., 2007). These effects are consistent with the idea that loss of *ine* increases extracellular carcinine and that excess extracellular carcinine is the cause of the *ine/rosA* mutant phenotype.

Since histamine is released from photoreceptor cells but metabolized in glia, recycling is likely to follow a fairly circuitous path, similar to that proposed for glutamate recycling through glia. Therefore, similar to System A and N in the glutamate:glutamine cycle, additional transporters are likely to be involved in histamine recycling. Some of these may

be novel members of SLC6. In addition to *ine*, insects express other distinct subfamilies of SLC6 including 6 insect-specific Nutrient Amino Acid Transporters (iNATs) (Boudko et al., 2005). Another 5 genes whose function remains obscure form another distinct subfamily of transporters in SLC6 (Romero-Calderón et al., 2007). mRNA expression of two members in this group (CG13794 and 5) as well as CG4476 are down-regulated by the loss of eye tissue, consistent with a possible function in the visual system (Romero-Calderón et al., 2007). Furthermore, mutation of CG4776 causes a defect in phototactic behavior (Romero-Calderón et al., 2007). We suspect that CG4476, CG13794 CG13795 and perhaps other novel members of SLC6 could participate in recycling histamine in the fly and other insects, but additional experiments are clearly needed to test this idea.

Future Directions

Structure-function studies in vivo

As described elsewhere in this issue, experiments using cultured cells have been invaluable for exploring the functional consequences of specific mutations in neurotransmitter transporters. In parallel, mouse knockouts and mutations in model organisms such as *C. elegans* and *Drosophila* have demonstrated the physiological importance of specific neurotransmitter transporters for a variety of processes. We suggest that flies and other model organisms will be very important for combining these strategies and that structure-function studies of neurotransmitter transporters in vivo will be an important future direction.

One use of this strategy is the expression of risk alleles for human disease in vivo. For rare or de novo mutations the relationship between a particular polymorphism and the disease phenotype can be difficult to ascertain. In the absence of a pedigree or the association of a phenotype with multiple de novo events, it is often impossible to determine if a given polymorphism or copy number variant has a relationship to the phenotype of the patient. In some cases, it may be possible to demonstrate molecular or cellular phenotypes for a candidate gene, as shown for disease-associated mutations in ion channels. However, it is also possible that the cellular phenotype of a disease allele may not reflect its previously defined physiological function, e.g. a cellular phenotype of a rare mutation associated with Autism Spectrum Disorders (ASDs) is dendritic retraction (Krey et al., 2013). This phenotype was not predicted from the known function of the gene as a calcium channel subunit and does not appear to be associated with alterations in calcium flux (Krey et al., 2013).

The potential pathogenic mechanisms associated with polymorphisms in neurotransmitter transporters are also likely to be complex, and it may be difficult to predict how a subtle change in the structure of a neurotransmitter transporter could increase risk for a complex illness such as autism or ADHD. As discussed above, the potential use of flies to examine these questions is exemplified by the analysis of a *dDAT* allele associated with autism (Hamilton et al., 2013). We anticipate that similar studies will be very useful to explore the consequences of other risk alleles such as those in SERT linked to autism. To date only one of the SERT mutants has been tested in mice (Ye and Blakely, 2011; Veenstra-VanderWeele et al., 2012). Generating mutant lines in flies or worms may be used for detailed mechanistic

studies, or alternatively, large panels of potentially relevant polymorphisms may be screened in model invertebrates to determine which ones should be studied in more detail in mammals.

More generally, structure-function studies in vivo will be useful to determine the downstream consequences of other mutations in neurotransmitter transporters. Mutants predicted to affect transport activity, post-translational regulatory processes or trafficking can be easily expressed in a loss of function mutant background to determine their effects on synaptic transmission and behavior. As noted above, this strategy has already been used to determine the in vivo importance of phosphoacceptor sites in dDAT (Pizzo et al., 2013).

We believe that this strategy will be particularly useful to understand the functional importance of transporter trafficking. A large number of studies have already dissected some of the signals and pathways required for the internalization of vesicular transporters and their targeting to secretory vesicles (reviewed in (Fei et al., 2008)). Similarly, a number of studies have explored the signals that allow internalization of plasma membrane transporters (Eriksen et al., 2010; Quick, 2006; Robertson et al., 2009; Sager and Torres, 2011; Steiner et al., 2008). However, it is not known how disruption of these pathways might affect the function of the nervous system as a whole. We are currently investigating these questions using trafficking mutants in *dVMAT*. We have focused on *dVMAT* in part because it localizes to two distinct types of vesicles, SVs, required for rapid release at the nerve terminal and LDCVs, thought to be required for neuromodulatory signaling. The contribution of amine release from SV versus LDCVs for specific behaviors remains surprisingly obscure. We are using mutations that shift *DVMAT* between SVs and LDCVs to explore this question (manuscript under review). We suggest that a similar analysis may be useful to explore the functional significance of mutations that disrupt the internalization of plasma membrane transporters.

Forward Genetic Screens for Interacting Genes and Proteins

Most psychiatric illnesses are likely to follow a complex pattern of inheritance with large numbers of genes contributing to the phenotype, and additional environmental factors also playing a role (Sullivan et al., 2012). Genetic modifiers alter the phenotype of the ASD mutation in SERT when expressed in mice, but the identities of these interacting genes are not known (Kerr et al., 2013). We suggest that “modifier” a.k.a. “enhancer/suppressor” screens in model invertebrates such as *C. elegans* and *Drosophila* represent a powerful and essentially untapped method to identify specific genes that interact with risk alleles in SERT, and more generally to identify genes that interact with neurotransmitter transporters.

Modifier screens may be used to identify new loci that interact with any type of mutation, provided it generates a genetically tractable phenotype. In common genetic parlance, the mutation is used to first generate a “sensitized genetic background” which may consist of an anatomic or behavioral defect, or simply a decrease in survival. A panel of mutations in other genes-- so-called “second-site mutations”-- are then crossed into the sensitized genetic background. The resulting progeny are screened to determine whether the phenotype of the parent strain-- the sensitized genetic background-- has been modified by any of the

additional mutations. The defect seen in the parent strain may be worsened or “enhanced”. Alternatively, the defect might improve, and the phenotypic defect “suppressed”.

Classic screens for molecules that modulate the function of the oncogene *ras* provide an important example of this strategy in flies (Gaul et al., 1993). Suppression or enhancement of defects in *Drosophila* eye development caused by mutations in *ras* were used to identify genes relevant to *ras* and *ras*-dependent pathways. Although eye development in flies was used as a screening phenotype, the molecules that emerged from these screens were not specific for eye development or *Drosophila* but rather represented *general* components of the *ras* signaling pathway. Flies and worms may similarly be used to screen for genes that modulate the function of neurotransmitter transporters. The first step will be to identify a genetically tractable phenotype caused by mutation of the transporter in order to generate a sensitized genetic background. Importantly, the nature of the phenotype need not be particularly interesting, just consistent, robust and easily scored.

In *C. elegans*, mutations in VACHT/*unc-17* have already been used to screen for interacting loci (Mathews et al., 2013; Sandoval et al., 2006). One screen identified a mutant allele of synaptobrevin and a second screen identified the novel gene *sup-1* (Mathews et al., 2013; Sandoval et al., 2006). The nature of these second site mutant alleles suggest that in both cases the interactions are likely to be mediated by direct protein-protein binding to VACHT. However, this need not always be the case. One advantage of this type of forward genetic screen is that it can detect indirect interactions. Another advantage is that it is not strictly hypothesis based. The authors of the VACHT interaction studies did not know that they would detect synaptobrevin, much less *sup-1* (Mathews et al., 2013; Sandoval et al., 2006). Similarly, we suggest that identification of molecules that indirectly influence the function of neurotransmitter transporters *in ways that we are unable to predict* will be a very important future use for *Drosophila*.

Forward genetic screens in *C. elegans* have also been used to identify novel genes that regulate DA signaling and could potentially regulate dopamine transport. Mutations in the *C. elegans* ortholog DAT-1 generate a genetically tractable phenotype termed Swimming-Induced Paralysis (SWIP) (Hardaway et al., 2013; McDonald et al., 2007). Some DAT-1 mutations that cause SWIP decrease DAT-1 localization to the synapse, and the SWIP phenotype is thought to result from activation of extrasynaptic DA receptors by DA spillover (Hardaway et al., 2013; McDonald et al., 2007). Thus, screens for mutations that modify SWIP have the potential to identify novel genes required for DAT trafficking as well as other aspects of DAT activity. Although a classical modifier screen using a weak allele of DAT-1 has not yet been reported, a recent study reported a screen for genes that would mimic or phenocopy the DAT-1 mutant and cause SWIP. In addition to new alleles of DAT-1, the screen identified two novel second-site mutations, one of which appears to genetically interact with DAT-1 (Hardaway et al., 2013). It is possible that modifiers of *Drosophila fmn* could be similarly identified using sleep or the response to amphetamines as a screening phenotype.

Drug screens

Although still a nascent field, a number of labs have begun to use flies as an *in vivo* platform for drug screens (Tickoo and Russell, 2002). One strategy, based on classical forward genetic screens is to select a phenotype of interest, e.g. sleep (Nall and Sehgal, 2013) or feeding (Gasque et al., 2013), then screen for drugs that are responsible for or enhance that phenotype. Some of the identified targets of these screens may be neurotransmitter transporters, e.g. the VMAT inhibitor reserpine was identified in a screen for small molecules that increase sleep in flies (Nall and Sehgal, 2013). A more efficient strategy to identify agents that affect the function of a given neurotransmitter transporter may be to screen for drugs that modify the phenotype caused by mutation or over-expression of the transporter, akin to screens to identify genetic modifiers. Similar to genetic modifier screens, this strategy again takes advantage of the fact that all of the regulatory machinery is in place, and drug targets may be novel elements of the regulatory machinery rather than the transporter itself. We suggest that this approach might expand the way we think about drugs that affect transporters. In addition, we suggest that this represents a potential way to identify drugs that may *increase* transport activity. There are few if any drugs that make neurotransmitters transporters work more effectively. Targeting upstream proteins that *inhibit* transporter function could accomplish this.

We have taken this approach to identify new molecules that might potentiate the function of VMAT *in vivo* (Lawal et al., 2014). Using a partially functional *dVMAT* mutant background, we screened larvae for drugs that would increase signaling in the octopaminergic circuit required for baseline larval locomotion. The results of this screen yielded several molecules that *a priori* would not be classified as aminergic drugs and are unlikely to directly bind dVMAT (Lawal et al., 2014). Rather, we suggest that some of the identified drugs may act on upstream targets to indirectly increase VMAT activity, and we are currently testing this hypothesis. We suggest that similar screens might be used to identify drugs that modify the function of other neurotransmitter transporters.

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Abbreviations used in the text include

5HT	5-Hydroxytryptamine (Serotonin)
ACh	Acetylcholine
ASD	Autism Spectrum Disorder
ChT	Choline Transporter
ChAT	Choline Acetyl Transferase

DA	Dopamine
Ddc	Dopa decarboxylase
EAAT	Excitatory Amino Acid Transporter
GABA	Gamma Amino Butyric Acid
GAT	GABA transporter
KC	Kenyon Cell
LDCV	Large Dense Core Vesicle
Mas	Manduca sexta (Tobacco Hornworm)
MAO	Monoamine Oxidase
MB	Mushroom Body
MDMA	3,4-methylenedioxy-N-methylamphetamine (Ecstasy)
OA	Octopamine
NMJ	Neuromuscular Junction
SV	Synaptic Vesicle
TNT	Tetanus Toxin
<i>T. ni</i> or <i>Trn</i>	Trichoplusia ni (Cabbage Looper Caterpillar/Moth)
TβH	Tyramine β Hydroxylase
Tdc	Tyrosine decarboxylase
TH	Tyrosine Hydroxylase
VGAT	Vesicular GABA Transporter
VGLUT	Vesicular Glutamate Transporter
VMAT	Vesicular Monoamine Transporter
UAS	(Yeast) Upstream Activating Sequence

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Highlights

Drosophila express orthologs of most mammalian neurotransmitter transporters.
Fly genetics provides a powerful tool-set to explore transporter function in vivo.
Screens for genes and drugs that modify transport function are possible in the fly.

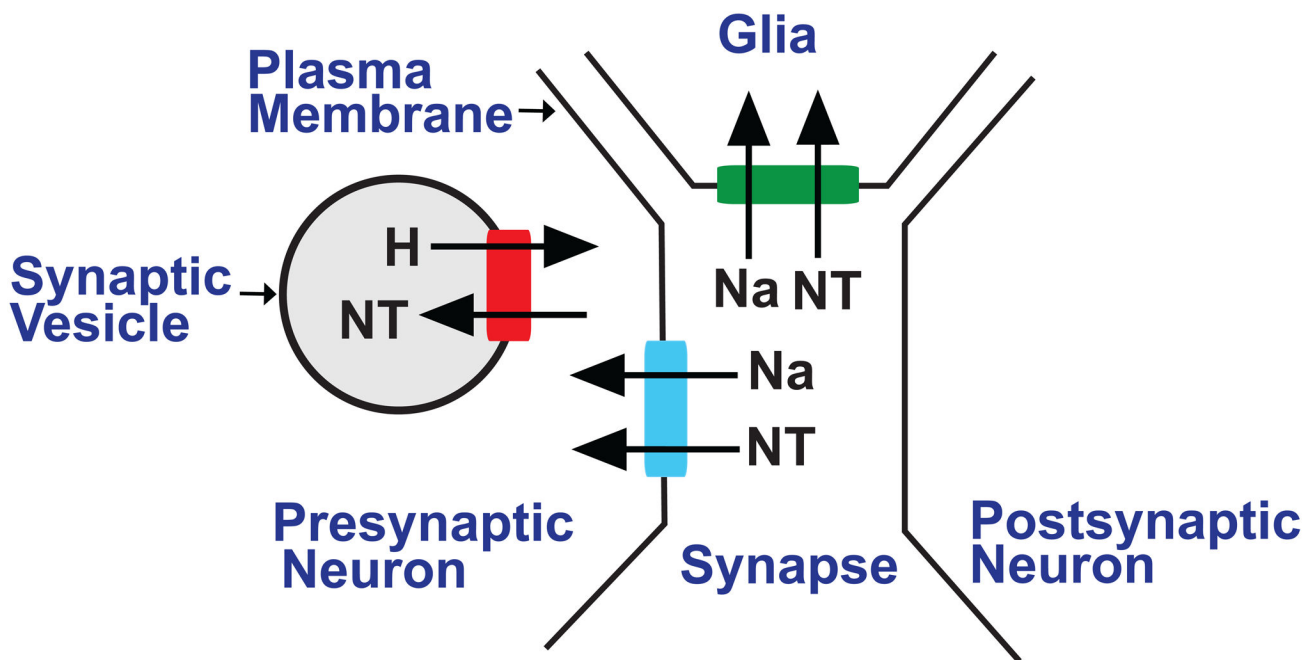


Figure 1. Vesicular and plasma membrane neurotransmitter transporters

Vesicular and plasma membrane neurotransmitter transporters differ in several respects, including their localization and bioenergetics. In general, vesicular transporters localize to secretory vesicles, including synaptic vesicles in the presynaptic neurons that synthesize and release neurotransmitter. Depending on their subtype (see text) plasma membrane transporters may localize to either presynaptic neurons or surrounding glia. The movement of neurotransmitter via vesicular and plasma membrane transporters are coupled to proton and sodium gradients respectively. Vesicular transporters move protons (**H**) and neurotransmitter (**NT**) in opposite directions (antiport), using the high concentration of luminal protons to drive transport. At the plasma membrane, sodium (**Na**) and neurotransmitters move in the same direction (symport).

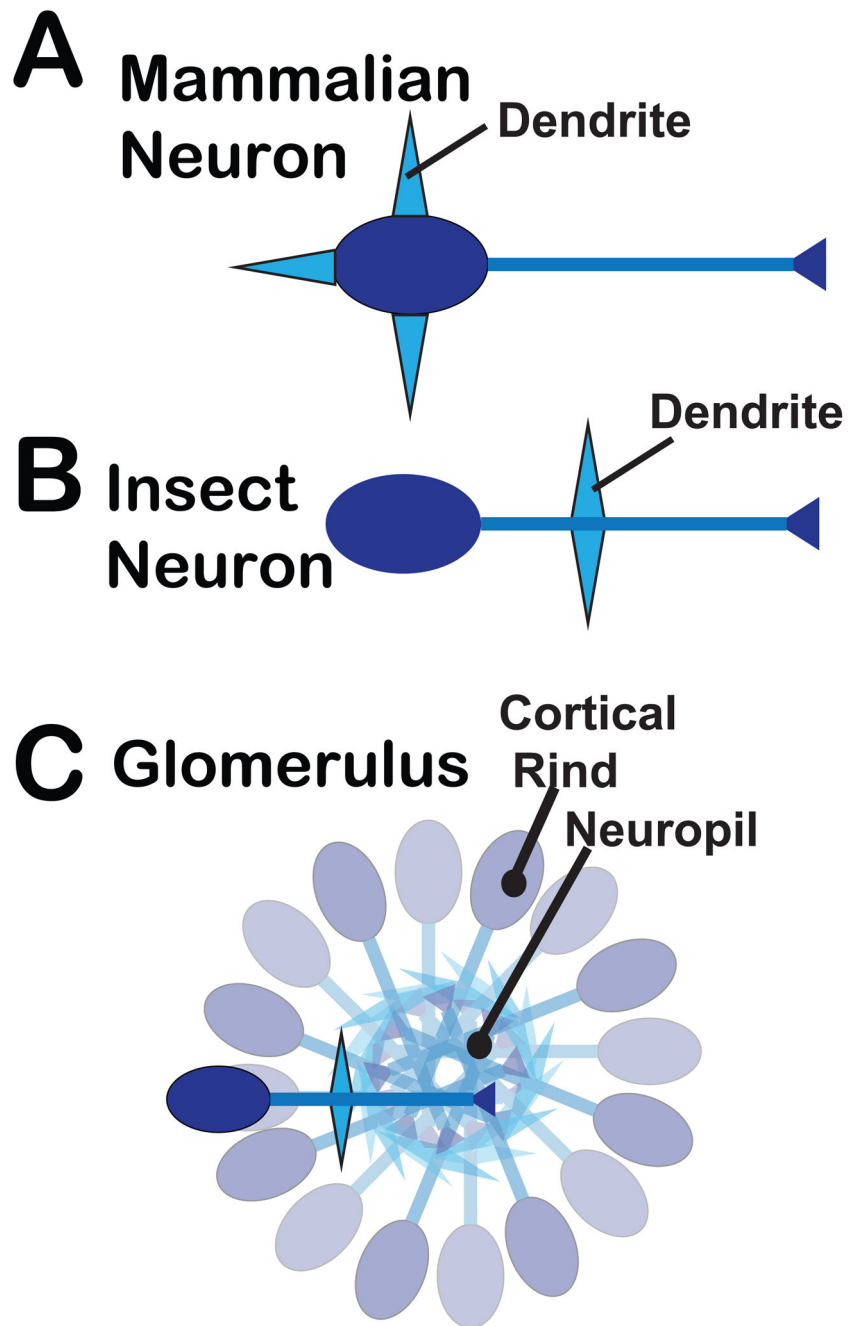


Figure 2. The morphology and organization of mammalian and insect neurons

A canonical mammalian (A) and insect neuron (B) are indicated, highlighting differences in the location of dendrites: on the cell body of mammalian neurons versus a single process emanating from the soma of insect neurons that also gives rise to the axon. This morphology allows the characteristic organization of insect neurons into glomeruli (C), or larger but similarly organized ganglia, which include a cortical rind and neuropil as indicated.

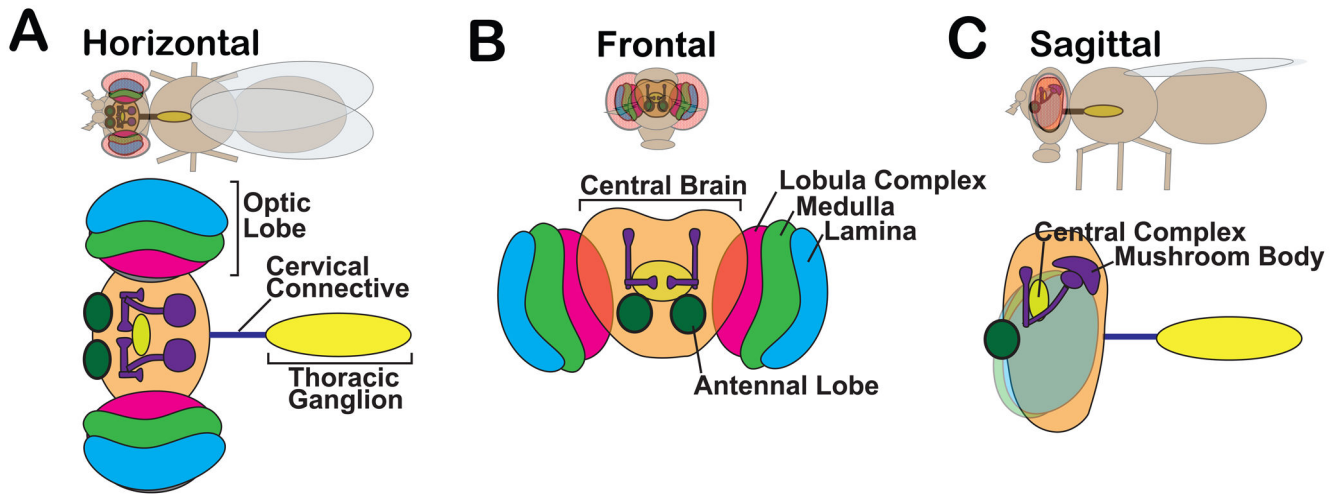


Figure 3. The neuroanatomy of the adult fly brain

Cartoons show (A) horizontal, (B) frontal and (C) sagittal views of the fly and corresponding diagrams of the central nervous system. For simplicity, structures are labeled in one panel but color-coded to allow identification in all panels. Labeled structures include the central brain (orange), bilaterally symmetric optic lobes, the thoracic ganglion (yellow) and the cervical connective (blue), which contains both ascending and descending fibers. Ganglia within the optic lobes include the lamina (blue) and medulla (green) both of which receive projections from photoreceptors in the retina. Cells in both the lamina and medulla project to the lobula and lobula plate, indicated here as the lobular complex (pink). Major structures within the central brain include the mushroom bodies (purple), required for olfactory learning and memory and the central complex (yellow ellipsoid), which regulates some motor behaviors.

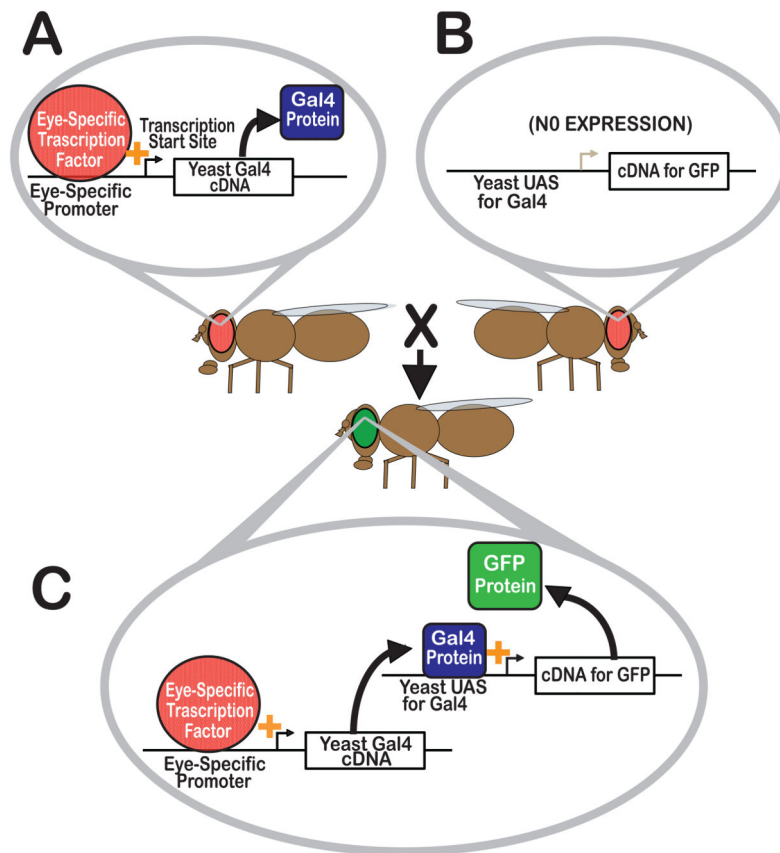


Figure 4. The Gal4/UAS System

The bipartite Gal4/UAS System employs separate transgenes to (A) drive expression and (B) allow specific cDNAs to be expressed, e.g. for Green Fluorescent Protein (GFP) as shown here. The Gal4 “driver” consists of a cell or tissue specific promoter e.g. for cells in the eye as shown in (A) immediately upstream of the cDNA encoding the yeast Gal4 transcription factor. Mating these lines results in a fly that contains both the Gal4 and UAS transgenes (C). In these progeny, Gal4 is expressed and binds to its UAS, which in turn drives expression of GFP in cells defined by the Gal4 promoter.

Table 1

Mammalian and *Drosophila* vesicular transporters

In both mammals and flies, known vesicular transporters are members of the SLC17, 18 or 32 subfamilies. See text for details.

SLC17		SLC18		SLC32	
Mammals	<i>Drosophila</i>	Mammals	<i>Drosophila</i>	Mammals	<i>Drosophila</i>
VGLUT1	<i>dVGLUT</i>	VMAT1	<i>dVMAT</i>	VGAT	<i>dVGAT</i>
VGLUT2		VMAT2			
VGLUT3		VACHT	<i>dVACHT</i>		
VNUT (SLC17A9)	<i>CG15438</i>		<i>prt</i>		

Table 2

Mammalian and *Drosophila* plasma membrane transporters

In both mammals and flies, known plasma membrane neurotransmitter transporters are members of the SLC1 and 6 subfamilies. As described in the text, dEAAAT2 may not function as a glutamate transporter. The choline transporter is a member of SLC5, and although it is not a neurotransmitter transporter per se, it is responsible for reuptake of choline into the presynaptic neuron after acetylcholine is metabolized in the synapse. The predicted fly gene CG11880 appears to be most similar to mammalian choline transporters, but has not been characterized. CG5549 is listed as a putative glycine transporter based on its similarity to mammalian glycine (and proline) transporters, but its function is not known; it also remains unclear whether glycine functions as a neurotransmitter in the fly. The gene CG7075, recently named *Neurotransmitter Transporter-Like (Ntl)* is also a putative glycine transporter but found only in male germline cells (Chatterjee et al., 2011). SLC6 also includes amino acid transporters required for nutrient uptake and several additional members whose functions are not known (not shown) as well as *inebriated*, which may transport a metabolite of histamine (see text).

SLC1		SLC6		(SLC5)	
Mammals	<i>Drosophila</i>	Mammals	<i>Drosophila</i>	Mammals	<i>Drosophila</i>
EAAT1		DAT	<i>dDAT</i>	ChT1	CG11880?
EAAT2		SERT	<i>dSERT</i>		
EAAT3	<i>dEAAT1</i> <i>dEAAT2</i>	GAT1			
EAAT4		GAT2	<i>dGAT</i>		
EAAT5		GAT3			
		GlyT1			
		GlyT2	(CG7075) CG5549?		
		NET			
			(<i>inebriated</i>)		