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CrossTalk rebuttal

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The two novel and controversial aspects of the glymphatic hypothesis are: (i) convective fluid transport in brain parenchyma; and (ii) a major role for AQP4 in CSF/ISF exchange under normal physiological conditions. In their cross-talk article, Iliff and Simon (2019) agree that these are the main areas of controversy. Regarding (i), it seems we are now largely in agreement that transport in grey matter is best described by non-directional, parenchymal diffusion coupled to fast solute transport in the perivascular spaces. The direction and rate of solute transport in the perivascular spaces remain to be determined. Recent studies demonstrate pulsation-driven, directional solute transport in the subarachnoid space around pial vessels (Bedussi *et al.*, 2018); however, this transport appears to occur in a separate compartment from that within the leptomeningeal vessel sheath which facilitates solute transport into and out of the brain (Pizzo *et al.*, 2018).

Substantial differences of opinion remain with respect to (ii), the role of AQP4 in brain fluid transport. Conventional thinking makes it difficult to understand how a water-selective transporter in a mammalian cell membrane could facilitate directional, hydrostatically driven transport of fluid, which consists of both solutes and water (Smith *et al.*, 2015). Experimental observations on the effect of AQP4 deletion on transport of cisternally injected solutes from CSF into brain parenchyma are highly variable among labs; indeed, this variability exceeds any purported effects of AQP4 deletion (Smith *et al.*, 2017, Mestre *et al.*, 2018). Given the uncertainty in experimental results, and the lack of a plausible mechanism of how AQP4 could be the rate-limiting step for solute movement from subarachnoid space to parenchyma (Jin *et al.*, 2016), we consider the proposed role of AQP4 in ‘glymphatic’ solute influx at best conjectural.

The glymphatic hypothesis assumes that solute clearance following direct parenchymal injection is driven by CSF influx from the subarachnoid space, but in our arguably more plausible model (Smith & Verkman, 2019) this is not the case. Iliff and Simon (2019) cite studies in which AQP4 deletion retards clearance of solutes following injection of relatively large, 0.5–1.0 μl solution volumes into mouse striatum. These injections displace a substantial fraction of striatal interstitial fluid, having total volume of 1.0–1.5 μl, such that

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fluid convection during the injection determines solute distribution. Effects of AQP4 deletion under these conditions cannot provide evidence for AQP4-dependent parenchymal convection from arteries to veins under normal physiological conditions. Because AQP4 deletion causes multiple structural and functional alterations in the brain, including increased extracellular volume (Yao *et al.*, 2008) and impaired reabsorption of exogenous fluid (Papadopoulos *et al.*, 2004), various nonglymphatic mechanisms can explain any apparent effect of AQP4 deletion on solute clearance.

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