LETTER

REPLY TO VAN SWINDEREN AND HINES:

Drosophila model establishes the lipid membrane as a target of anesthetics

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We thank van Swinderen and Hines (1) for their response to our recent article, where we find that anesthetics disrupt lipid rafts and activate phospholipase D (PLD) through a membrane-mediated mechanism (2). Here we take the opportunity to address some minor misunderstandings and clarify our experimental procedures.

Our chloroform induction in *Drosophila* is part of a larger study (3) which we reference in our study (reference 40 in ref. 2), referring the reader to a detailed description of the animal experiments, including the genotype of the wild-type flies (w1118), and full dose–response curves for three anesthetics (chloroform, isoflurane, and diethyl ether). The number of animals used in our study can be read from our survival curves—eight events, so eight animals, for each curve ($n = 8$) (2). Each animal is a separate experiment. The request for error bars on a survival curve (figure 6B in ref. 2) is highly uncustomary. For a description of the PLD^{null} animals, we referred the reader to Thakur et al. (4) (reference 41 in ref. 2). As described in our methods, the fly brains for imaging were incubated in a saturated solution of chloroform (2). The average apparent raft size was calculated from the direct observation of ∼16,000 rafts imaged from two brains and an unspecified number of cells. We inadvertently left out the number of fly brains used for imaging, and we thank van Swinderen and Hines for pointing out this error (two treated and two control brains). Each raft is a quantitative individual measurement of raft diameter resulting in a large number of data points. The representative images are an unspecified region of the fly brain. Lastly, we note that a nonanesthetic (nonimmobilizer, F6) was tested, and had no effect on PLD2 activity at concentrations previously predicted by the Meyer–Overton correlation (see figure 5*B* in ref. 2).

Our study answers the question, is the membrane a target of anesthesia? This was a pressing 100-y-old question in the field, and we believe it is the reason why the paper has drawn considerable attention, including from medical practitioners and the public. We establish that an anesthetic perturbation of the membrane directly activates PLD, and thus PLD's mutation in *Drosophila* is an excellent experiment to establish a membrane-mediated role for anesthesia in vivo. We made no claim that PLD activates a TREK-1 channel in flies. Many proteins could be downstream effectors and contribute to diverse aspects of general anesthesia, including amnesia, but none of those proteins are known to be directly regulated by an anesthetic perturbation to the membrane, and thus they are poor subjects for establishing the membrane as a target of anesthesia.

We contend our chloroform experiments with PLD^{null} flies validate the membrane as a target of general anesthesia and establish a raft-associated protein activation mechanism of general anesthesia.

1 B. v. Swinderen, A. D. Hines, Turning to *Drosophila* for help in resolving general anesthesia. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 24627–24628 (2020).

2 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).

3 E. N. Petersen, K. R. Clowes, S. B. Hansen, Measuring anesthetic resistance in *Drosophila* by VAAPR. *bioRxiv*:10.1101/797209 (10 October 2019).

4 R. Thakur *et al.*, Phospholipase D activity couples plasma membrane endocytosis with retromer dependent recycling. *eLife* **5**, 1–23 (2016).

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The authors declare no competing interest.

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