Turning to *Drosophila* for help in resolving general anesthesia

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A recent study (1) uses *Drosophila* flies to support a mechanism for general anesthesia. The authors find a specific effect on TWIK-related K^+ (TREK-1) channels (a class of potassium leak channels) that results from a nonspecific effect on cholesterol-rich rafts in neuronal membranes. Using primarily in vitro assays, the study shows how this might work: General anesthetics appear to disrupt lipid raft architecture, causing an embedded enzyme (phospholipase D [PLD]) to be released. Increased PLD in the proximity of TREK-1 channels raises the local concentration of phosphatidic acid (PA), activating TREK-1 channels and causing an outflow of potassium, hyperpolarizing the cell. This could, in principle, contribute to general anesthesia (2). One way to refute this hypothesis would be to test a nonanesthetic analog alongside its anesthetic counterpart (3)—both might disrupt lipid rafts. Instead, the authors switch gears and seek behavioral relevance, turning to fruit flies for help.

Experiments in animals are what distinguishes a study on general anesthesia from a study on the diverse molecular and cellular effects of general anesthetics, hence the potential value of ending this work with *Drosophila*. PLD and TREK-1 work the same way in flies, so demonstrating behavioral resistance to general anesthetics in a fly mutant lacking PLD protein is potentially valuable, as would be evidence of disrupted lipid rafts in intact fly brains exposed to these drugs. It is unfortunate, however, that making the crucial link to animals and brains does not always seem to require the same level of scientific rigor as biochemical and cellular work. In figure 6 of ref. 1, we are shown a single anesthesia induction experiment on one drug (chloroform) with no error bars and no sample sizes, compared to an unnamed wild-type strain; we are then provided with two images of lipid rafts of unclear provenance, followed by a single *t* test on >16,000 data points extracted from an unstated number of fly brains of unknown genotype exposed to an undetermined concentration of chloroform. *Drosophila* experiments can add value to a molecular story, but not at the cost of lowered standards. We invite the authors to redress that standard, in a letter.

Whether TREK-1 channels play an important role in general anesthesia, as has been posited before (2), still remains to be determined. Considering that synaptic communication is key to how the brain works, it would not be surprising that a variety of target mechanisms affect brain function, including TREK-1 but also other channels (4), and even synaptic release mechanisms themselves (5, 6). Alongside a variety of postsynaptic targets, most notably, GABA(A) receptors (7), the likely scenario is that these heterogenous mechanisms together produce the successive end points we term "general anesthesia," which always need validation in animals or whole-brain readouts. Which mechanisms are most relevant to loss of consciousness? Which are most relevant to loss of responsiveness, or to amnesia, or to recovery kinetics? These questions can be effectively addressed in *Drosophila* or other animal models (8–10), with ideally the same rigor as applied to in vitro systems.

1 M. A. Pavel, E. N. Petersen, H. Wang, R. A. Lerner, S. B. Hansen, Studies on the mechanism of general anesthesia. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 13757–13766 (2020).

2 E. A. Steinberg, K. A. Wafford, S. G. Brickley, N. P. Franks, W. Wisden, The role of K_2p channels in anaesthesia and sleep. *Pflugers Arch.* **467**, 907–916 (2015).

3 E. Pejo *et al.*, Analogues of etomidate: Modifications around etomidate's chiral carbon and the impact on in vitro and in vivo pharmacology. *Anesthesiology* **121**, 290–301 (2014).

4 H. C. Hemmings Jr. *et al.*, Towards a comprehensive understanding of anesthetic mechanisms of action: A decade of discovery. *Trends Pharmacol. Sci.* **40**, 464–481 (2019).

5 A. T. Bademosi *et al.*, Trapping of syntaxin1a in presynaptic nanoclusters by a clinically relevant general anesthetic. *Cell Rep.* **22**, 427–440 (2018).

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6 B. E. Herring, Z. Xie, J. Marks, A. P. Fox, Isoflurane inhibits the neurotransmitter release machinery. *J. Neurophysiol.* **102**, 1265–1273 (2009).
7 N. P. Franks, General anaesthesia: From molecular targets to neuronal pathways of sleep and arousal. *Nat. Rev. Neurosci.* **9**, 370–386 (2008).
8 H. A. Nash, In vivo genetics of anaesthetic action. *Br. J. Anaesth.* **89**, 143–155 (2002).
9 O. Zalucki, B. van Swinderen, What is unconsciousness in a fly or a worm? A review of general anesthesia in different animal models. *Conscious. Cogn.* **44**, 72–88 (2016).
10 L. M. Steele, P. G. Morgan, General genetic strategies. *Methods Enzymol.* **602**, 113–132 (2018).