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1. Background

Valves in the venous vasculature and peripheral lymphatic systems convert pressure oscillations to directed flows. Still, valve structures have not been identified in either the periarterial or the perivenous spaces of the glymphatic system [1]. Pressure oscillations occur at several timescales in brain fluids, ranging from arterial pulsations several times per second [2] over respirations [3] to slow vasomotion every several seconds [4], and would provide a driving force for bulk flow if coupled to a valve.

Astrocytic vascular endfeet plastered around the cerebral vasculature are connected by gap junctions and may form valves [5]. In this hypothesis, the valves open when artery dilation causes an increased pressure at the astrocytic endfeet and valves close when artery constriction causes a corresponding pressure drop. Since astrocyte endfeet (i) naturally protect the extracellular matrix, (ii) are likely to regulate their stiffness with brain states (e.g. via laminin and aquaporin rafts [6,7]), and (iii) provide a probable route for cerebrospinal influx [8], we propose that astrocyte endfeet may also function as valves to turn pressure oscillations into forward fluid flow.

2. The modelled endfoot valve under pressure oscillations

The recent model of viscous flow in a slit between two elastic plates by members of our team, Christensen and Jensen [9], provides a natural minimal framework, figure 1. In their set-up, two flexible plates have a narrow slit between them, like
astrocyte endfeet gaps. A key assumption is that the thickness of the plates is much greater than the distance between them. Though precise in vivo measurements of astrocyte endfoot dimensions are unfortunately unavailable, they can be estimated from 2-photon imaging of vascular cross-sections (such as those by Enger et al. [10]) and ex vivo histology (such as that by Wang et al. [8]) (table 1). Even considering the wide margins of measurement uncertainty, the endfoot circumference is much greater than their thickness which is much greater than their separation. Though their short radius approaches the limits of its assumptions, this simplified two-dimensional mathematical model is an effective approach to reasoning about asymmetric flow between endfeet (see electronic supplementary material, information).

The endfeet may be asymmetric in several ways, but we examine the simplest here. The endfoot gap can be slightly asymmetric, with a narrower slit at the interstitial side than the perivascular side, figure 1. Other asymmetries which may enable valve-function include tethering with interstitial extracellular matrix proteins, internal structure of the cytoskeleton, such as glial fibrillary acidic protein (GFAP) or anchoring to perivascular protein complexes [6]. Here we focus on what is perhaps the simplest realization. Mathematically, we add a single parameter to the original model, the slit height $h_2$ on the narrower interstitial side, figure 1.

Aside from the static geometry of the endfeet, the model depends on the stiffness of the plate, known as Young’s modulus, and weakly on the ratio of transverse to axial strain, or Poisson’s ratio. Despite considerable advances in the measurements of brain mechanical properties, there is a large interval of realistic values possible for both parameters [13–15]. For our calculations here, we will apply both ends of the spectrum of human in vivo measurements of macroscopic brain tissue stiffness, table 1, and check results against the softest estimates made (in vitro glia cells [15] see electronic supplementary material, figure S2).

Finally, the model requires a pressure gradient to drive the flow. The pressure oscillations will be most pronounced near arterioles, and we here focus on those periarterial spaces. To best test the valve-function’s ability to selectively

**Figure 1.** An asymmetry in endfoot gaps may favour inwards over outwards fluid flow. (a) An astrocyte (green) near an artery will extend processes with endfeet to cover the periarterial space around the artery (created with BioRender.com). (b) Using the large parameters (table 1), the endfoot drawn to scale bends only little under the investigated pressure differences of $\Delta p = 0.2$ mmHg. (b(ii)) When the interstitial pressure is greater than perivascular pressure, fluid will be driven out into the perivascular space (with rate $Q$) and endfeet will be pushed together towards closing. (b(iii)) When pressures are equal, there will be no flow. (b(iii)) When the perivascular pressure exceeds the interstitial pressure, fluid will be driven into tissue. (c) The magnified endfoot gap has mid-height of $h_0$, narrowest height $h_2$, and thickness $t$. (d) Drawn to scale, the small endfoot has a relatively small slit opening facing the interstitial side compared with the radius of the endfoot (dashed line, length $\ell$). (e) At larger positive pressures ($\Delta p = 3.4$ mmHg), the large endfoot gap closes and prevents a fast inflow of cerebrospinal fluid to the interstitial space.

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**Table 1.** Summary of model parameters.

<table>
<thead>
<tr>
<th>parameter</th>
<th>symbol</th>
<th>small</th>
<th>large</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>gap mean height</td>
<td>$h_0$</td>
<td>0.04 µm</td>
<td>1.0 µm</td>
<td>Wang et al. [8]</td>
</tr>
<tr>
<td>gap narrow height</td>
<td>$h_2$</td>
<td>0.02 µm</td>
<td>0.5 µm</td>
<td>Enger et al. [10]</td>
</tr>
<tr>
<td>endfoot thickness</td>
<td>$T$</td>
<td>2 µm</td>
<td>6 µm</td>
<td>Wang et al. [8]</td>
</tr>
<tr>
<td>endfoot radius</td>
<td>$\ell$</td>
<td>1.5 µm</td>
<td>6.5 µm</td>
<td>Wang et al. [8]</td>
</tr>
<tr>
<td>endfoot perimeter</td>
<td>$W$</td>
<td>10 µm</td>
<td>40 µm</td>
<td>Wang et al. [8]</td>
</tr>
<tr>
<td>fluid viscosity</td>
<td>$\eta$</td>
<td>$0.693 \times 10^{-4}$ Pa s</td>
<td>$0.693 \times 10^{-3}$ Pa s</td>
<td>Mestre et al. [2]</td>
</tr>
<tr>
<td>Poisson’s ratio</td>
<td>$\nu$</td>
<td>0.5</td>
<td>0.5</td>
<td>Goriely et al. [11]</td>
</tr>
<tr>
<td>Young’s modulus</td>
<td>$E$</td>
<td>$2.65 \pm 0.55 \times 10^3$ Pa</td>
<td>$2.65 \pm 0.55 \times 10^3$ Pa</td>
<td>Green et al. [12]</td>
</tr>
</tbody>
</table>
allow forward flow, we use a pure oscillation with a pressure varying sinusoidally from −0.2 to 0.2 mmHg and with a zero mean based on poroelastic simulations [16] and 2-photon imaging of perivascular spaces [17]. A pressure difference of 0.2 mmHg over the small thickness of the endfoot is large compared with the 1.5 mmHg m⁻¹ gradient often considered realistic for cerebrospinal fluid [18], but realistic in light of the local arterial dilation and contraction [16,17,19] and squeeze-flow approximations (see electronic supplementary material, information).

### 3. Astrocyte endfeet may act as valves to convert pressure oscillations to glymphatic flow

The relationship between flow around the astrocyte endfoot and the pressure gradient is complex and depends on the size and shape of the endfoot, with the size of the gaps between the endfeet being the most important parameter. Due to the considerable measurement uncertainty and the artefacts related to ex-vivo histology, we summarize our findings in two scenarios, figure 2a. In the small scenario, we choose the lower-bounds for gap width, endfoot thickness and radius (figure 2 top row), and in the large scenario, we choose the corresponding upper-bounds (figure 2 bottom row). Both small and large endfoot show the asymmetry, but backflow through the small endfoot gaps is more significantly reduced (figure 2a).

In figure 2b, we show the flow resulting from letting the pressure on the endfoot vary sinusoidally from −0.2 to 0.2 mmHg (assuming quasi-static conditions, see electronic supplementary material, information). As expected from their larger gaps, large endfeet provide for greater absolute flow levels. For both large and small endfeet, the forward flow during positive pressure on the endfoot is greater than the corresponding backward flow during negative pressure.

The ability to turn pressure oscillations into forward flow forward flow can be quantified as the ratio of forward to backward flow across the pure oscillation, which depends on Young’s modulus or stiffness. Both small and large endfeet can turn pressure oscillations into a driver for forward flow, but small endfeet are more effective.

**Figure 2.** Flexible astrocytes can act as valves to convert pressure oscillations to forward flow. (a) In this model, steady flow (Q) around small (red, top row) and large (blue, bottom row) astrocyte endfeet depends asymmetrically on pressure (Δp). Normalized to the maximal flow rate (Q_max) the pressure–flow relationship is similar for large and small endfeet. (b) When exposed to pure pressure oscillations with zero mean, the forward flow is greater than the backward flow for both small and large endfeet. The small endfeet nearly close at maximal backwards pressure, especially when they are soft. Shaded areas show the effect of varying endfoot stiffness within the measurement uncertainty interval (table 1), with softer endfeet allowing greater levels of forward flow than stiff endfoot. (c) The effectiveness with which endfeet turn pressure oscillations into forward flow can be quantified as the ratio of forward to backward flow across the pure oscillation, which depends on Young’s modulus or stiffness. Both small and large endfeet can turn pressure oscillations into a driver for forward flow, but small endfeet are more effective.

### 4. Discussion

Since several fluid oscillations of considerable amplitude and frequency are present in the live brain, it is important to determine whether any valve mechanisms exist, as the oscillations could then drive directed bulk flow in addition to contributing dispersive clearance [20]. We here drew on recent modelling of fluid mechanics, ex vivo quantifications of astrocyte endfeet, and in vivo elasticity measurements to argue that endfeet are a realistic valve candidate. We used a geometric asymmetry in the shape of the gap between endfeet, but several sources of asymmetry could promote valve-like behaviour.

Since aquaporins in the endfoot membrane allow faster fluid transport across the membrane, they might contribute
to endfoot flexibility [21]. This would connect the circadian localization of AQP4 to the membrane in preparation for sleep [7] with the enhanced fluid flow around the endfoot membrane. However, aquaporins may be anchored to the dystrophin-associated complex via alpha-syntrophin [6] and dystrophin is associated with cell stiffness rather than flexibility [22]. The biomechanical consequences of the aquaporin-associated complexes are important to determine since their regulation may enable a direct mechanical valve function and explain the observed glymphatic dependence on aquaporins.

Efforts to measure the astrocyte endfoot structure along with its mechanical properties in vivo are required for progress on this hypothesis. The dynamical deformations necessary for the valve mechanism are relatively small (0.1 µm for large endfeet at 0.2 mmHg pressure), or below the ex vivo resolution of 2-photon-imaging (approx. 0.2 µm), which in the live brain is further reduced by brain constant movements. Beyond the arteriolar perivascular spaces modelled here, the mechanism may contribute to flow regulation towards the capillary level depending on glia coverage [8,23]. In principle, a reversed asymmetry of the endfoot could also promote efflux rather than influx along arteries as proposed in the iPAD model [24,25]. Until novel imaging approaches can test the valve model or make precise measurements of endfoot mechanical properties, we propose that models of brain fluid flow should consider the possibility that astrocyte endfeet act as valves to convert fluid oscillations to unidirectional glymphatic flow.

5. Additional material

The supplementary information with calculations referenced above is available in PDF as Additional File 1. The Calculation Code used to generate the figures is available as a .zip-file collection of Julia code scripts in Additional File 2.

Since in vivo measurement of astrocyte endfoot dimensions are lacking and probably vary over orders of magnitude between small and large endfeet [8], we made two parameter scenarios corresponding to the smallest and largest values estimated from the given references. Viscosity is taken as that of water at body temperature and the Poisson’s ratio was taken at 0.5, as is common in the literature (with little effect on the calculations [11,26]). Young’s modulus was measured in vivo in humans with MR elastography in white and grey matter and we here consider the range from mean minus the standard deviation to mean plus the standard deviation [12].

Data accessibility. The data are provided in the electronic supplementary material [27].

Authors’ contributions. P.A.R.B.: conceptualization, formal analysis, investigation, methodology, project administration, software, visualization, writing—original draft, writing—review and editing; A.H.C.: formal analysis, investigation, methodology, software, visualization, writing—original draft, writing—review and editing; A.L.: conceptualization, data curation, writing—review and editing; K.H.J.: conceptualization, formal analysis, investigation, methodology, writing—review and editing; M.N.: conceptualization, funding acquisition, investigation, resources, supervision, writing—original draft, writing—review and editing; T.B.: conceptualization, formal analysis, investigation, methodology, supervision, visualization, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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