

Drosophila models of vesicular transport function

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Abstract. Over-expression of *Drosophila* vesicular transporters can have dramatic effects on behavior. To identify additional phenotypes for further genetic screens, we are now studying the effects of mutating endogenous transporter genes including the vesicular monoamine transporter, dVMAT. (www.actabiomedica.it)

Key words: Dopamine, *Drosophila*, transporter, neurodegeneration

Introduction

Model genetic organisms such as *C. elegans* and *Drosophila melanogaster* may be useful for studying how changes in vesicular transport activities affect complex behavior, and to develop genetic models to identify regulatory pathways. *Drosophila* expresses a single variant of the vesicular glutamate transporter (DV-GLUT) (1), and two vesicular monoamine splice variants (DVMAT-A and B), that differ at the C-terminus (2). DVMAT-A is expressed in all aminergic neurons of the fly CNS (2) and over-expression of DVMAT-A causes robust behavioral changes including an increase in locomotion and a decrease in the fly's behavioral response to cocaine (3). Similarly, over-expression of DVGLUT increases vesicular storage and release of glutamate at the neuromuscular junction (1). To complement these studies and to develop a background for future genetic screens, we have are testing the behavioral effects of mutating endogenous transporter genes including dVMAT.

Material and methods

Transgenes containing the DVMAT-A cDNA were generated using the GAL4/UAS systems (3, 4).

A P-element insert in the dVMAT gene was obtained from a previous screen for mutations on the second chromosome (5). HPLC, Westerns and behavioral analyses were performed as described (3, 6).

Outcomes

The behavioral phenotypes of DVMAT over-expression are likely to be due to an increase in the exocytosis and extracellular concentration of dopamine and serotonin. Conversely, reduction of DVMAT activity would be predicted to decrease monoamine storage and amine-linked behaviors. To test this hypothesis, we have obtained a line (5) in which the coding region of dVMAT is interrupted by a transposable P-element, thus functionally deleting the last two transmembrane domains and the cytoplasmic C-terminus (Fig. 1A). Using an antibody to the N-terminus of DVMAT, we find that the P insertion causes a dramatic decrease in DVMAT protein expression (Fig. 1B) and a decrease in adult survival (Fig. 1C). Ubiquitous expression of DVMAT-A using the da-GAL4 driver rescues the decrease in survival, indicating that this phenotype is a result of disrupting dVMAT (Fig. 1C). Further phenotypic analysis shows that mutation of the dVMAT gene decreases the storage of both se-

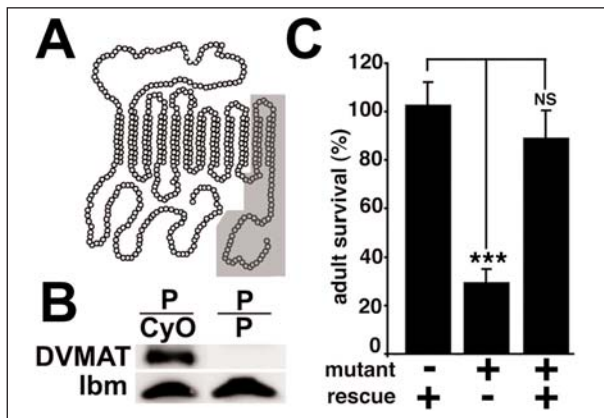


Figure 1. Mutation of the dVMAT gene. A) An insertion of a P element into the last exon of dVMAT causes a functional deletion of the C-terminus, with deleted segments shaded in gray. B) Using an antibody to the DVMAT amino-terminus to probe Western blots, we see a dramatic decrease in protein expression. Identical samples were probed using an antiserum to the late bloomer protein (lbn) to control for sample loading. C) Homozygote dVMAT mutants (“mutant”) show a decrease in adult survival, with 36% of animals surviving in the culture conditions shown here. Ubiquitous expression of UAS-DVMAT-A using the daughterless-GAL4 driver rescues the lethality of dVMAT mutants (“rescue”), one way ANOVA, $p < 0.0001$, $n = 6$ crosses, Bonferroni’s Multiple Comparison Test, *** $p < 0.001$.

rotonin and dopamine (Fig. 2) and generates several easily observable behaviors. These include a decrease in larval locomotion (Fig. 3A). In adults, we have used a counter-current apparatus (6) to measure the number of flies that move away from a light source (Fig. 3B). Although the flies are able to locomote (not shown), they show a defect in their ability to move away from the light (Fig. 3B), suggesting an increase in their attraction to this stimulus.

Conclusions

Mutations in mouse VMAT2 and the *C. elegans* ortholog of VMAT show reduced serotonergic and dopaminergic neurotransmission and severe behavioral deficits (7). We see similar changes in the behavior of dVMAT mutants. Octopamine regulates the release of glutamate at the larval neuromuscular junction and the decrease in larval locomotion we observe may be due to reduced octopamine release (8). Serotoner-

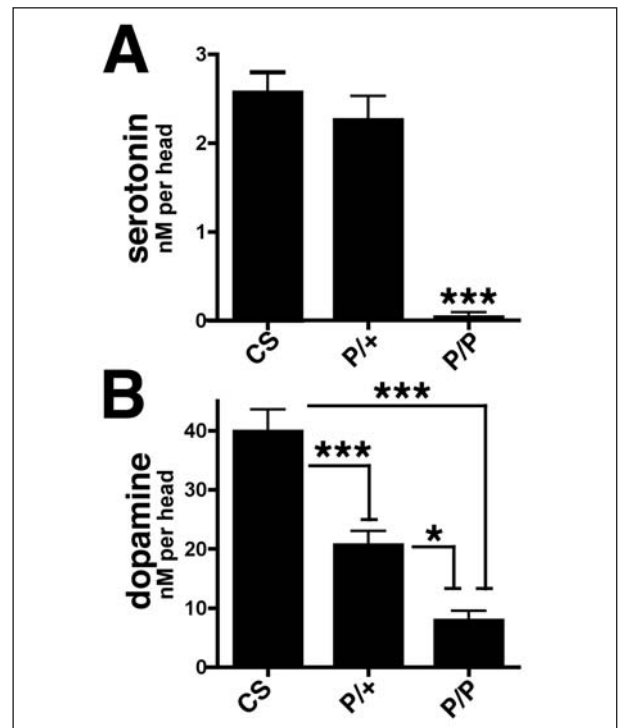


Figure 2. Decreased dopamine and serotonin content in dVMAT mutants. Extracts from heads of homozygous female dVMAT mutants (P/P) heterozygotes (P/+) and wild type (Canton S, CS) controls were analyzed for serotonin and dopamine content ($n = 6$ samples/genotype, 3 heads/sample) as described (3). Homozygous dVMAT mutants show reduced serotonin (A) and dopamine (B) levels, one way ANOVA for serotonin and dopamine $p < 0.0001$, Bonferroni’s Multiple Comparison Test, * $p < 0.05$, *** $p < 0.001$.

gic, octopaminergic and dopaminergic processes are present in the adult optic ganglia and defects in their release from nerve terminals expressing DVMAT-A may cause the changes in visual behavior that we observe. Alternatively, the effects of mutating dVMAT on visual behavior may be the result of decreased expression of the DVMAT-B variant. Surprisingly, DVMAT-B is expressed in a subset of glia just beneath the adult retina (data not shown). The expression of DVMAT-A and -B in specific cell types using the well established GAL4/UAS system and available promoters for both aminergic subtypes and glia will allow us to tease apart these possibilities. Furthermore, the behavioral phenotypes we observe will provide a useful background for genetic screens to identify pathways that regulate the function of VMAT.

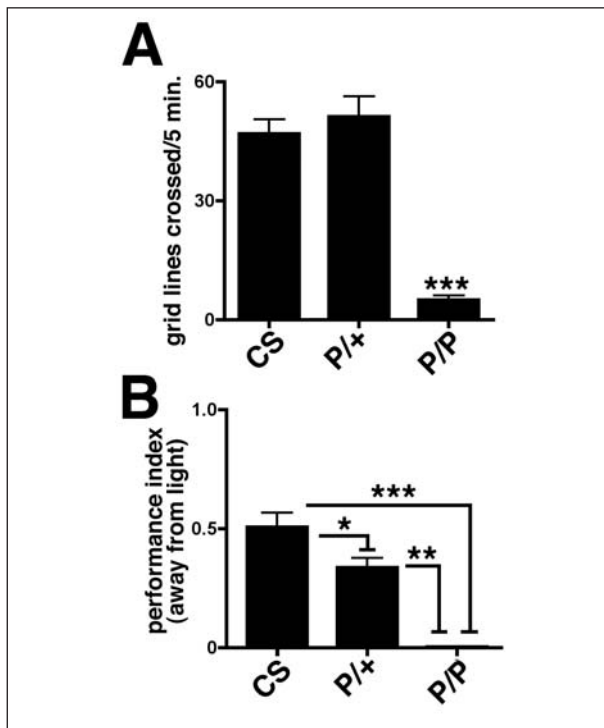


Figure 3. Behavioral phenotype of dVMAT mutants. A) Larval locomotion was quantified by counting the number of grid lines crossed in a 5 minute period, similar to previous assays of adult locomotion (3). Homozygous dVMAT mutants move significantly less than either wild type (CS) or heterozygotes (P/+), one way ANOVA, $p < 0.0001$, Bonferroni's Multiple Comparison Test, *** $p < 0.001$. B) Homozygous dVMAT mutants that survived to adulthood were tested in a variant of the fast phototaxis assay in which the number of flies that move away from a light source is measured in a counter-current apparatus (6). Homozygous dVMAT mutants appear to show an increased attraction to light, relative to controls, one way ANOVA, $p < 0.0001$, $n = 3$ experiments, 100 flies/experiment, Bonferroni's Multiple Comparison Test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

References

1. Daniels RW, Collins CA, Gelfand MV, et al. Increased expression of the *Drosophila* vesicular glutamate transporter leads to excess glutamate release and a compensatory decrease in quantal content. *J Neurosci* 2004;24:10466-74.
2. Greer CL, Grygoruk A, Patton DE, et al. A splice variant of the *Drosophila* vesicular monoamine transporter contains a conserved trafficking domain and functions in the storage of dopamine, serotonin and octopamine. *J Neurobiol* 2005; 64:239-258.
3. Chang H-Y, Grygoruk A, Brooks ES, et al. Over-expression of the *Drosophila* vesicular monoamine transporter increases motor activity and courtship but decreases the behavioral response to cocaine. *Molecular Psychiatry* 2006;11:99-113.
4. Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 1993;118:401-15.
5. Oh SW, Kingsley T, Shin HH, et al. A P-element insertion screen identified mutations in 455 novel essential genes in *Drosophila*. *Genetics* 2003;163:195-201.
6. Simon AF, Liang DT, Krantz DE. Differential decline in behavioral performance of *Drosophila melanogaster* with age. *Mech Ageing Dev* 2006;127:647-51.
7. Eiden LE, Schafer MK, Weihe E, Schutz B. The vesicular amine transporter family (SLC18): amine/proton antiporters required for vesicular accumulation and regulated exocytotic secretion of monoamines and acetylcholine. *Pflugers Arch* 2004;447:636-40.
8. Monastirioti M. Biogenic amine systems in the fruit fly *Drosophila melanogaster*. *Microscop Res Tech* 1999;45:106-121.

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