Neuron *46*, 1–9, January 5, 2006 ©2006 Elsevier Inc.

Vesicular Monogamy?

Vesicular neurotransmitter transporters package transmitter into the lumen of synaptic vesicles for quantal release. However, the number of transporters that localize to each vesicle is not known. In this issue of *Neuron*, a study by Daniels et al. using the *Drosophila* neuromuscular junction and mutations of the vesicular glutamate transporter suggests that one transporter may suffice to fill each vesicle.

Some 50 years ago, Bernard Katz and colleagues demonstrated that neurotransmitter is released as packets, or quanta, and a wealth of subsequent data have shown that synaptic vesicles (SVs) are the physical correlate of these quanta (Atwood and Karunanithi, 2002). Vesicular neurotransmitter transporters are responsible for packaging neurotransmitter into the lumen of the vesicle and thereby generating the concentration and absolute quantity of transmitter that makes up each quanta (see Hediger et al., 2004 and accompanying articles). But how much neurotransmitter is contained in the lumen of each vesicle? Is that number absolutely fixed, or might it vary depending on the activity of the transporter and the number of transporters that localize to each vesicle? And finally, how many transporters are required to localize to an individual SV in order to fill the lumen of the vesicle? The molecular-genetic analysis of vesicular transporters has begun to yield answers to some of these questions (Colliver et al., 2000; Fremeau et al., 2004; Pothos et al., 2000; Wilson et al., 2005; Wojcik et al., 2004), and in this issue of *Neuron*, a paper from the DiAntonio lab suggests that the number of transporters required to fill a vesicle may be as few as one (Daniels et al., 2006).

The success of these experiments and others that track transmitter storage and release depends on the ability to accurately quantitate the amount of transmitter in a single vesicle. Carbon fiber electrode amperometry can directly measure the content of monoamine neurotransmitters such as dopamine and serotonin from individual secretory vesicles (Bruns and Jahn, 1995; Colliver et al., 2000; Pothos et al., 2000). In contrast, for neurotransmitters that are not as easily oxidized, transmitter release from single SVs must be measured indirectly by recording the electrophysiologic response of a postsynaptic cell. The electrophysiologic response to transmitter released by a single vesicle corresponds to the miniature excitatory postsynaptic potential or "mini" first described by Katz at the frog neuromuscular junction (NMJ). In flies, as in vertebrates, minis can be reliably quantified at the NMJ, thus giving a readily available metric for assessing the amount of transmitter in each vesicle (Atwood and Karunanithi, 2002). Furthermore, the electrophysiological preparations for recording at the fly NMJ are extremely robust and have been used extensively to model neurotransmission at an intact synapse (Atwood and Karunanithi, 2002; Karunanithi et al., 2002). In contrast to mammals and other vertebrates, which release acetylcholine at the NMJ, the primary neurotransmitter released at the fly NMJ is glutamate, and the vesicular glutamate transporter (VGLUT) is responsible for packaging the transmitter in the fly motoneuron. In this paper and another recent report, the DiAntonio group have used the fly NMJ to determine how changes in VGLUT expression might contribute to the size of a mini (Daniels et al., 2006; Daniels et al., 2004).

Although three *VGLUT* genes have been identified in mammals, the genome of *Drosophila* is more parsimonious and contains a single isoform, thus facilitating the analysis of DVGLUT using classical genetic techniques. Indeed, the novelty of the study by Daniels et al. emerges from the interplay between fly genetics and electrophysiology. Rather than "knocking out" the *dvglut* gene and completely eliminating its function as was reported recently for mouse *VGLUT1* (Fremeau et al., 2004; Wojcik et al., 2004), Daniels et al. generated an allelic series of *dvglut* mutants in which the flies show a graded decrease in the amount of transporter that is expressed in glutamatergic motoneurons. The use of these weak or "hypomorphic" alleles reduced but did not eliminate VGLUT expression and allowed the authors to target a reduced quantity of functional transporters to the SVs at the nerve terminal of the NMJ.

The key to exploring the relationship between transmitter content and transport function is to know how many transporters are on an individual vesicle. It is possible that increasingly sophisticated proteomic techniques may someday answer this question directly. In the absence of that ability, the DiAntonio lab relied on what amounts to the genetic equivalent of limiting dilution to force that number to one. They reasoned that if the number of SVs remains relatively intact in *dvglut* mutants, then as the number of transporters is decreased, fewer and fewer transporters will localize to each vesicle. In the extreme case, as in a limiting dilution, the presence of a large number of vesicles without a transporter suggests that the SVs that do have a transporter will have only one copy. Using quantitative electron microscopy, they show that the number of SVs is not dramatically reduced in the *dvglut* mutants. In contrast, the number of minis recorded is significantly reduced as the amount of the DVGLUT expressed at each terminal declines. These data suggest that a large fraction of the vesicles are devoid of transmitter and thus unable to produce a mini. However, this finding in itself does not tell us the number of transporters that reside on a normal vesicle. Rather, the key finding in this paper is the size of the minis generated by SVs that have only one vesicular transporter. Relative to wild-type flies, the size of the minis in the *dvglut* mutants is unchanged. That is, even as you limit the number of transporters such that each SV is likely to have one functional unit of DVGLUT, all SVs fill to the same capacity and fill equivalently to wild-type vesicles. The authors conclude that a single functional unit of DVGLUT is sufficient to fill an SV (Figure 1).

The idea that one and only one functional transport unit may reside on an SV is at odds with some

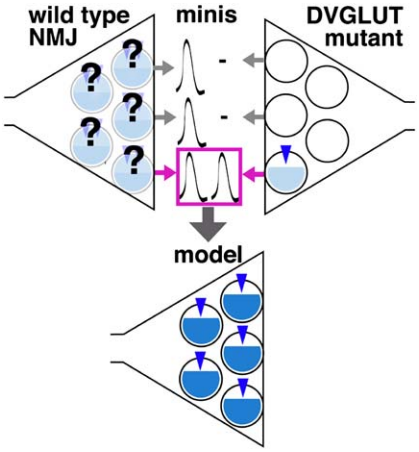

Figure 1. How Many Transporters Does a Vesicle Need?

The number of vesicular transporters required to fill a synaptic vesicle with neurotransmitter is not known. Mutations in the *dvglut* gene decrease transporter expression such that most vesicles are empty and do not produce minis. It is therefore likely that vesicles with only one transporter (blue pyramid) produce the few minis that remain in the mutant NMJ. *dvglut* mutant minis are equal in size to wild-type (see box), suggesting a model in which one transporter normally fills a synaptic vesicle to capacity.

mammalian studies (Wojcik et al., 2004). Therefore, the authors performed a series of critical controls to rule out other explanations for the results. One possible reason for the maintenance of SV filling in the face of diminished DVGLUT expression is that another transporter compensates for the deficit. If this were true, then this gene should also compensate for the absence of DVGLUT in more extreme null *dvglut* alleles. Since minis are absent in the null animals, it is unlikely that another transporter can substitute for DVGLUT at the NMJ. Another potential explanation for the maintenance of mini size is that postsynaptic receptors are upregulated or more sensitive to lower concentrations of transmitter release; however, the authors did not detect a change in the function of postsynaptic receptors. Finally, the results could be biased by a change in the release properties of empty or partially filled vesicles or a change in vesicle biogenesis in the absence of DVGLUT. *VGLUT1* knockout mice do indeed show a 50% change in the number of vesicles at the synapse, and it is possible that *VGLUT1* knockout mice show defects in at least a portion of recycling SVs (Fremeau et al., 2004). However, *dvglut* hypomorphs show only a minimal change in vesicle number, and experiments using the lipid dye FM1-43 indicate that SVs recycle normally in *dvglut* mutant larva.

These data support the surprising idea that a single functional unit of DVGLUT is sufficient to fill an SV to its normal capacity. It remains to be determined whether a functional unit of DVGLUT in fact represents a DVGLUT monomer. For plasma membrane transporters, abundant biochemical evidence and more recent crystallographic studies indicate that many members of this family form stable oligomers (Sonders et al., 2005). Although there is no clear evidence that vesicular transporters form similar, stable complexes, it remains possible that monomers could loosely associate to form an active, functional unit.

The possibility of VGLUT oligomers aside, the finding that a single transporter unit may be necessary and sufficient to fill a single vesicle has a variety of interesting consequences. First, it suggests that trafficking of vesicular transporters may be exquisitely regulated to maintain the appropriate ratio of 1:1. Alternatively, if the synapse is less stringent, there may be a large number of empty vesicles in the average neuron that do not receive their single allocation of transporter and thus do not contain neurotransmitter. The precise matching of transporter to vesicle might be further stressed during prolonged stimulation and increased turnover of SVs at the nerve terminal. In this scenario, the relative number of empty vesicles might be further increased and perhaps contribute to the rundown of transmitter release. These possibilities remain highly speculative. Nonetheless, the results of these and other recent studies (Wilson et al., 2005) highlight the potential importance of vesicular transporters in regulating synaptic transmission and their use in unraveling its fundamental properties.

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Selected Reading

Atwood, H.L., and Karunanithi, S. (2002). Nat. Rev. Neurosci. *3*, 497–516.

Bruns, D., and Jahn, R. (1995). Nature *377*, 62–65.

Colliver, T.L., Pyott, S.J., Achalabun, M., and Ewing, A.G. (2000). J. Neurosci. *20*, 5276–5282.

Daniels, R.W., Collins, C.A., Gelfand, M.V., Dant, J., Brooks, E.S., Krantz, D.E., and DiAntonio, A. (2004). J. Neurosci. *24*, 10466–10474.

Daniels, R.W., Collins, C.A., Chen, K., Gelfand, M.V., Featherstone, D.E., and DiAntonio, A. (2006). Neuron *49*, this issue, 11–16.

Fremeau, R.T., Jr., Kam, K., Qureshi, T., Johnson, J., Copenhagen, D.R., Storm-Mathisen, J., Chaudhry, F.A., Nicoll, R.A., and Edwards, R.H. (2004). Science *304*, 1815–1819.

Hediger, M.A., Romero, M.F., Peng, J.B., Rolfs, A., Takanaga, H., and Bruford, E.A. (2004). Pflügers Arch. *447*, 465–468.

Karunanithi, S., Marin, L., Wong, K., and Atwood, H.L. (2002). J. Neurosci. *22*, 10267–10276.

Pothos, E.N., Larsen, K.E., Krantz, D.E., Liu, Y., Haycock, J.W., Setlik, W., Gershon, M.D., Edwards, R.H., and Sulzer, D. (2000). J. Neurosci. *20*, 7297–7306.

Sonders, M.S., Quick, M., and Javitch, J.A. (2005). Curr. Opin. Neurobiol. *15*, 296–304.

Wilson, N.R., Kang, J., Hueske, E.V., Leung, T., Varoqui, H., Murnick, J.G., Erickson, J.D., and Liu, G. (2005). J. Neurosci. *25*, 6221–6234.

Wojcik, S.M., Rhee, J.S., Herzog, E., Sigler, A., Jahn, R., Takamori, S., Brose, N., and Rosenmund, C. (2004). Proc. Natl. Acad. Sci. USA *101*, 7158–7163.

DOI 10.1016/j.neuron.2005.12.013