

SPOTLIGHT

EVs move messes, not messages, at the synapse

Marina N. Bostelman¹ and Heather T. Broihier¹ 

Extracellular vesicles are known for intercellular signaling roles but can also serve to simply dispose of unwanted cargoes. In this issue, Bostelman and Broihier discuss new work from Rodal and colleagues (<https://doi.org/10.1083/jcb.202405025>) that refutes prior work by showing that extracellular vesicles at *Drosophila* neuromuscular junctions are not required for signaling and instead likely serve a proteostasis role.

EVs (electric vehicles) are widespread on roads around the world, where they move people, packages, and even garbage. Perhaps similarly, extracellular vesicles (EVs hereafter) are widespread in metazoans, where they also transport a broad range of cargoes. What has been less clear is what the function of this intercellular EV traffic is. On one hand, EVs are proposed to carry key intercellular signaling proteins between cells, and on the other, to serve primarily as a waste disposal mechanism. The current study from the Rodal lab convincingly demonstrates that for two different signaling molecules at the *Drosophila* neuromuscular junction (NMJ), neurons release EVs most likely as a disposal mechanism—not to signal across the synapse (1).

EVs are a heterogeneous vesicle population responsible for releasing a wide variety of cargoes from many cell types, including neurons and glia. However, the underlying functions of EV release in physiological and pathological contexts are contested. Typically, studies of neuronal EV function purify the vesicles from donor neurons and then exogenously apply them to recipient cells (2), which tests the effects of high concentrations of EVs on target cells, but not necessarily what these vesicles actually do in vivo. A rigorous assessment of neuronal EV function requires (1) an in vivo system permitting independent genetic manipulation of signal-sending and signal-receiving cells, (2) genetic mutations that block EV

biogenesis, and (3) putative EV-mediated signaling pathways with well-defined neuronal functions. The *Drosophila* NMJ fulfills these three criteria and is an ideal in vivo model in which to investigate EV signaling.

Dresselhaus et al. (1) sought to exploit the myriad experimental strengths of the fly NMJ model to test whether two putative trans-synaptic signals, evenness interrupted/Wntless/Sprinter (Evi) and Synaptotagmin-4 (Syt4), require EV release, as proposed. Both proteins appear to be carried across the synapse from pre-synaptic neuron to post-synaptic muscle in EVs (3–5). Moreover, elegant phenotypic analysis has established that they serve key functions at the NMJ (6, 7). Evi traffics Wg (Wingless)/Wnt to presynaptic terminals and wg and evi mutants display characteristic defects in NMJ maturation. Syt4, on the other hand, is best known for its roles in activity-dependent plasticity. However, whether their functions depend on EV release has not been definitively established.

Before tackling this question, the authors of the current study first needed to identify genetic backgrounds with impaired EV biogenesis. Exosomes, a subtype of EV, derive from structures called multivesicular endosomes (MVEs). An important pathway through which EVs are formed from MVEs is the endosomal sorting complex required for traffic (ESCRT) family: ESCRT-0, -1, and -2 aggregate EV cargoes and disrupt the membrane of the MVE, then recruit

ESCRT-3 to drive fission of the MVE membrane and form intraluminal vesicles (8). When the MVE membrane fuses with the plasma membrane, the vesicles are released as exosomes into the extracellular space and can then be taken up along with their cargoes by neighboring cells to signal or undergo phagocytosis.

Because ESCRTs are not involved in EV biogenesis in all cell types and their roles at the NMJ were unknown, Dresselhaus et al. (1) tested if ESCRT machinery is required for EV release in this model. They find that presynaptic knockdown of the ESCRT-1 component Tsg101 leads to accumulation of multiple EV cargoes on the presynaptic side and virtually eliminated their release. Importantly, they observe a comparable impairment of EV release when two other ESCRT components are mutated: the ESCRT-0 complex component Hrs and the ESCRT-III homolog Shrub. Thus, the authors conclude that ESCRT machinery is indeed required for EV release at the NMJ, thereby establishing the *Drosophila* NMJ as an ideal system to study endogenous roles of EV release.

A possible caveat to inferring EV functions from analysis of ESCRT component mutants is that these proteins are important not only for EV biogenesis, but also broadly in neuronal membrane traffic. Specifically, they regulate autophagy and autophagic flux in different cell types (9). The authors addressed this concern in several ways. First, they tested if neuronal Tsg101 RNAi-

¹Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH, USA.

Correspondence to Heather T. Broihier: heather.broihier@case.edu.

© 2024 Bostelman and Broihier. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>).

mediated knockdown (KD) affects autophagy and indeed found a dramatic build-up of autophagic intermediates in Tsg101-KD presynaptic terminals, suggesting a compensatory induction of autophagic machinery. Importantly however, loss of Hrs did not similarly impair autophagy. Given that EV release is blocked in both backgrounds, the defect in EV release does not necessarily reflect broader defects in membrane traffic. Second, the authors tested whether EV release is disrupted when the core autophagy components Atg1 and Atg2 are lost and found that it is not. Thus, the role of the ESCRT pathway in EV release is at least partially separable from its involvement in autophagy.

Dresselhaus et al. (1) next investigated their central question: is EV release necessary for trans-synaptic signaling? They turned first to the EV cargo Evi/Wntless, a carrier protein required for Wg secretion. Unexpectedly, loss of ESCRT components does not phenocopy the reduction in bouton number observed in *wg* or *Evi* mutants. Interestingly, loss of one of the ESCRT components (Tsg101) does result in an increase in immature boutons like that observed upon Wg/Evi loss; however, Hrs/ESCRT-0 mutants do not display this phenotype. Similarly, a post-synaptic Wg signaling reporter indicates active Wnt signaling in the Hrs/ESCRT-0 mutant, but not upon loss of Tsg101. In the future, it will be interesting to pursue the function of Tsg101 in Wg

signaling, but the absence of Wg phenotypes in Hrs mutants indicates that it is not due to impaired EV release. Might EVs still be essential for the action of another EV cargo at the NMJ? To investigate this question, the authors tested whether their ESCRT mutants phenocopy the defects in synaptic plasticity observed in Syt4 mutants. Similarly, they find that Syt4-dependent structural and functional plasticity is not impaired upon loss of the ESCRT pathway. Together, these data indicate that EV release is dispensable for Wg and Syt4 signaling and suggest that these proteins are also released by a canonical secretory pathway or that they act cell-autonomously in the neuron.

If EVs carrying Wg/Evi and Syt4 do not play a primary signaling role, what is their function? The simplest hypothesis is that motoneurons release EVs as debris to maintain proteostasis. In this event, the EVs are likely to be engulfed and phagocytosed by surrounding cells. Thus, the authors tested whether levels of secreted/post-synaptic Syt4 are elevated when phagocytosis is impaired in Draper-KD animals. Draper is a key phagocytosis receptor important for clearing neuronal debris at the NMJ (10). The authors found a dramatic increase in secreted Syt4 in Draper-KD animals, arguing that the normal destination of Syt4-positive EVs is phagocytosis by cells in the environment.

This important work demands a broader re-evaluation of proposed signaling roles for EVs and their cargoes with a focus on in vivo systems and loss-of-function approaches. It should also be emphasized that while this study calls into question roles of signaling EVs at the *Drosophila* NMJ, there are other contexts where compelling evidence exists for signaling and pathological EVs (11). Moving forward, as more EV cargoes are defined, it will be critical to rigorously evaluate if EV-mediated release is specifically required for their functions.

References

1. Dresselhaus, E.C., et al. 2024. *J. Cell Biol.* <https://doi.org/10.1083/jcb.202405025>
2. Welsh, J.A., et al. 2024. *J. Extracell. Vesicles.* <https://doi.org/10.1002/jev2.12404>
3. Korkut, C., et al. 2009. *Cell.* <https://doi.org/10.1016/j.cell.2009.07.051>
4. Korkut, C., et al. 2013. *Neuron.* <https://doi.org/10.1016/j.neuron.2013.01.013>
5. Koles, K., et al. 2012. *J. Biol. Chem.* <https://doi.org/10.1074/jbc.M112.342667>
6. Miech, C., et al. 2008. *J. Neurosci.* <https://doi.org/10.1523/JNEUROSCI.0164-08.2008>
7. Yoshihara, M., et al. 2005. *Science.* <https://doi.org/10.1126/science.1117541>
8. van Niel, G., et al. 2018. *Nat. Rev. Mol. Cell Biol.* <https://doi.org/10.1038/nrm.2017.125>
9. Leidal, A.M., and J. Debnath. 2021. *FASEB Bioadv.* <https://doi.org/10.1096/fba.2020-00138>
10. Fuentes-Medel, Y., et al. 2009. *PLoS Biol.* <https://doi.org/10.1371/journal.pbio.1000184>
11. Gassama, Y., and A. Favereaux. 2021. *Front. Cell. Neurosci.* <https://doi.org/10.3389/fncel.2021.626043>