



Perivascular pumping in the mouse brain: Improved boundary conditions reconcile theory, simulation, and experiment

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ABSTRACT

Cerebrospinal fluid (CSF) flows through the perivascular spaces (PVSs) surrounding cerebral arteries. Revealing the mechanisms driving that flow could bring improved understanding of brain waste transport and insights for disorders including Alzheimer's disease and stroke. In vivo velocity measurements of CSF in surface PVSs in mice have been used to argue that flow is driven primarily by the pulsatile motion of artery walls — perivascular pumping. However, fluid dynamics theory and simulation have predicted that perivascular pumping produces flows differing from in vivo observations starkly, particularly in the phase and relative amplitude of flow oscillation. We show that coupling theoretical and simulated flows to more realistic end boundary conditions, using resistance and compliance values measured in mice instead of using periodic boundaries, results in velocities that match observations more closely in phase and relative amplitude of oscillation, while preserving the existing agreement in mean flow speed. This quantitative agreement among theory, simulation, and in vivo measurement further supports the idea that perivascular pumping is an important CSF driver in physiological conditions.

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1. Introduction

Cerebrospinal fluid (CSF) has long been known to flow in the perivascular spaces (PVSs) that surround arteries in the brain (Rennels et al., 1985; Rennels et al., 1990). A brain-wide fluid pathway, the glymphatic system (Iliff et al., 2012), has been proposed to transport fluid close to much or all of the brain parenchyma, enabling waste evacuation and nutrient / neurotransmitter delivery at rates more rapid than would be possible with diffusion alone, and affecting the brain parenchyma primarily during sleep. Observations in mice (Xie et al., 2013), rats (Ratner et al., May 2017; Koundal et al., 2020), pigs (Bèchet, 2021), and humans (Eide et al., 2018; Ringstad et al., 2018; Eide et al., 2021) have produced data consistent with that proposal, though other studies have raised questions (Joan Abbott et al., 2018; Smith and Verkman, 2018; Ma et al., 2019). CSF flow has also been implicated in early edema after stroke (Mestre et al., 2020) and proposed as a pathway for drug delivery (Plog et al., 2018; Lilius et al., 2019). We will focus here on surface (pial) PVSs, where detailed flow mea-

surements are possible via in vivo imaging (Mestre et al., 2018; Bedussi et al., 2017). Recent reviews (Benveniste, 2018; Ray and Heys, 2019; Thomas, 2019; Nedergaard and Goldman, 2020; Rasmussen et al., 2022) summarize current knowledge of CSF flow and mass transport in the brain, including surface PVSs.

Measurements in surface PVSs show flow pulsing in synchrony with the cardiac cycle, suggesting a causal link to blood flow. Hadaczek et al. (2006) proposed that the dilations and constrictions traveling along artery walls with each heart beat might drive CSF in a peristalsis-like mechanism they dubbed “perivascular pumping.” As evidence, they presented experimental results showing that macromolecules injected into the central nervous systems of rats were transported further in animals with beating hearts than in animals whose hearts had recently been stopped. Iliff et al. (2013) presented additional evidence in support of the hypothesis. More recent theoretical (Schley et al., 2006; Wang et al., 2011) and numerical (Bilston et al., 2003; Asgari et al., 2016; Kedarasetti et al., 2020) studies have indeed shown that perivascular pumping can plausibly drive net fluid motion locally (except when dilations and constrictions do not travel Rey and Sarntinoranont, 2018).

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Though it may be plausible for perivascular pumping to drive CSF, theoretical studies that use reasonable approximations and realistic parameters predict flows that differ from in vivo observations in surface PVSs. In a pioneering study, Schley et al. (2006) produced an analytic prediction of the flow due to perivascular pumping in an open, two-dimensional, Cartesian space, which is based on the lubrication approximation and is rigorous in the case of long wavelengths. For sinusoidal dilations and constrictions with a $b = 0.3 \mu\text{m}$ half-amplitude traveling at $c = 1 \text{ m/s}$ on one wall of a channel with width $H = 40 \mu\text{m}$, their theory predicts a flow in which the mean downstream velocity (that is, the velocity along the length of the PVS, parallel to blood flow) is $0.034 \mu\text{m/s}$. Later in vivo measurements found a mean downstream velocity of $18.7 \mu\text{m/s}$ (Mestre et al., 2018). Uncertainty in the input parameters, along with the analytic simplifications involved, particularly the geometric differences between a two-dimensional Cartesian space and a three-dimensional annular space, may explain some of the discrepancy in the mean flow. Harder to explain, however, are the discrepancies in phase and relative amplitude of oscillation. Flow oscillation is predicted to lag the wall velocity (which we define as the rate of PVS channel constriction, consistent with Mestre et al. (2018)) by $\varphi = 270^\circ$, but in vivo observations indicate flow oscillations lag wall velocity by $\varphi = 353^\circ$. The ratio of oscillatory to mean flow predicted analytically is $\gamma = 22,200$, but in observations, dividing the peak root-mean-square velocity oscillation by the mean downstream velocity yields $\gamma = 0.53$. Thus if the mean flow were the same, oscillations in observed flows would need to be about 40,000 times faster in order to match the prediction.

Wang et al. (2011), also using lubrication theory and the long-wavelength approximation, produced an analytic prediction of the flow due to perivascular pumping in a cylindrical annulus filled with a porous medium. For sinusoidal dilations and constrictions with the same $0.3 \mu\text{m}$ half-amplitude and the same speed 1 m/s , traveling on the inner wall of an annulus with inner radius $r_1 = 30 \mu\text{m}$ and outer radius $r_2 = 70 \mu\text{m}$, with porosity $\varepsilon = 1$, their theory predicts a flow with mean downstream velocity $10.13 \mu\text{m/s}$, quite close to the $18.7 \mu\text{m/s}$ observed value. But disagreement again arises on oscillation phase and amplitude. Like Schley et al. (2006), Wang and Olbricht predict a $\varphi = 270^\circ$ phase lag from wall velocity to flow oscillations, disagreeing with observations. The Wang and Olbricht theory predicts $\gamma = 443$, far from $\gamma = 0.53$, as observed in vivo.

Perivascular pumping has also been studied using numerical simulations, which likewise predicted flows that differ starkly from in vivo observations. Kedarasetti et al. (2020) recently performed a series of simulations. The first set considered axisymmetric flows in an open (not porous) cylindrical annulus with inner radius $30 \mu\text{m}$ and outer radius $70 \mu\text{m}$. Sinusoidal dilations and constrictions with half-amplitude on the order of $0.3 \mu\text{m}$, speed 1 m/s , and frequency 8.67 Hz propagated on the inner wall. The computational domain was one wavelength long, with periodic end boundaries. Though the authors did not report the mean flow speed or volume flow rate, they did state that for realistic speeds, the phase of flow oscillations lagged wall velocity by $\varphi = 270^\circ$, agreeing with predictions from lubrication theory (Schley et al., 2006; Wang et al., 2011) but not with in vivo observations (Mestre et al., 2018). The authors also stated that $\gamma \sim 100$, again disagreeing with in vivo observations.

The second set of simulations by Kedarasetti et al. (2020) considered flow in a three-dimensional domain whose cross-sectional size and shape are similar to in vivo observations (Iliff et al., 2012; Bedussi et al., 2017; Mestre et al., 2018) and similar to annular shapes that have minimum hydraulic resistance (Tithof et al., 2019). Dilations and constrictions on the inner wall

propagated at $c = 1 \text{ m/s}$ with frequency $f = 8.67 \text{ Hz}$ but were not sinusoidal; rather, their shape and amplitude were taken from the in vivo observations of Mestre et al. (2018). The pressure was set to zero at the end boundaries. The simulations predicted a time-averaged centerline velocity of $102.1 \mu\text{m/s}$, in reasonable agreement with the $18.7 \mu\text{m/s}$ observed in vivo. The phase difference between wall velocity and flow oscillations is not stated, but judging from Fig. 3c in Kedarasetti et al. (2020), flow oscillations lag wall velocity by $\varphi \approx 330^\circ$, significantly different from 353° . And the ratio of oscillations to steady flow was $\gamma = 290$, strikingly different than $\gamma = 0.53$ as observed in vivo. Kedarasetti et al. (2020) also presented a third set of simulations, to be discussed below.

Repeatedly, analytic and numerical predictions of the mean flow caused by perivascular pumping agree reasonably well (if not perfectly) with each other and with mean flows observed in vivo. Analytic and numerical predictions agree that flow oscillations lag wall velocity by a substantial phase difference (270° to 330°), but in vivo observations indicate nearly zero (or equivalently, nearly 360°) phase difference. And when considering the relative amplitude of oscillation γ , though the values vary, theory and simulations have consistently predicted that perivascular pumping would drive far stronger oscillations than have been observed in vivo.

One explanation, as Kedarasetti et al. (2020) and others (Asgari et al., 2016; Diem et al., 2017; Smith et al., 2017; Faghih et al., 2018) have argued, is that perivascular pumping is not an important driver of flows observed in vivo. CSF production by choroid plexus and uptake by arachnoid villi and other efflux routes almost certainly drive some flow. Non-physiological flow induced by injection of tracer particles has been offered as an explanation (Kedarasetti et al., 2020; Faghih et al., 2018; Smith et al., 2017; Croci et al., 2019; Veluw et al., 2020; Bakker et al., 2019), but a recent publication showed that withdrawing an equal amount of fluid while injecting tracer particles leaves perivascular flows unchanged, implying they are unlikely to be artifacts (Raghunandan et al., 2021). Mestre et al. (2018) demonstrated that altering the artery wall motion substantially changed CSF flow characteristics and significantly reduced the mean flow speed, suggesting that perivascular pumping does play some role. An explanation of the discrepancies among theory, simulation, and experimental observation is badly needed.

We present evidence that the discrepancies may be resolved via the end boundary conditions. The flow produced by a perivascular pump depends on the pathways coupled to the pump, into which the pumped fluid must pass. Those pathways can be characterized with simple lumped parameters: hydraulic resistance and compliance. Hydraulic resistance quantifies the viscous effects along a fluid pathway, which tend to slow CSF flow, especially when it passes through vessels or interstitial spaces that are small. Compliance quantifies elastic effects along a fluid pathway: when walls and boundaries stretch, fluid can be stored temporarily. Though this two-parameter representation of a large set of pathways is a drastic simplification, it is nonetheless more realistic than the periodic, zero-pressure, or infinite boundary conditions used previously. We present in vivo measurements that approximate both parameters, then demonstrate that coupling existing analytic and numerical perivascular pumping models to a lumped-parameter pathway model produces flows that more closely match in vivo observations.

The paper continues with a discussion of the lumped-parameter model in Section 2. Measured values of the hydraulic resistance and compliance are presented in Section 3. We couple the lumped-parameter model to existing analytic predictions in Section 4 and to an existing numerical simulation in Section 5.

2. Lumped-parameter model for boundary conditions

The intricacy of the brain makes it impossible to study the global CSF pathway in full detail. Some mechanisms are unknown, some processes occur at length and time scales unmeasurable with current technology, and a full numerical simulation would overwhelm supercomputers. Thus it is practical to separate the CSF pathway into components that can be considered independently, among them the surface PVSs. Perivascular pumping in a PVS is most simply represented as a source that produces a volume flow rate q_0 . Considered in isolation, it can be represented by the closed-loop fluid pathway sketched in Fig. 1a. This uncoupled pathway is the lumped-parameter representation of perivascular pumping as considered by all past theoretical and computational studies, including those described above. Periodic end boundary conditions, zero-pressure boundary conditions, and infinite domains are equivalent, in the lumped-parameter characterization, to making a direct connection between the PVS inlet and outlet.

Realistic modeling, however, eventually requires accounting for interactions when components are connected. To understand how a peristaltic pump interacts with the rest of the CSF pathway, additional lumped parameters must be introduced, as sketched in Fig. 1b. We will characterize the CSF pathway beyond surface PVSs using resistance R and compliance C (a two-element Windkessel model). The resistance of a component (or pathway) is defined as the pressure difference across the component (or pathway) divided by the volume flow rate through it, and is analogous to electrical resistance. The compliance is defined as the flow rate divided by the rate of change of the pressure difference, and is analogous to electrical capacitance. Including both a compliance and a resistance is essential because we are interested in pulsatile flows and need to account for the characteristic timescale of the CSF pathway: RC . (Some studies discuss the same mechanics in terms of the elastance C^{-1} .)

The values of R and C are constant if the pathway behaves linearly; otherwise, their values vary with the pulsation frequency f . The compliance is well-approximated as constant if the walls of the pathway behave like linear, elastic solids, which is typically the case when strains are small. In surface PVSs, the strain is approximately $b/2/r_1 = 0.5\%$, where b is the half-amplitude of dilations and constrictions, and $r_1 = 30 \mu\text{m}$ is the inner radius of the annulus; so we take C to be constant. If the Womersley number $Wo = H\sqrt{2\pi f}/v$ is small (1 or less), the flow is well predicted by a steady laminar (Poiseuille) flow, and the resistance is well-approximated as constant. Again using $H = 40 \mu\text{m}$ and $f = 8.67 \text{ Hz}$, and using $v = 0.697 \times 10^{-6} \text{ m}^2/\text{s}$ (the kinematic viscosity of water at 36.8°C), we find $Wo = 0.35$, so we take R to be constant. On a brain-wide scale, when studying intracranial CSF dynamics,

the assumption of a pressure-independent resistance and a constant compliance has been shown to be valid for physiological values of ICP (Andersson et al., 2008; Qvarlander et al., 2010). In summary, the values of R and C relevant to steady flow are also relevant to pulsatile flow as observed in vivo.

With components modeled as in Fig. 1b, the volume flow rate q_1 through the rest of the CSF pathway must satisfy

$$\frac{\partial q_1}{\partial t} + \frac{q_1}{RC} = \frac{q_0}{RC}. \quad (1)$$

One way to arrive at this expression is to note that mass conservation requires inflows and outflows at node A to sum to zero ($q_c + q_1 = q_0$), and to note that energy conservation requires the pressure across C to equal the pressure across R ($P = P_c = P_R$). The pressure across R is given by Ohm's law ($P = q_1 R$), and the pressure-flow relationship for the capacitor is $q_c = C \partial P / \partial t$. Thus, equation (1) is obtained after replacing q_c in terms of q_1 , R , and C . We want to remark that our approach represents a simplification of the PVSs assuming that the flow across the glial boundary can be neglected, and thus we can apply conservation of mass. However, we anticipate that this assumption would not affect our results since these flows are estimated to be some orders of magnitude smaller than the mean downstream velocity and can be safely neglected (Romanò et al., 2020). Therefore, our lumped-parameter characterizations of perivascular pumping and of the rest of the CSF pathway make it possible to predict the flow in the coupled system from the flow in the uncoupled system, if the resistance R and compliance C can be estimated.

3. In vivo resistance and compliance measurements

Lumped-parameter characterizations of the typical CSF pathway beyond a surface PVS have been limited due to a lack of knowledge of the pathways' connectivity and mechanical properties. Neither the resistance nor the compliance has been measured, and the possible roles of flow through brain parenchyma and/or capillary PVSs are topics of ongoing investigation and debate. We estimated R and C using the brain-wide resistance and compliance, which we could measure directly. Those quantities are likely to differ from the resistance and compliance experienced by any particular surface PVS, but can serve as initial estimates and are likely to be more accurate than the $R = 0$ and $C = 0$ values implied by periodic, zero-pressure, or infinite boundary conditions. The effects of variation in their values are discussed in more detail below.

We performed bolus-injection experiments in 11 mice, as described in Methods, using the setup shown in Fig. 2. The resulting variation of ICP over time is shown in Fig. 3. In $N = 7$ mice, we measured ICP in the cisterna magna; in $N = 4$ others, we measured

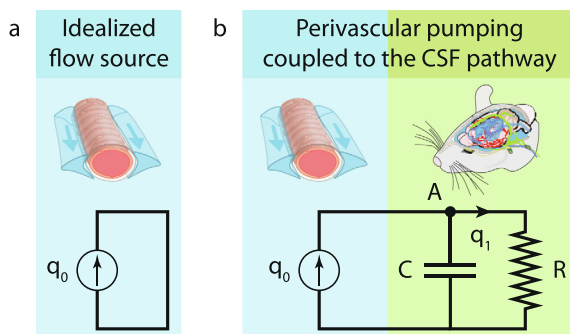


Fig. 1. (a), A lumped-parameter characterization of perivascular pumping, uncoupled from other fluid pathways. (b), A lumped-parameter characterization of perivascular pumping coupled to other fluid pathways.

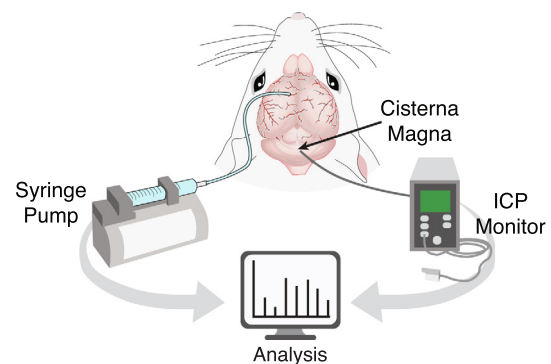


Fig. 2. Experimental setup. By injecting artificial CSF into the brain of an anesthetized mouse, we measured the resulting intracranial pressure (ICP) to determine the resistance and compliance of brain CSF spaces.

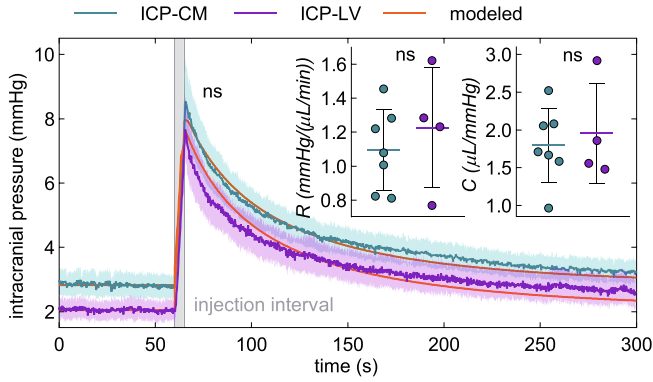


Fig. 3. Resistance and compliance of the cerebrospinal fluid pathway in mice, measured in vivo. After a brief and rapid fluid injection (1 $\mu\text{L/s}$ for 5 s), intracranial pressure decays with dynamics well-modeled by an RC boundary condition, as sketched in Fig. 1. From pressure variations measured in the cisterna magna in $N = 7$ animals (ICP-CM) we calculate resistance $R = 1.097 \pm 0.090$ mmHg/($\mu\text{L}/\text{min}$) and compliance $C = 1.798 \pm 0.185$ $\mu\text{L}/\text{mmHg}$. From pressure variations measured in the ventricle in $N = 4$ other animals (ICP-LV) we calculate resistance $R = 1.227 \pm 0.176$ mmHg/($\mu\text{L}/\text{min}$) and compliance $C = 1.953 \pm 0.332$ $\mu\text{L}/\text{mmHg}$. Repeated measures two-way ANOVA was performed for pressure variations; ns, not significant. The solid lines indicate the mean, and the shaded region represents the standard error of the mean (SEM). Unpaired Student's t-test was performed for resistance and compliance; ns, not significant; mean $\pm$ SEM.

ICP in the right lateral ventricle. From its value before injection, the ICP increased suddenly to a maximum value P_{max} , then decayed gradually. The decay was nearly exponential, as we would expect from a linear RC system. We calculated the compliance C from the pressure–volume index (PVI), as described in Methods. The resulting R and C values are shown in Fig. 3. When measured in the cisterna magna, the resistance is $R = (8.772 \pm 0.722) \times 10^{12} \text{ Pa} \cdot \text{s}/\text{m}^3 = 1.097 \pm 0.090$ mmHg/($\mu\text{L}/\text{min}$) (mean $\pm$ standard error of the mean), and the compliance is $C = (1.349 \pm 0.139) \times 10^{-11} \text{ m}^3/\text{Pa} = 1.798 \pm 0.185$ $\mu\text{L}/\text{mmHg}$. The corresponding time constant is $RC = 118.3$ s. When measured in the ventricle, the resistance is $R = (9.815 \pm 1.408) \times 10^{12} \text{ Pa} \cdot \text{s}/\text{m}^3 = 1.227 \pm 0.176$ mmHg/($\mu\text{L}/\text{min}$), and the compliance is $C = (1.465 \pm 0.249) \times 10^{-11} \text{ m}^3/\text{Pa} = 1.953 \pm 0.332$ $\mu\text{L}/\text{mmHg}$. The corresponding time constant is $RC = 143.8$ s. Differences in R and C between the two locations are not significant ($P = 0.478$ and 0.665 , respectively; Fig. 3). Below, we shall refer to values measured during cisterna magna injection, a protocol used more commonly for introducing tracers because the cisterna magna is more accessible than the ventricles.

Other studies have determined the resistance and compliance of the CSF pathway. Jones (1985) used a constant-rate infusion technique to measure the resistance of CSF spaces during development in normal and hydrocephalus mice. The author measured a resistance of 1.88 ± 0.37 mmHg/($\mu\text{L}/\text{min}$) in 5-week-old mice, in good agreement with the R value reported here. The marginally higher value reported by Jones (1985) may be due to the infusion method. The bolus injection method is known to underestimate the resistance derived by the constant-rate infusion method (Kosteljanetz, 1985; Eklund et al., 2007). We also measured the resistance using the constant-rate infusion method and obtained a value of $R = 1.927 \pm 0.315$ mmHg/($\mu\text{L}/\text{min}$) which closely matches the value reported by Jones (1985). Oshio et al. (2005) measured a resistance of 5.149 ± 1.103 mmHg/($\mu\text{L}/\text{min}$) in CD-1 wild-type mice using a similar constant-rate infusion method. This higher resistance also explains their elevated resting ICP (6.988 ± 1.030 mmHg) as compared to other studies with lower ICP levels (≈ 4 mmHg) (Yang et al., 2013; Moazen et al., 2016; Wang et al., 2020). This overestimation of the resistance and resting ICP may be due to the high pressure gradient established by the

authors while the pipette was in the brain parenchyma to assess ventricle puncture (≈ 29 mmHg). In another study from the same group, Papadopoulos et al. (2004) measured the PVI in CD-1 wild-type mice using the bolus injection method. They reported a value of $\text{PVI} \approx 19$ μL , higher than the values measured here ($\text{PVI} \approx 10$ μL). However, based on their resting ICP, their compliance would be $C = 1.12$ $\mu\text{L}/\text{mmHg}$. This is in the range of our C value but smaller which agrees with exponential behavior of the CSF volume–pressure curve and a higher resting ICP (Marmarou et al., 1978).

4. Theoretical predictions with RC end boundary conditions

Having characterized the perivascular pump and the CSF pathway in terms of the parameters R and C , we can now use Eq. (1) to determine the flow rate q_1 in the coupled system if the uncoupled flow rate q_0 is known. We will first determine q_1 from two analytic predictions of q_0 .

Schley et al. (2006) considered a two-dimensional Cartesian domain in which one wall dilates and constricts such that the channel width varies over time and space. Here we consider the general case of sinusoidal wall motion that follows $h = \mathcal{R}\{H + ibe^{i2\pi f(\frac{x}{c}-t)}\}$, where H is the mean channel width, b is the half-amplitude of dilation and constriction, c is the wave speed, x is the streamwise spatial coordinate, and $\mathcal{R}\{\cdot\}$ denotes the real part. Henceforth, whenever complex quantities appear, we consider only their real part, dropping the $\mathcal{R}\{\cdot\}$ notation. Applying lubrication theory and considering the long-wavelength case, Schley et al. found that perivascular pumping in the uncoupled system produces flow rate $\hat{q}_0 = c(h - h_0)$, where $h_0 = \bar{h}^{-2}/\bar{h}^{-3}$. From this expression, the quantities tabulated above can be calculated directly. The mean downstream velocity is $\bar{q}_0/H = 0.034$ $\mu\text{m}/\text{s}$. The ratio of the amplitude of the oscillatory component to the amplitude of the steady component is $\gamma = b/(H - h_0) = 22,200$. The phase of the oscillatory component of $\hat{q}_0$ is identical to the phase of h and therefore lags the wall velocity $-\partial h/\partial t$ by $\varphi = 270^\circ$.

Because the system is two-dimensional, $\hat{q}_0$ is an area (not volume) flow rate and Eq. (1) becomes

$$\frac{\partial \hat{q}_1}{\partial t} + \frac{\hat{q}_1}{\hat{R}\hat{C}} = \frac{\hat{q}_0}{\hat{R}\hat{C}}, \quad (2)$$

where $\hat{q}_1$ is the area flow rate in the coupled system, $\hat{R} = R/w$, $\hat{C} = C/w$, and w is the width of the channel in the third dimension. Since w was not part of the original theory, we must choose it. Imagining extending the two-dimensional domain to produce a rectangular channel, we match its cross-sectional area to that of the annular channel considered by Kedarasetti et al. (2020): $w = \pi(r_2^2 - r_1^2)/H = 94$ μm . The solution to Eq. (2) is

$$\hat{q}_1 = c(H - h_0) - \frac{bc}{2\pi f \hat{R} \hat{C} + i} e^{i2\pi f(\frac{x}{c}-t)} + \hat{q}_2 e^{-\frac{x}{RC}}. \quad (3)$$

The last term is a starting transient that decays over time. Focusing our attention on fully-developed dynamics, we choose the integration constant $\hat{q}_2 = 0$. The wall velocity $\partial h/\partial t$, the uncoupled flow rate $\hat{q}_0$, and the coupled flow rate $\hat{q}_1$ are shown in Fig. 4.

The first term in Eq. (3) gives the steady component of the flow, unchanged from the uncoupled case. The second term gives the oscillatory component, which lags the wall velocity $-\partial h/\partial t = 2\pi f b e^{i2\pi f(x/c-t)}$ by $\varphi = \arg(-bc(2\pi f \hat{R} \hat{C} + i))^{-1} - \arg 2\pi f b = \arctan(-2\pi f \hat{R} \hat{C})^{-1} - 0 = 0$. Coupling the perivascular pump to the rest of the CSF pathway shifts the phase of oscillation

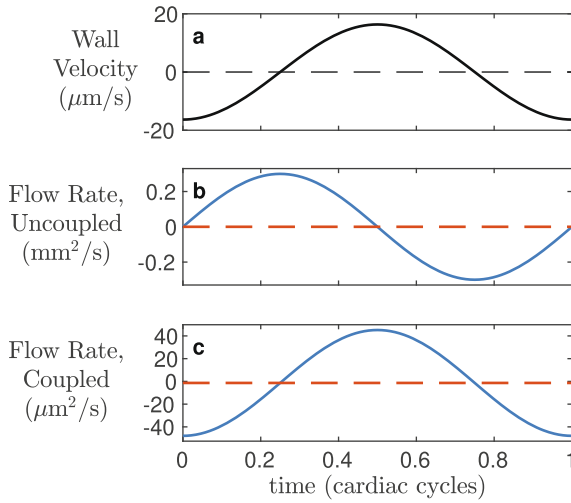


Fig. 4. RC boundary conditions alter the phase and relative amplitude of flow pulsations in the [Schley et al. \(2006\)](#) solution for peristaltic pumping. (a), Artery wall velocity at $x = 0$, over one cycle. (b), Flow rate $\dot{q}_0$ when the peristaltic pump is uncoupled from the CSF pathway, at $x = 0$, over one cycle. (c), Flow rate $\dot{q}_1$ when the pump is coupled to the CSF pathway, at $x = 0$, over one cycle. Note that different units are used in panels (b) and (c). The phase and relative amplitude of flow oscillation agree closely with in vivo observations when coupled, but not when uncoupled.

by 90° , so that the flow oscillates at nearly the same phase as the wall velocity. That phase shift is consistent with our expectations from the lumped-parameter model shown in [Fig. 1](#): the CSF pathway acts like a first-order lowpass filter with cutoff frequency $(\hat{R}\hat{C})^{-1}$. Since $f \gg (\hat{R}\hat{C})^{-1}$, the phase shift imposed by the filter is well-approximated by $\arctan 2\pi f \hat{R}\hat{C} = 89.96^\circ$. Because of that shift, the analytic solution of [Schley et al. \(2006\)](#), when coupled to the rest of the CSF pathway, predicts that wall velocity and flow oscillations will have nearly the same phase, as observed in vivo.

The ratio of the amplitudes of the oscillatory and steady terms in Eq. (3) is $\gamma = b(H - h_0)^{-1} (4\pi^2 \hat{R}^2 \hat{C}^2 f^2 + 1)^{-1/2} = 34.5$. Coupling the perivascular pump to the rest of the CSF pathway decreases γ by a factor of more than 600. That decrease is consistent with our expectations from the lumped-parameter model shown in [Fig. 1](#). Since $f \gg (\hat{R}\hat{C})^{-1}$, the gain of the lowpass filter at frequency f is well-approximated by $(1 + 2\pi f \hat{R}\hat{C})^{-1} = 1.551 \times 10^{-4} = 1/645$. Without coupling, $\gamma = 22,200$, disagreeing by many orders of magnitude with $\gamma = 0.53$ measured in vivo. Coupling the analytic prediction of [Schley et al. \(2006\)](#) to the rest of the CSF pathway, however, brings much closer agreement to in vivo observations, especially considering that the theory is two-dimensional and Cartesian.

[Wang et al. \(2011\)](#) considered a porous, axisymmetric cylindrical annulus in which the inner wall dilates and constricts such that the channel width (distance between inner and outer walls) varies over time according to $h = r_2 - r_1 + ibe^{i2\pi f(\frac{x}{L} - t)}$. Applying lubrication theory and considering the long-wavelength case, they found that perivascular pumping in the uncoupled system and in the absence of other pressure gradients produces flow rate $q_0 = -2\pi\epsilon cr_2^2/(\alpha_- + \alpha_+) + \pi\epsilon c(r_2^2 - h^2)$, where ϵ is the porosity of the space, which we presume to be open ($\epsilon = 1$), and $\alpha_\pm = ((1 \pm r_1/r_2)^2 - (b/r_2)^2)^{-1/2}$. From these expressions, the quantities tabulated above can be calculated directly. The mean downstream velocity is $\bar{q}_0/\pi/(r_2^2 - r_1^2) = 10.13 \mu\text{m/s}$. The ratio of

the amplitude of the oscillatory component to the steady component is $\gamma = 443$. The phase of the oscillatory component of q_0 is identical to the phase of h and therefore lags the wall velocity $-\partial h/\partial t$ by $\phi = 270^\circ$.

Using Eq. (1), we can solve for q_1 . The result is plotted in [Fig. 5](#), along with the wall velocity $-\partial h/\partial t$ and the uncoupled flow rate q_0 . (The analytic form of q_1 is lengthy, so we do not repeat it here.) Again, we neglect the transient term, and the mean downstream velocity is not changed by coupling the perivascular pump to the rest of the CSF pathway. The oscillatory component of q_1 lags the wall velocity $\partial h/\partial t$ by $\phi = 359.9^\circ$, agreeing well with in vivo observations. The ratio of the amplitude of the oscillatory component to the steady component is $\gamma = 0.069$, agreeing well with $\gamma = 0.53$ observed in vivo.

5. Simulation predictions with RC end boundary conditions

Having demonstrated the effects of RC end boundary conditions on two existing theoretical predictions, we now demonstrate the effect on existing predictions from simulation. As described above, the second set of simulations presented by [Kedarasetti et al. \(2020\)](#) considered flow in a three-dimensional domain whose cross-sectional shape and size are similar to in vivo observations. The inner wall was made to dilate and constrict according to wall velocity measured in vivo ([Mestre et al., 2018](#)); the wall velocity is plotted in [Fig. 6a](#). The pressure was set to zero at end boundaries, again with the system isolated from the rest of the CSF pathway. Perivascular pumping produced the centerline velocity shown in [Fig. 6b](#). As mentioned above, the time-averaged centerline velocity was $102.1 \mu\text{m/s}$, the flow oscillations lag wall velocity by $\phi \approx 330^\circ$, and the ratio of oscillations to steady flow was $\gamma = 290$.

The cross-sectional mean velocity is not given by [Kedarasetti et al. \(2020\)](#), but it is surely similar to the centerline velocity, perhaps smaller than the centerline velocity by 20–40%. Approximating the mean velocity as the centerline velocity, we can use the data shown in [Fig. 6b](#) to solve Eq. (1) numerically with a simple forward-Euler scheme. The cross-sectional area that relates mean velocities to volume flow rates is arbitrary, being the same for both q_0 and q_1 . The result, shown in [Fig. 6c](#), shows the centerline velocity predicted by the [Kedarasetti et al. \(2020\)](#) simulation with RC end boundary conditions, accounting for coupling to the rest of

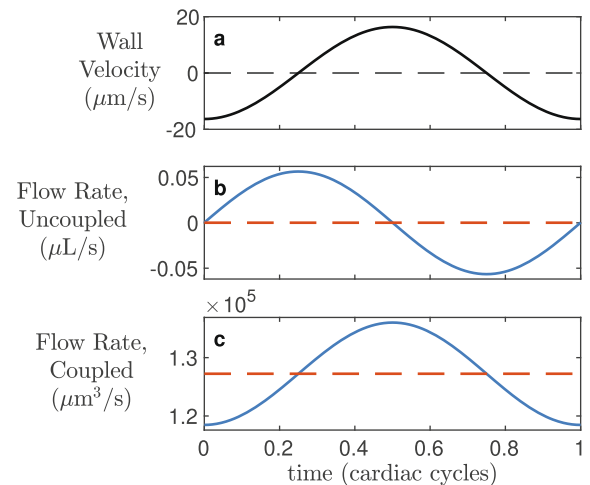


Fig. 5. RC boundary conditions alter the phase and relative amplitude of flow pulsations in the [Wang et al. \(2011\)](#) solution for peristaltic pumping. (a), Artery wall velocity over one cycle. (b), Flow rate $\dot{q}_0$ when the peristaltic pump is uncoupled from the CSF pathway, at $x = 0$, over one cycle. (c), Flow rate $\dot{q}_1$ when the pump is coupled to the CSF pathway, at $x = 0$, over one cycle. Note that different units are used in panels (b) and (c). The phase and relative amplitude of flow oscillation agree closely with in vivo observations when coupled, but not when uncoupled.

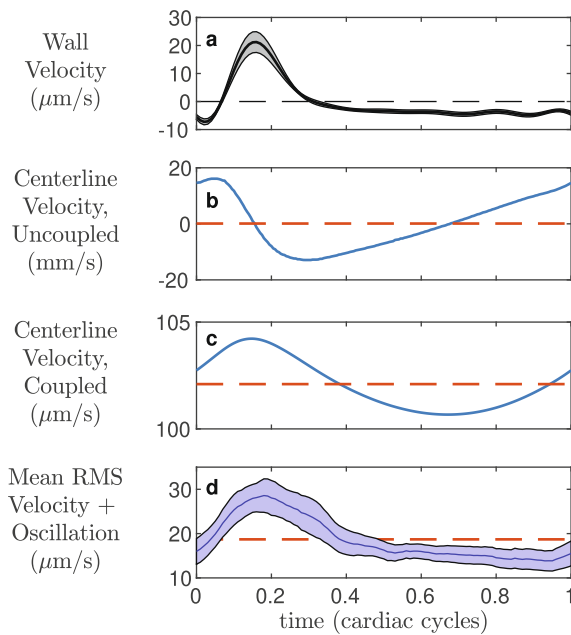


Fig. 6. RC boundary conditions bring good agreement between the fluid dynamical simulations of Kedarasetti et al. (2020) and in vivo measurements. (a), In vivo measurements of artery wall velocity in the peri-arterial space surrounding the middle cerebral arteries of mice. The curve indicates the mean, and the shaded region indicates the standard error of the mean, over 7 mice. From Mestre et al. (2018). (b), Centerline fluid velocity in the simulations of Kedarasetti et al. (2020), as driven by the wall velocity shown in (a). (c), Centerline fluid velocity after coupling to RC boundary conditions, calculated numerically using Eq. (1), from the simulation results in (b). (d), In vivo measurements of oscillation of the root-mean-square velocity, in the same 7 experiments as in (a). The curve indicates the mean, and the shaded region indicates the standard error of the mean. Note that different units are used in panels (b) and (c). With RC boundary conditions, the phase, relative oscillation amplitude, and oscillation shape are similar in simulations and in vivo observations.

the CSF pathway. The time-averaged centerline velocity is $102.1 \mu\text{m/s}$, unchanged from the uncoupled case and in reasonable (though not exact) agreement with the roughly $40 \mu\text{m/s}$ centerline velocity implied by the $18.7 \mu\text{m/s}$ mean observed in vivo. The peak of the centerline velocity lags the peak of the wall velocity by $\varphi = 356^\circ$, similar to the in vivo observations. The ratio of the amplitude of oscillations to steady flow is $\gamma = 0.021$, similar to the $\gamma = 0.53$ observed in vivo. For comparison, Fig. 6 shows the oscillatory velocity as measured in vivo. Its magnitude, phase, zero-crossing, and shape all resemble the prediction we can make by coupling the simulation results to RC end boundary conditions.

6. Discussion

Noting discrepancies between in vivo observations of flow in surface PVSs and flows predicted by theory and simulation, we have considered the effects of coupling to an RC end boundary condition. We estimated R and C by measuring the brain-wide hydraulic resistance and compliance via bolus injections in live mice. Coupling two published theoretical models and one published simulation to the new boundary conditions, we found that in all three cases, two key discrepancies were largely resolved: the ratio of oscillatory flow to mean flow was reduced, and the phase difference between wall motion and fluid motion was changed, so that both more nearly matched in vivo observations.

Our quantitative results are summarized in Table 1. Uncoupled, predictions from theory and simulation all produce mean speeds roughly similar to in vivo observations, phase shifts much larger than in vivo observations, and oscillation ratios much larger than

in vivo observations. Coupling to realistic, lumped-parameter boundary conditions, based on our in vivo measurements, brings agreement in phase and oscillation ratio, in addition to mean speed. This broad agreement among four independent studies supports the hypothesis that perivascular pumping is indeed an important driver of CSF flow in PVSs under physiological conditions.

One key implication of our findings is the general importance of using RC boundary conditions when making predictions from theory or simulation. To the extent that the dynamics are linear, a lumped-parameter model can be coupled to theory or simulation a posteriori, as we have done here. However, in a case where non-linear behaviors are appreciable, likely if the Reynolds number is large, accuracy requires including RC boundary conditions in the theory or simulation itself. Lumped-parameter models are used routinely in simulations of cardiovascular flows, either as standalone models of the circulatory physiology, or coupled to hydrodynamic models as boundary conditions (Stergiopoulos et al., 1992; Vignon-Clementel et al., 2006; Tran et al., 2017). Unfortunately, the $RC = 118.3\text{s} \gg f^{-1}$ time constant we measure presents a particular challenge when simulating the CSF pathway. Transients decay on the RC timescale (see Eq. (3)), so observing fully-developed dynamics will require simulating many cardiac cycles, at substantial and perhaps impractical computational expense. A posteriori coupling may be the more viable approach.

The lumped-parameter model used in this study was the simplest two-element Windkessel model; the model successfully captures the decay constant and phase relation in our study of perivascular flows. The three-element model, which adds a resistance (or impedance) in series with the RC circuit, captures high-frequency dynamics measured for aortic impedance in vivo, and hence is used widely in the cardiovascular community (Burkhardt et al., 1988; Westerhof et al., 1969; Westerhof et al., 1971). Without measurements of glymphatic impedance over a wide frequency range, however, the need for a more complex model for perivascular flow is currently speculative, and may be the subject of future work.

Kedarasetti et al. (2020) presented a third set of simulations in which a traveling wave propagated on the outer PVS wall as well, to explore the implications of surrounding tissue being deformed by pressure oscillations. Also included was a prescribed pressure difference of order 0.01 mmHg , which drove mean flow through the low-resistance PVS. However, that boundary condition is again unrealistic, because the pressure at the ends of the PVS would be affected by coupling to the rest of the CSF pathway. Surface PVSs connect to a network of distal PVSs and interstitial space with higher resistance, implying that greater pressure differences would be required to drive flow.

Our findings are subject to caveats. Most importantly, we have approximated the resistance R and compliance C of the rest of the CSF pathway — that is, the components to which a surface PVS is connected, directly or indirectly — with the measured brain-wide resistance and compliance. Measuring R and C more locally, in a way that distinguishes the resistance and compliance of the CSF pathway connected to a surface PVS from other CSF pathways, would be more accurate. That said, the predicted phase lag would nonetheless be nearly zero for any case in which $RC \gg f^{-1}$. Since heart rates vary little (perhaps a factor of two or three, not more), and since the measured RC value is larger than the cardiac time by four orders of magnitude, even if the value of RC were two orders of magnitude smaller than the value used above (1.438 s instead of 143.8 s), agreement in φ among theory, simulation, and in vivo observations would hold. On the other hand, the predicted oscillation ratio depends more sensitively on RC, so agreement in γ may be a better test of the accuracy of RC.

Table 1

Summarized flow characteristics from theoretical predictions, simulation predictions, and in vivo observations. The velocity $\bar{u}$ is averaged over the channel, except in the case of the simulation predictions, where centerline velocity was given.

	$\bar{u}(\mu\text{m/s})$	φ	γ
uncoupled Schley prediction	0.034	270°	22,200
uncoupled Wang prediction	10.13	270°	443
uncoupled Kedarasetti prediction	102.1	330°	290
coupled Schley prediction	0.034	0°	34.5
coupled Wang prediction	10.13	359.9°	0.069
coupled Kedarasetti prediction	102.1	356.4°	0.021
in vivo observations	18.7	353°	0.53

Another important caveat is the length of each segment of perivascular space. We presented results of coupled flows in domains that are at least one wavelength long, but shorter domains produce different predictions. [Asgari et al. \(2016\)](#) simulated a domain with length just 0.1 to 0.2% of the wavelength, which is more physiologically realistic. Similar to others ([Schley et al., 2006](#); [Wang et al., 2011](#); [Kedarasetti et al., 2020](#)), they predict a flow rate with large $\gamma = 4280$. However, their flow rate is nearly in phase with the wall velocity, in agreement with the in vivo measurements of [Mestre et al. \(2018\)](#), even without an RC boundary condition. The domain length likely caused the phase shift, which was also observed by [Kedarasetti et al.](#) in their simulation of a short domain ([Kedarasetti et al., 2020](#)). Though the detailed effects of varying domain length are beyond the scope of this paper, we plan to study it more deeply in the future.

Our approach is built on the assumption that surface PVSs do connect to an extended CSF pathway. If there were no such connection, and surface PVSs were merely isolated annular spaces whose inlets and outlets connected to large fluid chambers like the sub-arachnoid space, then the lumped parameter boundary conditions we propose would not apply. However, it is generally believed that they do connect ([Ray et al., 2019](#); [Wardlaw et al., 2020](#)). Our approach does not rely on assumptions about the location or nature of the connected CSF pathway, except that it has compliance and resistance. A pathway through the brain parenchyma, as proposed by the glymphatic hypothesis and supported by tracer influx studies ([Xie et al., 2013](#); [Ratner et al., May 2017](#); [Koundal et al., 2020](#); [Eide et al., 2018](#); [Ringstad et al., 2018](#)), would have such properties, but other pathways would have them as well. Identifying and characterizing CSF pathways, including the particular anatomy that provides their resistance and compliance, is an important topic of ongoing work.

The simple model sketched in [Fig. 1b](#) implies that the flow is q_0 at locations left of point A and q_1 at locations right of point A. Compliance and resistance affect the flow elsewhere in the system, but not immediately adjacent to the idealized source. If the situation in surface perivascular spaces is truly this simple, we would expect measurements made immediately adjacent to the flow source to find velocities matching those predicted with periodic boundary conditions. Existing in vivo measurements do not. We conclude that those measurements were not made immediately adjacent to the flow source, that instead, significant perivascular pumping and compliance occur at locations proximal to the measurement locations. Future work could test this idea. Future work could also expand the simple model of [Fig. 1b](#) to include multiple sources and multiple compliances, thereby accounting for more intricacies of the network of PVSs.

Our findings suggest that if CSF passes through brain tissue as proposed by the glymphatic hypothesis, then not only the mean flow $\bar{u}$, but also the phase φ and the normalized oscillation amplitude may vary with the state of wakefulness. Iontophoresis measurements have shown that the interstitial space in murine brain parenchyma increases 60% during sleep, and tracer measurements

showed that mass transport through brain tissue increased by an order of magnitude ([Xie et al., 2013](#)). Thus it seems the mean flow increases during sleep. We hypothesize that the expanded interstitial space lowers the resistance R of the CSF pathway and therefore changes γ and φ as well, as expected from [Eq. \(1\)](#). We expect γ to be more sensitive to wakefulness state than φ , because the phase shift of a lowpass RC filter is nearly flat when $RC \gg f^{-1}$. Future work might test this hypothesis. Other physiological changes that resize interstitial spaces, such as altering the osmotic potential ([Plog et al., 2018](#)), are likely to have similar effects. That said, the focus of this paper is modeling flow in surface PVSs, and how boundary conditions affect outcomes, regardless of whether or not CSF passes through brain tissue.

It is well-established that aging typically stiffens arteries, especially the major arteries, and their reduced compliance significantly affects cardiovascular flows ([O'Rourke, 1990](#); [O'Rourke and Hashimoto, 2007](#)). In particular, the reduced RC time implies a higher filter cutoff frequency and correspondingly increased the ratio of oscillatory to mean flow in blood. Our results suggest that aging may also alter CSF flow via changes in compliance. On the one hand, reduced arterial compliance would imply a higher filter cutoff frequency and tend to increase the ratio of oscillatory to mean flow in CSF (γ), just as in blood flow. On the other hand, reduced arterial compliance would reduce artery wall motion and the corresponding perivascular pumping, reducing both the mean flow and the pulsations. One recent study ([Kress et al., 2014](#)) shows reduced CSF flow in aged mice, which might be explained in part by arterial stiffening, though other mechanisms are likely to play a role as well. Future work might quantify them and suggest corresponding clinical interventions.

Accumulation of amyloid- β and τ plaques associated with Alzheimer's disease may also reduce compliance, with the same effects. Vascular pulse wave velocity has been found to change with amyloid- β deposition in humans ([Hughes et al., 2013](#)). Changes in the compliance and effective mass of the artery wall, because of plaques accumulating there, would tend to change the wave velocity and also the CSF flow. Future studies might explore the extent to which plaque accumulation in Alzheimer's affects compliance and therefore CSF flow.

An improved understanding of the mechanisms that drive CSF flow in the brain remains an important topic for future work. We have shown here that results from theory, simulation, and experiment are all consistent with perivascular pumping being a primary driver in physiological conditions. We hope our analysis will lead to more precise quantification of flows and driving mechanisms. Neuroimaging studies have shown that the ICP pressure waveform has different components including the cardiac cycle, respiration cycle, and slow vasomotion ([Vesa Kiviniemi et al., 2016](#)), and other mechanisms are known to dominate in pathological conditions like stroke ([Mestre et al., 2020](#)). Seeking flow and mechanisms at frequencies other than the heart rate, including the 0.05 Hz range of ventricular flow observed by [Fultz et al. \(2019\)](#), is a promising topic for future study. With RC boundary conditions, first-principles simulations might be precise enough to quantify what fraction of the mean flow, if any, cannot be driven by arterial pulsations.

7. Methods

7.1. Animals and surgical preparation

All animal experiments presented in the manuscript were approved and in accordance with relevant guidelines and regulations by the University of Rochester Medical Center Committee on Animal Resources (Protocol No. 2011-023), certified by Association for Assessment and Accreditation of Laboratory Animal Care,

and reported according to the Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines. All of the University of Rochester's animal holding rooms are maintained within temperature (18–26°C) and humidity ranges (30–70%) described in the ILAR Guide for the Care and Use of Laboratory Animals (1996). All efforts were made to keep animal usage to a minimum. C57BL/6 mice ages 2–4 months (25–30 g) were purchased from Charles River Laboratories (Wilmington, MA) with exact animal numbers stated in the results and figure legends. In all experiments, animals were anesthetized with a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally. Depth of anesthesia was determined by the pedal reflex test. The pedal reflex was tested every 5 to 10 min during the infusion experiment to ensure proper anesthesia throughout the study. If the mouse responded to toe pinch, an additional 1/10 of the initial dosage was given and the infusion experiment was delayed until full unconsciousness was obtained. Body temperature was maintained at 37.5°C with a rectal probe-controlled heated platform (Harvard Apparatus). Anesthetized mice were fixed in a stereotaxic frame, and two cannulae were implanted into the right lateral ventricle (0.85 mm lateral, 2.10 mm ventral and 0.22 mm caudal to bregma) and the cisterna magna, as previously described (Xavier et al., 2018).

7.2. Evaluation of CSF dynamics

We measured hydraulic resistance and compliance using bolus injection, an approach introduced by Marmarou et al. (1978). We injected fluid briefly and rapidly, measuring the resulting change in intracranial pressure (ICP), to estimate an impulse response, approximating the CSF pathway as a linear RC system. In one set of experiments, using a computer-controlled syringe pump (Harvard Apparatus Pump 11 Elite), we injected $V = 5\mu\text{L}$ of artificial CSF (126 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4 , 2 mM MgSO_4 , 2 mM CaCl_2 , 10 mM glucose, 26 mM NaHCO_3 ; pH 7.4 when gassed with 95% O_2 and 5% CO_2) at 1 $\mu\text{L/s}$ into the right lateral ventricle. We monitored ICP via the cisterna magna cannula connected to a transducer attached to a pressure monitor (BP-1, World Precision Instruments Inc., Sarasota, FL). In another set of experiments, we instead injected into the cisterna magna and monitored ICP in the right lateral ventricle, keeping other parts of the procedure unchanged. ECG and respiratory rate were also acquired using a small animal physiological monitoring device (Harvard Apparatus). All the signals were recorded at 1 kHz and digitized with a Digidata 1550A digitizer and AxoScope software (Axon Instruments).

We calculated the compliance C from the pressure–volume index (PVI): $C = \log_{10} e \cdot \text{PVI}/P_0$, where e is the base of the natural logarithm. The PVI is defined as the volume of fluid required to cause a tenfold pressure increase during bolus injection:

$$\text{PVI} = \frac{V}{\log_{10} \frac{P_{\max}}{P_0}}. \quad (4)$$

The resistance R can be estimated as

$$R = \frac{tP_0}{\text{PVI} \log_{10} \frac{P(t)(P_{\max}-P_0)}{P_{\max}(P(t)-P_0)}}, \quad (5)$$

where $P(t)$ is the pressure measured at time t . We expect R to be nearly constant, but to increase accuracy, we estimate R for each animal by averaging the results of Eq. at five evenly-spaced times during the experiment.

7.3. Statistical analysis

All statistical analyses were done in GraphPad Prism 8 (GraphPad Software). Data in all corresponding graphs are plotted as mean $\pm$ standard error of the mean (SEM). Parametric and non-

parametric tests were selected based on normality testing (Shapiro–Wilk test). Sphericity was not assumed in the repeated measure two-way ANOVA and a Geisser–Greenhouse correction was performed. All hypothesis testing was two-tailed, and significance was determined at an $\alpha = 0.05$.

Author contributions statement

ALG carried out the laboratory experiments and analyzed the resulting data. JKS carried out the simulations and analyzed the resulting data. MN participated in the design of the study and revised the manuscript. DHK conceived of the study, designed the study, and drafted the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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