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DRIVERS OF FLUID AND METABOLITE TRANSPORT IN THE CENTRAL NERVOUS SYSTEM

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by
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Non-Technical Abstract

The brain is one of the most metabolically active organs in the mammalian body. For proper brain function, the metabolic waste has to be removed continuously. Accumulation of metabolites in the brain extracellular space (ECS), such as Amyloid-β and Tau, has been linked to Alzheimer’s disease, which currently affects 5–7% of the population in developed countries. While metabolic waste from other major organs in mammals is cleared by lymph vessels, which have valves to prevent backflow, the brain does not contain any lymph vessels. Instead, the brain relies on metabolite clearance through interstitial fluid (ISF) and the movement of cerebrospinal fluid (CSF). This thesis is an investigation on the possible drivers of ISF and CSF movement and the clearance of metabolites from the brain parenchyma.

One of the major obstacles to understanding fluid flow in the brain is the sensitivity of the intracranial pressure and fluid flow in the brain to invasive experimental procedures. To partially compensate for this problem, I used mathematical models to predict the fluid flow in the brain in response to the movement of cerebral arteries and tested the predictions of the models with minimally invasive experiments. One of the main conclusions of my models and experiments is that functional hyperemia, or changes to blood volume in the brain in response to local neural activity, can aid the clearance of metabolic waste from the brain. The models also showed that the cerebral blood volume changes observed during rapid eye movement (REM) sleep can appreciably improve fluid movement in and metabolite clearance from the brain. These results suggest that neural activity and REM sleep can hold the key to the prevention and treatment of neurodegenerative diseases like Alzheimer’s.
Technical Abstract

It has been known that the movement of interstitial fluid (ISF) and cerebrospinal fluid (CSF) contribute to metabolite clearance from the central nervous system (CNS). However, the exact mechanisms and drivers of fluid movement and metabolite transport in the brain remain the subject of intense debates. The last decade has seen the rise of the glymphatic system hypothesis (Fig.0.1a), which postulates the existence of a distinct pathway for fluid circulation in the brain parenchyma. It is hypothesized that CSF flows along this pathway from the subarachnoid space (SAS) along penetrating arteries into the brain, enters the parenchymal extracellular space (ECS) through Aquaporin-4 (AQP4) water channels on astrocytic end-feet and exits into the venous paravascular space (PVS) again through and between astrocyte end-feet. My Ph.D. thesis work was focused on understanding the drivers of transport in the glymphatic pathway, particularly the PVS surrounding cerebral arteries.

Previously published research on the topic of fluid flow in the PVS, based on some experimental evidence, posited that fluid movement in the PVS was driven by pulsations of arteries due to the heartbeat. Using finite element simulations of fluid flow in the PVS of adult mice with realistic geometries and boundary conditions, based on experimental data, I found that under physiological conditions, heartbeat pulsations do not drive transport in the PVS, thus leading to the conclusion that some other mechanism must move fluid in the brain. I improved upon the existing models of fluid flow in the PVS by performing fluid-structure interaction simulations, where the fluid flow in the PVS was coupled to the elastic response of the surrounding brain tissue and showed that functional hyperemia, or the dilation of cerebral arteries in response to local neural activity, could drive metabolite transport in the PVS (Fig. 0.1b). My simulations showed that the deformation of the brain tissue plays an important role in the fluid flow in the PVS (Fig.
0.1c). I verified the predictions of my fluid-structure interaction models by measuring the deformation of the brain tissue surrounding arteries in awake and freely behaving mice (Fig. 0.1d). Using two-photon laser scanning microscopy, I obtained images of the arteriolar lumen of cerebral arteries and the neural processes (axons and dendrites) in surrounding brain tissue (Fig. 0.1e). I also developed a novel image processing pipeline for reliably measuring brain tissue deformation from the noisy images collected using awake mice (Fig. 0.1f). I extended the fluid-structure interaction models to understand the how the arteriolar dilation during sleep affects the CSF flow in the PVS and the SAS. The work in this thesis demonstrates that a combination of theoretical models and minimally-invasive experiments is necessary to properly understand the nature and drivers of transport in the CNS.
**Figure 0.1: Graphical Abstract**

**a.** A schematic of the glymphatic pathway for CSF flow in the brain. CSF enters through the para-arterial spaces (left), flows through the brain parenchyma and clears the extracellular waste (black dots) through the para-venous space (right). Taken from Nedergaard, "Garbage truck of the brain." Science (2013)

**b.** Fluid-structure interaction simulations show the CSF flow driven by functional hyperemia in the paravascular space (PVS) and the subarachnoid space (SAS). The green lines show fluid entering the PVS and blue lines show the fluid leaving the PVS.

**c.** Cross section of the brain tissue in simulations shows that when the fluid leaves the PVS (shown by arrows), the pressure changes in the PVS deform the brain tissue.

**d.** Experimental set up to collect images of brain tissue and blood vessels in awake mice, head-fixed and placed on a spherical treadmill under the objective (lens) of a two-photon laser scanning microscope.

**e.** Sample image used for measuring the displacement of the arteriole wall and brain tissue. The brain tissue is fluorescent from yellow fluorescent protein (YFP) expressed in a subset of neurons (Thy1 promoter), shown in green. The lumen of an arteriole penetrating into the brain tissue is fluorescent from injection of dye (Texas red), shown in magenta.

**f.** The simultaneous measurements of displacement of the vessel wall (outlined in white in e) and the brain tissue (at the location marked by the cross in e), confirms the prediction of the fluid structure interaction models that the pressure changes in the PVS deform the brain tissue and that the brain tissue deformation plays an important role in CSF flow.
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List of Abbreviations

2PLSM  Two-photon laser scanning microscope
ALE   Arbitrary-Lagrangian-Eulerian
AQP4   Aquaporin-4
BDF    backward difference formula
CBF    cerebral blood flow
CBV    cerebral blood volume
CMRO$_2$ cerebral metabolic oxygen rate
CNS    central nervous system
DFT    discrete Fourier transform
EEG    electroencephalogram
EMG    electromyogram
FC     frontal cortex
FL/HL  fore limb/hind limb
FRAP   fluorescence recovery after photobleaching
GFP    green fluorescent protein
IOS    intrinsic optical signal
LFP    local field potential
PDE    partial differential equation
PET    positron emission tomography
PoRTS  polished reinforced thin-skulled
REM    rapid-eye-movement
YFP    yellow fluorescent protein
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This dissertation is dedicated to my mom.
Chapter 1: Motivation, Background and Objectives

1.1 Motivation – prevention and treatment of neurological disorders

The first thing that comes to mind when one mentions the brain are the neurons, which are the functional units of the brain that communicate through electrical signals. However, it can be argued that the interstitial fluid (ISF), which occupies the extracellular space (ECS) of the brain parenchyma, plays an equally important role in the functioning of the brain. The contrast between the ionic concentrations in the ISF and the cytoplasm of the neurons is directly responsible for the membrane potentials of neurons and therefore is part of the basis for the electric communication in the brain. The ISF also contains several proteins and amino acids that play a major role in the communication between the cells across different regions of the brain. Moreover, movement of the ISF is thought to transport harmful metabolites that are released into the ECS through neural activity, away from the neurons. The concentrations of ions, proteins, amino acids and metabolites in the ISF are maintained through its secretion at the brain capillaries and through its interaction with the cerebrospinal fluid (CSF) that surrounds the brain. In this thesis, we focus on the CSF-ISF interactions in the subarachnoid space (SAS) around brain, and in the fluid filled paravascular spaces (PVS) that surround the blood vessels in the brain.

The activity of neurons releases into the ECS neurotransmitters like glutamate, whose accumulation can lead to neurotoxicity (Bak, Schousboe, and Waagepetersen...
2006; Schousboe, Bak, and Waagepetersen 2013; Robinson and Jackson 2016), and metabolites like Amyloid-β, whose accumulation has been linked to the onset and progression of Alzheimer’s disease (Hardy and Higgins 1992; LaFerla, Green, and Oddo 2007). Unlike other organs in the mammalian body, the brain does not contain any lymphatic vessels to clear extracellular waste, and CSF plays a major role in metabolite clearance (Weller 1998). Understanding of the mechanisms and drivers of CSF-ISF interactions, and how they vary during different physiological states, could lead to better prevention and treatment of Alzheimer’s Disease. With 4.7 million Americans suffering from Alzheimer’s disease in 2010, and with this number projected to go up to 13.8 million by 2050 (Hebert et al. 2013), it is important to understand the drivers of fluid flow and metabolite transport in the brain.

The CSF-ISF interactions can also play a major role in drug delivery to the brain. The capillaries of the brain form the blood-brain-barrier (BBB), which tightly controls the transport of ions, molecules, proteins and cells between the ISF and the capillaries (Abbott 2005), effectively shielding the brain from the fluctuations of ionic and molecular concentrations in the blood. Due to its control of molecular transport into the brain, the BBB is a major stumbling block in treating neurological disorders, as it prevents the entry of many drugs into the brain through the bloodstream. Disrupting the integrity of the BBB to transfer compounds into the brain is not recommended, since it can cause serious disturbances to the brain homeostasis. An alternative method of drug delivery to the brain is through convection enhanced delivery (Allard, Passirani, and Benoit 2009; Bobo et al. 1994; Voges et al. 2003), where drugs are injected directly into the CSF, from which they can be transported to the ISF. By understanding the mechanisms and drivers of CSF-ISF interactions in the CNS, this thesis aims to contribute to the development of promising new avenues of treating neurological disorders like convection enhanced delivery.
1.2 Choice of model animal

Understanding the drivers and mechanism of CSF-ISF interactions, and the role of CSF flow in transporting solutes in the SAS and the PVS is a fundamental physiological question whose answer could hold great promise in the treatment of neurodegenerative diseases. However, CSF dynamics in the cranial space are highly sensitive to experimental manipulations (Anthony Marmarou, Shulman, and Rosende 1978) and therefore it is extremely difficult to study CSF flow in the CNS through experiments. In this thesis, the problem of the sensitivity of CSF dynamics to experimental manipulations was tackled by using mathematical models based on the known anatomy of the CNS and by employing minimally invasive experimental methods to test the model predictions. This could only be achieved by choosing a model animal, with sufficient anatomical and physiological information available for building mathematical models, and with established experimental methods to effectively test the predictions of the mathematical models. Therefore, adult mice were chosen as the model animal for this thesis.

The choice of mice as the model animal is in-line with much of the recently published research in the field of CSF production and circulation, as-well-as neuroscience in general, which makes extensive use of mice. The relatively small size of mice (average adult mouse weighs 30 g) and the small gap between consecutive generations of mice (10 weeks), compared to other mammals make them a convenient choice for studying biological systems. The relatively flat shape of the calvaria (the top part of the skull) in mice compared to other mammals make them ideal for high-resolution in vivo imaging of brain tissue using light microscopy because large sections of the brain surface can be captured in the same focal plane. The flat skull also makes it relatively easy to obtain optical access for imaging through a thin-skulled window (Drew et al. 2010), where a portion of the skull is thinned until it is translucent and the thinned portion is reinforced
with a glass cover. There is also a wide variety of transgenic mice (cf. https://www.jax.org/, The Jackson Laboratory), particular ones that express fluorescent reporter proteins in a subset of cells that enable the visualization of various cell types in the brain tissue.

1.3 Background

This section is a summary of the published literature that provided a basis for the design of models and experiments used in this thesis for investigating the drivers of fluid flow and metabolite transport in the brain. We begin with examining the deformable properties of the brain tissue and discuss how the properties can be used in models of fluid flow. We describe the fluid in the CNS and its sources, and explain why interaction between CSF and ISF is essential for the transport of metabolites in the brain. The anatomical locations where these interactions are possible are described. The available methods of investigation of fluid and metabolite transport in the brain and the associated caveats are discussed. Finally, we present the existing evidence of fluid and metabolite transport in the brain.

1.3.1 Elasticity of the brain and its possible effect on fluid flow

In this thesis, we use mathematical models to investigate fluid flow surrounding the brain tissue, which is an extremely deformable, porous tissue located inside the skull and surrounded by fluid. Brain tissue is softer than any other tissue in the mammalian body (Fig.1.1) (Budday et al. 2019), and the deformability of the brain tissue can affect pressure-driven flow around it. This phenomenon of the deformability of a solid affecting fluid flow around it has been studied thoroughly in the context of blood flow through the arteries and veins in mammals (Torii et al. 2006; Greenshields and Weller 2005; Bazilevs et al. 2010; Toro 2016; Müller and Toro 2014b; 2014a), and it is widely agreed that the blood flow is affected by the deformability of the blood vessels. Since the brain tissue is a few orders of
magnitude softer than the blood vessels (Fig.1.1) (Buday et al. 2019), we should expect that fluid flow surrounding the brain to be affected by the deformability of the brain. Therefore, we need to understand the elastic properties of the brain tissue and how to include these properties in models of fluid flow surrounding the brain.

The brain tissue has very complex elastic properties. For example, the apparent stiffness of brain tissue is higher during compressive loading than tensile loading (Buday et al. 2017; Franceschini et al. 2006; Jin et al. 2013), depends on the strain rate (Clayton, Genin, and Bayly 2012; Donnelly and Medige 1997; Nie et al. 2013) and is higher during loading than unloading (Buday et al. 2017; Franceschini et al. 2006). In terms of modeling, a constitutive model describes a material’s response to various loading conditions, from which the stress-strain relationship can be derived (W. F. Chen et al. 1993). Several constitutive models capturing the hyperelastic, viscoelastic and poroelastic behavior of the brain tissue are available and it is prudent to select a constitutive model depending on the complexity of the simulations (Buday et al. 2019). For example, for models of traumatic brain injury during high impact loading, viscoelastic models, where the stress depends not only on the strain but also on the strain rate are used (El Sayed et al. 2008), while relatively simple hyperelastic models can adequately simulate the elastic response of the brain during automated neurosurgery (Leizea et al. 2015; Wittek, Hawkins, and Miller 2009). For the cases considered herein, which are based on physiological conditions in the healthy brain, we consider relatively small strains and strain rates. Therefore, in this thesis, we use relatively simple models of elasticity, namely the Saint-Venant-Kirchhoff model for compressible elasticity and the neo-Hookean model for incompressible elasticity with a shear modulus in the order of 1 kPa (Buday et al. 2017; Franceschini et al. 2006; Goriely et al. 2015; Mihai et al. 2017; Sack et al. 2009, 2011;
Streitberger et al. 2011; Weickenmeier et al. 2018). These constitutive models are convenient as they require a very limited number of parameters for their description.

Simulations of fluid filled channels surrounded by pliable walls use fluid-structure interaction formulations, which are based in the continuity of displacement, velocity and traction at the interface of the solid and the fluid (Wick 2011; Fernández et al. 2009; Greenshields and Weller 2005). We will look at fluid-structure interaction models in chapters three and five.

1.3.2 Fluid in the CNS and its sources

The brain tissue is surrounded by the CSF, and the extracellular spaces of the brain contain the ISF. The CSF and ISF are clear liquids with the density and viscosity of water (Støverud et al. 2013; Yetkin et al. 2010). They are both aqueous solutions containing Na\(^+\), K\(^+\), Ca\(^+\), Mg\(^+\), Cl\(^-\) and HCO\(_3\)^- ions in concentrations in the range of 1mM to 100mM (Abbott 2005). The CSF and ISF are stored in the cranial space at a slightly higher
pressure relative to the atmospheric pressure, called the intracranial pressure (ICP). The ICP in normal healthy mice is in the range of 6-8mmHg (Oshio et al. 2005; Johanson et al. 2008). Physiological fluctuations of ICP (up to 40mmHg in mice (Norwood et al. 2019)), do not appreciably affect the density of water, and therefore for all the calculations in this document, the fluid in the brain is assumed to be incompressible.

Most CSF is secreted by the choroid plexus (Brown et al. 2004; Wolburg and Paulus 2010; Abbott 2005), a specialized structure present in the ventricles of the brain (Fig. 1.2a). The ventricles are a system of interconnected cisterns (or cavities) in the brain (Shoykhet and Clark 2011). The CSF secreted by choroid plexus flows through the ventricular system and enters the cisterna magna, from where it moves into the SAS of the brain and the spinal cord (Abbott et al. 2018; Kimelberg 2004), surrounding the organs of the CNS. The CSF leaves the cranial space through the lymph vessels and venous sinuses (Fig. 1.2a) located in the meninges and the spinal column, along the optic nerve, the fossa in the base of the skull or through the cribriform plate along the olfactory nerves (Norwood et al. 2019; Ma et al. 2017; Louveau et al. 2015).

Most ISF is secreted by brain capillaries (Abbott 2004). Compared to other organs of the mammalian body, the ISF (or lymph for other organs) in the brain is secreted at a slower rate through the gaps between the endothelial cells of the capillaries (Fig. 1.2b). The endothelial cells of the brain capillaries are connected through tight junctions and form the blood-brain barrier (BBB). It is believed that the BBB is formed and maintained through the interaction between the endothelial cells and the astrocytic endfeet processes that surround the endothelial cells (Abbott, Rönnbäck, and Hansson 2006). The transport of ions and other polar solutes through the BBB is facilitated only by means of specific channels, transporters and ion pumps in the endothelial cells of the brain capillaries. The low permeability of the BBB to ions and polar solutes means that water leaving the brain
capillaries due to hydrostatic pressure generated by the heart will be pulled back into the capillaries due to osmotic pressure (Daneman and Prat 2015). It is important to note that the BBB does not affect the transport of gaseous species like $O_2$ and NO, which diffuse through the cell membranes (Popel 1989; Denicola et al. 1996).

The nature of transport of solutes in CSF and ISF can be inferred from their rates of production and removal from the CNS. The mouse brain contains a total volume of nearly 40$\mu$l of CSF, which is produced at a rate of 0.3-0.4 $\mu$l/min, turning over the 40$\mu$l volume of CSF 12–14 times per day on average (Oshio et al. 2005). The turnover rate indicates that solutes in the CSF can be cleared from the CNS through bulk flow (convection). Besides the constant secretion of the CSF and its removal from the cranial space, bulk flow of CSF in the SAS of the brain and the spinal cord is driven by the changes in ICP due to respiration and heartbeat (Dreha-Kulaczewski et al. 2015; Sweetman et al.)
2011; Sweetman and Linninger 2011; Norwood et al. 2019; Ma et al. 2017). On the other hand, a total ISF volume in the mouse brain of ~100µl can be estimated from the published data, which suggests that ISF occupies roughly 15–20% of the volume (Korogod, Petersen, and Knott 2015; Lehmenkühler et al. 1993; Sykova et al. 2008) in the brain parenchyma, which has a volume of 500-600µl (Maheswaran et al. 2009). The ISF production rate is about 10–20% that of the CSF (Orešković and Klarica 2010; Redzic 2011; Hladky and Barrand 2014), which implies that a complete turnover of 100µl of ISF takes longer than a day. This turnover rate suggests that the transport of solutes in the ISF is diffusion-dominated. Calculations of fluid flow using realistic geometries of the ECS (Holter et al. 2017; Jin, Smith, and Verkman 2016) and conservative over-estimates of ICP differences in the brain also agree that the transport of solutes in the ISF occurs mainly through diffusion.

1.3.3 Sites of CSF flow and CSF-ISF exchange

The analysis of the turnover rates and modes of transport of CSF and ISF indicates that for the effective clearance of metabolites from the CNS, there needs to be a flow of CSF in the CNS and an exchange of solutes and fluid between the ISF and CSF. The flow of CSF and the exchange of solutes and fluid between the ISF and CSF will remain the main focus of this thesis. Therefore, we need to examine the anatomical locations where the flow of CSF and CSF-ISF exchange is possible.

In this section, we discuss the anatomy of the meninges and the blood vessels in the brain parenchyma. The meninges are of interest as they contain the spaces that allow CSF flow around the brain, and house several structures for the drainage of the CSF from the cranial space (Weller et al. 2018). The anatomy of blood vessels is important for us because there is evidence suggesting blood vessels, especially arteries, play a major role in solute exchange between CSF and ISF (Mestre, Tithof, et al. 2018; Hladky and Barrand
2014; Abbott et al. 2018; van Veluw et al. 2020). Amyloid plaques have been observed in the fluid-filled spaces within the arterial basement membrane and around the blood vessels in the brain of Alzheimer’s patients, suggesting that blood vessels play a role in fluid exchange and metabolite transport in the brain (Hladky and Barrand 2014; Weller, Boche, and Nicoll 2009). There is also evidence suggesting that dementia is often accompanied by vascular dysfunction (Dickstein et al. 2010; Di Marco, Farkas, et al. 2015; Di Marco, Venneri, et al. 2015; Sweeney et al. 2019), which suggests a possible role of blood vessels in the clearance of metabolites.

1.3.3.1 The meninges — sites of CSF flow and drainage

   The meninges are composed of the dura, the arachnoid membrane, the subarachnoid space (SAS) and the pial membrane. The outermost layer of the meninges is the dura, which is connected to the skull. The dura consists of three layers (Mack, Squier, and Eastman 2009), the periosteal dura, the meningeal dura and a layer of border cells (Fig.1.3a). The periosteal dura is directly connected to the skull bone. The meningeal dura contains blood vessels, including the superior sagittal sinus and the venous sinus that drain deoxygenated blood. These sinuses, along with some lymphatic vessels provide a route of exit for the CSF from the cranial space (Ma et al. 2017; Louveau et al. 2016; Mack, Squier, and Eastman 2009). The arachnoid membrane consists of three to four layers of leptomeningeal cells, collagen fibers and desmosomes (Johanson 1988; Lopes and Mair 1974). The arachnoid membrane also contains arachnoid granulations, valve like structures that allow for CSF drainage into the sinuses and the lymphatic vessels (Upton and Weller 1985). The SAS surrounds the brain and the spinal cord and contains CSF and collagen bundles. The blood vessels supplying blood to the brain tissue traverse through the SAS (Zhang, Inman, and Weller 1990) (Fig.1.3b). The pia covers the surface
of the brain parenchyma, and is composed of a layer of fibroblasts connected through desmosomes (Weller 2005).

1.3.3.2 Cerebral arteries and sites of CSF-ISF exchange

All the blood to the rodent brain is supplied by the left and right carotid arteries and the two vertebral arteries (Blinder et al. 2010a; Hirsch et al. 2012; Scremin 2015). The two vertebral arteries fuse to form the basilar artery. The basilar artery along with the two carotid arteries feed into the circle of Willis at the base of the brain (Fig.1.4a). The middle, anterior and posterior cerebral arteries originate from the circle of Willis (Hur, Blaise, and Limon 2016) and supply the blood to the cerebral cortex (Blinder et al. 2010b; Adams et al. 2018). We are interested in the blood supply to the cortex, specifically the somatosensory cortex, because it is the region most accessible for imaging in vivo (Shih et al. 2012; Drew et al. 2010; Mateo et al. 2017; Thrane et al. 2011; Iliff et al. 2012; Iliff et al. 2013). Most of the blood to the somatosensory cortex is supplied by the middle cerebral artery (MCA) (Fig.1.4b). Its location in the brain (Bedussi, Van Der Wel, et al. 2017) suggests that most of the arteries imaged under two-photon microscopy are branches of the MCA. The branches of the MCA pass through the SAS and enter the brain tissue.
perpendicular to the surface of the brain (Blinder et al. 2013; Gagnon et al. 2015) (Fig. 1.4c).

The smooth muscle of pial arteries is surrounded by a layer of leptomeningeal cells that form the leptomeningeal sheath or the adventitial cell layer (Pizzo et al. 2018; Abbott et al. 2018). When arteries penetrate into the brain parenchyma, they are surrounded by glial endfeet (Fig.1.5a). The continuous space between the smooth muscle and the leptomeningeal layer in the subarachnoid space and smooth muscle and the glial endfeet in the parenchyma is filled with fluid and is called the paravascular space (PVS) (Fig.1.5a).

The PVS surrounding major branches of the MCA can be up to 40 µm wide in the mouse brain (Bedussi, Almasian, et al. 2017; Mestre, Tithof, et al. 2018), while the average radius of these arteries is around 30 µm. The PVS is narrower around arterioles penetrating into the brain parenchyma, with a width of 2–5 µm around vessels of diameter 20 µm (Iliff et al.

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**Figure 1.4: Blood supply to the cerebral cortex**

a. Schematic diagram of the ventral view of the mouse brain (as indicated by the arrows in inset) shows the circle of Willis formed by the Basilar artery (BA) and the vertebral arteries (VA). The MCA, ACA and the PCA originate from the circle of Willis. Modified from Hur et al. 2016.

b. Schematic of the mouse brain showing the location of the MCA and its branches with respect to the cerebral cortex. Modified from Snyder et al. 2018.

c. A 3D reconstruction of cortical vasculature (corresponding region shown in the box in b) in mice. The branches of MCA traverse the SAS and enter the parenchyma perpendicular to the brain surface. Taken from Gagon et al. 2015.
An example of the relative size of the PVS around pial and penetrating arterioles was shown by Schain et al. (Schain et al. 2017) (Fig. 1.5b). The PVS is believed to play a major role in allowing fluids and metabolite transport into and out of the brain (Iliff et al. 2012). The arterial wall consists of endothelial cells, surrounded by up to three layers of smooth muscle (Ratz 2016). The endothelial cells are separated from the smooth muscle by the basement membrane of arteries (Bakker et al. 2016). The basement membrane is a thin sheet of extracellular matrix (collagenous fibers) between layers of tissue. The basement membrane is also present between the layers of smooth muscle. This gap between the vascular connective tissue can be up to 100 nm in thickness and is filled with fluid (Carare et al. 2008). This fluid-filled space, called the perivascular (as opposed to paravascular) space (Fig. 1.5a), is one of the regions of Amyloid-β accumulation in Alzheimer’s disease and is believed to be a conduit for extracellular waste clearance (Weller et al. 2008; Carare et al. 2008). In this thesis, we are mostly concerned with fluid flow in the paravascular space (PVS), which is the larger of the two fluid filled spaces surrounding blood vessels.

Since we are interested in the fluid flow in the PVS, i.e., the space between the smooth muscle and the leptomeningeal layer or the basement membrane of the glia limitans, the movement of the arterial walls relative to the SAS on the pial surface and the glial limitans in the parenchyma is of special interest to us. When the arterial walls move during vessel dilation and contraction, or during heartbeat-driven pulsations, they could displace the fluid in the PVS, thus driving fluid and metabolite transport. The movement of arteries and the related changes to blood flow have been measured in mice and other mammals, including humans. In mice, the arterial wall movement has been measured directly using confocal (Tomita et al. 2005) and two-photon microscopy (Gao, Greene, and
Drew 2015; Shih et al. 2012). The arterial wall movements and their effect on fluid and metabolite transport in the brain will be further discussed in chapters two through five.

1.3.4 Methods of investigating CSF-ISF exchange

Based on our understanding of the fluid turnover rates and the anatomy of the brain, it is clear that, to understand fluid flow and metabolite clearance in the brain, one needs to examine CSF flow and CSF-ISF exchange in the SAS, in the PVS of cerebral arteries and in the brain parenchyma itself. The most intuitive way to examine transport of solutes and fluids is to inject a tracer, preferably in a way that does not alter the existing flow, and to observe the movement of the tracer. This was indeed the basic principle used in a large number of studies examining CSF flow and CSF-ISF exchange in the SAS (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017; van Veluw et al. 2020), the PVS (Iliff et al. 2012; Ma et al. 2017; Mestre, Hablitz, et al. 2018; Schain et al. 2017; Xie

Figure 1.5: Paravascular and perivascular pathways of CSF-ISF exchange flow

a. A schematic diagram of the paravascular (blue arrows) and perivascular pathways (green arrows) for CSF-ISF exchange. The paravascular space (PVS) is outside the arterial wall, while the perivascular space is within the different layers of the arterial wall. Modified from Bakker et al. 2016.

b. The PVS around pial arteries and penetrating arteries visualized by injecting Texas red dye into the cisterna magna of live, CAG-GFP mice (mice expressing GFP with a β-actin promoter). The images were collected under a two-photon microscope with a two-channel photomultiplier setup. The top frames show the PVS of arterioles at the pial surface and 30µm below the pial surface. The bottom frame is a slice of an image stack. The PVS at the pial surface is relatively large compared to PVS in the parenchyma. Modified from Schain et al. 2016.
et al. 2013) and the brain parenchyma (Smith et al. 2017a). In this section we will discuss the methods available for visualizing the anatomical regions of interest (brain tissue, blood vessels, the SAS and the PVS) and for injecting tracers into the ISF and the CSF.

1.3.4.1 Visualizing the brain and sites of CSF-ISF exchange

The major obstacle to visualizing the brain and fluid flow in the cranial space directly is the skull. One obvious way around this is to inject the tracers into the CSF or ISF compartments of the brain, euthanize the mice and observe the tracer penetration in the fixed brain ex vivo (Mestre, Hablitz, et al. 2018; Ma et al. 2017). However, this method only provides one snapshot of the tracer movement per-animal and therefore offers no temporal information. Moreover, the fixation process alters the CSF dynamics and causes a 10-fold reduction in the size of the PVS (Mestre, Tithof, et al. 2018; Korogod, Petersen, and Knott 2015). There are some methods available to visualize the brain in live animals and for quantifying the fluid flow and solute movement in the brain without disturbing the skull, such as contrast-enhanced magnetic resonance imaging (Ringstad, Vatnehol, and Eide 2017; Ringstad et al. 2018) and transcranial wide-field imaging (Plog et al. 2018). However, these methods only have a spatial resolution of a 0.1-1 mm, which is insufficient to observe fluid flow in the PVS, which has a width of around 40µm around pial arteries (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017; Schain et al. 2017) and around 5µm around penetrating arterioles (Iliff et al. 2012; Schain et al. 2017). Therefore, to observe CSF-ISF exchange, we need to employ high-resolution optical imaging methods.

There are methods available to obtain optical access to the brain without completely removing the skull. One method involves removing a portion of the skull, and replacing it with a cover made of transparent glass to contain the brain and the CSF. This implantation is referred to as a cranial window (Navari et al. 1978). One of the major
problems with completely removing a portion of the skull, is that the skull is attached to
the dura, which is connected to the meningeal layers, that are filled with CSF (Fig.1.6a). Disturbing the meninges by removing the skull and the dura is therefore not a reliable
method of studying CSF flow in the brain (Fig.1.6b). Moreover, cranial windows can result
in serious inflammation of the brain tissue (Xu et al. 2007) and axonal degeneration (Harris
1960), and affect the neuronal response (Holtmaat et al. 2009; Forcelli, Kalikhman, and
Gale 2013), arterial pulsatility (Plog et al. 2020), BBB permeability (Olesen 1987) and
deformability of the brain tissue (Hatashita and Hoff 1987; Cole et al. 2011). Another
method of obtaining optical access to the brain, is to implant a polished reinforced thin-
skulled (PoRTS) window in the skull of mice (Drew et al. 2010). In this method, a portion
of the skull is thinned using a dental drill until it is translucent. This part of the implantation
is similar to the thin-skulled window (Grutzendler, Kasthuri, and Gan 2002). The thinned
portion is then reinforced with cover glass that is kept in place using cyanoacrylate glue
and dental cement between the edges of the glass and the surrounding skull. The dura is
relatively undisturbed in this process and the inflammatory response is minimized (Fig.
1.6c). PoRTS windows can provide optical access to the brain for months following the
surgery.
To observe the CSF-ISF exchange, we need to obtain images of the brain parenchyma and the PVS of penetrating arterioles beneath the surface of the brain. Using traditional light microscopes, one can only obtain images of the surface of the brain. The images of the brain tissue below the surface can be obtained by using the principles of confocal fluorescence microscopy (Inoué 2006). In confocal fluorescence microscopy, a fluorophore located below the surface of the sample is excited by using a focused laser tuned to the excitation wavelength of the fluorophore, and the resulting fluorescence is measured by a photodetector tuned to the emission wavelength of the fluorophore. By using the same objective lens for excitation and detection of fluorescence, the focus for the exciting laser and the photodetector are maintained at the same point (hence the name confocal), and the fluorescence of a point in the sample that is beneath the surface can be measured. By scanning the focal point, 2D or 3D images and videos of the fluorescence in a sample can be collected. The depth of images obtained by confocal microscopy is limited by light scattering in the sample and by the increased chance of fluorescence out

Figure 1.6: PoRTS windows provide stable optical access to the brain

a. An illustration of the intact skull containing the Dura, arachnoid matter, the fluid filled SAS and the pia matter, along with the brain tissue and blood vessels. The skull bone is the major obstacle to obtaining optical access to the brain.

b. An illustration of the cranial window where the skull and the dura are removed. This leads to damage of the arachnoid matter and the fluid containing SAS, along with the pia matter, making it unsuitable for investigation of fluid flow in the CNS. Cranial windows also lead to inflammation of brain tissue.

c. Thin-skulled windows do not completely remove the skull. The skull is thinned till it is translucent and a few 10’s of microns thick. The thin-skulled windows can provide optical access to the brain with minimal damage to the dura and the meningeal layers.
of the focal plane being detected with a high-intensity light source. Images of deeper regions in the tissue can be obtained using two-photon laser scanning microscopy (2PLSM), where the fluorophores are excited by an intense beam of wavelength that is twice the characteristic excitation wavelength of the fluorophore (which is typically in the near infrared spectrum), instead of light at the excitation wavelength used in traditional confocal microscopy (Helmchen and Denk 2005). The chance of exciting a fluorophore with two photons of twice the excitation wavelength varies non-linearly with the intensity of the incident light (Inoué 2006; Shih et al. 2012) and therefore, out of plane fluorescence is drastically reduced in 2PLSM compared to traditional confocal microscopy (Fig. 1.7a inset). A simplified schematic of 2PLSM setup is shown Fig.1.7a. The setup includes a tunable near infrared laser emitter, which controls the duration and frequency of the light pulses in the laser beam. The width and intensity of the laser beam (shown in light red in Fig.1.7a) can be controlled before coupling the laser to the microscope. Within the microscope, the beam is scanned in a raster pattern using a software-controlled setup. The width of the beam at the back aperture of the objective lens is maintained by a combination of lenses (scan lens and tube lens in Fig.1.7a) in the path of the laser. The fluorescence emitted from the sample (shown in green in Fig.1.7a) passes through the same objective lens and then is focused into photomultipliers tubes (PMT) through a collection lens. Through the precise control of the imaging setup offered through all these components, images of the brain tissue up to 1mm below the brain surface with sub-micron resolution (an example is shown in Fig.1.7b) can be obtained using 2PLSM (Shih et al. 2012; Crowe and Ellis-Davies 2014).
Injecting tracers into ISF and CSF

We are ultimately interested in the movement of solutes (metabolites and neurotransmitters) from the ISF to the CSF, and therefore, it might seem that the best way to observe this is to inject tracers directly into the ECS of the brain. However, there are several caveats to observing fluid flow by injecting tracers into the ECS. The most direct method to track fluid flow is by injecting fluorescent microspheres. This method cannot be used in the brain parenchyma, because microspheres that can be tracked reliably with the current technology have a size of around 1µm (Mestre, Tithof, et al. 2018; Kelley and...
Ouellette 2011; Bedussi, Almasian, et al. 2017; Bedussi, Van Der Wel, et al. 2017) and cannot enter the brain extracellular spaces that have a typical channel width in the order of 0.1 µm. Another way is to inject dyes into the brain parenchyma and track dye movement. One has to insert a needle through the skull and the meninges, in order to inject dyes into the brain parenchyma which can affect CSF flow in the meninges and therefore affect the CSF-ISF exchange (Mestre, Hablitz, et al. 2018). Inserting a needle into the brain parenchyma would also result in inflammation of brain tissue and scar formation, which would affect the ISF movement. Moreover, injecting dye into a ISF surrounded by soft brain tissue would deform the brain tissue surrounding the point of injection and some time has to be allowed between the injection and the measurement to allow for the tissue to reach equilibrium (Senjuntichai and Rajapakse 1993) and by then, the dye might already spread over the entire region of interest and make it hard to track movement. One way around this is to track fluorescence recovery after photobleaching (FRAP). In this method, a fluorescent dye is injected into the sample and allowed to settle, till the local pressure at the site of injection returns to its normal state. Then, the fluorophore is visualized with a low intensity light and a baseline image is taken, while a section of the sample is photobleached by using a high-power laser. This creates a region lacking fluorophore, that appears as a region of decreased fluorescence. The recovery of fluorescence in this dark spot can provide information about the nature of transport of dye in the sample. For convection-driven transport, the recovery of fluorescence is directional as the fluorophore is carried into the bleached region by bulk flow, and independent of molecular weight of the fluorophore. For diffusion-dominated transport, the fluorescence recovery is uniform in all directions and slower for heavier molecules.

An alternate way to examine CSF-ISF exchange, while avoiding injury to the meninges and the brain tissue to is inject dyes or particles into the fluid-filled ventricles or
the cisterna magna and study fluid movement at the sites of possible ISF-CSF exchange. This method has been used in several studies to study fluid flow in the SAS (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017) and the PVS (Iliff et al. 2012; Schain et al. 2017; Xie et al. 2013; Mestre, Hablitz, et al. 2018; Ma et al. 2017). There is one major caveat in this method — the injection rate used to introduce tracers and particles into the CSF in all these studies is in the range of 1-2µL/min, which is appreciably higher than the CSF production rate of 0.3-0.4µL/min (Oshio et al. 2005) in healthy mice. When Norwood et al. (Norwood et al. 2019) infused tracers at a slower rate (0.2µL/min), the path of tracer flow (Fig.1.8a) was different from the tracer flow observed with the 1µL/min infusions by Ma et al. (Ma et al. 2017). Specifically, Ma et al. observed tracer influx into the cerebral cortex (Fig.1.8b) through the PVS, while data from Norwood et al. shows negligible tracer movement into the cortex.

![a. The image shows a sagittal plane-cut cross section of the spinal cord, brain and nasal cavity, after decalcifying the skull and the spinal column, following a dye (Evans blue) injection into the cisterna magna at a slower rate than the CSF production rate. The dye does not enter the cerebral cortex of mice (red ellipse). Taken from Norwood et al. 2019. Scale bar 1mm.](a)

**Figure 1.8: Possible effect of dye infusion rate on CSF flow pathways**

- **b.** The dorsal view of an *ex vivo* brain after a fluorescent dye (P40D680) is infused into the ventricles at a rate faster rate compared to the CSF production rate. The mice, from left to right, are euthanized 10, 30 and 60mins after infusion. The dye enters the cortex through the paravascular spaces of arteries. This is not likely an artifact of injecting dye through the ventricles, as other studies using high rates of infusion through the cisterna magna show a similar pathway for CSF flow. Taken from Ma et al. 2017. The scale bar is 200µm.
In the next section, we present the existing evidence concerning the fluid and metabolite transport in the brain. This evidence should be viewed with the caveats regarding dye infusion into the brain discussed here.

1.3.5 Evidence for fluid and metabolite transport in the CNS

Transport of CSF in the PVS and through the brain extracellular spaces has received considerable interest in the last decade since the proposal of the “glymphatic hypothesis.” In 2012, Iliff et al. (Iliff et al. 2012) published the results of experiments where a fluorescent dye was injected into the cisterna magna of anesthetized mice (green in Fig.1.9a), while a different dye was injected into the bloodstream (red in Fig.1.9a), and the dye movement was observed under a two-photon microscope. The dye was observed in the PVS surrounding the arteries near the surface of the cerebral cortex within 5 minutes after injection (Fig.1.9a). In the next 5 minutes (Fig.1.9b), the dye was observed to enter the PVS surrounding penetrating arterioles, from where it entered the brain parenchyma. Based on the observation that the dye transport was not significantly affected by the molecular weight of the dye, Iliff et al. (Iliff et al. 2012) proposed that the flow in the paravascular pathway is convection dominated. This influx of dye into the parenchyma was significantly reduced in Aqp4 knockout mice. Since Aqp4 is strongly expressed in the glial endfeet and because the glial endfeet surround a large portion of the PVS, this pathway of CSF flow was termed the glymphatic (glia-lymphatic) pathway (Nedergaard 2013), where directed CSF flow is proposed to occur from the cisterna magna along the PVS of arteries and through astrocytic endfeet (Fig.1.9c). According to this hypothesis, once CSF enters through the astrocytic endfeet, it flows through the brain parenchyma, clearing metabolites in the ECS, and flows out through the PVS of cerebral veins. It should be noted that Iliff et al. injected the dye into the cisterna magna of mice at a rate of 2µl/min, a rate much higher than the normal CSF production rate (0.3-0.4µl/min), and it is possible
that the directed CSF flow observed in their experiments might be an artifact of the high injection rate.

The concept of directed CSF flow in the PVS and the brain ECS has been challenged by subsequent experimental and theoretical studies. Smith et al. (Smith et al. 2017a) injected tracers directly into the brain parenchyma and observed that the dye movement was dependent on the molecular weight, as predicted by the diffusive transport hypothesis. In the same study, Smith et al. also used FRAP methods to establish that the transport in the brain extracellular space is diffusion-dominated. It should be noted that the experimental results of Smith et al., might be influenced by directly injecting dyes into the brain parenchyma, which can affect the flow in the CSF in the meningeal layers. However, the findings of Smith et al., are corroborated by models of fluid flow in the brain extracellular space based on 2D and 3D reconstructions of ECS, which also suggest that under physiological conditions, the transport in the brain parenchyma is diffusion-dominated.

Figure 1.9: Possible effect of dye infusion rate on CSF flow pathways

The concept of directed CSF flow in the PVS and the brain ECS has been challenged by subsequent experimental and theoretical studies. Smith et al. (Smith et al. 2017a) injected tracers directly into the brain parenchyma and observed that the dye movement was dependent on the molecular weight, as predicted by the diffusive transport hypothesis. In the same study, Smith et al. also used FRAP methods to establish that the transport in the brain extracellular space is diffusion-dominated. It should be noted that the experimental results of Smith et al., might be influenced by directly injecting dyes into the brain parenchyma, which can affect the flow in the CSF in the meningeal layers. However, the findings of Smith et al., are corroborated by models of fluid flow in the brain extracellular space based on 2D and 3D reconstructions of ECS, which also suggest that under physiological conditions, the transport in the brain parenchyma is diffusion-dominated.
dominated (Holter et al. 2017; Jin, Smith, and Verkman 2016). A review of all the experimental work on the topic of glymphatic flow by Abbot et al. (Abbott et al. 2018) concluded that diffusion is the likely mechanism of transport in the parenchyma.

The mechanism driving fluid and metabolite transport in the glymphatic pathway has also been unclear. Iliff et al. (Iliff et al. 2013) proposed that heartbeat pulsations can drive directional fluid flow into the brain through the peristaltic motion of arterial walls. This hypothesis, called the peristaltic pumping hypothesis, is supported by the recent evidence that CSF flow in the PVS of arteries is pulsatile and follows the frequency of heartbeat (Bedussi, Almasian, et al. 2017; Mestre, Tithof, et al. 2018). It should be noted that in the experiments by Bedussi et al. and Mestre et al., directed CSF flow was observed only in the PVS of pial arteries but not penetrating arterioles. There have been several computational models used to understand the nature of transport in the PVS and the parenchyma. Early models (Wang and Olbricht 2011; Schley et al. 2006; Bilston et al. 2003) suggested that there is a possibility of pulsation-driven directional flow of CSF in the PVS. But a more recent model by Asgari et al. (Asgari, De Zélicourt, and Kurtcuoglu 2016) showed that under physiological conditions, heartbeat does not drive appreciable directed CSF flow in the PVS. In a recent review of all the computational studies on the topic of CSF flow in the PVS by Martinac and Bilston (Martinac and Bilston 2019) concluded that heartbeat driven pulsation is unlikely to be the sole driver of fluid flow.

Currently, there are several unanswered questions regarding the nature and drivers of fluid and metabolite transport in the brain. The possibility of directional flow of CSF through the brain, with arterial pulsations a driver of the directional flow is one of the highly debated topics. The mechanisms of how pulsations and functional hyperemia affect metabolite transport in the PVS and the SAS is not clear. The role of sleep and anesthesia in the metabolite clearance from the brain is also unclear. This thesis attempts to answer
these questions through a combination of mathematical models and experiments. The new contributions of this thesis pertain to the nature of transport in the PVS and the possible drivers of fluid and metabolite transport through the PVS and the SAS. Before defining the main objectives of the thesis, we will examine the principles of diffusion, convection and dispersion, and their application to the fluid and metabolite transport in the fluid filled regions of the cranial space.

1.4 Modes of transport

1.4.1 Diffusion

Diffusion is the movement of a constituent of a mixture relative to the movement of the mixture. At a molecular level, diffusion can be modeled as Brownian motion of particles, a random motion which is a function of the absolute temperature of the mixture. This random motion (or random walk) of particles can be used to explain the diffusion of a species that is initially highly concentrated over a subdomain of a solution to, in the end, achieving a uniform distribution. As per the traditional understanding of Brownian motion, while each particle moves in a random direction at each point in time, the constituent as a whole spreads out uniformly in all directions until the concentration throughout the solution is equalized. In a traditional context in which solute and solvent occupy a domain that does not move on average, each component must move past the other to preserve the null mean motion constraint. However, since here we are dealing with dilute solutions with peak concentrations around 1mM, we shall only consider the diffusion of the solutes and assume that the volume of the fluid part of the mixture is occupied entirely by the solvent (water). In the absence of any net movement of the solution, the flux of a solute, $\alpha$, is given by Eq.1.1.

$$J_\alpha = - \left( RT \frac{c_\alpha}{d_\alpha} \right) \nabla c_\alpha$$  

1.1
\[ J_\alpha = -D_\alpha \nabla c_\alpha \quad ; \quad D_\alpha = \left( \frac{RT c_\alpha}{d_\alpha} \right) \]

In Eq.1.1, \( c_\alpha \) is the concentration of the solute and \( d_\alpha \) is the frictional coefficient between the solute \( \alpha \) and all the other components of the solution. \( R \) and \( T \) are the universal gas constant and the absolute temperature, respectively. The term in the bracket of Eq.1.1, is more familiarly called as the diffusion coefficient in Fick’s first law of diffusion, Eq.1.2, and is inversely related to the molecular size and mass. For example, the diffusion coefficient in water for glucose with a molecular weight of 180 is \( 6.0 \times 10^{-10} \text{m}^2/\text{s} \), while for insulin with a molecular weight of 12,000, the diffusion coefficient is \( 1.5 \times 10^{-10} \text{m}^2/\text{s} \) (Hober et al. 1946).

Fick’s second law of diffusion Eq.1.3 gives the rate of change of the molar concentration of a species that is being produced at a rate of \( \dot{c}_\alpha \).

\[ \frac{\partial c_\alpha}{\partial t} = \dot{c}_\alpha - \nabla \cdot J_\alpha \]

In the brain, diffusion is one of the chief modes of transport. Neurons, the functional units of the CNS, communicate with each other through diffusion of neurotransmitters released at the synapses. While diffusion is a quick and effective means of transport over small distances like synaptic clefts that are on the order of 100 nm, over larger distances diffusion is a very slow process. This is reflected in the units of the diffusion coefficient \( (\text{m}^2/\text{s}) \), which means that the time taken for diffusion increases as the square of the distance.

Diffusion through the fluid portion of a porous solid, such as the brain extracellular space, is affected by many factors including the curvature of the fluid spaces and the presence of dead spaces and obstructions (Sykova et al. 2008). For the purposes of macro-scale calculations and measurements, diffusion through porous spaces can be modelled as being like diffusion through open spaces but with a reduced effective diffusion
The effect of the hindered diffusion can be captured by dividing the diffusion coefficient by the square of tortuosity (Eq.1.4).

\[ D^* = \frac{D}{\lambda^2} \] \hspace{1cm} 1.4

The tortuosity (\(\lambda\) in Eq.1.4) can be considered as the extra length (as a fraction) that the diffusing solute molecule has to travel as a result of the hindrances.

1.4.2 Convection

The movement of the solvent (water in physiological systems) in the absence of semipermeable membranes is driven by hydrostatic pressure. The solvent flows along the direction of decreasing pressure gradient. This kind of directional flow is called convection. The magnitude of pressure changes in physiological systems do not appreciably affect the density of water, and therefore for all the calculations in this document, water is assumed to be incompressible. Fluid incompressibility, in a region with no secretion or absorption of fluid, is expressed by requiring that the divergence of fluid velocity, \(v_f\) (Eq.1.5), be equal to zero.

\[ \nabla \cdot v_f = 0 \] \hspace{1cm} 1.5

The pressure change driving the flow of the solvent has to balance the resistance to flow from the inertial and viscous forces of the fluid. The ratio of the inertial forces to viscous forces can be estimated by the Reynolds number. For a fluid with density \(\rho_f\) and viscosity \(\mu_f\) flowing through a channel of area of cross-section \(A\), with a flow rate \(Q\), the Reynolds number is given by Eq.1.6.

\[ Re = \frac{\rho_f Q \bar{d}}{\mu_f A} \] \hspace{1cm} 1.6
In Eq.1.6, \( \bar{d} \) is the hydraulic diameter, which is equal to the diameter for a circular channel. For the flow of CSF in the cranial space of mice, the Reynolds number is usually a few orders of magnitude smaller than 1 (Mestre, Tithof, et al. 2018), indicating that most of the flow resistance is offered by the viscous forces.

At the molecular level, the resistance to viscous flow can be explained by intermolecular forces of attraction. Here, we are concerned with flow of the solvent (water) in the SAS, the PVS and the extracellular spaces. In all these cases, the substrate for water flow is the phospholipid bilayer of cell membranes and the collagen fibers that form the extracellular matrix. The hydrophilic head of the phospholipid bilayer, as well as the collagen fibers that are in contact with the solution have a strong affinity for water, which makes the layer of water molecules immediately adjacent to the solid substrate move with the solid. Away from the membrane, the resistance to flow is offered by the forces of attraction among the water molecules. This results in a flow with the velocity increasing in magnitude as we move away from the membranes and the fluid moving in a layer-like fashion. The resistance to the shear between these layers of the fluid on a macro scale is given by the viscosity of the fluid, \( \mu_f \). Having assumed the fluid to be incompressible and linear viscous, the flow of the fluid in an open channel is modelled by the Navier-Stokes equations, whose balance of momentum is given by Eq.1.7.

\[
\frac{\partial \mathbf{v}_f}{\partial t} + (\nabla \mathbf{v}_f) \mathbf{v}_f - \frac{1}{\rho_f} \nabla \cdot \mathbf{\sigma}_f = \mathbf{0} \tag{1.7}
\]

\[
\mathbf{\sigma}_f = -p_f \mathbf{I} + \mu_f (\nabla \mathbf{v}_f + (\nabla \mathbf{v}_f)^T) \tag{1.8}
\]

The fluid stress, \( \mathbf{\sigma}_f \), is given by Eq.1.8, where \( p_f \) is the hydrostatic pressure, \( \mathbf{I} \) is the identity tensor and the superscript “\( T \)” is used for the transpose of a tensor. Eqs.1.5 and 1.7 together, form the set of governing equations for the incompressible Navier-Stokes
Laminar flows in straight pipes with pressure gradients only in the axial direction have a parabolic velocity profile (Fig. 1.10a), with the velocity increasing as the square of distance from the walls of the channel.

For flow through porous solids with complicated pore structure, the flow within each pore is given by the Navier-Stokes equations. However, the tedious task of calculating flow through the complicated pore shapes, sizes and the paths in a porous solid can be replaced by simply calculating the average fluid velocity through the porous solid. One of the most commonly used models for flow through porous solids is the Darcy flow model. In this model, the average velocity changes linearly with the pressure gradient. For Darcy flows through a straight pipe with pressure gradients only in the axial direction, the average flow velocity has a flat profile (Fig. 1.10b) instead of the parabolic profile seen in Navier-Stokes flow. The Darcy permeability of a porous solid with fluid volume fraction \( \zeta_f (0 < \zeta_f < 1) \), is given by \( k_s \), which has a unit of \( \text{m}^2 \). The flow of an incompressible fluid in the Darcy flow model is governed by Eqs. 1.5 and 1.9.

\[
\frac{\partial \mathbf{v}_f}{\partial t} + (\nabla \mathbf{v}_f) \mathbf{v}_f + \frac{1}{\rho_f} \nabla p_f + \frac{\zeta_f \mu_f}{\rho_f k_s} \mathbf{v}_f = \mathbf{0} \quad 1.9
\]

While the Darcy flow model works well for porous solids with low fluid volume fractions and permeabilities, the Brinkman correction is necessary to accurately model the flow in solids with large void fractions and high fluid permeability (Neale and Nader 1974). The Brinkman correction is particularly necessary to model flow at the boundary where a porous solid meets an impermeable wall or an open channel. The velocity transition from the no-slip velocity on the impermeable wall or the free flow velocity in an open channel to the velocity in the porous solid occurs at a length of the order \( \sqrt{k_s} \). Therefore, to model the flow through a porous solid with dimensions comparable to \( \sqrt{k_s} \), it is appropriate to include the Brinkman correction to
Darcy’s law. The flow of an incompressible fluid governed by the Darcy-Brinkman’s model is given by Eqs. 1.5 and 1.10.

\[
\frac{\partial \mathbf{v}_f}{\partial t} + (\nabla \mathbf{v}_f) \mathbf{v}_f + \frac{1}{\rho_f} \nabla \cdot \mathbf{\sigma}_f + \frac{\zeta_f \mu_f}{\rho_f k_s} \mathbf{v}_f = 0
\]

1.10

The fluid stress here includes the shear among the fluid layers and is the same as the one described in Eq. 1.8. For Darcy-Brinkman flow through a straight pipe with pressure gradient only in the axial direction, the velocity profile is between the parabolic profile of Navier-Stokes and the flat profile of Darcy flow (Fig. 1.10c).

When the solvent undergoes convective flow, the solute has a convective flux along with the diffusive flux from Eq. 1.1. The spatio-temporal evolution of concentration of the solute in this case is given by Eq. 1.11, called the convection-diffusion equation.

\[
\frac{\partial c_\alpha}{\partial t} + \mathbf{v}_f \cdot \nabla c_\alpha = \dot{c}_\alpha - \nabla \cdot (D_\alpha \nabla c_\alpha)
\]

1.11

The time taken for convective transport (for a fixed velocity of the solvent) increases linearly with distance, while time taken for diffusive transport increases with the square of distance. Unlike diffusion, the convective transport is independent of the molecular weight of the solute (except in cases where the solute is filtered by the porous solid, which is not considered in this thesis). The ratio of convective to diffusive transport rate of a solute is given by the Peclet number (Eq. 1.12).

\[
P_e = \frac{v_{ave} L}{D_\alpha}
\]

1.12

In Eq. 1.12, \( L \) is a characteristic length, which is a length parameter indicating the typical distance over which the two modes of transport are being compared and \( v_{ave} \) is the fluid speed in the direction of interest averaged over the area of cross-section.
1.4.3 Dispersion

Transport of solutes can be improved to rates faster than diffusion without directed movement of the solvent. Solute transport can be enhanced through oscillatory movement of the solvent which results in a combination of diffusion and fluid mixing, also called as dispersion. Consider a 2D “toy-model” of the SAS-PVS system (Fig. 1.11), a system of two fluid-filled porous channels meeting at right angles (representing the PVS of arteries traversing the SAS and entering the brain parenchyma perpendicular to the brain surface,

Figure 1.10: Fluid velocity profiles for Navier-Stokes, Darcy and Darcy-Brinkman flow
similar to Fig. 1.5a). The channel on the top has a directed fluid flow from left to right and the channel on the bottom only has oscillatory fluid flow up and down. A solute (representing the metabolites) is initially present at a higher concentration in the channel with the oscillatory flow. The clearance rate of the solute from the latter channel is higher when the oscillatory flow is present in the PVS space as compared to the case where there is no oscillatory flow, as shown in Fig. 1.11. We will consider additional models of dispersion-based metabolite clearance in chapters three and five. When the channel with directed flow contains much more fluid as compared to the latter channel or if the magnitude of the directed flow is large compared to the magnitude of the oscillatory flow, it is common practice to assume that the solute concentration at the interface of the two channels as fixed (Asgari, De Zélicourt, and Kurtcuoglu 2016; Sharp, Carare, and Martin 2019), rather than explicitly modeling the channel with the directed flow.
1.5 Objectives

The objective of this thesis is to understand the drivers of CSF flow and metabolite transport in the sites of possible CSF-ISF exchange, namely the SAS and the PVS of arteries. We will use mathematical models of transport based on the anatomical features of the SAS and the PVS and the principles of fluid and solute transport described in this chapter. The predictions of the mathematical models will be tested against existing experimental data, or in some cases, minimally-invasive experiments will be designed to test the predictions of the models. By using mathematical models based on established principles of transport, well-understood anatomy and by using minimally invasive
experiments, we expect to study the highly sensitive fluid spaces of the CNS in their natural state. The objective of this thesis is to answer the following questions.

1. **Can arterial pulsations drive directional pumping of CSF in the PVS?** We will test the peristaltic pumping hypothesis using mathematical models based on the detailed experimental data about the PVS shape, size and arterial wall movements published by Mestre et al. (Mestre, Tithof, et al. 2018) and compare the predictions of the models to the fluid velocities presented in the same article.

2. **In the absence of directional flow, how do arterial wall movements and brain tissue deformability influence CSF-ISF exchange?** We will build mathematical models of fluid movement in the PVS and the SAS driven by arterial wall movements in the cortex, coupled with the deformability of the brain tissue. The predictions of the models will be tested by measuring brain tissue deformation in Thy1-YFP mice (Fig.1.7b).

3. **How do sleep states influence CSF-ISF exchange?** We will extend our models of fluid transport in the PVS to understand the influence of arterial dilations during REM and NREM sleep on CSF-ISF exchange.

4. **Is metabolite clearance a primary purpose of arterial wall movements?** It has been traditionally believed that the purpose of arterial dilations in regions of high neural activity is to increase oxygen demand of the tissue, while our research suggests that the main purpose of arterial wall movements is the metabolite clearance in the brain. We will test the former hypothesis by studying how arterial dilations affect tissue oxygenation in the mouse brain.
Chapter 2:
Peristaltic pumping of CSF in the arterial paravascular space¹

2.1 Introduction

One of the leading theories in support of bulk flow of cerebrospinal fluid (CSF) the paravascular space (PVS) identifies “peristaltic pumping” as the flow driver, i.e., the idea that heartbeat-driven pulsations pump CSF in the PVS. Peristaltic pumping in a deformable tube is achieved by repeated contractions and dilations propagating along the walls of the tube. In the context of CSF flow in the PVS, a peristaltic movement is known to exist due to the pulse wave propagation along arteries. The periodic pressure changes from the heart travel down the arterial tree, causing repeated, periodic contractions and dilations of arterial walls. The phenomenon of a travelling pulse wave driven by heart contractions has been observed in mice (Herold et al. 2009; Williams et al. 2007) and in humans (Gladdish and Rajkumar 2002; Allen and Murray 2002). Recently Mestre et al. (Mestre, Tithof, et al. 2018) and Bedussi et al. (Bedussi, Almasian, et al. 2017) used in vivo two-photon microscopy (Shih et al. 2012) to simultaneously measure arterial pulsations and the flow of CSF in the PVS of the branches of middle cerebral artery (MCA) by tracking the motion of fluorescent microspheres. They found that movement of CSF in the PVS had two components, a constant flow in the direction of blood flow with a mean flow speed of approximately 20µm/sec, and an oscillatory flow in phase with the arterial

pulsations (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017), with a peak velocity of approximately 10µm/sec. Based on these observations, it has been proposed that peristaltic motion of the arterial wall generates a “pumping” force that drives the net flow of CSF parallel to the direction of the pulse wave propagation.

In fluid dynamics, peristaltic pumping is a well-understood mechanism of fluid transport. The mechanism of peristaltic pumping of fluids was first demonstrated by Latham (Latham 1966). Further work on the peristaltic pumping of fluids has encompassed a wide range of scenarios (Jaffrin and Shapiro 1971; Shapiro et al. 1969; Abdelsalam and Bhatti 2018; Fung and Yih 1964). Calculations made using fluid dynamic principles can make very accurate predictions of fluid flow under peristalsis, and have been used in designing artificial peristaltic pumps (Hartley 1998; Sorensen and Akkas 2001; Peclat 2000).

In this chapter, we apply the well-established fluid dynamic principles of peristalsis to study the nature of fluid flow in the PVS, aiming to bridge the gap between experimental observations and hypotheses. As previous studies of CSF flow in the PVS disagree on both the direction and the flow rates (Wang and Olbricht 2011; Schley et al. 2006; Asgari, De Zélicourt, and Kurtcuoglu 2016), we started our calculations by revisiting the mechanism of peristaltic pumping using time-dependent fluid dynamic simulations with fluid particle tracking in a deforming domain. By emphasizing the mechanism of peristatic pumping, we aimed at providing a clear physical interpretation for our calculations. We then performed fluid dynamic simulations on more realistic models of the PVS. Our simulations suggest that peristalsis with physiologically-plausible arterial pulsations cannot drive the experimentally-observed fluid flow. However, we found that a small, constant pressure gradient (of order 0.01mmHg/mm) can account for the net forward movement observed experimentally. These results suggest that the observed directional
movement of CSF in the PVS is generated by naturally occurring and/or experimenter-generated pressure differences, but not by arterial pulsations.

2.2 Material and Methods

2.2.1 Model equations and boundary conditions

We use a standard time-dependent finite element method to solve the equations of fluid motion in the PVS. These equations are formulated to account for the deformation of the PVS. Specifically, we write the equations in Arbitrary-Lagrangian-Eulerian (ALE) coordinates. In this chapter, the equations are presented in their more familiar Eulerian form. The ALE form is presented in Appendix B. As is well-known, ALE formulations are able to account for the deformation of the solution’s domain at the expense of having to determine an auxiliary motion typically referred to as the “mesh motion” (Formaggia, Quarteroni, and Veneziani 2010; Donea et al. 2004; Fernández et al. 2009). The governing equations for the fluid and the mesh movement are written in their weak, tensor form (see Appendix B) and converted to their component form using a custom program in Wolfram Mathematica. These component form equations are implemented in COMSOL Multiphysics (Burlington, MA) using the “Weak Form PDE” interface, where PDE stands for partial differential equation. Therefore, the overall solution scheme is our own, and COMSOL Multiphysics simply provides a high-level integrated programming environment within which said scheme is implemented.

The fluid (CSF) velocity and pressure are governed by the incompressible Navier-Stokes equations (Eqs.2.1 – 2.3). We solve for the fluid velocity ($v_f$) and pressure ($p_f$) in the PVS as a function of time ($t$).

\[
\frac{\partial v_f}{\partial t} + (v_f \cdot \nabla) v_f - \frac{1}{\rho_f} \nabla \cdot \sigma_f = 0
\]  

2.1
∇ \cdot \mathbf{v}_f = 0 \quad \text{2.2}

\mathbf{\sigma}_f = -\rho_f \mathbf{I} + \mu_f (\nabla \mathbf{v}_f + (\nabla \mathbf{v}_f)^T) \quad \text{2.3}

In Eq. 2.1, $\rho_f$ and $\mathbf{\sigma}_f$ are the fluid's mass density and Cauchy stress, respectively. In Eq. 2.3, $\mu_f$ is the fluid's dynamic viscosity.

The governing equation for the mesh motion in the deforming fluid domain is dictated by convenience and, where necessary, by the problem's geometric constraints. In the problems in this chapter, the deformation of the solution's domain (PVS) is relatively mild and therefore the mesh motion equation, with primary unknown given by the mesh displacement $\mathbf{u}_m$, is chosen to be a linear elliptic model (Wick 2011), namely the Laplace equation (Eq. 2.4):

\[ \nabla \cdot (\nabla \mathbf{u}_m) = 0 \quad \text{2.4} \]

A no-slip boundary condition is used at the inner and outer walls of the PVS, i.e., fluid velocity is equal to the wall velocity in all simulations (Eq. 2.5).

\[ \text{at } r = R_1 \text{ and } r = R_2, \quad \mathbf{v}_f = \frac{\partial \mathbf{u}_m}{\partial t} \quad \text{2.5} \]

For the axisymmetric simulations, the inner walls have a baseline radius of $R_1$ and the outer walls have a fixed radius of $R_2$. The movement of the inner walls is given by a travelling sinusoidal wave (Eq. 2.6).

\[ \text{at } r = R_1, \mathbf{u}_{mr} = \alpha R_1 \sin \left( \frac{2\pi}{\lambda} (z - ct) \right), \quad \mathbf{u}_{mz} = 0 \quad \text{2.6} \]

There is no wall movement at the outer wall (Eq. 2.7).

\[ \text{at } r = R_2, \mathbf{u}_{mz} = 0, \quad \mathbf{u}_m = 0 \quad \text{2.7} \]
The total length of the tube is taken equal to the wavelength ($\lambda$) of the peristaltic wave and periodic boundary conditions are used at the two ends of the tube (Eq. 2.8).

$$v_f\big|_{z=0} = v_f\big|_{z=\lambda}; \quad p_f\big|_{z=0} = p_f\big|_{z=\lambda} \quad 2.8$$

To obtain a unique pressure solution, a global constrain is applied for the total pressure (Eq. 2.9).

$$\int p_f = 0 \quad 2.9$$

In Eqs. 2.6-2.7, $u_{m_r}$ and $u_{m_z}$ are the r and z components of the mesh displacement ($u_m$). \(\alpha\) is the half wave amplitude of the peristaltic wave, as a fraction of the baseline diameter $R_1$. \(c\) is the speed of the peristaltic wave. The integration in Eq. 2.9 is performed over the entire computational domain.

For the 3D simulations presented in this chapter, the cross section of the PVS geometry is made to resemble the geometries observed in vivo (Tithof et al. 2019; Mestre, Tithof, et al. 2018). The inner wall of the cross section is a circle of radius $R_1$. The outer wall of the cross section is an ellipse with major axis $R_2$ and minor axis $0.8R_1$. The intersection of the circle with the ellipse is smoothened with a fillet of radius $0.08R_1$. The boundaries can be divided into three regions:

I. The inner walls of the PVS (the walls facing the arteries, or the circular face), where a dilation of the arterial wall will cause a deformation of the PVS in the direction opposite to the unit outward normal, $n$ (Eq. 2.10).

$$\text{at inner walls: } u_m = -an \ [ct - z]n \quad 2.10$$

II. The outer walls of the PVS (wall facing the SAS or the brain tissue or the elliptical face), where the pressure is higher when the vessel dilates and lower when the
vessel contracts (the relation between pressure and arterial wall movement can be observed in Fig.2.6e). Therefore, when the vessel dilates, the outer walls of the PVS deform in the direction of the outward normal, \( n \) (Eq.2.11).

\[
\text{at outer walls: } u_m = \varphi an \left[ ct - z \right]n
\]  

III. The part of the wall between these two regions, (the transition region or the fillet region). Here, the displacement is transitioned using the \textit{step} function available in COMSOL Multiphysics, which creates a smooth transition between two values over a desired interval with continuous first and second order derivatives.

In Eqs.2.10 and 2.11, ‘\( an \)’ is a periodic function with a time period of \( 1/f \), where \( f \) is the heart rate frequency. The waveform of ‘\( an \)’ is interpolated from the pulsation waveform reported by Mestre et al (Mestre, Tithof, et al. 2018). The value of \( \varphi \) (SAS displacement parameter) is 0 for most of the simulations presented in this chapter.

For most of the 3D simulations presented in this chapter, no traction was applied at the axial ends of the PVS (Eqs.2.12-2.13).

\[
\text{at } z = 0, \quad \sigma_f \cdot n = 0 \tag{2.12}
\]

\[
\text{at } z = \lambda, \quad \sigma_f \cdot n = 0 \tag{2.13}
\]

This change (from the case of periodic boundary conditions used for axisymmetric simulations in Eq.2.8) was useful to understand the magnitude of pressure changes in the PVS. For the simulations where a pressure difference is applied across the ends, no traction is applied at the distal end of the PVS (\( z = L_a \), where \( L_a \) is the length of the MCA). This is similar to Eq.2.13. At the proximal end of the PVS, a pressure like traction is applied (Eq.2.14).

\[
\text{at } z = 0, \quad \sigma_f \cdot n = -p_1 n \tag{2.14}
\]
The parameter \( p_1 \) in Eq. 2.14 is the pressure difference across the length of the PVS. On the peripheral walls of the PVS, the fluid velocity is equal to the wall velocity (as in Eq. 2.5).

The Reynolds number for all the simulations is calculated using the formula for flow in a pipe (Eq. 2.15).

\[
Re = \frac{\rho_f Q D_h}{\mu A}, \quad D_h = \frac{4A}{P}
\]

In Eq. 2.15, \( D_h \) is the hydraulic diameter, which is calculated using the area \( A \) and the perimeter \( P \). \( Q \) is the flow rate. The Péclet number is calculated using the diffusion (D) coefficient for Amyloid-β in water (Eq. 2.16) and \( v_{ave} \), the mean downstream speed of the fluid at the center of the PVS (\( r = (R_1 + R_2)/2 \)).

\[
Pe = \frac{v_{ave} L_a}{D}
\]

The details about particle tracking in ALE are explained in Appendix A. The particle tracking calculations and movies were made using a MATLAB code. All the code for Wolfram Mathematica, COMSOL Multiphysics, and MATLAB are available to download on GitHub (https://github.com/DrewLab/Peristaltic-pumping-of-CSF.git).

### 2.2.2 Model parameters

All the parameters of the models were selected to represent the anatomy of mice. The dimensions of the cross-section of the PVS and the pulsation waveform of the arteries were taken from Mestre et al. (Mestre, Tithof, et al. 2018), to emulate their experimental results. All the parameters used in the model are listed in Table 2.1.
Table 2.1: Parameters for models of peristaltic pumping

<table>
<thead>
<tr>
<th>Parameter Name</th>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial radius</td>
<td>$R_1$</td>
<td>30</td>
<td>$\mu$m</td>
<td>(Mestre, Tithof, et al. 2018)</td>
</tr>
<tr>
<td>PVS width</td>
<td>$wd$</td>
<td>40</td>
<td>$\mu$m</td>
<td>(Mestre, Tithof, et al. 2018)</td>
</tr>
<tr>
<td>PVS outer radius</td>
<td>$R_2$</td>
<td>70</td>
<td>$\mu$m</td>
<td>$R_1 + wd$</td>
</tr>
<tr>
<td>CSF viscosity</td>
<td>$\mu_f$</td>
<td>0.001</td>
<td>Pa.s</td>
<td>(Støverud et al. 2013; Yetkin et al. 2010)</td>
</tr>
<tr>
<td>CSF Density</td>
<td>$\rho_f$</td>
<td>1000</td>
<td>kg/m$^3$</td>
<td>(Støverud et al. 2013; Yetkin et al. 2010)</td>
</tr>
<tr>
<td>Pulsation Frequency</td>
<td>$f$</td>
<td>8.67</td>
<td>Hz</td>
<td>(Mestre, Tithof, et al. 2018)</td>
</tr>
<tr>
<td>Pulse wave speed</td>
<td>$c$</td>
<td>1</td>
<td>m/s</td>
<td>(Herold et al. 2009; Williams et al. 2007)</td>
</tr>
<tr>
<td>Pulse wave wavelength</td>
<td>$\lambda$</td>
<td>0.115</td>
<td>m</td>
<td>$c/f$</td>
</tr>
<tr>
<td>MCA Length</td>
<td>$L_a$</td>
<td>5</td>
<td>mm</td>
<td>(Bedussi, Almasian, et al. 2017; X. Chen et al. 2011; Adams et al. 2018)</td>
</tr>
<tr>
<td>Diffusion coefficient</td>
<td>$D$</td>
<td>$1.4 \times 10^{-6}$</td>
<td>cm$^2$/s</td>
<td>(Massi et al. 2001; Tseng et al. 1999)</td>
</tr>
</tbody>
</table>

2.2.3 Anisotropic non-dimensionalization

One of the major concerns when using finite element simulations to study flow in the PVS is the long and narrow geometry of the PVS. For example, the domain used for the axisymmetric simulations has a length of one wavelength of the peristaltic wave (115.3 mm or 115,340$\mu$m), which is nearly 3000 times the width of the PVS (40$\mu$m). Simulating the geometry with these dimensions would need a large number of elements, making it computationally expensively to solve or create elements with poor aspect ratios. To deal with this problem, we non-dimensionalized the equations with different scaling factors in the x, y (or r for axisymmetric simulations), and z directions. All the equations from the mesh coordinates ($X_m$) are rewritten in these non-dimensional coordinates ($X_c$) (Eq.2.17).

In Eq.2.17, the coordinates are written in the conventional order, i.e., $(x, y, z)$ for Cartesian and $(r, \theta, z)$ for cylindrical coordinates.

$$X_m = L_o \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & g_3 / f \end{pmatrix} X_c \quad 2.17$$
The characteristic length, $L_0$, was chosen to be equal to the arterial wall radius ($R_1$). The scaling factor, $g_3$, was chosen so that the axial (z) length of the domain in the non-dimensionalized coordinates is 10. This resulted in a scaling factor ($g_3$) value of approximately 400 in all the simulations. To verify the validity of this choice of parameter, we solved the axisymmetric models (Eqs. 2.1-2.9), with a peristaltic wave amplitude equal to 1% of the arterial radius. Fig. 2.1 shows the z and r components of the velocity gradient in the mesh coordinates and non-dimensional coordinates at time, $t = 4/f$, where $f$ is the frequency of pulsations. In the mesh coordinates, the velocity gradients (for the radial and the axial component) were nearly three orders of magnitude higher in the r direction compared to the z direction. Our choice of scaling factor results in velocity gradients in the computational coordinates of similar magnitude, which means that for meshes of aspect ratio close to 1 in the non-dimensionalized coordinates, the approximation and interpolation errors are rather contained (for low Reynold’s number flows).

Figure 2.1: Use of anisotropic non-dimensionalization ensures that the approximation and interpolation errors are minimized

The results presented in this figure are at $t = 4/f$, where $f$ is the frequency of pulsations, with a sinusoidal peristaltic wave, whose peak-to-peak amplitude is 1% of the arterial radius.
2.2.4 Finite element implementation

The governing equations and traction boundary conditions were written in their weak form (see Appendix B) and implemented using the “Weak Form PDE” interface in COMSOL Multiphysics (Burlington, MA). The mesh consisted of rectangular elements with second order Lagrange polynomials for fluid velocity ($v_f$) and mesh displacement ($u_m$) and first order Lagrange polynomials for fluid pressure ($p_f$).

The mesh used in the axisymmetric simulations had 10 elements in the radial direction (graded distribution) with a higher density at the inner and outer walls of the annular section representing the PVS and 100 elements in the axial direction (uniform distribution) (Fig.2.2a). We chose the ratio of the number of elements so that the aspect ratio of the elements in the computational domain was nearly one. We started with a mesh with just 5 elements and 50 elements in the radial and axial directions respectively. Then we refined the mesh by a factor of 2 for each iteration of the mesh and calculated the relative $L_2$ error norm in velocity and pressure for each refinement (Eqs.2.18-2.19) at the end of four heartbeat cycles $t = 4/f$, where $f = c/\lambda$, is the frequency of heartbeat.

\[
L2Err_v = \frac{\sqrt{\int (v_{f,hz=1/5m} - v_{f,hz=1/10m})^2}}{\sqrt{\int v_{f,hz=1/10m}^2}} \bigg|_{t = 4/f} \tag{2.18}
\]

\[
L2Err_p = \frac{\sqrt{\int (p_{f,hz=1/5m} - p_{f,hz=1/10m})^2}}{\sqrt{\int p_{f,hz=1/5m}^2}} \bigg|_{t = 4/f} \tag{2.19}
\]

In Eqs. 2.18-2.19, $h_z$ is the height of each element in the z direction. We start with $h_z = 10/50 = 1/5$ and estimate the error for the values of $m = 1, 2, 4, 8$. We found that for a
value of $m = 2$, the error norms for both velocity and pressure were below 0.1% (Fig. 2.2b). Therefore, we performed all the axisymmetric simulations with 10 elements in the radial direction and 100 elements in the axial direction. For the 3D simulations, we kept the same number of elements axially and created a graded rectangular mesh in the XY plane (Fig.2.2c). The models had 9,553 and 1,290,356 degrees of freedom for the axisymmetric and 3D calculations respectively.

The time stepping for the time-dependent problems was performed using the backward difference formula with minimum order set to 1 and maximum order set to 5. The maximum time step was set to $1/1000^{th}$ of the heartbeat cycle. The norm of the change relative to the norm of the solution obtained by changing the time step from $0.001/f$ to $0.0005/f$ (cf. Eqs.2.18 and 2.19), was less than $10^{-8}\%$.

**Figure 2.2:** Finite element mesh and mesh convergence

- **a.** The finite element mesh for axisymmetric calculations. The mesh contains 10 elements in the radial direction and 100 elements in the axial direction. The dimensions are shown in the computational domain.
- **b.** The relative L2 error in velocity and pressure fields (eq. M17-18) plotted against the mesh refinement factor, $m$. The initial mesh ($m = 1$) had 5 elements in the radial direction and 50 elements in the axial direction. For $m = 2$, the relative error is less than 0.1%. The errors and the norms are calculated at the completion of 4 heartbeat cycles.
- **c.** The mesh used for 3D calculations. The dimensions are shown in the computational domain.
2.3 Results

We first examine how peristaltic motion affects the flow of an incompressible fluid in a two-dimensional tube. Consider a fluid-filled tube with deformable walls and no pressure difference across its two ends. When the position of the walls is fixed, there is no pressure gradient, and therefore no fluid flow (Fig. 2.3a). When the walls move inward due to a peristaltic wave propagating to the right, the fluid-filled domain deforms and the fluid is displaced. When the direction of the fluid flow is the same as the peristaltic wave, the motion is said to be anterograde, otherwise it is said to be retrograde. The flow in both directions is a result of the fluid pressure distribution, shown in Fig. 2.3b. The pressure is maximum at the location of the moving neck and is minimum at the two ends of the tube. Therefore, the fluid that is displaced by the wall is subject to the same pressure difference (Δp) in either direction (Δpₐ = Δpᵣ, where the subscripts ‘a’ and ‘r’ denote the anterograde and retrograde flows, respectively). However, since the width of the tube (h) is smaller in one direction (hₐ > hᵣ), there is more resistance for retrograde flow than anterograde flow (since flow resistance R scales with width of the tube $R \propto 1/h^3$ for 2D flow, $Rₐ < Rᵣ$). As a result of this difference in flow resistance, the anterograde flow is greater than the retrograde flow (flow rate, $Q = Δp/R, Qₐ > Qᵣ$). Thus, while peristalsis drives both anterograde and retrograde flows, a net flow in the direction of the peristaltic wave emerges. This example is a simplified version of peristalsis, where, the walls of the fluid-filled tube only contract. An example with a periodic contraction and expansion of the walls is shown in Fig. 2.4.
Our model also shows that the textbook picture of peristalsis (Silverthorn et al. 2004; J. E. Hall 2015), derived from the transport of solid matter in the esophagus and the gastro-intestinal tract cannot be directly translated to the peristalsis of fluids. When solid matter is transported by peristalsis, all of the material moves in the direction of the peristaltic wave (Fig.2.3c). This differs from the case of fluid transport by peristalsis, which generates both anterograde and retrograde flows (Figs. 2.3b, 2.4a). Moreover, the peristaltic transport of solids is independent of the magnitude of wall motion and the length of the tube. In contrast, the nature of fluid flow in a tube driven by peristaltic motion of the walls is highly dependent on the magnitude of both wall motion and tube length (Martinac and Bilston 2019), which will be demonstrated in this chapter. This understanding of the mechanism of peristaltic transport of fluids is crucial to interpreting the results of fluid
dynamic models of the PVS. The assumptions of the shape, size and the deformation of the PVS may vary between the models, but the mechanism of peristaltic transport remains the same.

2.3.1 Pulsation amplitudes are insufficient for directional pumping

To understand the relation between arterial wall movement and fluid movement in the PVS, we created a model of peristaltic pumping. In our model, the geometry of the PVS is taken to be cylindrically symmetric, with the artery centered within the PVS (Fig. 2.5a). While the geometry of the fluid domain is simplified, the inner and outer radii are based on realistic values (see Section 2.2.1). We then imposed a sinusoidal peristaltic wave on the arterial wall, while keeping the outer wall of the PVS fixed, effectively making the brain tissue rigid. In order to capture the whole peristaltic wave, the length of the PVS...
used in the simulation was equal to one wavelength ($\lambda$) of the peristaltic wave. Since we are interested in studying the pumping generated by arterial wall movement alone, we used periodic boundary conditions at the axial ends of the PVS. This is equivalent to studying flow driven by peristalsis with no additional pressure differences (Shapiro et al. 1969; Jaffrin and Shapiro 1971; Wang and Olbricht 2011; Bilston et al. 2003). We tracked the motion of particles at the center of the PVS.

Peristaltic pumping of fluid is a result of lower flow resistance for anterograde flow and higher resistance for retrograde flow (see Figs. 2.3b and 2.5b). This explains the fluid velocities observed with respect to the arterial wall position and wall velocity (Fig. 2.5c-d). The phase difference between the arterial wall velocity and the downstream fluid velocity (axial velocity, $v_z$) remained the same throughout the length of the domain. Flow resistance ($R_{flow}$) of a tube with an annular cross-section decreases with approximately the fourth power of the internal radius (Tithof et al. 2019). For slow, laminar flows like those in the paravascular space, the flow resistance of a tube with annular cross-section is given by the equation:

$$Q = -\frac{\partial p}{\partial z} \div R_{flow} = \frac{8\mu}{\pi} \left[ r_2^4 - r_1^4 - \frac{(r_2^2 - r_1^2)^2}{\ln(r_2/r_1)} \right]^{-1}$$

In Eq.2.20, $Q$ is the flow rate, $p$ is the pressure and $\mu$ is the dynamic viscosity of the fluid. The internal and external radii of the annular region are given by $r_1$ and $r_2$ respectively. For our calculations, Reynolds numbers range from 0.13 to 10.67, well within the laminar flow regime. Given the strong dependence of fluid resistance on the diameter, it follows that the amplitude of pulsations (the change in internal radius) should have a large effect on the flow resistance changes and therefore the pumping generated by peristaltic motion.
We examined the relation between pulsation amplitude and the trajectory of the fluid particles in the PVS. To put our results in the context of experimental findings, the typical half wave amplitude of heartbeat pulsations is 0.5-2% of arterial diameter (Bedussi, Almasian, et al. 2017; Mestre, Tithof, et al. 2018). Our simulations show that such small amplitude pulsations generate little difference between forward and backward flow resistance, which resulted in oscillatory fluid flow with minimal net anterograde flow (Fig. 2.5e). However, the kind of fluid particle trajectories reported by Bedussi et al. (Bedussi, Almasian, et al. 2017) and Mestre et al. (Mestre, Tithof, et al. 2018) are very different from the ones simulated in Fig.2.5e. The fluid trajectories in the PVS observed in both studies are more similar to the ones shown in Fig.2.5f, where the net anterograde motion of the fluid is of the same order as the oscillatory motion. Sample CSF particles trajectories can be found in the supplementary videos of Mestre et al. (Mestre, Tithof, et al. 2018) and Fig. 3 of Bedussi et al. (Bedussi, Almasian, et al. 2017) This kind of fluid motion would require non-physiological amplitudes arterial pulsations, with half wave amplitudes around 25% of the arterial radius. To better understand the effect of pulsation amplitude on fluid flow, we examined the relation between the pulsation amplitude and the ratio of mean flow speed (or average anterograde velocity) to oscillatory velocity (difference between peak anterograde velocity to peak retrograde velocity) (Fig.2.5g). Measurements by Mestre et al. (Mestre, Tithof, et al. 2018) show that mean flow speed (~20µm/s) and oscillatory velocity (~10µm/s) are of the same order. Our simulations show that for heartbeat-driven pulsations (1-4% of arterial radius peak-to-peak (Bedussi, Almasian, et al. 2017; Huo, Smith, and Drew 2014; Mestre, Tithof, et al. 2018)), the mean flow speed is 2-3 orders of magnitude smaller than the oscillatory velocity (Fig. 2.5g). These results show that heartbeat-driven pulsations in an idealized model are too small to explain the directed flow of CSF seen in vivo.
While the shape of the PVS in the axisymmetric model was simplified, the model still provides important generalizable insights into the mechanism of peristalsis. Specifically, the axisymmetric model helps us understand the relation between the movement of the arterial wall and the flow of fluid. We found that the radial wall velocity and the anterograde fluid velocity are always out of phase (by $270^\circ$, Fig.2.5c-d), and that the kind of fluid trajectories observed in vivo (Bedussi, Almasian, et al. 2017; Mestre, Tithof, et al. 2018) would require large, non-physiological amplitudes for arterial pulsations. Next, we examined if these results held for a 3-dimensional model of peristalsis with a realistically shaped PVS and pulse waveform.

**Figure 2.5: Heart rate pulsations drive oscillatory, but not directional flow. Large non-physiological pulsations are required for appreciable peristaltic pumping**

a. Schematic of the axisymmetric peristatic pumping model. The arterial wall undergoes peristaltic movement, while the outer wall of the PVS is fixed. The length of the PVS is taken as one wavelength of the peristaltic wave ($\lambda$) with periodic boundary conditions at the two axial ends.

b. Schematic of the fluid motion during periodic peristalsis. The fluid displaced by the moving wall should move with lower resistance along the direction of the peristaltic wave than in the opposite direction. This difference in flow resistance results in net forward pumping.
2.3.2 Paravascular flow measurements are inconsistent with peristaltic pumping

To test if fluid flow is influenced by the details of the shape of the PVS or the waveform of heartbeat driven pulsations, we created a model with a realistically-sized and shaped PVS, with a cardiac waveform drawn from the experimental data by Mestre et al. (Mestre, Tithof, et al. 2018) (Fig. 2.6a-b). The outer wall of the PVS was assumed to be fixed, and the length of the domain was set to be equal to one wavelength ($\lambda$) of the peristaltic wave. We use a no pressure (traction) boundary condition at the boundaries in place of the periodic boundary condition used for the axisymmetric simulations. This is done to better estimate the pressure changes in the PVS (Fig. 2.6e).

The mean flow speed (anterograde velocity time-averaged over a complete cycle) of fluid particles at the centerline of the PVS in our simulation was 102.1\(\mu\)m/s. However,
this was accompanied by oscillatory fluid velocities of approximately 30,000 µm/sec (3 cm/s), well over a hundred times the mean flow speed (Fig. 2.6c-d). The simulations also show the phase relation between the arterial wall velocity (radial component of wall velocity) and the fluid anterograde velocity (axial component of fluid velocity). It is clear that the wall velocity and the fluid velocity are out of phase, i.e., when the arterial wall dilates, the fluid flows in the retrograde direction and, when the arterial wall constricts, the fluid flows in the retrograde direction. This phase relation between the velocities is consistent with our axisymmetric model (Fig. 2.5d). This is in contrast to the in vivo measurements by both Bedussi et al. (Bedussi, Almasian, et al. 2017) and Mestre et al. (Mestre, Tithof, et al. 2018) (Fig. 2.7d), where the fluid flows in phase with the arterial wall, i.e., when the artery dilates, the fluid flow is anterograde and vice versa. Viewed together with the results of the axisymmetric simulations, our simulations suggest that the shape of the PVS and the waveform of heartbeat pulsations cannot pump CSF in a model with a simplified geometry of the PVS.

These results show that a peristaltic pumping model is inconsistent with experimental findings, which suggests that there are some problems with the assumptions of the peristaltic pumping model. In the next section, we revisit these assumptions and attempt to match the results of the fluid dynamic calculations with experimental findings.
2.3.3 Pressure differences, not arterial pulsations drive bulk fluid flow

In order to better capture the geometry of the PVS, we made changes to our 3D model based on the anatomy of the brain, the subarachnoid space and cerebral vasculature. We shortened the length of the PVS to 5 mm, and made the outer wall of the PVS move with the pulsations. Previous peristaltic pumping models (Shapiro et al. 1969;
Jaffrin and Shapiro 1971; Wang and Olbricht 2011; Schley et al. 2006) have set the length of the fluid chamber to be equal to one wavelength of the peristaltic wave. However, the wavelength of the heartbeat-driven peristaltic motion of arterial walls is considerably larger than the length of the MCA in mice, which is the proposed source of peristaltic pumping of CSF. With a peristaltic wave speed of 0.5-2 m/s (Herold et al. 2009; Williams et al. 2007), and a heartbeat frequency of 6-10 beats/second in mice (Bedussi, Almasian, et al. 2017; Mestre, Tithof, et al. 2018; Winder et al. 2017; Mitchell, Jeron, and Koren 1998; Swoap et al. 2008), the wavelength of the peristaltic wave is between 50-160mm in mice, while the MCA is only 4-6 mm long in mice (Bedussi, Almasian, et al. 2017; Chen et al. 2011; Adams et al. 2018). This means that the pulse wave travels so fast across the PVS of mice that the entire length of the wall of the MCA moves in and out simultaneously and therefore there is no appreciable difference in the flow resistance for anterograde and retrograde flows (Fig.2.7b). We calculated the fluid particle trajectories 1 mm from the distal end of the PVS segment (z = 4 mm), which captures the geometry of the surface of the brain where the flow measurements were made. This corrected the inconsistency between the phase of the fluid downstream velocity and the arterial wall velocity found in the peristaltic pumping model with a length of one pulsation wavelength (Fig. 2.7). Our results are similar to the phase relation between arterial wall velocity and fluid velocity estimated by Asgari et al. (Asgari, De Zélicourt, and Kurtcuoglu 2016), who studied the flow driven by penetrating arterioles in a model with anatomically realistic dimensions.
Secondly, the fixing of the outer wall of the PVS in other models means that the brain tissue and the subarachnoid space that surround the PVS are rigid. This is not realistic because the brain tissue is very soft, with a shear modulus in the range of 1-8 kPa (Goriely et al. 2015; Mihai et al. 2015; 2017; S Budday et al. 2017; Weickenmeier et al. 2018) (7.5-60 mmHg) and the 80 mmHg pressure changes (Fig.2.6e) predicted by the peristaltic pumping model will cause substantial deformations. To include the effect of the
soft tissue, we moved outer wall of the PVS with the same frequency as the heartbeat driven pulsations (Fig. 2.8b). We applied these small, pressure-driven deformations in the direction of the outward normal of the surface of the PVS, because pressure-like forces act along the outward normal of a surface. Since the mechanical properties of the subarachnoid space are mostly unknown (Willinger, Taleb, and Pradoura 1995; Ruan, Khalil, and King 1993; Saboori and Sadegh 2011; Galford and McElhaney 1970), we adjusted the amplitude of these deformations so that the oscillatory fluid velocity matched that observed in vivo by Mestre et al. (Mestre, Tithof, et al. 2018)

Our simulations suggest that the wall movement itself can only drive oscillations in fluid flow with negligible (0.007 µm/s) mean anterograde flow. The time course of fluid velocity from our simulations and its relation to the arterial wall movement agrees very well with the measured values in both phase and magnitude (Fig. 2.8d-e). The phase relation between the arterial wall velocity and the fluid velocity is a direct result of correcting the length of the PVS, while the magnitude of fluid velocity is corrected by including movement of the outer walls of the PVS in the simulation. However, the simulations suggest that arterial pulsations generate very little net anterograde flow with a time averaged downstream velocity of 0.007 µm/s (Fig. 2.8f).

Finally, we tested the possibility that small pressure differences across the ends of the PVS can drive the bulk flow observed in the experimental studies. We calculated the fluid flow through the PVS while varying the imposed pressure difference over a physiologically plausible range. We found that a very small pressure difference, 0.01 mmHg across the length of the PVS (5 mm), was sufficient to drive a mean downstream speed of 24.4µm/sec (Fig.2.8g), close to the mean flow speed observed in vivo (Mestre, Tithof, et al. 2018) (Fig. 2.8h). The pressure difference value was also found using equations derived in another recent theoretical study that estimated the flow resistance of
paravascular spaces (Tithof et al. 2019). Such a small pressure difference is practically impossible to measure in live animals, due to the lack of instruments sensitive to such small changes (Oshio et al. 2005; Yang et al. 2013). The pressure differences could be normally present due to CSF production in the ventricles (Oshio et al. 2005) and drainage via meningeal lymphatic vessels (Louveau et al. 2016; Aspelund et al. 2015) and the cribriform plate (Norwood et al. 2019), or be generated by intracranial injections (Hladky and Barrand 2014; Marmarou, Takagi, and Shulman 1980) of the tracer spheres. We conclude that peristalsis cannot drive unidirectional fluid pumping in the PVS of cerebral arteries under physiological conditions and that the experimentally observed CSF flow in the PVS is probably due to pressure differences present in the system.
a. Schematic of the model. The length of the PVS is set to 5 mm to match the length of the MCA in adult mice (4-6mm (Bedussi, Almasian, et al. 2017; Adams et al. 2018; Chen et al. 2011)). An additional pressure difference between the inlet and outlet is applied.

b. The displacement of the arterial wall (orange) and the PVS wall (green) used in the simulation. The displacement is given in the direction of the surface normals shown in c.

c. Positive displacement direction at a cross-section of the PVS, this is the direction of displacement for the plots shown in b. The inner wall is shown in orange and the outer wall is shown in green. The displacement changes in amplitude and direction from the inner wall to the outer wall. This transition is carried out using the smooth, step function in COMSOL Multiphysics. The changing direction and length of the blue arrows indicates the smooth transition.

d. Plot showing arterial wall velocity and oscillations in fluid velocity measured in vivo. Adapted from Mestre et al. (Mestre, Tithof, et al. 2018)

e. Plot showing arterial wall velocity and oscillations in downstream fluid velocity our simulation matches with those in d.

f. Plot of the trajectory of a fluid particle in the z direction shows that there is very little pumping by arterial wall movement.

g. Plot showing the relation between an applied pressure difference across the ends of the PVS and the mean anterograde flow speed. An additional 0.01mmHg pressure difference across the PVS is required to achieve a mean flow speed of 24.4μm/s, similar to the mean flow speeds observed in vivo (h).

h. Mean flow speed in the PVS measured in vivo. Adapted from Mestre et al. (Mestre, Tithof, et al. 2018)
2.4 Discussion

Peristatic pumping has been hypothesized to drive directed movement of cerebrospinal fluid in the paravascular space. In this chapter, we tested the "peristaltic pumping" hypothesis, by using simulations of fluid dynamics to understand what experimental measurements tell us about bulk flow. We started with simple models to provide a physical interpretation to the process of peristalsis of fluids and built more physiologically realistic models informed by the results of these models. We were able to improve upon previously published computational models aimed at studying the flow of CSF in the PVS (Bilston et al. 2003; Wang and Olbricht 2011; Schley et al. 2006; Asgari, De Zélicourt, and Kurtcuoglu 2016), using the detailed anatomical and physiological information from the experiments by Mestre et. al. (Mestre, Tithof, et al. 2018). This experimental data provided information about the shape of the PVS around cerebral arteries and the amplitude and waveform of the heartbeat driven pulsations, which we used in our modeling. The experiments also had detailed information on the oscillatory and anterograde flow of CSF in the PVS. Our simulations show that the cardiac pulsation of arteries is only capable of driving the oscillatory motion of CSF in the PVS, and not the unidirectional bulk flow. Rather, the experimentally observed unidirectional flow is likely to be driven by pressure differences in the system.

Our simulations point to two main reasons why arterial pulsations cannot drive unidirectional fluid flow in the PVS. First, direct measurement of cortical arteriole diameters in mice using two-photon imaging shows that the amplitude of the heartbeat-driven pulsations is small (1-4% peak to peak change in arterial diameter (Bedussi, Almasian, et al. 2017; Mestre, Hablitz, et al. 2018; Huo, Smith, and Drew 2014)). In humans, CT angiography has shown that pulsations drive only a maximum of 4-6% (Umeda et al. 2011; Kuroda et al. 2012) change in the volume of the MCA (2-3% change in diameter assuming

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a cylindrical geometry). Our calculations show that substantially larger cardiac pulsations (roughly 50% peak-to-peak change in diameter) are required to drive significant directed motion of the fluid relative to the oscillatory motion. Second, the peristaltic motion of arteries cannot drive unidirectional fluid flow because the length of the PVS is substantially less than the wavelength of the peristaltic wave. The total length of the MCA is between 4-6 mm in mice (Bedussi, Almasian, et al. 2017; X. Chen et al. 2011; Adams et al. 2018), while the wavelength of the peristaltic wave is between 100-1000 mm (based on the pulse wave velocity of 1-5 m/s (Herold et al. 2009; Williams et al. 2007) and a heart rate of 6-12 Hz (Winder et al. 2017; Mitchell, Jeron, and Koren 1998)). This is over an order of magnitude difference between the length of the PVS and the wavelength of the peristaltic wave. In humans, the MCA is longer, (roughly 100 mm (Herculano-Houzel 2009)). However, while the pulse wave velocity (a function of arterial stiffness (Asmar et al. 1995; Blacher et al. 1999)), remains roughly the same in mice and humans (Gladdish and Rajkumar 2002; Allen and Murray 2002), the heart rate in humans is around 1-2 Hz, which makes the wavelength of the peristaltic wave 1-2 orders of magnitude higher than the length of the MCA in humans. Therefore, in mice as well as in humans, arterial pulsations are unlikely to drive unidirectional CSF flow. While arterial pulsations cannot cause directed flow in the PVS, it is possible that these pulsations improve metabolite transport in the brain through dispersion. Asgari et al. (Asgari, De Zélicourt, and Kurtcuoglu 2016) showed that dispersion could be a mechanism of transport in the PVS surrounding penetrating arteries in the absence of directed flow. Sharp et al. (Sharp, Carare, and Martin 2019) showed that oscillatory flow could result in faster metabolite transport compared to diffusion in the periarterial, paraarterial and the spinal subarachnoid space.

The models in this chapter assume that there is no inherent preference to directional flow in the PVS and that any flow in the PVS is a direct result of pressure
differences and incompressibility of CSF (fluid continuity equation). It is however possible that the PVS or other fluid filled chambers in the cranial space could contain valve-like structures that are responsible for a directional preference for flow. Valve like structures are present in the lymphatic vessels (Swartz 2001), veins (Caggiati et al. 2006) and arachnoid granulations (Weller 1998; Davson and Segal 1996) in mammals. To the best of our knowledge, no such valve-like have been observed in the PVS. Moreover, Mestre et al. (Mestre, Tithof, et al. 2018) showed that retrograde flow of CSF is observed during hypertension, which suggests that valve-like structures are either absent in the PVS or that these structures are impaired during hypertension.

Based on the experimental evidence available, we speculate that two possible mechanisms that could generate pressure differences to drive directional CSF flow in the PVS, namely, CSF production in the choroid plexus and osmotic pressure differences across astrocytic end feet. CSF flow through the PVS and into the brain is severely affected in aquaporin-4 (Aqp4) knockout mice (Iliff et al. 2012; Mestre, Hablitz, et al. 2018). The AQP4 channel is selectively permeable to water (Ho et al. 2009; Agre et al. 2002) and is present in the choroid plexus (Speake, Freeman, and Brown 2003) and the astrocytic endfeet (Iliff et al. 2012). The deletion of the AQP4 gene could reduce CSF production and osmotic flow through astrocytic endfeet. It is possible that a combination of the two factors drive CSF flow since the osmotic concentration gradients and the CSF production rate are interrelated (Brown et al. 2004). Alternatively, the observed flow in the PVS might be an caused by the infusion rate of 1-2µl/min used in the experiments to study CSF flow (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017), which is 3-5 times the typical rate of CSF production rate in mice (0.38µl/min (Oshio et al. 2005)). The infusion rate used in these experiments is known to increase intercranial pressure (Yang et al. 2013; Iliff et al. 2012), as pointed out by Hladky and Barrand (Hladky and Barrand
A detailed 3-D model of the whole brain with the PVS and the SAS, all modelled as poroelastic media (Costanzo and Miller 2017) would be needed to test the possibility of the observed flow being an artifact of the infusion.

An important result of our simulations is that the paravascular spaces around pial arterioles provide a crucial pathway for fluid transport in the brain due to their low flow resistance. A very small pressure difference (0.01 mmHg, Fig. 2.8g) across the length of the MCA (5 mm) can be sufficient to drive fluid through the PVS with a mean speed of ~20 µm/s. This pressure difference is equivalent to a pressure gradient of 2 mmHg/m, which is very close to the pressure gradient of 2.85 mmHg/m (Vinje et al. 2019) between the ventricular and subdural space measured in humans. This is in stark contrast to the much less permeable brain tissue, where a pressure gradient of 1 mmHg/mm can only generate fluid velocities in the order of 0.010 µm/s (Holter et al. 2017). However, the low flow resistance makes understanding the driving force for CSF movement in the PVS extremely difficult. A pressure difference in the range of 0.01 mmHg cannot be accurately measured with current instruments, which have a resolution of around 1 mmHg (Oshio et al. 2005; Uldall et al. 2017). Moreover, invasive access of the skull probes through the skull severely affects the flow through the PVS (Mestre, Hablitz, et al. 2018).

In terms of the objectives of this thesis, it is clear that CSF pumping into the brain through the PVS, driven by arterial pulsations is highly unlikely under physiological conditions.
Chapter 3: Functional hyperemia drives fluid exchange in the arterial paravascular space

3.1 Introduction

In this chapter, we examine the possible drivers of fluid movement and metabolite transport in the paravascular space (PVS) surrounding arterioles that penetrate into the brain parenchyma and examine how the wall motions of penetrating arterioles affect fluid flow and metabolite transport through the PVS. In addition to the heartbeat-driven pulsations, the movement of walls of the penetrating arterioles can be driven by changes in local neural activity. This phenomenon, called functional hyperemia, has been identified as a possible driver of metabolite clearance from the brain (von Holstein-Rathlou, Petersen, and Nedergaard 2018; van Veluw et al. 2020), although the mechanism of metabolite clearance through functional hyperemia is unknown. To understand the possible drivers and mechanisms of fluid and metabolite transport in the PVS of penetrating arteries, we developed realistic models of fluid transport in the PVS coupled to the deformation of the soft brain tissue surrounding the PVS, and the flow resistances of the brain parenchyma and the subarachnoid space (SAS). The physical dimensions and material parameters of our models were carefully chosen so as to make meaningful

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predictions about transport in the PVS of penetrating arterioles in the somatosensory cortex of adult mice.

We began our investigation here with the possibility of directional fluid movement in the PVS driven by the movement of arteriolar walls. We found that neither arteriolar pulsations, nor functional hyperemia could drive appreciable directional flow of CSF through the PVS. Next, we examined the possibility of metabolite clearance from the PVS through exchange of fluid between the PVS and SAS (cf. the dispersion mechanism discussed in section 1.4.3 and depicted in Fig. 1.11). Our simulations showed that functional hyperemia, but not arteriolar pulsations, can drive large fluid exchange between the PVS and the SAS. This fluid exchange could help remove metabolites from the CNS. Our simulations predicted that the difference in the volume of fluid exchanged by pulsations and hyperemia are partly because of the deformation of brain tissue in response to the pressure changes in the PVS. Finally, we examined how the model parameters affect the results of our simulations and found that the model predictions are valid for a wide range of parameters. These results suggest that in addition to its involvement in other processes, functional hyperemia can aid metabolite clearance in the brain through the exchange of CSF between the SAS and PVS and that the elastic response of the soft brain tissue needs to be considered while modeling fluid flow in the CNS.

3.2 Materials and methods

3.2.1 Choice of model and parameters

We are interested in understanding how the motions of the arteriolar walls drive fluid exchange between the PVS and SAS. We performed fluid mechanics simulations of the CSF in the PVS surrounding penetrating arterioles in adult mice. There is significant
uncertainty regarding several key parameters governing the fluid flow of the PVS, namely the permeability of the PVS, channel width of the PVS, and the flow resistance of the surrounding spaces (the brain parenchyma and the SAS). Keeping these ambiguities in mind, we performed simulations using a wide range of parameters (see Table 3.1) to ensure that our results are robust.

Table 3.1: Parameters used in axisymmetric fluid-structure interaction simulations

<table>
<thead>
<tr>
<th>Parameter Name</th>
<th>Symbol</th>
<th>Default</th>
<th>Range</th>
<th>Unit</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteriolar radius</td>
<td>$R_1$</td>
<td>12</td>
<td>5–20</td>
<td>µm</td>
<td>(Drew et al. 2010; Shih et al. 2012; Gao et al. 2017)</td>
</tr>
<tr>
<td>PVS length</td>
<td>$L_a$</td>
<td>250</td>
<td>250–500</td>
<td>µm</td>
<td>(Shih et al. 2012; Horton et al. 2013)</td>
</tr>
<tr>
<td>PVS width</td>
<td>$w_d$</td>
<td>3</td>
<td>2–10</td>
<td>µm</td>
<td>(Schain et al. 2017; Liiff et al. 2012; Mestre, Tithof, et al. 2018)</td>
</tr>
<tr>
<td>CSF viscosity</td>
<td>$\mu_f$</td>
<td>0.001</td>
<td>-</td>
<td>Pa.s</td>
<td>(Sløverud et al. 2013; Yetkin et al. 2010)</td>
</tr>
<tr>
<td>CSF Density</td>
<td>$\rho_f$</td>
<td>1000</td>
<td>-</td>
<td>kg/m³</td>
<td>(Sløverud et al. 2013; Yetkin et al. 2010)</td>
</tr>
<tr>
<td>PVS porosity</td>
<td>$\zeta$</td>
<td>0.8</td>
<td>0.5–0.9</td>
<td>-</td>
<td>(Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017; Vanlandewijck et al. 2018)</td>
</tr>
<tr>
<td>PVS permeability</td>
<td>$k_s$</td>
<td>$2\times10^{-14}$</td>
<td>$7\times10^{-13}$–$2\times10^{-15}$</td>
<td>m²</td>
<td>(Neeves et al. 2006; Smith and Humphrey 2007; Jin, Smith, and Verkman 2016; Holter et al. 2017)</td>
</tr>
<tr>
<td>Brain section radius</td>
<td>$R_3$</td>
<td>150</td>
<td>100–200</td>
<td>µm</td>
<td>(Horton et al. 2013; Adams et al. 2018)</td>
</tr>
<tr>
<td>Brain shear modulus</td>
<td>$\mu_s$</td>
<td>4</td>
<td>1–8</td>
<td>kPa</td>
<td>(Mihai et al. 2017; S Budday et al. 2017; Franceschini et al. 2006; Streitberger et al. 2011; Sack et al. 2011; 2009; Goriely et al. 2015; Weickenmeier et al. 2018)</td>
</tr>
<tr>
<td>Brain tissue density</td>
<td>$\rho_s$</td>
<td>1000</td>
<td>-</td>
<td>kg/m³</td>
<td>(Barber, Brockway, and Higgins 1970)</td>
</tr>
<tr>
<td>Pulsation amplitude (% arteriolar radius)</td>
<td>$b_1$</td>
<td>1</td>
<td>0.5–2</td>
<td>-</td>
<td>(Mestre, Tithof, et al. 2018)</td>
</tr>
<tr>
<td>Pulsation Frequency</td>
<td>$f$</td>
<td>10</td>
<td>7–14</td>
<td>Hz</td>
<td>(Winder et al. 2017)</td>
</tr>
<tr>
<td>Pulse wave speed</td>
<td>$c$</td>
<td>1</td>
<td>0.5–10</td>
<td>m/s</td>
<td>(Herold et al. 2009; Williams et al. 2007)</td>
</tr>
<tr>
<td>Pulse wave wavelength</td>
<td>$\lambda$</td>
<td>0.1</td>
<td>0.03–1.43</td>
<td>m</td>
<td>$c/f$</td>
</tr>
<tr>
<td>Diffusion coefficient</td>
<td>$D$</td>
<td>$1.4\times10^{-6}$</td>
<td>-</td>
<td>cm²/s</td>
<td>(Massi et al. 2001; Tseng et al. 1999)</td>
</tr>
</tbody>
</table>
We posit that fluid movement in the PVS is governed by the Darcy-Brinkman (Brinkman 1949) equations (one for the momentum balance and the other for volume conservation), which are used to simulate flow through highly porous regions (Vafai and Kim 1995). This choice is based on the experimental data available from recent studies that used intra-cisternal infusions to study the flow of CSF. These studies have shown unobstructed movement of 1 µm particles in the PVS surrounding arterioles on the surface of the brain (Bedussi, Almasian, et al. 2017; Mestre, Tithof, et al. 2018). While these relatively large particles do not enter the PVS surrounding penetrating arterioles, dye-conjugated dextrans (3-500kDa) with a hydrodynamic radius of 1-15nm (Armstrong et al. 2004) have been shown to enter the PVS around the penetrating arterioles (Schain et al. 2017; Bedussi, Van Der Wel, et al. 2017; Iliff et al. 2012). Based on these results, we modeled the PVS surrounding penetrating arterioles as a porous medium with higher porosity and fluid permeability than the brain tissue. The porosity (fraction of fluid volume to the total volume) of the PVS was assumed to be between 0.5–0.9. The fluid permeability of the PVS is taken from a range of possible values. The minimum value of PVS permeability we used was $2 \times 10^{-15} \text{m}^2$, the measured permeability of the brain tissue (Neeves et al. 2006; Smith and Humphrey 2007). We also performed simulations with infinite permeability, where the Darcy-Brinkman equations recover the standard Navier-Stokes equations. The default value of permeability was taken to be $2 \times 10^{-14} \text{m}^2$, where the PVS is 10 times more permeable than the brain parenchyma. This was chosen because dye injected through the cisterna magna enters the PVS nearly 10 times faster than it enters the parenchyma (<5 minutes vs. ~30 mins)(Iliff et al. 2012), presumably under a single source of difference in fluid pressure. The viscosity ($0.001 \text{Pa} \cdot \text{s}$) and density ($1000 \text{kg/m}^3$) of CSF were taken from experimentally determined values (Støverud et al. 2013; Yetkin et al. 2010).
The PVS is assumed to be 150–300µm long and 2–10µm wide for an arteriolar radius of 5–20µm (Gao et al. 2017; Drew et al. 2010; Shih et al. 2012). The length of 150–300µm is in the range of bifurcation-free length of penetrating arterioles in the mouse parenchyma. This is consistent with the length of the PVS used in previous studies that used an axisymmetric model of the PVS surrounding penetrating arterioles (Asgari, De Zélicourt, and Kurtcuoglu 2016). While the width of the PVS surrounding large pial arteries is in the range of 20–40µm (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017), the PVS around penetrating arterioles appears to be much smaller. The width of this section of the PVS is not explicitly mentioned in the literature. However, a width of 2–10µm can be calculated from the imaging data available from experimental studies (Schain et al. 2017; Iliff et al. 2012).

In the cerebral cortex of mice, the fluid leaving the PVS around penetrating arterioles has to enter the SAS or the PVS around pial arteries on the pial side (green arrows in Fig.3.1a) or the brain parenchyma or the para-capillary and para-venous spaces on the other side (magenta arrows in Fig.3.1a). To avoid confusion, we refer to the first set of fluid chambers as the SAS and the second set as the parenchyma. Due to the relatively large PVS surrounding the pial vessels (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017), the SAS region has a relatively low flow resistance compared to the PVS. Therefore, in our models the pial opening of the PVS is connected to a flow resistance with a resistance value $1/100^{th}$ (or $1/10^{th}$, see results) of the flow resistance of the PVS. The parenchyma is assumed to have a higher flow resistance, 10 times that of the PVS. There is evidence suggesting the anatomy, and therefore the flow path of CSF, is more complicated than what we modelled here. Potter et al. (Potter, Doubal, et al. 2015; Potter, Chappell, et al. 2015) showed that the PVS might be very small or non-existent in the healthy brain. Albargothy et al. (Albargothy et al. 2018) showed that CSF in the para-
arterial space mostly likely flows out through the periarterial basement membrane and not out of the venous PVS. There is also evidence showing that the PVS and the SAS are not contiguous fluid filled compartments but are connected through the stomata or pores in the leptomeningeal cell layer surrounding arterioles (Weller et al. 2018; Pizzo et al. 2018). Even though our assumptions do not exactly match these findings, the path of least resistance for the flow of CSF seems to be through the SAS and around penetrating arterioles (Potter, Doubal, et al. 2015; Albargothy et al. 2018; Weller et al. 2018; Pizzo et al. 2018; Potter, Chappell, et al. 2015), which was captured in our models.

![Figure 3.1: Schematic of the anatomical structure of a penetrating arteriole and surrounding tissue](image)

**Figure 3.1: Schematic of the anatomical structure of a penetrating arteriole and surrounding tissue**

- **a.** Depiction the fluid filled PVS between the arteriolar wall and the brain parenchyma, adapted from Abbot et al. (Abbott et al. 2018). The glia limitans covers the surface of the brain tissue and forms the brain-PVS interface. The subarachnoid space (SAS) and paravascular spaces (PVS) are interconnected fluid-filled compartments. The low resistance pathway for fluid flow to and from the PVS (along the SAS) is shown in green, while the high resistance pathway (through the brain parenchyma) is shown in magenta.

- **b.** Geometry of the computational model of a penetrating arteriole and the brain and fluid around it. The model is cylindrically symmetric around the penetrating arteriole, allowing us to use axisymmetric simulations.

We did not explicitly model fluid flow into the brain parenchyma, either through possible gaps in the glia limitans surrounding arterioles (Korogod, Petersen, and Knott
2015) or through the aquaporin channels in the astrocytic endfeet (Kacem et al. 1998; Iliff et al. 2012; Mestre, Hablitz, et al. 2018), because there is no agreement on the existence of bulk flow in the brain parenchyma (Smith et al. 2017a; Smith and Verkman 2019; Jin, Smith, and Verkman 2016; Holter et al. 2017; Mestre, Hablitz, et al. 2018; Mestre, Tithof, et al. 2018; Abbott et al. 2018). Models that have simulated the flow through astrocytic endfeet and the brain extracellular space concluded that transport through these pathways is dominated by diffusion and not bulk flow (Holter et al. 2017; Jin, Smith, and Verkman 2016). The flow through all these pathways is lumped together in a single effective parenchymal flow resistance. This is a limitation of our model, and our calculations of net flow into the brain need to be interpreted with this limitation in mind.

In models where we simulate the brain tissue as a deformable solid, the brain tissue was modelled as a compressible, Saint-Venant-Kirchhoff solid. A Poisson’s ratio of 0.45 was chosen, to match the known mechanical response of brain tissue under compression (Budday et al. 2019). We also performed these simulations with an incompressible Neo-Hookean elastic model for the brain tissue. These Saint-Venant-Kirchhoff and Neo-Hookean models are chosen to minimize the number of model parameters. These models have been shown to accurately estimate brain tissue deformation during craniotomies and automated surgeries (Leizea et al. 2015; Wittek, Hawkins, and Miller 2009). The elastic (shear) modulus of the brain tissue was taken to be between 1–8kPa, spanning the values found in the literature (Mihai et al. 2017; Budday et al. 2017; Franceschini et al. 2006; Streitberger et al. 2011; Sack et al. 2011, 2009). The radius of the simulated section of brain tissue was taken to be in the range of 100–200µm, half of the typical distance between two penetrating arterioles in the mouse cortex (Nishimura et al. 2007; Blinder et al. 2010a). In the models where the deformability of the tissue is modelled, we saw that the pressure changes in the PVS can cause deformation.
in the brain and affect fluid flow in the PVS. To model the pressure changes on the pial surface of the brain more accurately, we repeated our simulations where the flow resistance model at the pial opening of the PVS was replaced with a fluid filled SAS connected to the PVS over the brain surface.

The arteriolar dilations caused by heartbeats and those generated in response to local neural activity have very different temporal dynamics and amplitudes. While cardiac pulsations are small in size, the arteriolar dilations that accompany increases in local neural activity are substantially larger and longer lasting. Heartbeat drives changes of 0.5–3% in the radius of pial arteries in mice (Mestre, Tithof, et al. 2018). These pulsations travel at a speed of 0.5–10 m/s along the arterial tree (Herold et al. 2009; Williams et al. 2007). Mice have a heart rate of 7–14 Hz when they are unanesthetized and freely behaving (Winder et al. 2017). In contrast with arteriolar pulsations which occur at the heart rate, these neurally-induced arteriolar dilations take one to three seconds to peak and last for several seconds in response to a brief increase in neural activity. In response to increases in local neural activity, cerebral arterioles can dilate by 20% or more in non-anesthetized animals (Drew, Shih, and Kleinfeld 2011; Mishra et al. 2016; Hill et al. 2015; Rungta et al. 2018; Gao, Greene, and Drew 2015; Gao et al. 2017). These dilations induce blood flow changes that are the basis for the blood-oxygen-level dependent (BOLD), functional magnetic resonance imaging (fMRI) (Goense and Logothetis 2008; Hillman 2014; Logothetis 2008; Drew 2019) signal. Neural activity-driven changes in arteriolar diameter take place at a nominal frequency range of 0.1–0.3 Hz (Winder et al. 2017).

In this chapter, we used finite element simulations to model fluid flow in the PVS and the deformation of the brain tissue. To make the calculations and interpretation of results simpler, we assume a cylindrically symmetric geometry with the centerline of the arteriole as the axis of symmetry (Fig. 3.1b).
3.2.2 Modeling assumptions

Here, we present the boundary conditions and governing equations used in the simulations presented in this chapter. Arteriolar wall displacements and brain tissue deformability cause the PVS to be a time-dependent domain. To properly account for the motion of the PVS, we adopted an Arbitrary-Lagrangian–Eulerian approach (Fernández et al. 2009) (ALE). The motion of the PVS (often referred to as a mesh motion or ALE map) was modeled using a harmonic model (Wick 2011) to calculate the mesh displacement ($u_m$). This said, the equations are presented in this chapter in their more familiar Eulerian form for ease of readability (see Appendix C for the full mathematical description of the equations in the ALE form). The simulations were performed with the assumption of axisymmetric geometry (Fig. 3.1b).

The fluid flow in the PVS was modeled as incompressible Darcy-Brinkman flow through a highly porous region (Eqs. 3.1–3.3), with the fluid velocity ($v_f$) and pressure ($p_f$) as the primary unknowns.

\[
\frac{\partial v_f}{\partial t} + (v_f \cdot \nabla)v_f + \frac{\mu_f \zeta}{k_s \rho_f} v_f - \frac{1}{\rho_f} \nabla \cdot \sigma_f = 0 \tag{3.1}
\]

\[
\nabla \cdot v_f = 0 \tag{3.2}
\]

\[
\sigma_f = -p_f I + \mu_f (\nabla v_f + (\nabla v_f)^T) \tag{3.3}
\]

The density and dynamic viscosity of the fluid are given by $\rho_f$ and $\mu_f$ respectively. The porosity and permeability of the PVS are given by $\zeta$ and $k_s$, respectively. All the parameters are described in Table 3.1. The stress tensor for the fluid ($\sigma_f$) is given by Eq. 3.3.
For all the simulations, we used a no-slip boundary condition. This means that the fluid velocity at the arteriolar wall is given by the time derivative of the wall displacement. We assume that the arteriolar wall moves only in the radial direction. Therefore, the radial ($r$) and axial ($z$) components of fluid velocity at the arteriolar wall are given by Eq. 3.3. The waveform of the arteriolar wall deformation for pulsations and vasodilation are shown in Fig. 3.2a and Fig. 3.5a, respectively.

$$v_{fr} = \frac{du_{wall}}{dt}, v_{fz} = 0$$  \hspace{1cm} 3.4$$

The tissue displacement ($u_s$) and velocity ($v_s$) are the primary unknowns in the solid domain. The stress tensor for the solid ($\sigma_s$), given by Eq. 3.7, is a function of the Lagrange strain ($\epsilon_s$) and the Lamé parameters described in Eqs. 3.8 and 3.9, respectively.

$$\sigma_s = \frac{1}{\det F_s} F_s (\lambda_s Tr[\epsilon_s] I + 2\mu_s \epsilon_s) F_s^T$$  \hspace{1cm} 3.7$$

$$\epsilon_s = F_s^T F_s - I, \quad \text{where} \ F_s = I + \nabla \times u_s$$  \hspace{1cm} 3.8$$

$$\lambda_s = \frac{2\nu \mu_s}{(1-2\nu)}$$  \hspace{1cm} 3.9$$

The interaction between the flow in the PVS and the elastic deformation of the brain was implemented using a fluid-structure interaction model. The displacement of the PVS at the brain-PVS interface was made equal to the displacement of the brain tissue.
Similarly, the velocity of the fluid at the brain-PVS interface was made equal to the velocity of the brain tissue. The tractions applied by the fluid flow onto the PVS are applied as a boundary traction on the brain tissue at the brain-PVS interface. This coupling of displacements, velocity and tractions is implemented simultaneously to create a fully coupled fluid-structure interaction model (Gerbeau, Vidrascu, and Frey 2005) (Eqs. 3.10–3.12).

\[ u_m = u_s \]  
\[ v_f = v_s \]  
\[ \sigma_f \cdot n_f = \sigma_s \cdot n_s \]

Here, \( u_m \) is the mesh displacement (displacement of the fluid domain). The unit outward normals to the fluid and solid domains are represented as \( n_f \) and \( n_s \) respectively.

For the simulation where the brain tissue is assumed non-compliant (rigid), the equivalent versions of Eqs.3.10–3.11, \( u_m = 0 \) and \( v_f = 0 \), are used for this case.

For all the simulations shown in this chapter, the pial opening of the PVS was connected to a flow resistance, which models fluid moving into and out of the subarachnoid space. The flow resistance was implemented as a Robin boundary condition, i.e., a flowrate-dependent pressure-like traction was applied at the pial opening of the PVS. Simulations where the subarachnoid space (SAS) was modeled as a fluid filled, porous region connected to the PVS confirmed that the Robin boundary condition (Eriksson et al. 1996) is adequate to simulate the flow resistance of the SAS. The other axial end of the PVS is also modelled with a Robin boundary condition representing the flow resistance of the brain parenchyma.

All our models had a Reynolds number of less than 1 for flow in the PVS (Eq.3.13).
We also calculated the Péclet number (Eq. 3.14) based on the diffusion coefficient ($D$) for Amyloid-β (Massi et al. 2001; Tseng et al. 1999). The fluid flow rates $q_{f0}$, $q_{f50}$ in Eq.3.15 is calculated at the top surface of the PVS ($z = L_a$) and 50µm below the brain surface respectively ($z = L_a - 50$ µm).

$$Pe_0 = \frac{2q_{f0}wd}{D\pi((R_1 + wd)^2 - R_1^2)}$$

$$Pe_{50} = \frac{2q_{f50}wd}{D\pi((R_1 + wd)^2 - R_1^2)}$$

3.2.3 Model implementation

All the partial differential equations that govern the physics of the problem were implemented using the Galerkin finite element method (Eriksson et al. 1996). All the finite element simulations were performed using COMSOL Multiphysics. We used the Weak Form PDE (partial differential equation) interface in the Mathematics Module in COMSOL to implement the governing equations on an axisymmetric geometry. The vector equations for each problem are given in Appendix C. The equations are converted to a weak form in an axisymmetric ($r,z$) coordinate system using a custom program Wolfram Mathematica. A backward difference formula (BDF) scheme was used for the time-dependent problems in COMSOL. The fluid particle tracking in the PVS (see appendix A) is implemented using MATLAB.

3.3 Results

Note the geometry in all the figures in this section is depicted with an unequal aspect ratio in the radial ($r$) and axial ($z$) directions for viewing convenience.
3.3.1 Possibility of directional CSF flow in the PVS

We first investigated the hypothesis that arteriolar wall movements (heartbeat-driven pulsations, functional hyperemia-driven dilations) could drive directed CSF flow. Both experimental (Iliff et al. 2013; Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017; von Holstein-Rathlou, Petersen, and Nedergaard 2018) and computational (Wang and Olbricht 2011; Schley et al. 2006; Bilston et al. 2003) studies have suggested the possibility of directed CSF flow as a result arteriolar wall movements, especially heartbeat pulsations. In our models, the space between the penetrating arteriole (the inner wall of the PVS) and the brain (the outer wall of the PVS) is filled with fluid. Fluid enters or exits the PVS through the SAS or the parenchyma (Fig. 3.1a). The flow resistance of the SAS was 0.01 times (or 0.1 times) the flow resistance of the PVS. The flow resistance of the parenchyma was 10 times that of the PVS. To quantify the flow driven by arteriolar wall movements alone, we imposed no pressure difference across the two ends of the PVS. We started our simulations with the assumption that the outer wall of the PVS was fixed (implying that the brain tissue is non-compliant), as has been done in other models (Wang and Olbricht 2011; Asgari, De Zélicourt, and Kurtcuoglu 2016; Bilston et al. 2003). The balance laws and boundary conditions used in the simulations are described in methods.

3.3.1.1 Ignoring brain deformability leads to implausibly high pressures

We investigated the fluid flow in the PVS (with a non-compliant brain) driven by heartbeat pulsations, the smaller of the two arteriolar wall motions considered in this study. To simulate the peristaltic wall motion of arterioles due to the heartbeat, the position of the inner wall of the PVS was prescribed via a travelling sinusoidal wave whose amplitude (Mestre, Tithof, et al. 2018), frequency (Winder et al. 2017) and velocity (Brands et al. 1998; Gladdish and Rajkumar 2002) were taken from experimental observations in mice.
The results of the simulation with Darcy-Brinkman model are shown in Fig. 3.2 and Navier-Stokes model are shown in Fig. 3.3.

When the dimensions of the PVS in the simulations were of anatomically realistic size (3 µm wide and 250 µm long), we observed no appreciable net unidirectional movement of fluid. The average downstream velocity of fluid was $5.50 \times 10^{-4}$ µm/s ($1.84 \times 10^{-3}$ µm/s for Navier Stokes model) with an average flow rate of $0.14$ µm³/s ($0.47$ µm³/s for Navier Stokes). Instead of directional pumping, we saw periodic fluid movement in and out of PVS (Fig. 3.2b) with peak velocity magnitude in the range of 300 µm/s (Reynolds number, Re = $1.3\times10^{-3}$), resulting in an oscillatory flow with negligible net unidirectional movement. We also repeated the simulation without the flow resistances and found an average downstream velocity of $2.95 \times 10^{-3}$ µm/s. There was essentially no net fluid movement in these conditions because the wavelength of the cardiac pulsation (0.1 m in mice, see Table 3.1) is much longer than the PVS (150-300 µm). When the wavelength of the pulsation is substantially larger than the length of the PVS, the arteriolar wall movement cannot capture the shape of the peristaltic wave on the scale of cerebral arterioles. Effectively, the entire length of arteriolar wall moves in or out almost simultaneously. Our results are very similar, in terms of magnitude and direction of fluid
velocities, to those obtained by Asgari et al. (Asgari, De Zélicourt, and Kurtcuoglu 2016), who used a similar PVS geometry in their model.

\[ \text{Figure 3.2: Modeling Darcy-Brinkman fluid flows and induced pressures while ignoring brain deformability} \]

\( \text{a. Geometry of the PVS in our model. The outer wall of the arteriole is shown in dark orange and the boundary of the brain parenchyma is shown in pink. The dashed line represents the centerline of the arteriole. The inset shows the imposed heartbeat-driven pulsations in arteriolar radius (±0.5% of mean radius, } R_i \text{) at 10 Hz, the heart rate of an un-anesthetized mouse. The pulse wave travels at 1 meter per second along the arteriolar wall, into the brain (blue arrow). The flow through the SAS and the brain parenchyma was modelled by flow resistances (shown in green and magenta respectively).} \)

In \( \text{b and c}, \) a cross section of the PVS is shown together with the surrounding arteriolar wall (on the left) and brain tissue (on the right).

\( \text{b. Plot of the fluid velocity induced in the PVS by the arteriolar pulsation. Contour showing the axial velocity (velocity in the z-direction) in a cross-section of the PVS. The colors indicate the direction and magnitude of flow. Fluid velocity vectors (arrows) are provided to help the reader interpret the flow direction from the colors. Heartbeat pulsations drive negligible unidirectional flow with a mean flow speed (\( |v_z| \)) of } 5.5 \times 10^{-4} \text{ } \mu \text{m/s. To make the arteriolar wall movements clearly visible, we scaled the displacements by a factor of 10 in post-processing.} \)

\( \text{c. Fluid pressure in the PVS corresponding to the flow shown in } \text{b. Pressure changes due to fluid flow in the PVS reach several mmHg. These pressures will deform the soft brain tissue, which has a shear modulus of } 1–8 \text{kPa (8–60 mmHg). The dotted line shows the estimated deformation in the brain tissue (shear modulus } 4 \text{kPa – Kirchhoff/De Saint-Venant elasticity with Poisson ratio of } 0.45) \text{ from the pressure shown in the figure. Under these assumptions, the deformations in the brain tissue are 60 times bigger (3.59µm) in magnitude compared the peak of heartbeat driven pulsations (0.06µm – shown on inset in } \text{a). Therefore, the deformability of brain tissue cannot be neglected.} \)
Models where the brain-PVS interface is fixed in position presumes that the brain tissue is non-compliant. This assumption is only valid if the pressures produced are small relative to the elastic modulus of the brain. When the brain is presumed to be non-compliant, our simulations show that the peak pressures in the PVS during pulsations can reach 11 mmHg (Fig. 3.2c) for a Darcy-Brinkman flow and 0.32 mmHg (Fig. 3.3b) for Navier-Stokes flow. Given that the brain is a soft tissue with a shear modulus in the range of 1-8 kPa (Goriely et al. 2015; Weickenmeier et al. 2018; Mihai et al. 2017; Budday et al. 2017) (7-30 mmHg), we estimated that the peak displacement of the brain tissue induced by the pressure profile in Fig. 3.2c would be 3.59 µm (with a shear modulus of 4 kPa). The pressure profile for the Navier-Stokes model (Fig. 3.3b) predicts a displacement of 0.08 µm.
µm. This displacement cannot be ignored, because the arteriolar wall displacement driving the flow is only 0.06µm. We conclude that pressures induced by the flow demand that the mechanical properties of brain tissue and its deformability must be accounted for, to accurately simulate fluid dynamics.

3.3.1.2 Arteriolar wall motions cannot drive directed fluid flow in the PVS

We modified our model by treating the brain as a compliant, elastic solid (Fig. 3.4a). The pressure and the fluid shear forces in the PVS were coupled to the elastic deformation in the brain tissue using force-balance equations at the interface. We coupled the fluid velocity with the velocity of deforming brain tissue, to create a fully-coupled, fluid-structure interaction model (Fig. 3.4b). In this model, the pressure changes in the PVS directly affect the deformation of the brain tissue and have a feedback effect on the flow in the PVS. The balance laws and boundary conditions used in this problem are described in the methods.

We investigated how a compliant brain tissue model would respond to arteriolar pulsations. We imposed movement of the arteriolar wall with the same dynamics used in our previous model and visualized the resulting fluid flow in the axial direction (vz) (Fig. 3.4c). Throughout the pulsation cycle, most of the fluid in the PVS showed little to no movement (white). The flow observed in these simulations has a Reynolds number of 1.14 x 10⁻⁴. The average downstream velocity of fluid was 2.6 x 10⁻³µm/s.

To study the fluid flow in the PVS driven by functional hyperemia, we imposed arteriolar wall motion in our model that matched those observed in awake mice during a typical functional hyperemic event (Gao et al. 2017; Gao, Greene, and Drew 2015; Shih et al. 2012) (Fig. 3.5a). The mathematical formulation of this problem was identical to the previous simulation, with the exception that the arteriolar wall movement was given by a typical vasodilation profile instead of a heartbeat-driven peristaltic wave (Fig 3.5a).
Compared to the flow driven by arteriolar pulsations, functional hyperemia-driven flow in the PVS had substantially higher flow velocities (Fig.3.5b). The flow observed in these simulations has a Reynolds number of $3.15 \times 10^{-4}$. However, the average downstream velocity of fluid (over 10s) was 0.12 µm/s.

The flow observed in these simulations has a Reynolds number of $3.15 \times 10^{-4}$. However, the average downstream velocity of fluid driven by pulsations and hyperemia in the PVS remained less than 1 µm/s over a wide range of assumptions and parameters. Changing the brain tissue model from nearly incompressible (Poisson’s ratio of 0.45) to a completely incompressible (Poisson’s ratio of 0.5), Neo-Hookean model had minimal impact on the

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**Figure 3.4: Arteriolar pulsation-driven flow in the PVS in an arteriolar-brain model with realistic mechanical properties**

- **a.** The model of the penetrating arteriole. The brain tissue is modelled as a compliant solid. The subarachnoid space is modelled as a flow resistance ($R_s$) at the pial end of the PVS and the parenchyma is modelled as a flow resistance ($R_p$) at the other end.
- **b.** A schematic depicting the fluid-structure interaction model described in a. The arteriolar wall movement drives the fluid movement in the PVS. This fluid movement is coupled with the pressure changes. These pressure changes deform the brain tissue, changing the shape and volume of the PVS. These volume changes will affect the flow in the PVS, as demonstrated in c.
- **c.** Plot showing the axial fluid velocity (velocity in the z-direction) in a cross section of the PVS, when the arteriolar wall movement is given by periodic pulsations. The amplitude and frequency of the arteriolar pulsations are taken to be typical values for cerebral arterioles in mice. Fluid velocity vectors (arrows) are provided to help the reader interpret the flow direction from the colors. The region in white has little to no flow. These plots show that there is no significant flow into the PVS driven by arteriolar pulsations. Note: Arteriolar and brain tissue displacements induced by arteriolar pulsations are very small (<0.1 µm). To make the movements clearly visible, we scaled the displacements by a factor of 10 in post-processing.

These calculations were performed with fluid permeability, $k_s = 2 \times 10^{-14}$ m$^2$ and tissue shear modulus $\mu_s = 4$ kPa.

There was very little directional fluid flow in the PVS due to arteriolar wall motions.
fluid velocities. We also calculated directional fluid flow driven by pulsations and hyperemia with different values of PVS width, permeability and shear modulus of the brain tissue. When the flow in the PVS was modeled by Navier-Stokes equation, the average downstream velocity of fluid was 0.078 µm/s per heartbeat cycle and 0.16 µm/s for 10 seconds of functional hyperemia. Changing the flow resistance of the SAS from 0.01 times the resistance of the PVS to 0.1 times the PVS resistance further reduced the average downstream flow. Of all the simulations, the highest average downstream fluid velocity of 0.16 µm/s was obtained in a simulation of vasodilation, where the width of the PVS was 3 µm, and the fluid flow in the PVS was modeled by Navier-Stokes equations in a brain tissue with elastic modulus of 8 kPa. This average downstream velocity of 0.16 µm/s is two orders of magnitude smaller than the experimentally observed downstream velocities in the PVS of ~20 µm/s (Bedussi, Almasian, et al. 2017; Mestre, Tithof, et al. 2018). Our simulations suggest that the arteriolar pulsations and dilations cannot drive directed, net CSF flow into the PVS.

**Figure 3.5:** Functional hyperemia-driven flow in the PVS in an arteriolar-brain model with realistic mechanical properties

*a.* Contours showing the axial velocity (velocity in the z-direction) in a cross section of the PVS, when the arteriolar wall movement is given by a typical neural activity-driven vasodilation response. Compared to heartbeat-driven pulsations (Fig. 3.4c), vasodilation-driven fluid flow occurs through the entire length of the PVS and has substantially higher flow velocities. The model also predicts that the vasodilation can also cause appreciable deformation in the brain tissue. A portion of the vessel lumen is shown in red to provide a sense of vasodilation.

*b.* Plot of the prescribed arteriolar wall movement for functional hyperemia. All the other boundary conditions and parameters used in this simulation are similar to the ones shown in Fig. 3.4.
3.3.2 Fluid exchange between the PVS and the SAS

Our simulations suggested that the directional flow driven by arteriolar pulsations and functional hyperemia is negligible. Therefore, we considered a different paradigm of metabolite clearance from the PVS, fluid exchange between the PVS and the SAS, in the lines of the dispersion model depicted in Fig. 1.11. Here, we use the well-established CSF flow through the SAS (Cserr, Harling-Berg, and Knopf 1992; Bradbury, Cserr, and Westrop 1981; Aspelund et al. 2015; Weller, Kida, and Zhang 1992; Qiuhang et al. 2010; Norwood et al. 2019; Coles et al. 2017; Sweetman and Linninger 2011; Damkier, Brown, and Praetorius 2013; Dreha-Kulaczewski et al. 2015) as the basis for metabolite clearance from the PVS. We propose that the fluid exchanged between the PVS and the SAS could be carried away by the existing directional flow in the SAS, thus aiding the clearance of metabolites from the PVS. This assumption is similar to the fixed-concentration boundary condition used at the SAS–PVS interface in studies proposing dispersion as a mechanism for clearance of metabolites (Asgari, De Zélicourt, and Kurtcuoglu 2016). In order to quantify the fluid exchanged between the PVS and SAS, we calculated the volume exchange fraction, $Q_f$, driven by arteriolar wall movement. The volume exchange fraction was defined as the ratio of the maximum amount of fluid leaving the PVS to the total volume of fluid in the PVS. The volume exchange fraction is an indicator of the total volume change of the PVS. We use the volume exchange fraction as the metric for the fluid exchange between the SAS and the PVS and metabolite clearance from the PVS. The transfer of metabolites from the brain interstitial space to the PVS is not explicitly modelled here, and is assumed to occur via diffusion (Smith et al. 2017b; Abbott et al. 2018; Binder et al. 2004; Jin, Smith, and Verkman 2016; Holter et al. 2017).
3.3.2.1 Functional hyperemia but not pulsation drives appreciable fluid exchange

We measured the fluid exchange between the PVS and the SAS driven by the arteriolar pulsations and functional hyperemia from the models presented in the previous section. For the default parameters (Table 3.1), arteriolar pulsations driven by heartbeat cause a mere 0.21% ($Q_f = 0.0021$) of the fluid in the PVS to be exchanged with the SAS and the parenchyma per cardiac cycle. For the same parameters, a single brief hyperemic event could exchange nearly half ($Q_f = 0.4946$) of the fluid in the PVS with the SAS. This difference in the fluid exchange driven by pulsations and hyperemia can be inferred from the fluid velocities in the PVS shown in Figs. 3.4 and 3.5. The small flows in the PVS driven by pulsations were due to the compliance of the brain, as any pressure gradient that could generate substantial fluid movement will be dissipated on deforming the brain tissue instead. This result is in contrast to the calculations of Asgari et al. (Asgari, De Zélicourt, and Kurtcuoglu 2016), which suggested that the pulsatile flow in the PVS could improve metabolite clearance through dispersion. The relatively large pulsatile velocities calculated by Asgari et al., in the range of 120µm/s (as opposed to our calculations of less than 25 µm/s) can be attributed to not considering the elastic response of the brain tissue.

To understand the flow near the brain surface and into the PVS, we define two Péclet numbers, $P_{e0}$ and $P_{e50}$, near the surface of the brain ($z=La$) and 50µm below the surface ($z=La - 50µm$) of the brain respectively (see methods). For the default parameters, the values of $P_{e0}$ and $P_{e50}$ are 0.82 and 0.19 respectively for pulsation driven flow, confirming that transport in the PVS away from the surface of the brain appears to be diffusion-dominated. The values of $P_{e0}$ and $P_{e50}$ for functional hyperemia-driven flow are 2.97 and 1.96 respectively, showing that the fluid exchange caused by vasodilation can improve metabolite clearance compared to diffusion.
3.3.2.2 Fluid exchange by arteriolar pulsations is not compounded over time

Arteriolar pulsations occur at nearly two orders of magnitude higher frequency compared to functional hyperemia. Pulsations and hyperemia occur nominally 10 Hz and 0.2 Hz, respectively. If fluid exchange by arteriolar pulsations compounded with time, the fluid exchange between the PVS and the SAS would be similar for arteriolar pulsations and functional hyperemia over equal time periods. To test this possibility, we calculated fluid particle trajectories in the deforming geometry of the PVS (see Appendix A for full mathematical description of boundary value problem for particle tracking in a deforming domain). The blue-green dots in Fig.3.6a represent fluid in the PVS, with the colormap showing the initial position (depth) of the fluid particle in the PVS. Fluid particles near the SAS (red dots) are added once every 0.5 secs to the calculation to simulate the possibility of fluid exchange between the PVS and the SAS. The results of these calculations indicate that a single hyperemic event can cause substantially more fluid movement in the PVS compared to arteriolar pulsations over the same time (Fig.3.6, also see videos SV1 and SV2 of Kedarasetti *et al.*, 2020). These calculations suggest that when the flow in the PVS is modeled with coupled soft brain tissue mechanics, functional hyperemia can drive appreciably higher fluid exchange between the PVS and the SAS as compared to arteriolar pulsations over the same time period.
We compared the fluid particle trajectories for arteriolar pulsations and dilations only for a short 5 second time interval. This might not be a fair comparison because functional hyperemia might only occur occasionally, while heartbeat pulsations are perpetually present. Calculating fluid trajectories over larger time periods than what we showed here (5 s), while keeping the time step small enough to capture the details of peristaltic wave (pulsations traveling at 1 m/s would traverse an arteriole of length 250 µm in $2.5 \times 10^{-4}$ s) is challenging as the accuracy of longer simulations would be severely affected by the accumulation of numerical errors. Estimates of slow fluid drift from oscillatory flows can be obtained by semi-analytical methods (Nama, Huang, and Costanzo 2017) and by building representative experimental systems (Sánchez et al. 2018). However, our simulations suggest that there is almost no oscillatory flow in the PVS of penetrating arterioles. This is demonstrated by the majority of the PVS shown covered in white (indicating that there is no flow) in Fig. 3.4c.

Figure 3.6: Functional hyperemia but not arteriolar pulsation drives appreciable fluid exchange between the PVS and the SAS.
3.3.2.3 Brain tissue deformability affects fluid exchange

There are two main reasons why functional hyperemia drives large fluid exchange between the PVS and the SAS, while arteriolar pulsations are ineffective at driving fluid movement in the PVS. Firstly, heartbeat-driven changes in arteriolar diameter are very small (0.5-4% (Mestre, Tithof, et al. 2018)) in magnitude compared to neural activity-driven vasodilation (10-40% (Gao et al. 2017)) and therefore there is a large difference in the volume of fluid displaced by the two mechanisms. A difference in the magnitude of blood volume change driven by heartbeat and hyperemia has also been observed in macaques (Teichert et al. 2010) and humans (Dagli, Ingeholm, and Haxby 1999) using functional magnetic resonance imaging (fMRI). Secondly, there is a large difference in the frequency of pulsations (7-14 Hz (Winder et al. 2017) in mice, nominally 1 Hz in humans) and hyperemic (0.1-0.3 Hz (Gao, Greene, and Drew 2015; Huo, Gao, and Drew 2015)) motions of arteriolar walls. Fast (high frequency) movement of arteriolar walls cause larger changes in pressure, which will deform the brain tissue rather than driving fluid flow. Also, deformable (elastic) elements absorb more energy at higher frequencies. If the electrical circuit equivalent of flow through the PVS while ignoring brain deformation is analogous to a resistor, the equivalent of flow through the PVS with a deformable brain is analogous to a resistor and inductor in series (Fig. 3.7). In other words, arteriolar wall motion at higher frequencies drives less fluid movement compared to arteriolar wall movement at lower frequencies. A similar phenomenon has been studied extensively in the context of blood flow through deformable arteries and veins (Rideout and Dick 1967; Müller and Toro 2014a; 2014b; Vignon-Clementel et al. 2006). We compared the fluid exchange percentage for an arteriolar wall movement given by a sine wave (4% peak to peak) of different frequencies, and found that the fluid exchange percentage showed an inverse power law relationship to the frequency of the pulsation (f) \( Q_f = 2.33f^{-0.57} \% \) for the
default parameters, Fig. 3.7). These calculations show that slow frequency arteriolar motions can drive better fluid exchange between the SAS and the PVS, when the PVS is surrounded by a deformable brain tissue.

**Figure 3.7:** Brain tissue deformability affects the fluid exchange between the PVS and the SAS

- **a.** Geometry for a model in which the brain is a rigid boundary to the PVS (top) and the equivalent circuit diagram (bottom). The driver for fluid flow is the arteriolar wall motion. The flow resistance of the PVS can be modelled by a simple resistor and is independent of the frequency of the arteriolar wall movement.
- **b.** Geometry for the fluid-structure interaction model with a deformable brain(top) and the equivalent circuit diagram(bottom). The driver for fluid flow is the arteriolar wall motion. The total flow resistance of the system can be modelled by a resistance from the PVS and an inductance because of the deformable tissue. In this model, the flow resistance of the system increases with increase in the frequency of the arteriolar wall motion. This means that for arteriolar wall motion at high frequency, less fluid will be exchanged between the PVS and the SAS.
- **c.** Plot shows the relation between fluid exchange percentage and frequency of arteriolar wall motion. The arteriolar wall motion was given by a 4% peak-peak sinusoidal wave with different frequency values. The default values were used for all other parameters (see Table 3.1). For very low frequencies (<0.1 Hz), the fluid exchange driven by the arteriolar wall is same whether or not brain deformability is taken into account. For higher frequencies, the fluid exchange percentage has an inverse power law relation with the frequency of arteriolar wall motion.
3.3.3 Robustness of model predictions to assumptions and parameters

There is undeniable ambiguity regarding some of the modeling choices and parameters used for the simulations presented in this chapter. We provided some justification for the assumptions and default parameter used, in section 3.2.1. In this section, we examine how the model predictions are affected when the parameters are varied within the physiologically possible range and the model assumptions are switched. We examined the predicted fluid velocities and flow rates are affected, when the model assumptions are changed. The fluid exchange percentage driven by pulsations and hyperemia were compared for a wide range of parameters to test the robustness of the model to parameters.

In the simulations presented in Figs. 3.4-3.7, we assumed a compressible elastic model (Saint-Venant-Kirchhoff’s model with a Poisson’s ratio of 0.45) for the deformability of the brain tissue. However, there are several researchers that argue that the brain tissue should be modeled as an incompressible solid under physiological conditions (Brands, Peters, and Bovendeerd 2004; Laksari, Shafieian, and Darvish 2012). To examine the role of incompressibility, we repeated the simulations with an incompressible Neo-Hookean model, with a shear modulus of 4 kPa. The solid stress in Neo-Hookean elasticity, is determined by an unknown hydrostatic pressure-like term ($p_s$) in addition to the solid deformation (eq. 3.16). Incompressible elastic models were implemented in FEM using auxiliary variables for the Jacobian determinant ($J_s = \det F_s$) and a Lagrange multiplier ($q_s$) in the strain energy ($W_s$) function (Eq.3.17).

$$\sigma_s = p_s I + \mu_s \left( F_s F_s^T - \frac{1}{3} Tr(F_s F_s^T) \right)$$

3.16
\[ W_s = \frac{\mu_s}{2} (\text{Tr}(\tilde{F}_s \tilde{F}_s^T) - 3) + p_s (\det F_s - J_s) + q_s (J_s - 1) \]  

3.17

\[ F_s = \left( \frac{1}{\det F_s} \right)^{1/3} F_s \]  

3.18

The unit-determinant deformation gradients, \( \tilde{F}_s \), in Eq. 3.18 are useful for implementation using variational methods like FEM, as they result in the separation of the deviatoric and spherical parts of the stress tensor (Simo and Taylor 1991). The fluid velocities and flow rates predicted by simulations with incompressible elasticity were almost identical to the simulations with compressible elasticity for the default parameters (Figs. 3.8-3.9).

In the simulations presented in Figs. 3.3-3.9, the subarachnoid space was modeled as a flow resistance at the pial end (top) of the PVS. While this representation can model the effect that flow in the SAS has on the pressure in the PVS, it might not accurately represent the effect of pressure changes in the SAS on the deformability of the brain tissue. We simulated the flow in the PVS where the SAS was modeled explicitly as a fluid filled region. Including the SAS geometry in the simulations had a minimal effect on the flow in the PVS and the deformation of the brain tissue. This is expected based on the calculations in the previous chapter, where we showed that fluid flow in the SAS (which includes the relatively large PVS of the pial arteries) only requires pressure gradients of the order of 0.01mmHg/mm. The results of the simulations with SAS geometry are not shown here to avoid repetition. (Please see Figs S5 and S8 in Kedarasetti et al., 2020).
a. The imposed heartbeat-driven pulsations in arteriolar radius (±0.5% of mean radius, \(R\)) at 10 Hz, the heartbeat rate of an un-anesthetized mouse. The pulse wave travels at 1 meter per second along the arteriolar wall, into the brain.

b. Colors showing the axial velocity (velocity in the z-direction) in a cross section of the PVS, when the arteriolar wall movement is given by periodic pulsations. These plots (compare to those in Fig. 3.4c) show that there is no significant flow into the PVS driven by arteriolar pulsations.

c. Flow out of the PVS and into the subarachnoid space, through the pial opening of the PVS. The flow rates predicted by the model with nearly incompressible (Poisson’s ratio of 0.45) (magenta) and a completely incompressible, Neo-Hookean models (blue) were nearly identical.

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Figure 3.8: Pulsation-induced fluid flows in the PVS are small in an incompressible Neo-Hookean brain model

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a. Plot of the prescribed arteriolar wall movement, which is identical to the one shown in Fig 3.5a.

b. Plot showing the axial (z-direction) fluid velocity a cross section of the PVS, when the arteriolar wall movement is given by neural activity-driven vasodilation. These plots (very similar to the ones in Fig 3.5a) show that vasodilation-driven fluid flow occurs through the entire length of the PVS and has substantially higher flow velocities.

c. Flow out of the PVS and into the pia, through the top face of the PVS. The flow rates predicted by the model with nearly incompressible (SVK model with Poisson’s ratio of 0.45) brain tissue (magenta) and a completely incompressible, Neo-Hookean model (blue) are very similar.

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Figure 3.9: Vasodilation-induced PVS fluid flow in an incompressible, Neo-Hookean model was very similar to the compressible SVK model
We calculated the fluid exchange fraction for different values of shear modulus of the brain tissue (Fig. 3.10a), and width (Fig. 3.10b) and permeability (Fig. 3.10c) of the PVS. For all the tested parameters, functional hyperemia-like dilations drove substantial fluid movement in the PVS. Compared to arteriolar pulsations, the vasodilation-driven fluid exchange between PVS and SAS was two orders of magnitude higher under a wide range of model parameters (Fig. 3.10). When the fluid is modeled by the Navier-Stokes equations (infinite permeability in Fig. 3.10c), 69.8% of fluid in the PVS is exchanged with the SAS for a single, brief hyperemic event, whereas arterial pulsations only caused 1.37% of the fluid in the PVS to exchange with the SAS per cycle. Changing the flow resistance of the SAS from $1/100^{th}$ to $1/10^{th}$ of the resistance of the PVS had a minimal effect on the fluid exchange fraction. For example, in simulations where the SAS flow resistance was replaced by $1/10^{th}$ of the PVS resistance (instead of $1/100^{th}$), for the default parameters (see Table 3.1) one pulsation cycle drove fluid exchange of 0.13% (instead of 0.21%) and a single hyperemic event drove a fluid exchange of 48.0% (instead of 49.5%). This effect of changing the SAS flow resistance on the fluid exchange was much smaller compared to other parameters of interest (Fig. 3.10).
3.4 Discussion

While there have been several models investigating the fluid mechanics in the PVS (Schley et al. 2006; Wang and Olbricht 2011; Asgari, De Zélicourt, and Kurtcuoglu 2016), to our knowledge, none of them considered the impact of the soft, deformable brain tissue on CSF flow in the PVS. Our simulations show that fluid flow in a porous PVS resulting from the movement of the vessel walls can be affected by the deformability of the brain tissue. As far as we know, this is the first study to include deformability of the brain tissue in modeling fluid flow in the PVS and to use experiments to support the predictions of fluid dynamic simulations. For all the fluid-structure interaction models, the percentage of PVS fluid exchanged with the SAS is the main metric for metabolite clearance. The mean downstream speed (net directional flow velocity into the parenchyma) was negligible for all the models. A summary of the model parameters and main results of all the simulations in this article are shown in Table 3.2.

Figure 3.10: The effect of key model parameters on the fluid exchange between the SAS and the PVS

Vasodilation drives two orders of magnitude higher fluid exchange between the PVS and subarachnoid space compared to heartbeat driven pulsations. The plots show the changes in fluid exchange percentage, the percentage of fluid in the PVS exchanged with the SAS, with change of model parameters. The model predicts that compared to arteriolar pulsations; the vasodilation driven fluid exchange percentage is two orders of magnitude higher. This difference is similar for different values of elastic modulus of the brain (b), the width of the PVS (c) and the fluid permeability of the PVS (d). In (d), when the permeability is infinite, Darcy-Brinkman's law transforms into Navier-Stokes' law for fluid flow. All the plots are made on a log-log scale because the parameters were changed by 1-3 orders of magnitude.
Table 3.2: Summary of axisymmetric fluid-structure interaction simulations

<table>
<thead>
<tr>
<th>Fig</th>
<th>Brain model</th>
<th>Fluid Permeability (m²)</th>
<th>Arteriolar wall movement</th>
<th>Mean downstream speed (µm/s)</th>
<th>PVS fluid exchanged with SAS</th>
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<tr>
<td>3.2</td>
<td>Non-compliant</td>
<td>2x10⁻¹⁴</td>
<td>Heartbeat, ( \Delta R_{\text{max}} = 0.06 ) µm</td>
<td>5.5 x 10⁻⁴</td>
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<td>Conclusion: Expected brain deformation, 3.59 µm &gt; pulsation amplitude, 0.06 µm</td>
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<td>3.3</td>
<td>Non-compliant</td>
<td>( \propto ) (Navier-Stokes)</td>
<td>Heartbeat, ( \Delta R_{\text{max}} = 0.06 ) µm</td>
<td>1.8 x 10⁻³</td>
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<td>Conclusion: Expected brain deformation, 0.08 µm &gt; pulsation amplitude, 0.06 µm</td>
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<td>3.4</td>
<td>SVK, ( \mu_s = 4 ) kPa (compressible)</td>
<td>2x10⁻¹⁴</td>
<td>Heartbeat, ( \Delta R_{\text{max}} = 0.06 ) µm</td>
<td>2.6 x 10⁻³</td>
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<td>Conclusion: No appreciable fluid exchange by pulsations</td>
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<td>3.5</td>
<td>SVK, ( \mu_s = 4 ) kPa (compressible)</td>
<td>2x10⁻¹⁴</td>
<td>Hyperemia, ( \Delta R_{\text{max}} = 1.8 ) µm</td>
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<td>49.46%</td>
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<td>SVK, ( \mu_s = 4 ) kPa (compressible)</td>
<td>2x10⁻¹⁴</td>
<td>( \Delta R_{\text{max}} = 0.24 ) µm Frequency, ( f = 0.05-10)Hz</td>
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<td>( Q_f = 2.33f^{-0.57} )</td>
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<td>Conclusion: Fluid exchange is inversely related to frequency of arteriolar wall movement</td>
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<td>3.8</td>
<td>Neo-Hookean, ( \mu_s = ) 4kPa (Incompressible)</td>
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<td>Conclusion: No appreciable fluid exchange by pulsations</td>
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<td>3.9</td>
<td>Neo-Hookean, ( \mu_s = ) 4kPa (Incompressible)</td>
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<td>Hyperemia, ( \Delta R_{\text{max}} = 1.8 ) µm</td>
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<td>Conclusion: Appreciable fluid exchange by hyperemia</td>
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<td>3.10 a. SVK, ( \mu_s = 1-8 ) kPa (compressible)</td>
<td>2x10⁻¹⁴</td>
<td>Heartbeat/ Hyperemia, ( \Delta R_{\text{max}} = 0.06/1.8 ) µm</td>
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<td>Conclusion: Fluid exchange by hyperemia ( \approx 200 \times ) Fluid exchange by pulsations</td>
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Our model has several limitations, and the results should be interpreted with these limitations in mind. We assumed a cylindrically symmetric geometry for our model. In reality, the PVS around penetrating vessels can be eccentric and elliptical (Mestre, Tithof, et al. 2018). An eccentric annular region with a pulsating inner wall can cause a slow drift in the fluid particles (Sánchez et al. 2018). However, the drift caused by eccentricity is not
unidirectional, and the bulk movement of the particles is much smaller compared to the oscillations (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017). We did not explicitly model fluid flow within the brain parenchyma and the effect of the aquaporin-4 channels found on the astrocyte endfeet lining the brain-PVS interface (Abbott et al. 2018). The flow through the parenchyma was only simulated indirectly through the parenchymal flow resistance at one end of the PVS. This can be rectified in future studies by using models of poroelasticity (Costanzo and Miller 2017; Coussy 2011; Bowen 1980), which simultaneously simulate fluid movement through the extracellular space and the deformations in the brain tissue. Some authors have used poroelastic models to predict that oscillatory flows can enhance metabolite transport in the brain through dispersion (Keith Sharp, Carare, and Martin 2019). Poroelastic models can also explore the effect of static and dynamic occlusions to flow (Heil and Bertram 2016; Chou et al. 2016), which our fluid-structure model does not simulate. We also ignore the elastic response of the collagen fibers in the PVS and only simulate the fluid dynamics in this region.

Our results are in agreement with the findings of several experimental studies (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017; Smith et al. 2017b; von Holstein-Rathlou, Petersen, and Nedergaard 2018; Xie et al. 2013; Pizzo et al. 2018), though they cast their results in a new light. Several studies have used microspheres to visualize the CSF movement in the PVS around pial arteries (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017). Similar to their results, our simulations suggest that CSF oscillates with the frequency of heartbeat driven pulsations near the surface of the brain (Fig 3.3c). It can also be shown that larger arteriolar pulsations can cause larger oscillations in CSF flow, similar to the case of induced hypertension found by Mestre et al (Mestre, Tithof, et al. 2018). In contrast to the conclusions of these particle tracking studies (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017), our simulations suggest that
arteriolar pulsations do not provide a driving force for net unidirectional movement of CSF. Our results also agree with the findings that voluntary running, which increases neural activity (Nimmerjahn, Mukamel, and Schnitzer 2009; Dombeck et al. 2007) and induces functional hyperemia (Huo, Smith, & Drew, 2014; Huo et al., 2015) in several regions of the brain, enhances penetration of tracers in the brain parenchyma, when injected into the cisterna magna (von Holstein-Rathlou, Petersen, and Nedergaard 2018). The silencing of neural activity (Lamme, Zipser, and Spekreijse 1998; Alkire, Hudetz, and Tononi 2008) (and therefore vascular activity) by anesthetics (Aksenov et al. 2015; Andrea Pisauro et al. 2013) could explain diminished penetration of tracers in the brain parenchyma, when injected into the cisterna magna under anesthesia (Gakuba et al. 2018). The variability in results between groups may be influenced by anesthesia type and levels, both of which have large effects on the amplitude of the arteriolar dilations elicited during functional hyperemia (Gao et al. 2017). Finally, brain-wide hyperemia observed during REM sleep (Bergel et al. 2018) could explain improved tracer transport in the brain observed during sleep (Xie et al. 2013). We would like to point out that our model suggests that functional hyperemia enhances transport from the SAS to the parenchyma (and vice-versa) by fluid exchange through the PVS. We do not claim that functional hyperemia improves flow in the proposed “glymphatic” pathway (Nedergaard 2013), as we did not explicitly model flow through the brain extracellular space or the venous PVS.

Our results have implications for the development and treatment of CNS disorders and suggest that in addition to its other physiological roles, functional hyperemia may serve to improve transport into and out of the brain by driving exchange of fluid in the PVS with the SAS. Many studies support the idea that vascular dysfunction can be a precursor to neurodegenerative diseases (Kudo et al. 2000; la Torre 2002; Iadecola 2004). Our simulations suggest a mechanistic relation between neurovascular coupling and
metabolite clearance from the brain, which could explain the development of neurodegenerative diseases like Alzheimer’s. The response of our model to changes in key parameters can explain the effect of aging on clearance of metabolic waste from the brain. Some studies have shown that the elastic modulus of the brain decreases with aging (Sack et al. 2011; 2009), and our model predicts less fluid exchange between the SAS and the PVS when the elastic modulus is lowered (Fig. 3.10b). Finally, the increase of PVS width observed with aging (Ding et al. 2017) might be a reason for reduced clearance of metabolic waste from the brain (Fig. 3.10c). While these possibilities are admittedly speculative, they may be fertile ground for further investigation of the interaction of brain mechanics and health.

Taken together with the results of the Chapter 2, our models in this chapter suggest that arterial pulsations cannot improve metabolite clearance through the PVS by directional pumping or by dispersion, while functional hyperemia can drive fluid exchange between the PVS and the SAS.
Chapter 4: Measurement of Brain Tissue Deformation³

4.1 Introduction

In this chapter, we present the experiments used to verify the predictions of the fluid-structure interaction models, namely the deformation of the brain tissue in response to the dilation of arteries and the resulting changes of fluid pressure in the paravascular space (PVS). This prediction was verified by simultaneously measuring the displacement of the brain tissue and the arteriolar walls. The measurements were made in awake and head-fixed mice B6.Cg-Tg (Thy1-YFP)16Jrs/J (Jackson Laboratory) mice (Feng et al. 2000) implanted with a polished and reinforced thin-skulled window (PoRTS window, see section 1.3.4.1), using a two-photon laser scanning microscope. These transgenic mice express yellow fluorescent protein (YFP) in a sparse subset of pyramidal neurons, rendering their axons and dendrites highly fluorescent (Grutzendler, Kasthuri, and Gan 2002), which allowed the tracking of brain tissue. The movement of the arteriolar walls was tracked by injecting a dye (2.5% w/v Texas-red conjugated dextran in aCSF) into the blood stream. The experiments confirmed the prediction of the fluid-structure interaction models that the brain tissue can deform in response to the pressure changes in the PVS.

At first glance, it might seem that the most important prediction of the fluid-structure interaction models is the flow of cerebrospinal fluid (CSF) in the PVS and the subarachnoid space (SAS) and not the deformation of the brain tissue. However, there are several reasons why we chose to track displacement of brain tissue, instead of the fluid movement. The deformation of the soft brain tissue is the main reason for using fluid-structure models (section 3.3.1.1), and the difference in fluid exchange between the SAS and the PVS that is driven by pulsations and hyperemia are partly because of the deformation of brain tissue (section 3.3.2.3). Tracking the displacement of the brain tissue using transgenic mice requires no additional implantations other than the PoRTS window, which ensures that the highly sensitive fluid spaces in the CNS are unperturbed, in contrast to the experiments of fluid tracking, where the injection of dyes or particles can alter flow in the CNS (see section 1.3.4.2). There are also no methods currently available to track fluid movement in the PVS of penetrating arterioles. The fluid in the PVS cannot be tracked using large microspheres injected into the cisterna magna, because experiments demonstrated that the microspheres used for fluid tracking are excluded from the PVS of penetrating arterioles (Bedussi, Van Der Wel, et al. 2017; Bedussi, Almasian, et al. 2017; Mestre, Tithof, et al. 2018). The fluid flow in the PVS is mostly perpendicular to the surface of the brain and therefore perpendicular to the focal plane of the two-photon microscope and therefore fluorescence recovery after photobleaching (FRAP), which is only effective for identifying diffusion and convection in the focal plane of the microscope (Sykova et al. 2008; Smith et al. 2017a; Papadopoulos, Binder, and Verkman 2005; Binder et al. 2004), would not effective to test the predictions of the fluid-structure interaction models.

To find if pressure changes in the PVS are actually deforming the brain tissue, we considered two possible paradigms of brain deformation, a “non-compliant brain” model and a fluid-structure interaction model. We predict the two paradigms to yield completely
different results in terms of the displacement of the brain tissue observed *in vivo*. In the non-compliant brain model, the brain tissue will be unaffected by pressure changes in the PVS. In this model, pulsations and small dilations of arterioles would cause flow in the PVS but no displacement of the brain tissue (Fig. 4.1a). Only after the arteriolar wall comes in contact with the brain tissue (and the PVS has fully collapsed), arteriolar dilation would cause tissue displacement (Fig. 4.1b). Therefore, displacement in the brain tissue in this model would be either non-existent (for small dilations), or similar to a “trimmed” version of the displacement of the arteriolar wall (Fig. 4.1c). Alternatively, in the fluid-structure interaction model, any movement of the arteriolar wall that can drive fluid flow in the PVS will result in pressure changes in the PVS that are sufficient to deform the soft brain tissue, as predicted by our simulations in the previous chapter (Fig. 4.1d, 4.1e). Therefore, displacement should be observed in the brain tissue as soon as the arteriolar wall starts to dilate. In the fluid-structure interaction model, the radial displacement in the brain tissue would be a scaled version of the radial displacement of the arteriolar wall (Fig. 4.1f).
4.2 Materials and methods

4.2.1 Surgical procedures

All the surgeries were performed by Kevin L. Turner and Christina Echagarruga. All procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Pennsylvania State University. Mice were anesthetized with isoflurane (5% induction, 2% maintenance) for all surgical procedures. The scalp was resected, and the connective tissue removed from the surface of the skull. A custom-machined titanium headbar (https://github.com/KL-Turner/Mouse-Head-Fixation) was affixed with cyanoacrylate glue (32402, Vibra-Tite) immediately posterior to...
the lambda cranial suture. Three self-tapping, 3/32" #000 screws (J.I. Morris) were implanted into the skull, one in each frontal bone, and one in the contralateral parietal bone. A ~4mm x ~5mm polished and reinforced thinned-skull window was implanted over the right hemisphere somatosensory cortex as previously described (Drew et al. 2010; Shih et al. 2012). After thinning, the skull was polished with 3F and 4F grit, and a #0 glass coverslip (Electrode Microscopy Sciences, #72198) was attached to the thinned portion of the skull with cyanoacrylate glue. Dental cement (Ortho-Jet) was used to seal the edges of the window and connect the headbar and headscrews. At the conclusion of the surgery the mice were returned to their home cage and allowed 2 days of recovery before being habituated to head fixation. Mice were habituated to head-fixation on a spherical treadmill (60mm diameter) for 2-3 days before imaging. The mice were head-fixed for 30mins on the first session and the length of the session was increased to 90mins on the final session. The mice were monitored for any signs of distress during the period of habituation.

4.2.2 Two-photon imaging

Prior to imaging, mice were briefly anesthetized with isoflurane and retro-orbitally injected with 50µL of 2.5%w/v of Texas-red conjugated dextran (40kDa; Sigma-Aldrich), then head-fixed upon a spherical treadmill. The treadmill was coated with a slip-resistant tape and connected to a rotary encoder (US Digital, E7PD-720-118) to monitor changes in velocity of the treadmill. The changes in velocity (acceleration) were used to identify periods of rest and motion. Images were collected under a Sutter moveable objective microscope with either a 16x 0.8 NA objective or a 20x 1.0 NA objective (Nikon). A MaiTai HP laser tuned to 920nm was used to excite the YFP and the Texas-Red. The power exiting the objective was between 30-70mW. Arteries were visually identified by their more rapid blood flow, rapid temporal dynamics of their response to locomotion, and vasomotion
(Drew, Shih, and Kleinfeld 2011; Gao et al. 2017; Winder et al. 2017). A two-channel photomultiplier setup was used to collect fluorescence from YFP and Texas-red. Images were collected at a nominal frame rate of 3-8Hz. All the data was collected at a depth of 30-120µm below the pial surface, and none of the arterioles imaged bifurcated before the depth at which the measurements were made.

4.2.3 Image cleanup

The images collected from the method above had noise due to motion artifacts arising from imperfect head-restraints and the movement of the brain relative to the skull, photon shot noise (Wilt, Fitzgerald, and Schnitzer 2013) and crosstalk between the imaging channels. We used the red channel for motion correction (registration using discrete Fourier transform (Guizar-Sicairos, Thurman, and Fienup 2008)) to remove movement artifacts in order to generate movies where the center of the vessel was fixed. A 3D median filter (3,3 pixels in space and 5 frames in time) was used to remove shot noise. Due to crosstalk, the images on the red channel contained some YFP fluorescence (brain tissue) in addition to the Texas Red (vessel lumen) signal. To remove this crosstalk, we used linear model to remove the YFP signal from the red channel.

\[
\begin{align*}
    r_i(\text{Red Image}) &= r_f(\text{Red fluorescence}) + \alpha \cdot g_f(\text{Green fluorescence}) \\
    g_f &= g_i(\text{Green Image}) \\
    \alpha &= \min_{\alpha = (0,1.5)} \text{norm}(r_i - \alpha \cdot g_i)
\end{align*}
\]
Here, the image in the red channel, \( r_i \), was assumed to be a linear combination of the actual red fluorescence, \( r_f \), and a weighted version, \( \alpha \), of the green fluorescence, \( g_f \) (Eq. 4.1). The green image, \( g_i \), was assumed to be the actual representation of the green fluorescence (Eq.4.2). A constant (\( \alpha \)) was found, that minimized the total error under an inverse model, using MATLAB’s \textit{fminsearch} function (Eq.4.3). We then used Eq.4.1 to calculate the red fluorescence \( r_f \). A sample of the raw image and the cleaned-up image are shown in Fig. 4.2.

**Figure 4.2: Image cleanup sample**

Sample of the raw image obtained from the two-channel photomultiplier setup under a two-photon microscope(a) and the processes image(b). The image is obtained from a freely behaving, head-restrained Thy1-YFP mouse. The lumen of blood vessels is fluorescent as a result of a retro-orbital injection of Texas red dye. The cross-talk between the green and red channels is shown by the regions in white in a (example shown with yellow arrow).

### 4.2.4 Arteriole diameter measurement

We used the processed images to measure the changes in arteriolar diameter using the image sequence in the red channel. The section of the image containing the arteriole was cleaned-up using the thresholding in Radon space algorithm (Gao and Drew 2014). For this method, a rectangular region containing the arteriole was manually selected. The region was transformed into Radon space for angles between 0° and 180° in 1° increments. At each angle, the Radon transform value was rescaled between 0 and 1 to obtain a normalized value. A threshold value of 0.2 was chosen, and every value below this was set to zero. We then calculated the inverse Radon transform of the
normalized values into the image space. The area of the vessel was calculated from the inverse-transformed image using the regionprops function in MATLAB. The velocity data (collected from the rotary encoder on the spherical treadmill) was used to create a binary vector of movement/rest during each frame (Huo, Gao, and Drew 2015). The hemodynamic response function (HRF) between the binarized locomotion and the vessel diameter was calculated by fitting the parameters of a gamma distribution function (Lindquist et al. 2009). Only data sets where the goodness of fit ($R^2$) between the measured vessel data and the HRF-convolved function was $> 0.6$ were used for further analysis, so as to avoid noisy datasets.

4.2.5 Measurement of brain-tissue displacement

The displacement of brain tissue was calculated using a piecewise rigid motion model. A reference frame was chosen by averaging 10-30 seconds of data when the mice were stationary (not moving). The images from the green channel were broken down into overlapping boxes of 64 x 64 pixels with 48-pixel overlap in the x or y direction. The boxes containing no appreciable fluorescence were not used in calculating displacements. This was done by looking at the peak fluorescence in each box and only using boxes that were in the top 20 percentile of peak fluorescence. For each box, the displacement was calculated using image registration (Guizar-Sicairos, Thurman, and Fienup 2008) with the corresponding box in the reference frame. We used an iterative approach to calculate the displacements, meaning that each box was displaced by the negative value of the calculated displacement and the displacement between the reference and the corrected box was recalculated. This process was iterated five times for each box. The calculated displacement value was accepted only if the displacements converged, i.e., the displacement calculated in the last iteration was smaller than 1% of the total calculated displacement. This criterion was necessary because we observed several instances of
movement of the brain tissue out of the plane of imaging. The DFT registration algorithm gives out a displacement value even when the reference and target images do not match. An example of the iterative method at work is shown in Fig. 4.3.

Additionally, we use a threshold in the error (<70%) calculated by the DFT registration to accept or reject the calculated displacement. Above-threshold points were scrubbed from the time series and a median filter was used to fill in the scrubbed data points. A wavelet-based filter was used to denoise the resulting time series. A wavelet-based filter ("biorthogonal 3.3" wavelet in MATLAB) was used because it was found to be most efficient in extracting gamma-distribution function-like signal from noise.

4.2.6 Selection criteria

There are several sources of noise for the calculated displacement in the brain tissue. Therefore, only datasets of the calculated displacement time-series that met certain criteria were included in our results. Firstly, the direction of calculated the displacement should be radially outward (±30°) from the centerline of the vessel. Secondly, the displacement time-series should be well correlated with the time-series of vessel diameter.
changes (Pearson correlation coefficient >0.8). This criterion was required because the brain can move in the vertical direction (Kong, Little, and Cui 2016) (upto 5µm in some cases), which resulted in noisy results while processing our planar imaging data. We found that the Pearson correlation coefficient between the vessel response and tissue response in both fluid-structure interaction and non-compliant brain paradigms using pseudo data was always greater than 0.85 for signal-to-noise ratio between 2-50dB. One major drawback of this method is that we could have missed data sets where the brain tissue did not move at all. This problem can be rectified in future studies by using volumetric scans (Kong, Little, and Cui 2016) instead of planar scans, and this would require data collection higher frequencies than our 3Hz scans. Finally, we verified that the calculated displacement was visible in a projection of the image stack in time along the line of the calculated displacement. This last step was carried out in ImageJ (NIH). It is important to note that the displacements expected in both the “non-compliant brain” model and the fluid-structure interaction model meet all three criteria. A flowchart of the method used for calculating the displacement of the brain tissue, along with the selection criteria, is presented in Fig. 4.4.
4.3 Results

4.3.1 Validation of displacement-measurement algorithm

To validate our method, the displacement measuring algorithm was tested on pseudo-data generated using MATLAB. A base frame of 512x512 pixels was generated by randomly placing “bright” lines oriented in different directions. These lines are supposed to represent the axons and dendrites of neurons with the Thy1 promoter. The generated image was given a radially outward deformation with the temporal dynamics given by a gamma distribution function using the Matlab function imwarp. Varying levels of white noise, similar to shot noise were added to the images to determine the robustness of the algorithm. The displacement was then calculated using the procedure depicted in Fig. 4.4. We found that the displacements extracted by our method agree well with the input displacement, and were robust to high levels of noise. Increasing the level of noise...
(decreasing the signal-to-noise ratio) reduced the number of locations where the displacement could be detected but had a minimal effect on the waveform of displacement recovered using our algorithm (Fig. 4.5). The result demonstrates that our selection criteria (section 4.2.6) are crucial to obtaining reliable measurements from noisy data.

![Image of a computer generated image (512x512 pixels) with randomly oriented lines.](a)

b. The radially-outward displacement given to the image shown in a.

c. An image showing the radially-outward displacement at peak displacement (frame number 13). The initial position of the lines is shown in white and the displaced position is shown in blue.

d. The displacement extraction procedure is robust to noise and predicts the correct displacement. On the left, a case with low signal-to-noise ratio (0.59) is shown. The calculated displacements are very close to the actual displacement. The accuracy is comparable to the case with high signal-to-noise ratio (4.14) on the right. However, high noise results in a detection of displacement at fewer locations. The plot in the center shows that at low signal to noise ratio only 30% of the possible locations can be used for displacement calculations. Signal-to-noise ratio is calculated as the ratio of the mean signal value to the standard deviation in the noise.

Figure 4.5: Validation of displacement-measurement algorithm
4.3.2 Brain tissue deformation is consistent with a fluid-structure interaction model

We calculated the radial displacement of the arteriolar wall and the brain tissue in vivo (n = 21 vessels, 7 mice) using two-photon microscopy. A sample dataset with the resting frame, and the displacement are shown in Fig.4.6a-d. The displacement values (Fig.4.6d) calculated were subject to a rigorous set of tests (see section 4.2.6) to account for motion artifacts. To visualize the brain tissue displacements accompanying vasodilation, we plotted a kymogram taken along diameter line bisecting the arteriole and crossing neural processes (Fig.4.6b-c). Distance from the center of the arteriole is on the x-axis and time on the y-axis. Dilations appear as a widening of the vessel, while displacements of the brain tissue will show up as shifts on the x axis. This visualization was used as an additional step in validating the displacement values calculated by our method. The radial displacement of the brain tissue was between 20-80% of the radial displacement of the arteriolar wall.

The displacement of both the arteriolar wall and the brain tissue varied among vessels and also between each instance of voluntary movement within a trial. The simulations suggest that the relative displacement between the arteriolar wall and the brain tissue should vary, based on the heterogeneity in the width and depth of the PVS, the permeability of the PVS and the shear modulus of the brain tissue. This variability is depicted by the variation in the fluid exchange percentage discussed in section 4.3.3. Moreover, as we change the distance of the plane of imaging from the surface of the brain, we should expect a change in the relative displacement of the arteriolar wall and the brain tissue (Fig.4.7a-b). Despite the variation in the amplitude of displacement in the tissue, our simulations predict that the peak-normalized displacement response of the brain tissue should have the same temporal dynamics as the arterial dilation (Fig.4.7c). Therefore, we
used the peak-normalized waveform of the measured displacement to test the predictions of the model.

Figure 4.6: In vivo measurement of brain tissue-displacement suggests that the brain tissue can deform because of pressure changes in the PVS

d. The calculated radial displacement in the brain tissue in response to changes in arteriolar radius. The data suggests that the brain tissue deforms due to pressure changes in the PVS before the PVS completely collapses.

e. The average (7 mice, 21 vessels) peak-normalized impulse response of the radial displacement of the arteriolar wall (magenta) compared to the average peak-normalized impulse response of the radial displacement in the brain tissue (only one data point per vessel was used for this calculation). The data shows that there is no delay between displacement of arteriolar wall and the tissue, suggesting that the brain tissue deforms due to pressure changes in the PVS as predicted by the fluid-structure interaction model.

We calculated the peak normalized impulse response of the displacements to locomotion. For each of the datasets, the impulse response of the radial displacement of the arteriolar wall to locomotion events was first calculated using the method of deconvolution (Glover 1999). These impulse response functions were aligned so that the peak occurred at the same time and normalized to the peak value (L-infinity norm). The impulse response functions of the displacement of brain tissue to locomotion were
calculated, peak-normalized and applied the same time-lag as the corresponding arteriolar wall motion. The average, normalized radial displacement of the arteriolar wall and the brain tissue were then compared to consider the possibility of a “non-compliant brain” model or a fluid-structure interaction model (Fig. 4.6e). For calculating the average waveform of tissue displacement shown in Fig. 4.6e, only one of the calculated displacement values per vessel that could also be visually verified was used. The calculations of tissue displacement for each arteriole (an example is shown in Fig. 4.6d), as well as the normalized impulse response for the brain tissue (Fig. 4.6e) suggest that the displacement in the brain tissue started as soon as the arteriolar dilations started. This implies that the brain tissue can deform due to pressure changes in the PVS, as predicted by the fluid-structure interaction model.
These results are only an indirect proof of the fluid flow predicted by our models. A more direct investigation would require tracking the fluid movement. While the currently available fluorescent microparticles are too big to enter the porous PVS of penetrating arterioles, the ongoing research using nano-particles that can be delivered through light-activated vesicles (Xiong et al. 2020) might allow the direct tracking of fluid movement.

The displacements measured here are mired with the movement of the brain tissue perpendicular to the plane of imaging. This problem could be dealt with by obtaining 3D...
scans by using focus-tunable lenses in conjunction with the two-photon microscope (Kong, Little, and Cui 2016).

Interestingly, the fluid-structure interaction model predicted a negative radial displacement in the brain tissue, when the arteriole constricts or returns to its original diameter, which we did not observe in the in vivo measurements. This anomaly can be explained by the fact that the fluid-structure interaction model neglects the elastic forces in the connective tissue (extracellular matrix) in the PVS. The PVS contains collagen fibers and fibroblasts, that are continuous with the extracellular space of the surrounding tissue (Lam et al. 2017; Berliner et al. 2020). Collagen networks can have a highly non-linear elastic response when the loading is changed from compression to tension, and exhibit hysteresis during large, cyclic deformations (Liu et al. 2018; Haut and Little 1972). The elastic modulus of fibrous networks under tension can be 2-3 orders of magnitude higher than the elastic modulus in compression (Van Oosten et al. 2016; Vahabi et al. 2016; Storm et al. 2005). Connective tissue is made up of networks of fibers, and the energy cost of bending these fibers is several orders of magnitude smaller than stretching them. When the arteriole dilates, these fibers are subject to a compressive loading and they buckle (bend) rather than compress, and as a result generate very little elastic forces. However, when the arteriole constricts, these fibers are subjected to a tensile load and produce significantly higher (2-3 orders of magnitude higher) elastic forces. However, our model only considers the fluid-dynamic forces in the PVS and neglects the elastic forces. This is one of the shortcomings of our model, that can be corrected in the future using models of poroelasticity (Costanzo and Miller 2017; Coussy 2011; Bowen 1980) so as to account for the mutual interaction of flow and deformation within the PVS. Alternatively, the predicted negative radial displacement might be an artifact of modelling the brain tissue with a Poisson’s ratio of 0.45-0.5. While this range of Poisson’s ratio might be
adequate to simulate the elastic behavior of the brain under compression, the brain behaves like a solid with a Poisson’s ratio of 0.3 under tension (Budday et al. 2019).

The measured displacement of the brain tissue and its relation to the displacement of the arterial wall strongly suggests that the brain tissue can deform due to pressure changes in the PVS of penetrating arterioles. This is in-line with the prediction of our fluid-structure interaction models in chapter 3. The simulations and the experiments indicate that the deformation of the soft brain tissue plays a major role in fluid flow in the CNS, and therefore the deformability of the brain tissue should be included in models of fluid flow in the CNS.
Chapter 5:
The effect of REM sleep on fluid exchange with the subarachnoid space

5.1 Introduction

The role of sleep and the different stages of sleep in maintaining the health and homeostasis of the CNS has received a great deal of interest from researchers of all branches of neuroscience. Sleep is a state of mind and body marked by the lack of wakefulness and consciousness and can be broadly divided into of two distinct states, rapid eye movement (REM) sleep and non-REM (NREM) sleep. While it is well established that lack of sleep negatively affects learning and memory (Tononi and Cirelli 2014; Foster and Wilson 2006), development (Campbell and Feinberg 2009), decision making (Greer, Goldstein, and Walker 2013) and emotional responses (Goldstein and Walker 2014), the physiological processes underlying the restorative effects of sleep and the role of REM and NREM stages in the restorative process of sleep are not understood.

In mice (the model animal of this thesis), the REM and NREM sleep states can be distinguished from each other and from the awake state based on the neural activity in different regions of the brain, muscle tone and whisker movement. An example of the classification of the three states (awake, REM and NREM) based on the local field potential (LFP) in the hippocampus, whisker movement and neck muscle electromyogram (EMG) is shown in Fig. 5.1a (Turner et al. 2020). As the example in Fig. 5.1a demonstrates, sleep in mice is highly fragmented (compared to sleep in humans Fig. 5.1b), and mice switch rapidly between the three states, with a bout of REM sleep occurring roughly once every 10 to 20 mins (Weber and Dan 2016). Although the transitions between the three
states are seemingly random, an analysis of the transition probabilities reveals that transitions from the awake state to REM sleep is highly unlikely (Perez-Atencio et al. 2018) and therefore REM sleep, when it occurs, usually follows NREM sleep, except in cases of extreme sleep deprivation and narcolepsy (Saper et al. 2010).

Figure 5.1: Sleep in mice is highly fragmented and sleep/wake states can be identified by EMG, whisker movement and LFP

a. The bar on the top is a hypnogram identifying the sleep/wake states during a sample, 10-minute recording in mice. The measurements of EMG power, whisker angle, and hippocampal LFP power and the corresponding sleep/awake states are shown here. The awake state is characterized by a large amplitude EMG signal, arbitrary whisker movements and low power in the LFP signal. The sleep states in contrast have a lower EMG signal and a higher power across certain frequencies in the hippocampal LFP. REM can be differentiated from NREM based on rhythmic whisker movements and a low power in the delta band (1-4Hz) of the hippocampal LFP. Modified from Turner et al.

b. A comparison of typical hypnograms in humans and mice shows that sleep in mice is highly fragmented, with rapid transitions from NREM to REM occurring in 10-minute intervals. Modified from Weber and Dan, 2016.
One theory about the restorative function of sleep is that sleep enhances the clearance of metabolites from the brain parenchyma (Xie et al. 2013; Jessen et al. 2015; Kress et al. 2014). Xie et al. (Xie et al. 2013) injected fluorescent tracers into the cisterna magna of mice and observed the penetration of dye into the brain parenchyma while the mice were asleep, awake, or anesthetized (Fig.5.2a). Surprisingly, the dye penetration significantly increases during the sleep state (Fig. 5.2b). They also used real-time iontophoresis with tetramethyl ammonium to measure the extracellular volume fraction and the tortuosity of the extracellular space (Odackal et al. 2017). These measurements showed that, compared to the awake state, sleep significantly increased the brain extracellular volume without changing the tortuosity of the extracellular space. These results have been used by some researchers as an argument against the idea that diffusion is the main mode of transport in the brain parenchyma and in support of directed flow in the proposed “glymphatic pathway” (Jessen et al. 2015; Kress et al. 2014).

**Figure 5.2: Increased dye penetration into the brain parenchyma following cisterna magna injections in sleeping mice**

Taken from Xie et al., Science 2013

A. Schematic of the dye infusions and imaging set-up of Xie et al.. FITC dextran was injected into the cisterna magna of sleeping mice and the cerebral cortex was imaged under a two-photon microscope for 30 mins. The mice were woken up and Texas red dextran was infused to compare the dye penetration in the awake and sleep states.

B. The dye penetration in awake (orange) and sleep (green) states. The arrow points to a penetrating arteriole, around which the dye penetration is maximum.

The proponents of the glymphatic hypothesis have not considered the possibility of sleep-linked metabolite clearance being driven by the arterial dilation dynamics observed during sleep. A major feature of sleep, especially REM sleep, is a sustained
dilation of cerebral arteries. This phenomenon has been observed indirectly, as change in cerebral blood volume in humans (Braun et al. 1997; Townsend, Prinz, and Obrist 1973) and in mice (Bergel et al. 2018). More recently, Turner et al. (Turner et al. 2020) directly measured the changes in the blood volume and diameter of pial and penetrating arterioles in the barrel cortex of mice during wake, REM and NREM states. It was observed that compared to the wake state, NREM sleep engenders large amplitude oscillations in arterial diameters, while REM sleep causes slow and sustained arterial dilations. Based on these observations of arterial dynamics during sleep and the results of our fluid-structure interaction simulations in chapter three, which showed that the amplitude and frequency of arteriolar diameter changes can have a large impact on fluid exchange between the paravascular space (PVS) and the subarachnoid space (SAS), we propose that the increased dye penetration into the brain parenchyma following cisterna magna injections in sleeping mice (compared to wake mice) is driven by sleep-linked arterial dilation dynamics and the change in extracellular volume.

In this chapter, mathematical models to test the hypothesis of enhanced fluid and metabolite transport in the brain of sleeping mice via enhanced fluid exchange between the subarachnoid space (SAS), the paravascular space (PVS) and the brain extracellular space (ECS) are presented. The mathematical models that simulate sleep-driven fluid exchange in the CNS are presented in two parts in this chapter. In the first part, 3D fluid-structure interaction models that compare the fluid exchange between the PVS and the SAS in the cerebral cortex of mice during awake and sleep states are presented. The 3D models in this chapter are presented as an improvement over the axisymmetric models of chapter three, to better understand the fluid flow in the SAS and its interaction with fluid in the PVS. In the second part, we present the theoretical framework for poroelasticity that can be used in future models to study transport in the brain extracellular space (ECS)
along with the transport in the PVS and the SAS. This poroelastic framework could be applied to directly model the effect of increased extracellular volume (and corresponding increase in ECS permeability) in the brain parenchyma and therefore would be better suited to study the effect of sleep on diffusive and convective transport in the CNS, compared to the fluid-structure interaction framework.

5.2 Materials and methods

5.2.1 Vessel dynamics of awake and sleep states

The changes in the arteriole diameters and cerebral blood volume of the barrel cortex (the region of the somatosensory cortex that receives sensory input from the whiskers in mice) and the simultaneous movements of the whiskers in the awake and sleep states have been measured by Turner et al. (Turner et al. 2020). In their study, to monitor hemodynamics, mice were implanted with a thin-skulled window covering the barrel cortex. To monitor the arousal state, electrodes were implanted in the cortex and in the hippocampus, and electromyography (EMG) electrodes were implanted in the neck (Fig. 5.3a). These mice were head-fixed in a tube under an intrinsic optical imaging setup to measure the reflectance of the cortex to green (530 nm) light (Fig. 5.3b) for estimating changes in the hemoglobin concentration (Huo, Gao, and Drew 2015; Winder et al. 2017; Huo, Smith, and Drew 2014; Mayhew et al. 1996), or under a two-photon microscope, where the images of cerebral arterioles were collected following a retro-orbital injection of FITC dextran. The images of reflectance of the cortex or the arteriole segments were collected simultaneously with recordings of local field potential (LFP) with cerebral and hippocampal electrodes, neck muscle EMG, and videos of whisker position. A sample of the simultaneous recordings of EMG, whisker angle and the LFP in the hippocampus are shown in Fig. 5.1a. The recordings of the LFP, EMG and the whisker movement were used
to categorize the awake, NREM sleep and REM sleep states. A subset of the total data was used to manually classify the sleep/awake states and was used as the training data set to a random forest classifier algorithm. The rest of the data was classified into awake, non-REM sleep and REM sleep phases by the trained classifier. Turner et. al., reported that once habituated to the experimental setup, the mice rapidly transitioned between the awake and sleep states. Turner et al. collected over 350 hours of data from 14 mice using their experimental setup and reported that in total, mice spent 56% of the time awake, 38% of the time in NREM state and 6% of the time in REM state.

Based on the categorization of the experimental recordings into periods of awake, NREM and REM states, Turner et al. identified specific patterns in arteriolar diameters in the barrel cortex during these stages. An example of the changes in cerebral hemoglobin in the barrel cortex, during the different states in a 10-minute recording is shown in Fig. 5.3d. The baseline diameter of arterioles and the baseline cerebral hemoglobin concentration were calculated as the average diameter during an extended period of rest (no whisking) in the awake state. During the awake state, they observed that the arterioles in the barrel cortex dilated in response to whisker movement and fidgeting (fidgeting, or body movements of wake mice in an enclosed tube lead to slow vasodilation in the entire somatosensory cortex (Drew, Winder, and Zhang 2019; Winder et al. 2017)). In general, the awake state was accompanied by 10-20% changes in vessel diameter, occurring at a frequency of 0.1-0.3 Hz, depending on the level of whisking/fidgeting. During NREM state, continuous oscillations in the arteriolar diameter, with the diameter changing by 30-40% from the baseline were observed. REM sleep, when it occurred, drove slow and sustained dilations in the arterioles with diameters changing by upto 50% from the baseline. The dilations observed during the sleep states were followed by rapid transitions to the baseline diameter when the mice woke.
In this chapter, we want to implement 3D fluid-structure interaction models to study the effect of arterial diameter changes on the fluid-exchange between the PVS and the SAS during the awake and sleep states. Based on the fluid-structure models in chapter three, we expect the large amplitude, sustained (low frequency) dilations of arteries observed during REM sleep to have the biggest impact on fluid exchange between the SAS and the PVS. Therefore, in the simulations here, we compare the fluid exchange

Figure 5.3: Changes in cerebral hemoglobin concentration measured in awake and sleep states.

a. Schematic showing the implantations on mice, showing bilateral PoRTS windows, cortical and hippocampal stereotrodes for measurement of LFP and electrodes for the measurement of neck EMG.

b. Schematic showing parts of the experimental setup used for measuring the reflectance of the cortical surface.

c. A sample image showing the barrel cortex in the left and right hemisphere (marked in circles). The images were collected using a setup similar to b.

d. Hemoglobin concentration changes in the regions shown in c. NREM sleep is accompanied by large oscillations in the hemoglobin concentration, while REM sleep is accompanied by slow and sustained increase in cerebral hemoglobin. The transitions from sleep to awake state is accompanied by sharp decline in the cerebral hemoglobin (green ellipses).

Modified from Turner et al.
driven by arteriolar diameter changes representative of a bout of REM sleep with the arteriolar diameter changes representative of the wake state for the same time period.

For the simulations representing REM sleep, the arteriolar wall movements were prescribed based on the average arteriolar diameter changes measured under 2PLSM in 6 mice (Turner et al. 2020). From the data provided by Turner et al., it was found that the arteriole diameter changes during the first 70 seconds of REM sleep and in a 50 second period in the transition from REM sleep to the awake state followed a typical waveform (Fig. 5.4a-b). The combination of these waveforms, creating a 120-second-long waveform of arteriolar diameter changes was chosen to represent REM sleep. To create a self-contained time-dependent problem, we need the arteriole diameter in the model to be at the baseline state at the beginning and the end of the simulation. However, REM sleep is almost always preceded by NREM sleep (Perez-Atencio et al. 2018; Turner et al. 2020) and arteriole diameters did not have a typical waveform during NREM. Therefore, the 120-second-long waveform of arteriolar diameter changes representing sleep was padded by a 20 second period in the beginning where the arteriole diameter smoothly transitions from the baseline value to the value at the onset of REM. Similarly, a 10-second-long smooth transition of arteriolar diameter to the baseline value was added at the end, creating a 150-second-long waveform of arteriolar diameter changes representing a bout of REM sleep preceded and succeeded by the awake-and-resting state (Fig.5.4c).

The arteriole diameters in the barrel cortex, during the awake state change dynamically depending on whisker stimulation, volitional whisking and fidgeting behaviors (Turner et al. 2020). Using the data for whisker movement and the arteriole diameter changes during the awake state, Turner et al., calculated the mean response of arteriole diameter for a brief (1-5-second-long) whisking event (Fig.5.4d). We used a convolution of the mean response function with a randomly generated Poisson processes (Fig.5.4e)
to create generalized patterns of arteriole diameter changes for cases where the mice whisk for 10, 15 and 20% of the time in a 150-second-long trial (Fig. 5.4f). A trial size of 150 seconds was chosen so as to make a direct comparison between the fluid exchange during the awake and sleep states. It should be noted that it is highly unusual for mice spend 15-20% of the time whisking. A behavioral analysis of 12 mice that were habituated to their surroundings revealed that the median fraction of time spent in voluntary whisking is around 7% (Winder et al. 2017). Therefore, the case of whisking for 10% is a realistic representation of the awake state in general.

![Figure 5.4: Vessel diameter patterns used as boundary conditions for fluid-structure interaction models](image)

**a.** The mean diameter changes (n=6 mice) of arterioles in the barrel cortex during REM sleep. The REM sleep starts with the orange box. The narrow lines indicate the 95% confidence interval.

**b.** REM Wake

**c.** The mean diameter changes (n=6 mice) of arterioles in the barrel cortex when the mice wake up from REM sleep. The onset of the wake state is indicated by the blue box.

**d.** The mean impulse response (n=6 mice) of arterioles in the barrel cortex for 1-5 seconds of whisking. The onset of whisking is shown by the green rectangle. The narrow lines indicate the 95% confidence interval.

**e.** A sample, random Poisson process that represents a typical whisking pattern.

**f.** Representative awake state vessel diameter changes for 10%, 15% and 20% of the time spent whisking shown in blue, green and black respectively.

Note: Data for a, b and d were obtained from Turner et al., 2020. The 95% confidence intervals are shown to provide a sense of variability in the diameter changes across animals. All values are shown as percentage of resting diameter.
5.2.2 3D geometry and finite element mesh

The 3D model here was built to represent the PVS and the brain parenchyma in the barrel cortex of mice, which was the region of the cerebral cortex where Turner et al. measured changes in the vessel diameters. A 3D geometry was created of an arteriole passing through the SAS and penetrating the brain parenchyma using Autodesk Inventor (Fig. 5.5a). The actual arteriole was not included in the geometry, as we do not model the arteriole explicitly, but use the arteriole diameter changes as a boundary condition (as done in the simulations in chapters two and three). The geometry includes two distinct domains, a fluid domain representing the PVS and the SAS, and a solid domain representing the brain parenchyma. The pial segment of the arteriole is parallel to the y axis and it enters the brain tissue along the z axis. All the dimensions were taken to be typical values in the cerebral cortex of mice. The arteriole diameter of the arteriole at the pial surface (z>150µm) was 20µm (Turner et al. 2020; Kedarasetti et al. 2020). As the arteriole penetrated into the brain parenchyma, its diameter was reduced to 15 µm at 150 µm below the brain surface, because the baseline diameter of arterioles decreases as they penetrate deeper into the parenchyma (Gao, Greene, and Drew 2015). A section of brain parenchyma of dimensions 160 µm x 200 µm x 150 µm in the x, y and z directions respectively was created. The z extent of the subarachnoid space was 50 µm. The width of the PVS in the penetrating section of the arteriole was decreased as a function of distance from the pial surface, from 9 µm near the surface to 5.5 µm at 150 µm below the surface. The width of the PVS used here was larger than the values typical of the PVS typically seen around penetrating arterioles in the mouse cortex (Iliff et al. 2012; Schain et al. 2017). A larger PVS was required because the large vessel dilations during REM sleep created problems with numerical convergence with smaller sized PVS. The problems in
numerical convergence appeared because for large deformations of the fluid mesh, the Jacobian determinant of the deformation gradient can reach negative values, the latter being an inadmissible condition signaling the failure of the calculation (Wick 2011; Narain, Samii, and O’Brien 2012). Implementing a biharmonic model for mesh motion (Wick 2011) did not resolve the convergence issues. Smooth fillets were used to smoothen all the sharp transitions in the geometry. To save computational resources only half of the volume was explicitly included in the models, while the effect of the other half was simulated using symmetry boundary condition along the x-z plane of the model.

A mesh was generated using Altair Hypermesh software on the 3D geometry created (Fig. 5.5b). The mesh consists of 74,183 tetrahedral elements with 37,417 elements in the fluid domain and 36,766 elements in the solid domain. The mesh size was maintained to be 1.5–2 µm at the regions of transition from the solid to fluid domain and at all boundaries of the fluid domain and gradually increased in size to capture the variables of interest more accurately in the regions of transition. The minimum angle of the triangular faces was maintained to be 10° and the maximum angle was 150°. The tet-collapse, which is an indicator of the minimum height of the tetrahedron in relation to the base, was kept above 0.15. The maximum aspect ratio of elements was 8.08. The maximum length of the elements was 29.8 µm, occurring far from the regions of

Figure 5.5: Geometry and mesh used for 3D simulations of fluid-structure interaction
intersection between the domains. The mesh was converted to a format that can be imported into COMSOL multiphysics using a python script.

5.2.3 Model assumptions and parameters

The fluid flow is simulated by the incompressible Darcy-Brinkman flow equations and the brain tissue elasticity is simulated by compressible Saint-Venant Kirchhoff’s model. The mesh motion is simulated by a harmonic model. We solve for the fluid velocity ($v_f$), pressure ($p_f$) and mesh movement ($u_m$) in the fluid domain, whereas we solve for the tissue displacement ($u_s$) and velocity ($v_s$) in the solid domain. The governing equations of the fluid-structure interaction problem, and the continuity conditions for displacement, velocity and boundary forces (traction) at the interface of the two domains are as described in chapter 3. The boundary conditions are described below.

Symmetry boundary conditions are applied at $x = 0$ and $x = 80 \mu m$ (Eq. 5.1).

$$
\begin{align*}
\text{at } x = 0, x = 80: \quad & v_{fx} = 0, u_{mx} = 0, u_{sx} = 0, v_{sx} = 0
\end{align*}
$$

The fluid velocity is set to zero at the top of the SAS ($z = 200 \mu m$), which represents the dural surface (Eq.5.2).

$$
\begin{align*}
\text{at } z = 200: \quad & v_f = 0
\end{align*}
$$

A small pressure-like traction (Eq.5.3) with a magnitude of 0.01mmHg ($p_1$) is applied to the left end of the SAS ($y = -100 \mu m$). This, combined with the zero traction (Eq.5.4) at the right end ($y = 100 \mu m$) of the SAS generated a directional flow in the SAS, with a mean flow speed of 20 $\mu m/s$, comparable the CSF flow velocities observed in vivo (Mestre, Tithof, et al. 2018).

$$
\begin{align*}
\text{at } y = -100: \quad & \sigma_f \cdot n = -p_1 n
\end{align*}
$$
In Eqs. 5.3-5.4, $\sigma_f$ is the Cauchy stress of the fluid and $n$ is the unit outward normal.

The displacement of the arteriolar wall (shown in green in Fig. 5.5b) is prescribed in two sections, one for the arteriole on the pial surface ($z > 150\mu m$), and one for the penetrating section of the arteriole ($z \leq 150\mu m$). For the pial section of the arteriole, the wall movement is given simply in the direction of the outward normal (Eq. 5.5). For the tapered, penetrating section of the arteriole, the wall movement is prescribed in the $x$ and $y$ directions (Eq. 5.6).

\[
on \text{on arteriolar wall, for } z > 150, \quad u_m = -int1(t)R_0 n \tag{5.5} \]

\[
on \text{on arteriolar wall, for } z \leq 150, \quad u_{mx} = int1(t)x, \quad u_{my} = int1(t)y, \quad u_mz = 0 \tag{5.6} \]

In Eqs. 5.5 and 5.6, $int1(t)$ is a cubic spline interpolation of the waveforms shown in Fig. 5.4c and Fig. 5.4e respectively and $R_0$ is the resting radius of the arteriole (20$\mu m$) at the pial surface. A cubic spline interpolation was used for the diameter changes so that the waveforms and their time derivatives were smooth.

The fluid permeability of the PVS is taken to be $2 \times 10^{-14} m^2$, and the permeability of the SAS is $2 \times 10^{-12} m^2$. A relatively high value permeability was assigned to the SAS, because the SAS in our model represents a combination of the actual SAS and the PVS of the pial arteriole, and experiments suggest that the PVS of the pial arteriole has infinite Darcy permeability (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017; Thomas 2019). The transition in the value of permeability was achieved through the step function in COMSOL multiphysics, which provides a smooth transition with continuous first and second order derivatives, with a transition zone between $z = 145\mu m$ and $z = 155\mu m$. The elastic modulus of the brain tissue was taken to be 2 kPa. The density of both the
tissue and the fluid were taken to be 1000 kg/m$^3$. The flow resistance of the brain tissue was modelled by a lumped resistance (flow rate dependent pressure or a Robin boundary condition) at $z = 0$. The flow resistance of the brain tissue was assumed to be 10 times the flow resistance of an annular tube with inner diameter 15µm and outer diameter 28µm (the same dimensions as the PVS at $z = 0$) and length 150µm.

5.2.3 Model implementation

The equations were transformed into their weak, component form using a custom Wolfram Mathematica notebook and the model was implemented using COMSOL multiphysics. The problem was implemented in ALE coordinates. Time integration was setup using backward difference formula (BDF) with a maximum time step of 0.004 seconds. The order of BDF was adaptive with minimum order set to 1 and maximum order set to 5. MUMPS (MUltifrontal Massively Parallel Sparse direct Solver) was used as the direct solver. The problem had 666,532 degrees of freedom. Computations were performed on the Pennsylvania State University’s Institute for Computational and Data Sciences’ Roar supercomputer, with 8 cores and 64 GB of memory per core. The outputs of the 150-second-long simulations were saved in 15 parts containing consecutive 10-second-long intervals using the Java application programming interface (API) with COMSOL multiphysics. The fluid velocity, mesh displacement and solid displacement values at the grid points were exported to text files at every 0.05 seconds. These files were processed using a custom MATLAB script to calculate the trajectories of fluid particles in the SAS and the PVS for 150-second-long simulations. The details of particle tracking in ALE are given in Appendix A. The fluid particles in the PVS were generated so that they were equally spaced at the beginning of the simulation. A fluid exchange fraction was calculated as the ratio of the number of PVS particles exchanged with the SAS compared to the initial number of particles.
5.3 Sleep-linked arteriolar dilation dynamics drive higher fluid exchange compared to awake state dynamics

The 150-second-long simulations of PVS in the barrel cortex, representing the arteriole dilation dynamics of REM sleep and awake state were performed as described in the previous section. All the simulations were performed with the same parameters and boundary conditions except for the arteriolar wall motion. The simulations were performed with only one set of parameters in spite of the uncertainty in the PVS width, permeability and shear modulus of the brain tissue because the problem is computationally expensive. The results of the simulations clearly show that REM sleep drives appreciably higher fluid exchange between the PVS and the SAS. REM sleep drove 24.88% fluid in the PVS to exchange with the SAS, compared to 10.85%, 15.31% and 17.54% of PVS fluid exchanges in the awake state where the mice spend 10, 15 and 20% of the time whisking respectively. The difference in the fluid exchange is also apparent from the fluid trajectories in Fig.5.6. The fluid trajectories in Fig.5.6 are plotted in the coordinates of the undeformed PVS and the brain tissue, which are also called the computational coordinates.

The fluid exchange percentage observed in these calculations is lower than what was obtained in our axisymmetric calculations in chapter three, even though the arteriolar diameter changes are larger in this chapter. There are several reasons for this difference. The width of the PVS here was assumed to be 9µm, while in chapter three it the default value used was 3µm. The fluid exchanged between the PVS and the SAS is lower for a wider PVS, as described in section 3.3.3 and Fig.3.10b. The fluid was assumed to be
exchanged with the SAS as soon as it left the PVS in the calculations of chapter three, while here, fluid can return to the PVS due to a finite CSF flow velocity in the SAS.

It is important to note that there are several limitations to the fluid-structure interaction models presented. The models do not explicitly simulate transport through the brain parenchyma or the elastic response of the fibrous connective tissue in the PVS. Because the transport through the parenchyma is not explicitly simulated, the models here do not include the effect of increased extracellular space observed during sleep (Xie et al. 2013). The models here are also restricted in the size of the PVS that can be simulated. Most of these problems can be addressed by the use of poroelastic models based on mixture theory (Costanzo and Miller 2017), where the fluid and solid phases are modeled as a mixture occupying the same space. The diffusive and convective transport through the parenchyma and the changes in the extracellular volume can be directly included in
Poroelastic models. Poroelastic models can also simulate the elasticity of the connective tissue in the PVS. The solid part of the PVS could be modeled to be just as stiff as the brain when the fluid volume fractions in the brain and the PVS are comparable, thereby limiting the extent to which the PVS can be compressed, and eliminating the problems with numerical convergence. In the next section, we will discuss the progress made in the development of poroelastic models and some aspects of its application to the fluid and metabolite transport in the CNS.

5.4 Poroelastic theory

Here, we examine the basic concepts of mixture theory and their application to poroelasticity. A more detailed explanation of the development of the theory discussed here is provided by Costanzo and Miller (Costanzo and Miller 2017). Consider a mixture of an incompressible solid and an incompressible fluid. The true densities of the solid and fluid components are $\rho_s^*$ and $\rho_f^*$ respectively. The volume fraction of the solid and fluid phases at any given time are $\xi_s$ and $\xi_f$ respectively, and their sum is equal to unity ($\xi_s + \xi_f = 1$) indicating that the mixture is fully saturated with the fluid. As per the conventional method of solving problems involving deforming solids, all the equations here will be written in the coordinates of the undeformed solid. This reference coordinate system can be treated as an arbitrary Lagrange Eulerian (ALE) for the fluid and for the mixture as a whole. The deformation of the solid is defined by a continuous field of displacement $u_s$. The deformation gradient and the Jacobian determinant in the reference configuration of the solid are given by Eq.5.7.

$$F_s := I + \nabla u_s; \quad J_s := \det F_s$$ 5.7
Using Eq. 5.7, the volume fractions and the apparent densities of the solid and fluid components are given by Eqs. 5.8-5.9, in relation to \( \xi_s^R \) is the volume fraction of the solid in the undeformed or reference configuration, \( \xi_s^s \).

\[
\xi_s = \frac{\xi_s^R}{J_s}; \quad \xi_f = 1 - \xi_s = \frac{J_s - \xi_s^R}{J_s}
\]

\[
\rho_s = \rho_s^* \xi_s = \frac{\xi_s^R}{J_s} \rho_s^*; \quad \rho_f = \rho_f^* \xi_f = \left(1 - \frac{\xi_s^R}{J_s}\right) \rho_f^*
\]

The primary unknowns for the problem of an incompressible mixture of solid and fluid are:

1. The solid displacement, \( u_s \)
2. The solid velocity, \( v_s \)
3. The fluid velocity, \( v_f \)
4. The pressure, \( p \)

For flow through a porous solid, we use the Darcy model for the fluid flow. The equations of momentum and continuity for the components of the mixture, in the coordinates of the undeformed solid are written as follows (Costanzo and Miller 2017).

\[
\frac{\partial u_s}{\partial t} - v_s = 0
\]

\[
\frac{\xi_s^R \rho_s^*}{J_s} \left( \frac{\partial v_s}{\partial t} - b_s \right) + \xi_s^R F_s^{-T} \nabla p - \left(J_s - \xi_s^R\right)^2 \frac{\mu_f}{J_s k_s} (v_f - v_s) - \nabla P_s = 0
\]

\[
(J_s - \xi_s^R) \left[ \rho_f^*(\frac{\partial v_f}{\partial t} + \nabla v_f F_s^{-1}(v_f - v_s) - b_f) + F_s^{-T} \nabla p + \left(J_s - \xi_s^R\right) \frac{\mu_f}{J_s k_s} (v_f - v_s) \right]
- \nabla P_f = 0
\]

\[
F_s^{-T} : \nabla \left( \frac{\xi_s^R}{J_s} v_s + \left(1 - \frac{\xi_s^R}{J_s}\right) v_f \right) = 0
\]
\[ P_f = J_s \mu_f (\nabla \mathbf{v}_f \mathbf{F}_s^{-1} + (\nabla \mathbf{v}_f \mathbf{F}_s^{-1})^T \mathbf{F}_s^{-T} \]  

5.14

The parameters \( \mu_f \) and \( k_s \) above are the dynamic viscosity of the fluid and the fluid permeability of the porous solid respectively. \( P_s \) and \( P_f \) are the first Piola-Kirchhoff stresses of the solid and fluid phases respectively. The term \( P_f \) is absent in the poroelastic model of Costanzo and Miller because they did not consider the fluid viscous effects. It should be noted that for Darcy-Brinkman model of fluid flow, which is appropriate for fluid flow in the PVS and the SAS, an additional stress term that includes the gradient of relative velocity between the fluid and solid phases \( (\nabla (\mathbf{v}_f - \mathbf{v}_s)) \) should be used (dell'Isola, Madeo, and Seppecher 2009). The body force density for the solid and fluid phases (gravitational forces, for example) are given by the vectors \( \mathbf{b}_s \) and \( \mathbf{b}_f \) respectively. The colon (:) in Eq.5.13 is the inner product of two tensors. Eq.5.13 is the constraint of incompressibility and, for the ease of numerical implementation, can be recast by introducing an extra variable, which we call the spatial average velocity, defined by Eq. 5.15.

\[ v_{spc} := \frac{1}{J_s} (\xi^R_s \mathbf{v}_s + (J_s - \xi^R_s) \mathbf{v}_f) \]  

5.15

This simplified incompressibility constraint is given by Eq.5.16.

\[ F_{s}^{-T} \cdot \nabla v_{spc} = 0 \]  

5.16

Including the spatial average velocity, we now have five variables (four vector quantities and one scalar quantity) to solve for with five equations (Eqs.5.10-5.12, 5.15-5.16).

The choice of polynomial interpolation for each of the variables for their finite element implementation and their rates of convergence using the method of manufactured solutions were examined by Costanzo and Miller. The method of manufactured solutions is a way to test the finite element implementation of a formulation by comparing the finite
element solution to a known, exact solution. A known solution in the form of continuous variables is chosen for each of the variables and the corresponding body force densities and boundary conditions are calculated. A finite element model is then implemented with the obtained boundary conditions and forces. The solution of the finite element model (which is usually a polynomial interpolation) is then compared with the known solution to estimate the accuracy of the solution. Finally, the problem domain is discretized with finer elements to ensure the convergence of the solution.

We are interested in applying the formulation of poroelasticity to time-dependent finite element models to simulate fluid and metabolite transport in the combined system of the PVS, the SAS and the brain extracellular space. I examined two aspects for this implementation, namely the possible choices of constitutive models for the strain energy and the implementation of jump conditions. The constitutive model was chosen so that stable and unique solutions can be obtained for time-dependent problems that start with an undeformed reference configuration. The jump conditions are necessary because there is a discontinuity in the volume fraction of the solid at the interface between the mostly fluid filled spaces (PVS and SAS) and the brain parenchyma, where the extracellular fluid occupies roughly 15-20% of the volume (Tønnesen, Inavalli, and Nägerl 2018; Korogod, Petersen, and Knott 2015).

5.4.1 Choice of constitutive models

To study the appropriateness of constitutive models for time dependent problems, we need to consider a simple problem in poroelasticity, which also has a potential for experimental validation. I considered the case of consolidometer (or oedometer) experiments. In these experiments, a sample of tissue saturated with extracellular fluid, is placed in a practically rigid mold (a ring made of steel), between two porous plates that allow free drainage of water from the top and bottom faces (schematic in Fig.5.7a). The
bottom plate is fixed to the apparatus and the top plate is loaded with a weight. The
displacement of the top plate is measured at fixed intervals of time from loading and the
experiment is repeated with different weights. The consolidometer experiments are used
to determine the permeability, elastic modulus and the change of apparent elastic modulus
with consolidation (increased solid volume fraction as the sample is compressed). Such
experiments have been used to study the properties of human brain tissue (Franceschini
et al. 2006) and articular cartilage (Oloyede and Broom 1991; Oloyede et al. 2004).

A 2D finite element model, equivalent to the consolidometer experiments (Fig.
5.7b) was created. A rectangular domain containing a mixture of an incompressible solid
and fluid with a width of 1mm and a height of 5mm was subjected to the following boundary
conditions to emulate consolidometer experiments. A zero-displacement boundary
condition was applied perpendicular to the side walls and the bottom wall for the solid
phase (Eqs.5.17-5.18).

\[
\begin{align*}
for \ x = 0 \ and \ x = 1, \quad u_{sx} &= 0, \quad v_{sx} = 0 \\
for \ y = 0, \quad u_{sy} &= 0, \quad v_{sy} = 0
\end{align*}
\] 5.17 5.18

A pressure like traction was applied to the solid phase on the top wall. The amplitude of
the traction force was ramped up from a zero value to a value of \( p_1 \) over a period of 0.5
seconds using the step function in COMSOL Multiphysics (Eq.5.19).

\[
\begin{align*}
for \ y = 5, \quad (P_s - \xi_s p F^{-T})n &= \xi_s p_{in} F^{-T} n, \text{ where } p_{in} = p_1 \text{ step(t)} \quad 5.19
\end{align*}
\]

For the fluid, a no-slip boundary condition was applied on the side walls (Eq.5.20), and no
traction was applied to the top and bottom phases to simulate free drainage conditions
(Eq.5.21).

\[
\begin{align*}
for \ x = 0 \ and \ x = 1, \quad v_f &= 0 \quad 5.20
\end{align*}
\]
for \( y = 0 \) and \( y = 5 \), \( (P_f - (J_s - \xi_s^R)pF_s^T)n = 0 \)  \[5.21\]

The parameters used in the model are given in Table 5.1. The mesh is shown in Fig.5.7b. The time stepping was implemented by a backward difference formula with a minimum order of 2. The maximum time step was set to 0.01 seconds.

I first considered an incompressible elastic constitutive model for the strain energy of the porous solid component. The first Piola-Kirchoff stress is given in this case by Eq. 5.22, where \( \mu_s \) is the elastic modulus of the solid.

\[
W_s^1 = \frac{\mu_s}{2} (Tr[F_sF_s^T] - 3) \Rightarrow P_s = \xi_s^R \mu_s F_s \tag{5.22}
\]

This constitutive model did not yield stable solutions for the problem. The pressure solution diverged rapidly even when \( p_1 \) was assumed to be zero. It is interesting that the same choice of constitutive model yielded stable and convergent solutions for the problems considered by Costanzo and Miller (Costanzo and Miller 2017). This anomaly is probably because Costanzo and Miller did not start their time-dependent problems starting from an undeformed or zero-load situation, while our problem starts from undeformed state and slowly ramps up the load.

Table 5.1: Parameters for 2D consolidometer poroelastic model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu_s )</td>
<td>80</td>
<td>kPa</td>
</tr>
<tr>
<td>( \mu_f )</td>
<td>0.001</td>
<td>Pa s</td>
</tr>
<tr>
<td>( k_s )</td>
<td>(2 \times 10^{-15})</td>
<td>m²</td>
</tr>
<tr>
<td>( \rho_s )</td>
<td>1000</td>
<td>Kg/m³</td>
</tr>
<tr>
<td>( \rho_f )</td>
<td>1000</td>
<td>Kg/m³</td>
</tr>
<tr>
<td>( \xi_s^R )</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>( p_1 )</td>
<td>1000</td>
<td>Pa</td>
</tr>
</tbody>
</table>

I modified the constitutive model to one of compressible elasticity. The constitutive model and the elastic stress of the form given in Eq.5.23 yielded stable results.
\[ W_s^2 = \frac{\mu_s}{2} (\text{Tr}[F_sF_s^T] - \log f_s) \Rightarrow P_s = \mu_s \xi_s^R (F_s - F_s^{-T}) \]

5.23

The fluid flow patterns were similar to those observed during consolidometer experiments, with fluid flow dropping with time (Fig. 5.7c). The displacement at the top surface also follows the pattern expected from consolidometer experiments, changing rapidly as soon as the load is applied and slowly reaching an asymptotic value (Fig. 5.7d). It might seem paradoxical to employ a compressible constitutive model for a mixture of two incompressible components. This concept has been examined from a theoretical standpoint for the elasticity of swollen rubber, saturated and in equilibrium with the surrounding fluid (Treloar 1975). Treloar also provided a physical interpretation to justify the choice of a compressible constitutive model. When incompressible rubber, in its pure state is subjected to a hydrostatic pressure, there is no change in the volume or in the strain state. However, when the incompressible material exists in a saturated mixture with an incompressible liquid, application of hydrostatic pressure can change the volume and the liquid content of the mixture, by compressing the solid skeleton. Therefore, from a purely mathematical standpoint, incompressible solid in a saturated mixture with a liquid can be treated equivalent to a compressible solid.
5.4.2 Jump conditions

We are interested in simulating the fluid and metabolite transport through the PVS, SAS and the brain extracellular space simultaneously. These regions have varying fluid content and different structures contributing to the elastic response. For example, the PVS is mostly fluid filled and contains collagen fibers (Berliner et al. 2020), macrophages (Faraco et al. 2017) and fibroblast (Vanlandewijck et al. 2018) like cells, while the brain parenchyma is mostly occupied by neurons, glial cells and the extracellular collagen matrix. The different pore sizes and pore structures in these spaces would have a great

---

**Figure 5.7:** Results of consolidometer experiments can be replicated by a poroelastic model with a constitutive model for elasticity that is similar to compressible elasticity

- **c.** The colors show the displacement in the vertical (y) direction at t=1s and t=100s of the simulation. The magnitude of solid displacement increases while that of fluid velocity decreases with time.

- **d.** The plot shows the vertical displacement of the top plate of the consolidometer (blue line in b) with time. The pattern of the timecourse of displacement is similar to the displacements measured by Francescini et al., 2006.
impact on the fluid permeability. Moreover, experiments with fibrous networks of polymers suggest that the presence of volume conserving elements (van Oosten et al. 2019), such as cells would significantly impact the elastic properties. Therefore, to simultaneously simulate transport through the brain parenchyma, the PVS and the SAS, we need a formulation that accounts for interaction of porous mixtures with different properties. We refer to the boundary conditions at the interface of the porous mixtures as jump conditions, because these boundary conditions occur at an interface with a jump in physical properties, rather than a smooth and gradual transition.

Here, we present the jump conditions at the interface of two domains in a model, one domain representing a mixture of solid and fluid and the other domain representing an open channel, containing only fluid. The equations governing the solid displacement, solid velocity, the fluid velocity, pressure and the spatial average velocity in the mixture domain are the same as the ones presented in Eqs.5.10-5.12 and 5.15-5.16. For the fluid domain, we consider the fluid flow governed by an incompressible Navier-Stokes model. The mesh movement in the fluid domain is governed by a harmonic model (Wick 2011). The governing equations for the fluid velocity ($\mathbf{v}_c$), fluid pressure ($p_c$) and the mesh displacement ($\mathbf{u}_m$) are given in Eqs.5.24-5.27.

\[
J_m \rho_f \left( \frac{\partial \mathbf{v}_c}{\partial t} + \nabla \mathbf{v}_c \mathbf{F}_m^{-1} \left( \mathbf{v}_c - \frac{\partial \mathbf{u}_m}{\partial t} \right) - \mathbf{b}_c \right) - \nabla \cdot \mathbf{P}_c = 0
\]

\[F_m^{-T} : \nabla \mathbf{v}_c = 0\]

\[\nabla^2 \mathbf{u}_m = 0\]

\[
\mathbf{P}_c = J_m (-p_c \mathbf{I} + \mu_f (\nabla \mathbf{v}_f \mathbf{F}_m^{-1} + (\nabla \mathbf{v}_f \mathbf{F}_m^{-1}^T)) \mathbf{F}_m^{-T})
\]

The body force density on the fluid in the channel is given by $\mathbf{b}_c$. The mesh deformation gradient, $\mathbf{F}_m$ and the Jacobian determinant, $J_m$, are defined in a similar way as $\mathbf{F}_s$ and $J_s$. 
Eq. 5.7. The Navier-Stokes equations here are written in the coordinates of the computational (mesh) domain. The density ($\rho_f^*$) and dynamic viscosity ($\mu_f$) of the fluid in the channel are the same as those of the fluid in the mixture. The equations are presented in their ALE format, to be consistent with the equations of the poroelastic model.

We need to obtain jump conditions for fluid velocity and for momentum at the interface of the domains. A detailed theoretical derivation of the jump conditions was provided by dell’Isola et al. (dell’Isola, Madeo, and Seppecher 2009). Here we present the conditions derived by dell’Isola et al. for the specific case of an interface between a porous mixture and an open channel, and the finite element implementation of the boundary conditions. The jump conditions for the fluid velocity is given by the law of conservation of mass. Using the equations of kinematics and conservation of mass, dell’Isola et al. derived that the jump in filtration velocity should be zero. This leads to the condition that the fluid velocity in the channel is equal to the spatial average velocity in the mixture (Eq. 5.28).

$$v_c - v_s = \left(1 - \frac{\xi_s}{J_s}\right) (v_f - v_s) \Rightarrow v_c = v_{spc}$$  \hspace{1cm} 5.28

Intuitively, this can be understood as a continuity of divergence free fields in both the domains. In finite element models, this can be applied as a Dirichlet boundary condition at the interface, on the velocity in the open channel domain. A similar application of continuity as a Dirichlet boundary condition was also used in our fluid-structure interaction models.

The jump condition in momentum was derived by dell’Isola et al., using a combination of the law of conservation of momentum and the Rayleigh-Hamilton principle. In the computational coordinates, the jump condition is given by Eq.5.29, where $\mathbf{n}$ is the unit normal of the porous domain.
\[(P_s + P_f - J_s p F_s^T) n = P_c n + (\rho_f (J_s - \xi^R_s)(v_f - v_s).F_s^T n)(v_f - v_c) \] 5.29

The last term of Eq.5.28 is the force exerted due to the change in the velocity the fluid leaving the porous mixture. The term looks very similar to the force required to hold a fire hose due to the change in the velocity of water as it leaves the nozzle (Vera, Rivera, and Núñez 2018). However, considering the low velocities we expect for CSF flow this term will be negligible. Assuming CSF flow velocities changing in the order of 100μm/s at the interface of the PVS and the parenchyma (which is a conservative estimate), the last term of Eq.5.28 would account for a pressure difference in the order of 10^{-5} Pa, a negligible change even relative to the low elastic modulus of the brain tissue, which is in the order of 1-8kPa (Mihai et al. 2015; Budday et al. 2019; Goriely et al. 2015; Mihai et al. 2017). Therefore, in our finite element calculations, we neglect the last term and apply the traction from the channel flow on the solid and fluid phases of the porous mixture. The traction is divided between the solid and liquid phases based on the fraction of the surface occupied by each phase at the interface (Eqs.5.30-5.31).

\[P_s n = \frac{\xi^R_s}{J_s} P_c n + \xi^R_s p F_s^T n\] 5.30

\[P_f n = \frac{(J_s - \xi^R_s)}{J_s} P_c n + (J_s - \xi^R_s)p F_s^T n\] 5.31

The method of manufactured solutions was applied to create a 2D time-dependent problem. Sinusoidal functions were assumed to be the solutions for all the independent variables \((u_s, v_s, v_f, p, v_{spc}\) in the porous mixture domain and \(u_m, v_c, p_c\) for the open channel). The governing equations for the porous domain (Eqs.5.10-5.12 and 5.15-5.15) and the open channel (Eqs.5.24-5.26) were used to calculate the resulting body forces and boundary conditions from the manufactured solutions. The problem was solved using the finite element method in COMSOL multiphysics. Two domains, each a square of 1m,
sharing a common side were used with the square on the left representing the domain of
the porous mixture and the square on the right representing the domain of the open
channel. Dirichlet boundary conditions were applied on the top and bottom edges of both
domains (magenta in Fig 5.8a). The jump conditions, as described in Eqs. 5.27, 5.29 and
5.30 were applied on the common boundary (blue in Fig. 5.8a). Traction forces were
calculated based on the manufactured solutions and applied at the remaining boundaries
(green in Fig. 5.8a). A mesh of square elements with size 1/16 m was created and the
finite element was solved using a generalized-alpha time integration scheme. The
parameters used in the model are shown in Table 5.2.

Table 5.2: Parameters for method of manufactured solutions for jump conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
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</thead>
<tbody>
<tr>
<td>$\mu_s$</td>
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<td>MPa</td>
</tr>
<tr>
<td>$\mu_f$</td>
<td>1</td>
<td>Pa s</td>
</tr>
<tr>
<td>$k_s$</td>
<td>1x10^{-3}</td>
<td>m²</td>
</tr>
<tr>
<td>$\rho_s$</td>
<td>1</td>
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<tr>
<td>$\rho_f$</td>
<td>1</td>
<td>Kg/m³</td>
</tr>
<tr>
<td>$\xi^R_s$</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

The solution of the finite element model matched with the manufactured solution
for all the variables. The finite element solution of the fields $\nu_{spc}$ and $\nu_c$ and their
manufactured solutions are shown in Fig. 5.8b. The mesh size was varied using a
parameter, Mh, between 1/8 m to 1/64 m. The error between the calculated solution and
the manufactured solution were calculated by integrating the $L^2$-norm of the difference
between the fields. An example of the function for calculating error between the solid
displacement ($u_s$) and the corresponding manufactured solution ($u_s^{MS}$) in the porous
domain ($\Omega_p$) is shown in Eq. 5.32.
\[ \|e\|_{L^2}(u_s) = \sqrt{\int_{\Omega_p} (u_s - u_s^{MS}).(u_s - u_s^{MS})} \] 5.32

The logarithm of the error plotted against the mesh size parameter, h (Fig. 5.8c-d), shows that the finite element implementation of the jump conditions, along with the chosen governing equations is convergent.

**Figure 5.8:** Porous mixture and open channel with jump conditions form a convergent set of equations

**a.** The domains for porous mixture ($\Omega_p$) and the open channel ($\Omega_c$) and the finite element mesh. The interface with jump conditions is shown in blue. Dirichlet boundary conditions are applied on the magenta lines and traction boundary conditions are applied on the green lines.

**b.** The finite element solutions for $v_{spc}$ and $v_c$ (colored surface) and the manufactured solution (black grid) at $t=0.3s$ shows that the finite element solution matches the manufactures solution.

**c,d.** The $L^2$-error of the finite element solution with changing mesh size (h) in the porous mixture and the open channel domains.
5.5 Discussion

In this chapter, we examined the possible role of functional hyperemia during sleep in clearance of metabolites. We used the patterns of functional hyperemia observed during sleep and in awake mice by Turner et al., in simulations to understand how these large dilations could drive fluid exchange in the PVS. We performed 3D fluid-structure interaction simulations of PVS surrounding penetrating arterioles, surrounded by a soft brain tissue and an SAS with directional flow. Our simulations showed that the patterns of arteriolar dilations observed during REM sleep can drive appreciably larger fluid exchange between the SAS and PVS compared to arteriolar dilations during normal awake behavior. This fluid exchange could explain the difference in the dye movement between sleeping and awake mice observed experimentally (Xie et al. 2013).

It should be noted that based on the criteria used by Xie et al. (Xie et al. 2013) to identify sleep and awake states, it is possible that the mice in their experiments never entered the REM state. Xie et al. identified the sleep and awake states based on Electrocorticography (ECoG) recordings and defined wakefulness by desynchronized low-amplitude ECoG, while sleep states was identified by synchronized high amplitude ECoG dominated by low frequency waves (0-4 Hz) in artifact-free five-minute epochs. The length of the epoch chosen by Xie et al. and the observation that show low LFP power in the 0-4Hz frequencies during REM sleep (Turner et al. 2020) suggest that the mice were always in the NREM state. In our models here, a contrast between the fluid exchange between REM and awake states was simulated because of the large amplitude and sustained dilations observed during REM sleep had a better contrast with the arteriolar dilation patterns in the awake state and because the arteriolar dilations during REM sleep followed a specific pattern across different animals. For future simulations, a typical vasodilation pattern for NREM sleep should be defined and the fluid exchange between the SAS and
PVS during REM sleep should be compared to the awake state. It would also be worthwhile to repeat the experiments of Xie et al. during REM sleep.

One of the major limitations of our fluid-structure interaction models is that they do not simulate transport through the brain extracellular space, which can be overcome by using models of poroelasticity. Simulating transport through the brain extracellular space might be key to understanding the role of sleep in fluid flow and brain metabolite clearance because increase in extracellular volume is one of the changes observed during sleep (Xie et al. 2013). We made some progress towards implementing poroelastic models for simulation of fluid and metabolite transport through the combined system of the SAS, the PVS and the brain extracellular space in this chapter. We found a constitutive model for the elastic stress in a poroelastic mixture that is suited for time-dependent finite element simulations starting from the undeformed state. We also developed boundary conditions at the interface of a poroelastic mixture and an open channel. These jump conditions can be used to simulate the interface of the brain parenchyma with the fluid filled spaces of the PVS and the SAS. Further steps needed to implement finite element models of CSF flow in the parenchyma with poroelastic formulation will be discussed in chapter seven.
Chapter 6: The role of functional hyperemia in brain oxygenation

6.1 Introduction

The brain is one of the most metabolically active organs in the body and needs a constant and abundant supply of oxygen. This might be the reason why the brain receives 15-20% of cardiac output (Xing et al. 2017). Along the same line of reasoning, it has been hypothesized that the purpose of increase in local cerebral blood flow (CBF) based on neural activity (functional hyperemia), is to match the increased demand for oxygen by the metabolically active brain. This theory was supported by the observations that the baseline CBF of the whole brain changed with hyperoxia and hypoxia (Cohen, Alexander, and Wollman 1968). However, with the invention of positron emission tomography (PET) scanning technology in 1974, scientists could simultaneously measure glucose uptake, oxygen metabolism (cerebral metabolic oxygen rate or CMRO$_2$) and blood flow simultaneously, and this led to the finding that the changes in CBF were disproportionately high compared to the increases in CMRO$_2$ (nearly 30% increase in CBF was observed for 5% increase in CMRO$_2$) (Fox and Raichle 1986; Fox, Raichle, and Mintun 1988). Moreover, mathematical models of oxygen transport based on human PET scan data (Mintun et al. 2001) showed that adequate levels of oxygen could be maintained in the brain tissue without a change in the CBF.

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In this chapter, we re-examine the role of functional hyperemia in increasing oxygen supply to the metabolically active regions of the brain, using models of cerebral arterioles and oxygen transport in the brain. As in previous chapters, these models were created based on realistic anatomy of cerebral arterioles in mice and the results of the models were verified against experimental measurements of tissue oxygenation in the cerebral cortex of mice (Zhang et al. 2019). The results of the model match well with the experimental data and showed that during voluntary locomotion, the increased oxygenation of brain tissue is influenced more by respiration than by functional hyperemia. These results of these simulations, combined with the results of the chapter three, suggest the possibility that the primary purpose of functional hyperemia is the clearance of metabolic waste from active brain regions and not increasing oxygen supply to the metabolically active regions of the brain.

6.2 Experimental results

In this section, the results of the experiments by Zhang et al. (Zhang et al. 2019) that are relevant to the mathematical modeling of oxygen transport are presented. Note that these results are presented only to provide a context to the choice of boundary conditions and parameters for the models and I have not made any contributions to the experimental design, data collection or data processing presented in this section.

Zhang et al. used multiple experimental methods to measure changes in neural activity, cerebral blood volume (CBV), respiration rate and oxygenation of blood and tissue in awake and head-fixed mice on a spherical treadmill (Fig 6.1a). In all these experiments, events of locomotion were identified using a rotary encoder attached to the spherical treadmill.
Figure 6.1: Cortical oxygenation is modulated by respiration and not functional hyperemia

a. (top) Experimental setup for IOS imaging. Mice implanted with thin-skulled windows and head-fixed to spherical treadmill (bottom). Experimental setup for simultaneous measurement of respiration rate, LFP and tissue oxygenation.

b. Image of brain tissue and vasculature as seen through a thin-skulled window with the FC (blue) and the FL/HL (green) regions marked. Scale bar = 1mm.

c. The change in IOS reflectance percentage (ΔR/R₀) plotted vs percentage change in spike rate in FC (blue) and FL/HL (green) regions. The major and minor axes of the ellipses represent the standard deviation of the change in spike rate and the IOS reflectance respectively. The dots represent the mean of the samples. The line represents a case of linear relation between reflectance change and the spike rate change. The figure shows an inversion of functional hyperemia in the FC for locomotion.

d. The change in tissue oxygenation during locomotion (the start of locomotion is depicted by the gray box) in the FC and the FL/HL regions. The different colors indicate the different depths from the cortical surface. Oxygen concentration in both cortical regions increases in response to locomotion.

e. The peak cross-correlation between respiratory rate and oxygen tension in the somatosensory cortex during periods of rest (top) and full trial (bottom) shows that respiration correlates with tissue oxygenation in the somatosensory cortex.

f. The peak cross-correlation between gamma band power in the LFP signal and oxygen tension in the somatosensory cortex during periods of rest (top) and full trial (bottom) shows that neural activity correlates with tissue oxygenation in the somatosensory cortex only during locomotion, where the respiration rate increases.
In one experimental paradigm, the mice were implanted with PoRTS windows that granted visual access to the frontal cortex (FC) and the forelimb/hindlimb (FL/HL) region of the somatosensory cortex for IOS imaging (Fig. 6.1b), along with electrodes for the measurement of local field potential (LFP) in both regions. In these experiments, changes to the cerebral blood volume (CBV) were measured using intrinsic optical signaling (IOS) imaging (Huo, Gao, and Drew 2015), and changes to the spike rate were measured using electrophysiology. The IOS signal measures the reflectance of green light (530nm wavelength) at the surface of the brain, which is inversely related to the local blood volume. Therefore, a positive change in reflectance should be understood as a reduction in CBV and a negative change in the reflectance should be understood as an increase in CBV. During locomotion, neural activity (measured through spike rate in the LFP signal) increased in both the FC and the FL/HL regions of the brain. The locomotion induced neural activity was accompanied by an increase in CBV in the FL/HL region and a decrease in CBV in the FC region. Fig. 6.1c shows the peak percentage change in reflectance, 2–5s after the onset of locomotion on the y axis and the peak percentage change in spike rate change 0–2 s after the onset of locomotion on the x-axis for the FL/HL (green ellipse) and FC (blue ellipse) regions. This finding of inverted CBV-LFP coupling in the FC region has also been shown by previous studies (Huo, Smith, and Drew 2014). A subset of measurements under the same experimental setup were made in mice that were also implanted with cannulas in the brain parenchyma. When a solution of muscimol-CNQX-AP5 was infused through the cannulas to silence neural activity it almost completely ablated the changes in spike rate and the changes in CBV.

In a different experimental setup, the mice were implanted with oxygen probes to measure the locomotion evoked changes in tissue oxygen. These measurements revealed that locomotion was accompanied by an increase in tissue oxygenation in both
the FC and FL/HL regions (Fig. 6.1d), despite the decrease in blood volume in the FC region. The increases in tissue oxygenation persisted when the neural activity and the changes in CBV were blocked through the infusion of a Muscimol-CNQX-AP5 solution. These results suggested that something other than the CBV changes were responsible for increased brain tissue oxygenation during locomotion. By using k-type thermocouples placed near the nostrils of the mice, Zhang et al. measured the changes in respiration rates along with changes of tissue oxygenation and found that the tissue oxygenation correlated well with the changes respiration rate during rest and locomotion (Fig. 6.1e), but correlated with changes in neural activity (represented by changes in gamma band power in the LFP signal) only during locomotion (Fig. 6.1f). The increased correlation between the gamma band power and tissue oxygenation during locomotion was assumed to be a consequence of the increase in respiration rate and the resultant increase in blood oxygenation during locomotion. This assumption was confirmed by measurements of blood oxygenation using two-photon phosphorescence lifetime microscopy, made following an intravenous injection of Oxyphor 2P (Esipova et al. 2019), which showed that events of locomotion were accompanied by increases in blood oxygenation in cerebral arteries.

In summary the experiments by Zhang et al. found that the oxygen tension in the brain tissue increased with locomotion in mice in both the somatosensory cortex and the frontal cortex, irrespective of the changes in neural activity and CBV. These experiments also showed that changes in respiration rate and the corresponding change in blood oxygenation (but not functional hyperemia) drive the changes in cerebral oxygenation during locomotion in mice.

Using a model of oxygen transport in the arteries and the brain tissue, I confirmed that the changes in blood oxygenation are the major contributor to changes in tissue
oxygenation, which suggests that oxygen supply to active neural regions is not the main purpose of functional hyperemia.

6.3 Materials and methods

6.3.1 Model geometry and parameters

Time-dependent, axisymmetric, finite element models were used to simulate the coupled physics of blood flow and oxygen diffusion/consumption. All the simulations were performed in COMSOL Multiphysics (COMSOL Inc.) using the weak form partial differential equation module. The model was divided into two domains: the fluid domain and the solid domain (Fig. 6.2a). The fluid domain, which represents the arterial lumen, was assumed to be a cylinder of radius $R_1$ and a length of $L_a$. The vessel radius ($R_1$) was chosen to be in the range of the radii of penetrating arterioles in the somatosensory cortex (Drew et al. 2010; Shih et al. 2012; Gao, Greene, and Drew 2015). The length of the arteriole was selected to represent the depth of the cortex, where the oxygen is supplied directly by the arteriole and not by the capillaries. Therefore, an axial length of half the typical bifurcation free length of arterioles (Shih et al. 2012; Horton et al. 2013) was used for the models. The solid domain, which represents the tissue that is oxygenated by the arteriole, was assumed to be a cylinder of radius $R_2$ and of the same length $L_a$. The radius of the tissue cylinder was chosen based on previous studies of oxygen diffusion in the cortex (Linninger et al. 2013; Sakadžić et al. 2016).

The diffusion coefficient of oxygen in water (Lamkin-Kennard, Buerk, and Jaron 2004) was used for both fluid and solid domains ($D_{O_2}$), as oxygen can diffuse through the bilipid membrane of the cells. The oxygen concentration was converted from molar to partial pressures using the solubility coefficient of oxygen in water ($\rho$) (Goldman 2008; Lamkin-Kennard, Buerk, and Jaron 2004; Sakadžić et al. 2016). Blood viscosity ($\mu$) value
of 0.004 Pa.s was used (Pries, Neuhaus, and Gahtgens 1992). A shear modulus ($\mu_s$) of 4kPa was used for the brain tissue (Franceschini et al. 2006; Budday et al. 2017; Weickenmeier et al. 2018; Mihai et al. 2017). The resting oxygen tension at the surface of the brain ($P_{O_2}^{baseline}$) was taken from experimental measurements made using in vivo two-photon microscopy (Sakadžić et al. 2010). A resting oxygen consumption rate ($CMRO_2^{baseline}$) of 3 µmole.cm$^{-3}$.min$^{-1}$ was used for the tissue (Ni, Rudin, and Klohs 2018). As $\sim$75% of neural tissue oxygen consumption is activity dependent (Korey and Orchen 1959), muscimol/AP5/CNQx application was simulated by reducing the neuronally dependent portion of $CMRO_2$ by 82%, yielding a $CMRO_2^{MAC}$ value of 1.2 µmole.cm$^{-3}$.min$^{-1}$. Increases in $CMRO_2$ of 15% due to locomotion in FL/HL and a 4% in FC were used (proportionally scaled based on neural recordings in Fig 6.1c). The reflectance changes in the FL/HL and FC were made equivalent to 10% dilation and 5% constriction, respectively based on the measured relationship between reflectance and arteriole diameter (Huo, Gao, and Drew 2015). The locomotion evoked changes in arterial oxygenation were given by the same dynamics as the parametric model of Merkle and Srinivasan (Merkle and Srinivasan 2016). The effect of increased blood flow due to arteriolar dilation was simulated by using a constant pressure difference ($p_1$) between the top and bottom of the arteriole. The pressure difference $p_1$ was chosen so that the blood velocity of nearly 2mm/s (Shih et al. 2012) at baseline diameter. All the model parameters are listed in Table 6.1.
6.3.2 Model formulation

The aim of the simulations is to understand the contribution of changes in CBV, CBF, and respiration rate to tissue oxygenation. In the fluid domain ($\Omega_f$), the transport of free and hemoglobin-bound oxygen needs to be modeled along with the blood flow, while in the solid domain ($\Omega_s$), the transport and consumption of free oxygen needs to be modelled along with tissue deformation. Due to the low (µM) concentration of oxygen, it was assumed that the oxygen transport does not affect the momentum of the solid or the liquid phases and therefore, the concentration of oxygen was treated as a property of the

<table>
<thead>
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<th>Model parameter</th>
<th>Description</th>
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<td>$D_{O_2}$</td>
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<tr>
<td>$R_1$</td>
<td>vessel radius</td>
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<td>$R_2$</td>
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<td>$L_a$</td>
<td>Axial length</td>
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<td>Brain tissue shear modulus</td>
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<td>Resting CMRO$_2$</td>
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<td>solubility coefficient for O$_2$</td>
<td>1.39µM/mmHg</td>
</tr>
</tbody>
</table>

$$PO_{2,art} = \begin{cases} 
PO_{2,baseline} & \text{if } t < \tau \\
PO_{2,baseline} \left( A \frac{(t - \tau)^{\alpha - 1} e^{-\beta(t-\tau)}}{\Gamma(\alpha)} + 1 \right) & \text{if } t \geq \tau 
\end{cases}$$

| A               | 1                                        |
| $\alpha$        | 1.9                                      |
| $\beta$         | 0.3                                      |
| $\tau$ (delay)  | 1 second                                 |

Locomotion-evoked Δ vessel radius

<table>
<thead>
<tr>
<th></th>
<th>FL/HL</th>
<th>FC</th>
</tr>
</thead>
<tbody>
<tr>
<td>muscimol/AP5/CNQx</td>
<td>+10% (+0.9 µm)</td>
<td>-5% (-0.45 µm)</td>
</tr>
<tr>
<td></td>
<td>-20% (-1.8 µm)</td>
<td></td>
</tr>
</tbody>
</table>

Locomotion-evoked ΔCMRO$_2$

<table>
<thead>
<tr>
<th></th>
<th>FL/HL</th>
<th>FC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+15% (+0.45µmole.cm$^{-3}$.min$^{-1}$)</td>
<td>+4% (+0.12µmole.cm$^{-3}$.min$^{-1}$)</td>
</tr>
</tbody>
</table>
fluid and solid particles. To account for the dilation of arterioles, the equations of transport in the fluid domain were written in arbitrary Lagrangian-Eulerian (ALE) coordinates. In the solid domain, the equations are written in the Lagrangian coordinates, as it is convention for writing equations of motion in deforming solids.

The displacement of the arteriolar wall was used as a predetermined boundary condition based on experimental values and therefore, the displacement \( u_m \) of the mesh was calculated by a linear model (Eq. 6.1).

\[
\text{in } \Omega_f, \quad u_{m_r} = \frac{r}{R_1} \text{dilation}(t); \quad u_{m_z} = 0 \tag{6.1}
\]

In Eq. 6.1, \( u_{m_r} \) and \( u_{m_z} \) are the radial and axial components of mesh displacement and.

The time course of the function \text{dilation}(t)\ is shown in Fig.6.2c. The mesh deformation gradient and the Jacobian determinant were calculated from the displacement \( u_m \) (Eq. 6.2).

\[
F_m := I + \nabla u_m; \quad J_m := \text{det } F_m \tag{6.2}
\]

The blood flow was modeled by Stokes law to simulate the low Reynolds' number flow (Ku 1997) in the cerebral arterioles to calculate the fluid velocity (\( \nu \)) and pressure (\( p \)). Eq. 6.3 is Stokes law written in ALE coordinates and \( \sigma_f \) is the Cauchy stress on the fluid (Eq. 6.4).

\[
\text{in } \Omega_f, \quad \nabla \cdot (J_m \sigma_f F_m^{-T}) = 0, \quad F_m^{-T} : \nu = 0 \tag{6.3}
\]

\[
\sigma_f = -pI + \mu_f (\nabla \nu F_m^{-1} + (\nabla \nu F_m^{-1})^T) \tag{6.4}
\]

The transport of free and hemoglobin-bound oxygen can be written as a special case of two-species diffusion by Tezduyar and Park (Tezduyar and Park 1986) (Eq. 6.5-6.7), where \( c_F, c_B \) and \( c_T \) are the concentrations of free, bound and total oxygen respectively.
\[ in \ \Omega, \quad J_m \left( \frac{\partial c_T}{\partial t} + (\boldsymbol{v} - \frac{\partial u_m}{\partial t}) \cdot F_m^T \nabla c_T \right) = \nabla \cdot \left[ J_m D_{O2} F_m^{-1} F_m^T \nabla c_T \right] \]  \hspace{1cm} 6.5

where, \( c_T = c_B + c_F \)  \hspace{1cm} 6.6

and, \( c_B = 4 C_{Hb} S_{O2}(c_F) \)  \hspace{1cm} 6.7

In the model of transport shown in Eq. 6.5, free oxygen can be transported by both convection and diffusion, while hemoglobin-bound oxygen can only be transported by convection. The relation between bound and free oxygen is given by the blood-oxygen saturation relation (Eq.6.7), where \( C_{Hb} \) is the net Hemoglobin concentration (hematocrit x hemoglobin concentration in red blood cells). A value of 10mM was used for \( C_{Hb} \) (Rodgers, Fisher, and George 1975). The oxygen dissociation curve by Lobdell (Lobdell 2017) was used for the saturation curve \( S_{O2}(c_F) \), which is an invertible function and accurately represents experimental data (Roughton and Severinghaus 2017). Using a combination of Eqs.6.6-6.7, Eq.6.5 was written in terms of the free oxygen for the finite element models.

The displacement in the solid domain was given by the vector \( u_s \). This was used to calculate the deformation gradient and Jacobian determinant similar to Eq.6.2. The deformation of brain tissue was simulated by a quasi-static linear elastic model with a Poisson ratio (\( \nu \)) of 0.45 (Eqs.6.8-6.9). In Eq.6.8, \( P_s \) is the first Piola-Kirchhoff stress tensor.

\[ \nabla \cdot P_s = 0; \quad P_s = F_s (\lambda_s Tr[\varepsilon_s] I + 2\mu_s \varepsilon_s) \]  \hspace{1cm} 6.8

\[ \varepsilon_s = \frac{1}{2} (F_s + F_s^T) \quad ; \quad \lambda_s = \frac{2\nu \mu_s}{(1-2\nu)} \]  \hspace{1cm} 6.9

The transport of oxygen in the tissue was modeled by Fick’s law of diffusion, written in the Lagrangian coordinates of the deforming solid.

\[ in \ \Omega, \quad J_m \left( \frac{\partial c_F}{\partial t} + CMRO_2 \right) = \nabla \cdot \left[ J_m D_{O2} F_m^{-1} F_m^T \nabla c_F \right] \]  \hspace{1cm} 6.10
For the finite element model, the primary unknowns are the velocity \( \mathbf{v} \), pressure \( p \) and oxygen concentration \( c_F \) in the fluid domain \( \Omega_f \), and the solid displacement \( \mathbf{u}_s \) and oxygen concentration \( c_F \) in the solid domain \( \Omega_s \). The value of mesh displacement, given by the linear model (Eq. 6.1) was treated as a known variable.

### 6.3.2 Boundary and initial conditions

The fluid flow was dictated by a pressure difference across the two ends of the artery. This was achieved by applying a traction of magnitude \( p_1 \) at the inlet \( z = L_a \) of the artery and zero traction at the outlet \( z = 0 \) (Eqs. 6.11-6.12). Arterial dilation caused a decrease in the flow resistance that resulted in an increase in the flow velocity. In Eqs. 6.11-6.12, \( \mathbf{n} \) is the unit outward normal to the fluid domain.

\[
\begin{align*}
\text{at } z &= 0, \quad J_m \sigma_f F^{-T} \mathbf{n} = \mathbf{0} \quad 6.11 \\
\text{at } z &= L_a, \quad J_m \sigma_f F^{-T} \mathbf{n} = -J_m p_1 F^{-T} \mathbf{n} \quad 6.12
\end{align*}
\]

No slip-boundary condition was used at the tissue-artery interface \( r = R_1 \).

\[
\text{at } r = R_1, \quad \mathbf{v}_f = \frac{\partial \mathbf{u}_m}{\partial t} \quad 6.13
\]

The radial component of velocity was set to zero at the centerline \( r = 0 \).

\[
\text{at } r = 0, \quad v_{fr} = 0 \quad 6.14
\]

The concentration of free oxygen (and consequently the bound oxygen) was fixed at the inlet of the artery \( z = L_a \). In Eq. 6.15 \( \rho \) is the solubility constant of oxygen and \( P_{O_2}^{\text{baseline}} \) is the oxygen tension in the baseline condition (see Table 6.1).

\[
\text{at } z = L_a, \quad c_F = \rho P_{O_2}^{\text{baseline}} \quad 6.15
\]
The displacement at the tissue-artery interface ($r = R_1$) followed the same time course as the arterial dilation.

$$at \ r = R_1, \quad u_s = u_m \quad 6.16$$

No-traction and no-flux boundary conditions were used at the three open ends ($z = 0$, $z = L_a$ and $r = R_2$) of the tissue for the displacement and diffusion equations respectively (Eqs.6.17-6.18).

$$\text{in} \ \Omega_s, \text{at} \ z = 0, z = L_a, \text{and} \ r = R_2, \quad P_s n = 0 \quad 6.17$$

$$\text{in} \ \Omega_s, \text{at} \ z = 0, z = L_a, \text{and} \ r = R_2, \quad J_m D_{O_2} F_m^{-1} F_m^{-T} \nabla c_F \cdot n = 0 \quad 6.18$$

The model was solved with a stationary solver to obtain the baseline values of all the variables. The results of the stationary solution are shown in Fig.6.2b. The arteriolar dilation/constriction, increased metabolism ($\Delta$CMRO2), and the respiration related change in the oxygen tension at the arteriolar inlet ($P_{O_2,art}$) were applied in the time dependent problem, whose initial conditions were given by the solution to the stationary problem. Smooth functions with continuous first and second order derivatives (Fig.6.2c) were used for the transitions in arteriolar diameter, blood oxygenation and oxygen consumption in the tissue.
6.4 Results

6.4.1 Respiration is the major contributor to locomotion-driven PtO₂ changes

The simulations were first implemented with the combination of boundary conditions presented in Fig. 6.2 to represent the locomotion evoked changes of blood oxygenation, metabolic rate and blood volume in FC and FL/HL regions of the cortex. The oxygen concentration at the middle of the tissue cylinder \( (z = L_a/2, r = (R_1 + R_2)/2) \) in the model were compared to the experimental results. The changes in tissue oxygen concentrations predicted by the model (Fig. 6.3a, black curves) matched experimental measurements in the FC and FL/HL (Fig. 6.3a, blue and green curves) at 100 µm from the...
brain surface. Then, the cases equivalent to the muscimol/CNQX/AP5 infusion, with reduced baseline metabolic rate, and no changes in locomotion driven metabolic rate or vessel diameter were simulated. These models still included the arteriolar oxygen tension changes corresponding to respiration. The levels of baseline tissue oxygen changed in the simulations of muscimol/CNQX/AP5 infusion, but a locomotion-evoked increase in tissue oxygenation was still observed, in-line with the experimental findings (Fig. 6.3a, red curves).

The model was then used to tease out the relative contributions of vasodilation (and the corresponding increase in blood flow) and increased arterial oxygenation to tissue oxygen changes in the FC and FL/HL (Fig. 6.3b). Here, the changes in CMRO$_2$, vessel diameter, arterial oxygenation were applied one at a time, keeping the other two values fixed. In the FL/HL region, the reduced tissue oxygenation due to the large increase in CMRO$_2$ during locomotion is offset by approximately equal increases in O$_2$ due to vasodilation and increase in arterial oxygenation, whereas in the FC, the small decrease in tissue oxygen due to increase in CMRO$_2$ and vasoconstriction is totally offset by the increase in arterial O$_2$. These simulations show that vasodilation and increased blood flow do not solely cause the oxygenation increase in highly active brain regions during behavior and increases in respiration play an important role in increasing the tissue oxygenation. The increased arterial O$_2$ that accompanies increases in respiration can lead to increases in tissue oxygenation even in brain regions experiencing vasoconstriction.
Increased tissue oxygenation of the frontal cortex cannot be explained without respiration

The experimental measurements clearly show a decrease in CBV in the frontal cortex in response to locomotion. The results of both the experiments and the models suggest that the increase in tissue oxygenation accompanied by vasoconstriction is due to the increase in respiration rate and the subsequent increase in blood oxygenation. Another possible way to increase tissue oxygenation without increasing blood oxygenation is by reducing the oxygen consumption in the frontal cortex during locomotion. This scenario is unlikely because there is an increase in neural activity during locomotion, and therefore increase in metabolism and oxygen consumption in the frontal cortex (see Fig. 6.1c). To rule out this unlikely possibility, the model for the frontal cortex was modified by keeping the blood oxygenation in the arteriole constant and decreasing the CMRO$_2$. The reduction in the vessel diameter was kept the same as the original model (Fig.6.2c, top-left). The amount of reduction of CMRO$_2$ in the model was adjusted iteratively so that the...
tissue oxygenation changes in the model matched with the experimentally measured values. The model showed that the measured oxygen tension change in the tissue in the frontal cortex is possible for a 15% reduction in CMRO$_2$ (Fig. 6.4). However, such a large reduction in metabolism is only observed during deep sleep (Madsen et al. 1991) and anesthesia (PIERCE et al. 1962) but not under the awake state. These calculations confirmed the hypothesis that respiration is the most likely contributor to the increased oxygenation observed in the frontal cortex during locomotion.

![Figure 6.4: The observed increase in oxygen tension around constricting vessels cannot be explained by physiological changes in metabolism (CMRO$_2$)](image)

6.5 Discussion

This chapter is a slight departure from the rest of the thesis in that the fluid flow in the paravascular space or the metabolite clearance from the brain parenchyma wasn’t directly examined in any of the models presented in this chapter. Nevertheless, the models of this chapter shed a new light on the role of functional hyperemia in the health and functioning of the brain and therefore are important to our hypothesis that clearance of metabolic waste is one the primary roles of functional hyperemia. Conversely, the models here also raise the question of the role of respiration and cerebral oxygenation on vessel diameters and the clearance of metabolic waste in the brain. It has already been shown
that respiration is a major contributor to cerebrospinal fluid flow in the subarachnoid space (Vinje et al. 2019). Experimental studies (Steinback and Poulin 2008; Ray et al. 2002; Hoiland et al. 2017) and numerical models (Mintun et al. 2001; Haselden, Kedarasetti, and Drew 2020) have also demonstrated that levels of blood oxygenation affect the diameter of cerebral arterioles. The effect of changes in respiration during voluntary exercise (Eldridge et al. 1985) and sleep (A. Xie 2012; Douglas et al. 1982) and their effect on the fluid exchange between the paravascular space and the subarachnoid space, and the resistance of the paravascular spaces might be an interesting topic for future studies.

There are some limitations to the models presented here. The effect of capillaries on tissue oxygenation is not addressed in these models. The model also represents a relatively small region of the brain. To study the effects of the capillary bed on tissue oxygenation and to study oxygenation changes over a large section of the cortex, a 3D model (Fang et al. 2008) of vasculature and brain tissue, with changes in CBV and CBF changes would be required. The model and the experiments only study the changes in tissue oxygenation during locomotion. Locomotion was an ideal candidate for the models here because of the large dilation of arterioles in the somatosensory cortex and simultaneous constriction of vessels in the frontal cortex driven by locomotion. It would, however, be interesting to model changes in tissue oxygenation in the cortex during whisker stimulation or in the presence of visual stimuli and examine how the model predictions agree with experimental data.
Chapter 7: Conclusions and future directions

7.1 Conclusions

In this thesis, the nature of transport of cerebrospinal fluid (CSF) in the paravascular space (PVS) and the effect of arterial and arteriolar wall motions on fluid and metabolite transport through the PVS and the subarachnoid space (SAS) were examined. Models of fluid flow in the PVS were built using anatomically realistic geometries and boundary conditions representing the physiology of the cerebral cortex of mice, and the predictions of the models were compared to new and existing experimental data from mice. The following are the main conclusions of the thesis:

1. **Heartbeat-driven pulsations of arteries are unlikely to drive directional movement of CSF through the PVS and into the brain.** The hypothesis of peristaltic pumping of CSF by arterial pulsations (Wang and Olbricht 2011; Iliff et al. 2013; Mestre, Tithof, et al. 2018) was tested from a theoretical standpoint in chapter two. The results of the theoretical models matched the published experimental results (Mestre, Tithof, et al. 2018; Bedussi, Almasian, et al. 2017). The models showed that due to the small amplitude pulsations of arteries (compared to the size of the PVS of pial arteries) and the small length of the arteries (compared to the wavelength of the peristaltic motion of arteries), pulsations can only drive oscillatory flow of CSF but not directed flow.

2. **Functional hyperemia can drive metabolite clearance via fluid exchange between the PVS and the SAS.** Fluid-structure interaction models of flow in the PVS of penetrating arterioles surrounded by a compliant brain tissue were built in chapter three. These models showed that in comparison to the heartbeat-driven
pulsations, vasodilation due to changes in local neural activity can drive appreciable fluid exchange between the PVS and the SAS.

3. The brain tissue can deform due to pressure changes in the fluid-filled spaces of the CNS and therefore models of fluid flow in the CNS should consider the effect of brain tissue deformation. The brain one of the softest organs in the mammalian body and the deformation of the brain can affect the flow in and around the brain. This prediction of fluid-structure interaction models in chapter three was verified by the measurements of brain tissue displacement presented in chapter four.

4. The increased solute exchange between the cerebrospinal fluid (CSF) and interstitial fluid (ISF) observed during sleep (Xie et al. 2013) might be the result of the large dilation of cerebral arteries during sleep (Turner et al. 2020). The improved penetration of dyes injected into the brain parenchyma in the sleep state compared to the awake state, when injected into the cisterna magna was attributed to the increased extracellular space (reduced flow resistance) and the unknown driving force of the hypothesized glymphatic system (Jessen et al. 2015). Fluid-structure interaction models in chapter five, which incorporated realistic geometries of the SAS, the PVS and the brain parenchyma, showed that the arterial dilations observed during sleep can play a major role in the fluid exchange and solute transport between the PVS and the SAS.

5. Functional hyperemia is not sufficient to explain the increased tissue oxygenation observed in mice during locomotion, suggesting that the purpose of hyperemia might not be to match the oxygen supply in the brain to the metabolic demand. Models of oxygen transport in arteries and the brain parenchyma in chapter six replicated the experimental results of Zhang et al. (Zhang et al. 2019) and showed that respiration, is a major contributor to cerebral
oxygenation. In the context of the results of chapters three and five, the models of oxygen transport suggest that the main purpose of functional hyperemia is the clearance of metabolic waste from the PVS and not increasing oxygen supply to active brain regions.

7.2 Future directions

There is a pressing need for further investigation on the topic of fluid and metabolite transport in the brain, as it might be key to understanding the mechanisms of prevention and treatment of neurological disorders. While there are several possible aspects of transport in the brain that warrant further research, I will focus on two major aspects: 1) poroelasticity and transport through the extracellular space 2) transport through the glial syncytium.

7.2.1 Poroelasticity and transport through the extracellular space

Poroelastic models can be used for modeling transport in the brain extracellular space (ECS) along with the transport in the PVS and the SAS, and make predictions of flow in the ECS, which could be suited for experimental verification by examining fluorescence recovery after photobleaching (FRAP). FRAP can be employed to differentiate between diffusive and convective transport by photobleaching an area filled by a fluorescent dye and observing the recovery of the dye by photobleaching (Lippincott-Schwartz, Snapp, and Phair 2018). FRAP is generally investigated with confocal or two-photon microscopy, where the light source activating the fluorophore in the dye is used at a high-power setting to exhaust the fluorescence of the fluorophore (photobleach) in a portion of the sample, and then at a low-power setting to collect images of the recovery of fluorescence in the bleached region. The photobleaching process can usually only be performed over a small region and needs to be performed quickly, to prevent fluorescence
recovery before the completion of the photobleaching. Because fluorescence activation in confocal and two-photon microscopy has better spatial specificity in the focal plane of the light objective and because current technology only allows fast raster scanning of a spatial region in the focal plane of the objective (Inoué 2006), FRAP is only effective in differentiating between convective and diffusive transport in the focal plane of the objective (Sykova et al. 2008; A. J. Smith et al. 2017a; Papadopoulos, Binder, and Verkman 2005; Binder et al. 2004). The PoRTS windows used for visualizing the brain are parallel to the surface of the brain, which means that the focal plane of the light objective is parallel to the surface of the brain. The flow in the PVS predicted by our fluid-structure interaction models is mostly perpendicular to the surface of the brain (see chapters three and five) and therefore perpendicular to focal plane of the light objective, which means that FRAP would be ineffective to study flow in the PVS. The predictions of flow in the brain parenchyma from poroelastic models in the future could be tested with FRAP experiments, as this flow could be in the focal plane of the light objective.

In chapter five, we saw some advances in time-dependent finite element models of poroelasticity made through this thesis, focused on modeling the fluid and metabolite transport through the ECS. However, an equally important aspect of poroelastic models are the elastic properties of the connective tissue in PVS and the SAS. Tissue that is mostly composed of fibrous elements has very interesting elastic properties. For example, fibrous networks have relatively high tensile versus compressive strength (Vahabi et al. 2016) and exhibit hysteresis (Liu et al. 2018; Haut and Little 1972) during cyclic loading and unloading. There are some existing constitutive models of poroelasticity in fibrous networks, particularly for collagen gels (Lanir 1983; Stylianopoulos and Barocas 2007; Niizato and Gunji 2011). The constitutive models of elasticity in the PVS and the SAS need to be incorporated along with the poroelastic models of the brain using jump conditions.
similar to the ones presented in chapter five. Including the elastic properties of the PVS would also allow us to simulate the actual geometry of the PVS. Furthermore, the PVS will not be perfectly symmetric annular region between two concentric circles as simulated in our models. The main obstacle to simulating the actual geometry of the PVS in fluid-structure interaction models is the possibility of obtaining a negative Jacobian determinant of the mesh deformation, when the PVS is compressed over a certain extent. By simulating the elastic properties of the collagen fibers occupying the PVS, this problem can be avoided, since the PVS would become very stiff as the solid volume fraction \( \xi_s = \xi_s^R / J_s \), see section 5.4) approaches unity, which would happen before the Jacobian determinant, \( J_s \), reaches zero.

Images of the actual geometry of the PVS can be obtained by using two-photon microscopy with mice expressing enhanced green fluorescent protein (EGFP) with β-actin promoter (Okabe et al. 1997), or CAG-EGFP transgenic mice, that are available through the Jackson laboratory (https://www.jax.org/strain/003291). High levels of actin are present in the arteriolar walls (Nakajima-Iijima et al. 1985) and astrocytic endfeet (Nicchia et al. 2008), and therefore PVS can be identified as the dark space between two bright spots (Fig. 7.1a). By using a lens with adjustable focus, fast 3D scans can be obtained to visualize the 3D shape of the penetrating arterioles and the surrounding PVS during locomotion. A sample dataset of fast 3D scans in CAG-EGFP transgenic mice is shown in Fig.7.1b-e. Images of the lumen of the vessel and the PVS geometry can be obtained simultaneously by injecting Texas-Red dye into the bloodstream of CAG-EGFP transgenic mice. There are established methods of tracking the changes to the vessel lumen from images obtained using two-photon microscopy (Gao and Drew 2014). Similar methods could be developed for measuring the changes to PVS geometry.
It is also important to use appropriate models of diffusive transport along with poroelastic models. The poroelastic models of the brain tissue and the PVS and the SAS discussed in chapter five only consider the transport of two species, namely, the solid skeleton (tissue) and the fluid solvent (water). The models of poroelasticity need to be extended to include the transport of solutes and consider the possibility of exclusion of...
large molecules in the brain extracellular space or the varying transport properties in
different direction etc., which can have a huge impact on transport (Ambrosi, Preziosi, and
Vitale 2010; Hall and Rajagopal 2012; Taylor, Bert, and Bowen 1990). Models of mixtures
of solid, fluid and solute can be useful to study the effect of Amyloid-β molecules on the
transport in the parenchyma and probably even the formation of plaques.

7.2.2 Transport in the glial syncytium

There is ample evidence suggesting that astrocytes can drive fluid flow in the ECS.
Astrocytes are capable of changing the ECS volume of brain tissue by up to 30% in the
sensorimotor cortex of cats (Dietzel et al. 1980). This change in ECS volume has also
been observed in brain slices (Niermann et al. 2001; Holthoff and Witte 2000) and is
believed to play a major role in potassium buffering (Kofuji and Newman 2004), a process
by which the glial syncytium alleviates local increases in extracellular potassium due to
spiking activity of neurons. The astrocytes achieve this buffering by siphoning the
extracellular potassium from locations of high concentration and quickly distributing it
uniformly over large regions of the brain. Both experimental (Simard and Nedergaard
2004; Verkhratsky, Nedergaard, and Hertz 2014) and theoretical (Dietzel, Heinemann, and
Lux 1989; Chen and Nicholson 2000; Murakami and Kurachi 2016) studies have shown
that potassium buffering is an essential function of astrocytes to prevent severe and
localized increases in neural excitability, which could lead to seizures and spreading
depressions (Thrane et al. 2013). In spite of this evidence, the published models of fluid
and metabolite transport in the brain have either not considered astrocytes and aquaporins
in fluid movement, or have treated them as passive elements that allow flow in response
to hydrostatic pressure differences (Asgari, De Zélicourt, and Kurtcuoglu 2015; Jin, Smith,
and Verkman 2016; Asgari, De Zélicourt, and Kurtcuoglu 2016; Wang and Olbricht 2011;
Schley et al. 2006; Kedarasetti, Drew, and Costanzo 2020; Kedarasetti et al. 2020)
A number of experimental studies have pointed towards an association between potassium buffering, changes in ECS volume, and large-molecule clearance from the CNS (Fig. 7.2). The extent of dye penetration into the brain parenchyma, when injected into the cisterna magna, co-varies with the level of Aqp4 expression (Mestre, Hablitz, et al. 2018) (Fig. 7.2a). Similarly, increasing Aqp4 expression using arginine vasopressin (Liu et al. 2010) increases extracellular potassium clearance and ECS volume changes (Niermann et al. 2001), following neural activity (Fig. 7.2b). Microdialysis measurements in live rats (Wang et al. 2018) showed that infusion of vasopressin increased the clearance of acetaminophen and ibuprofen from the CNS. Disrupting the high expression of Aqp4 on astrocytic endfeet has been shown to delay the clearance of extracellular potassium.
(Amiry-Moghaddam et al. 2003) (Fig. 7.2c) and to slow down the dye movement in the PVS (Mestre, Hablitz, et al. 2018). Increased potassium buffering and PVS flow have been reported in mice during voluntary running (Rasmussen et al. 2019; von Holstein-Rathlou, Petersen, and Nedergaard 2018) and sleep (Ding et al. 2016). All this evidence indicates that there is a strong correlation between potassium buffering by astrocytes and flow in the PVS, and suggests a possible role of astrocytes in driving fluid and metabolite transport in the CNS.

Several mathematical models where the astrocytic membrane potential is coupled to ion and water transport have been built to understand the role of astrocytes in potassium buffering and changes to ECS volume. Early mathematical models (Gardner-Medwin 1983; K. C. Chen and Nicholson 2000; Dietzel, Heinemann, and Lux 1989) of the glial syncytium only considered the movement of ionic species ($Na^+$, $K^+$ and $Cl^-$), without modeling water transport. While more recent models of the glial syncytium have accounted for water movement across the glial membrane (Østby et al. 2009; Wei, Ullah, and Schiff 2014; Murakami and Kurachi 2016; O’Connell and Mori 2016), they only considered its effect on cell swelling and ECS volume changes. To date, none of the published models of the glial syncytium accounted for water flow within the intracellular and extracellular compartments. Models of coupled intracellular and extracellular water flow have been developed in the field of botany (Molz and Hornberger 1973; Molz and Ikenberry 1974). In these models, cell swelling, intracellular and extracellular flows are calculated by coupling the cell turgor pressure, and the differences in osmotic and hydrostatic pressures across the intracellular and extracellular compartments. These botanical models can be modified to model coupled intracellular and extracellular ionic transport in the glial syncytium.

Several of the parameters for these models need to be determined experimentally. The elastic tension of the astrocyte cytoskeleton, which would be the equivalent of cell
turgor pressure (Hill, Shachar-Hill, and Shachar-Hill 2004) in plant cells is an important parameter for modeling intracellular and transmembrane water flow. The elastic modulus of the cytoskeleton can be estimated by volume changes of astrocytes in response to changes in extracellular osmolarity. Such experiments have been performed with astrocyte cell cultures in the past (Morales and Schousboe 1988; O'Connor and Kimelberg 1993; Bender, Neary, and Norenberg 1993). These experiments would have to be repeated in brain slices, because astrocytes in the brain differ from cultured astrocytes in terms of morphology and the density of transporters and channels (Lange et al. 2012). Astrocyte specific fluorescent markers like sulphorhodamine 101 (Nimmerjahn et al. 2004; Rungta et al. 2015) can be used to measure volume changes in brain slices. Another crucial parameter required for the modeling of transport in the glial syncytium is the permeability of the gap junctions.

7.3 Closing Remarks

This thesis has demonstrated that a combination of theoretical and experimental approaches is required to properly understand the drivers and mechanisms of fluid flow and metabolite transport in the CNS. Theoretical models of transport, based on the anatomical and physiological features of the fluid spaces of the CNS should be used to understand the isolated and combined effect of different drivers of transport, and to make predictions that can be verified against experimental data. Research on the topic of fluid flow in the CNS also provides a scope for the development of new experimental and modeling techniques, and for the improvement of existing techniques, which can be applied to other topics of research. A simple example for this is the method of fluid particle tracking in ALE coordinates (Appendix A), which can be used to model fluid movement in a wide variety of biological systems including blood vessels and lymph vessels, where the deformation of the walls affects the fluid flow.
The findings of this thesis emphasize the importance of regular exercise and adequate sleep in maintaining long-term mental health. The models and experiments presented in thesis strongly suggest that dilation of cerebral arteries could provide a driving force for the clearance of metabolites from the PVS, and there is strong evidence showing that physical activity and sleep are accompanied by large vasodilation of arteries throughout the cerebral cortex (Huo, Gao, and Drew 2015; Turner et al. 2020; Willie et al. 2011; Bergel et al. 2018). There is direct evidence showing that solute transport between the SAS and the PVS is influenced by physical exercise (locomotion) (von Holstein-Rathlou, Petersen, and Nedergaard 2018) and sleep (Xie et al. 2013) in mice. Studies using transgenic mouse models of Alzheimer’s disease have shown that voluntary exercise reduces amyloid-β concentration and number of amyloid-β plaques (Yuede et al. 2009; Adlard et al. 2005), while sleep deprivation increases amyloid-β concentration in ISF (Kang et al. 2009). Moreover, there is indirect evidence showing that sleep disorders (Peter-Derex et al. 2015) and dysfunction of neurovascular coupling (Di Marco, Farkas, et al. 2015; Di Marco, Venneri, et al. 2015; Iadecola 2004) are precursors to Alzheimer’s disease. Further research into mechanisms of functional hyperemia-driven metabolite clearance in the brain is required to understand the exact role of exercise and sleep in the prevention and treatment of Alzheimer’s.
Appendix A: Kinematics and Particle Tracking in ALE

A1 Kinematics in ALE

We start with two configurations of the fluid, the deformed configuration and the reference configuration \( B_t \) and \( B_p \) respectively. The points in the deformed and reference configurations are denoted by \( x, X_p \) respectively. The deformed configuration \( B_t \) is the image of the reference configuration \( B_p \) under the map \( \chi_p(X_p, t) \). The displacement of material particles, \( u \), is given by Eq.A1.

\[
        u(X_p, t) := x(X_p, t) - X_p
\]  

A1

One can also use the Eulerian description of the displacement (Eq.A2), where \( X_p(x, t) \) is the image of \( x \) under the inverse map \( \chi_p^{-1}(x, t) \).

\[
        u(x, t) := x - X_p(x, t)
\]  

A2

The velocity \( v \) is the time derivative of the displacement. The relation between the Eulerian description of displacement and the velocity is given by Eq.A3, where \( \nabla x \) is the gradient with respect to the deformed coordinates.

\[
        v(x, t) := D_t u = \frac{\partial u}{\partial t} + (\nabla x u)v
\]  

A3

For calculations in ALE, we additionally define the mesh configuration \( B_m \), where the points are denoted by \( X_m \). The deformed configuration \( B_t \) is related to the mesh configuration \( B_m \) by the map \( \chi_m \). The displacement of the mesh domain (mesh motion for finite element calculations) is given by Eq.A4.

\[
        u_m(X_m, t) := x(X_m, t) - X_m
\]  

A4
The definitions of the deformation gradient and the Jacobian determinant are given in Eq. A5.

\[
F_m := I + \nabla_{x_m} u_m; \quad J_m := det F_m
\]  

A5

For the description of fields in the mesh coordinates, we use the 'hat' notation (Eq. A6).

\[
\hat{f}(X_m, t) = f(x, t) = f(x(X_m, t), t)
\]  

A6

The deformation gradient and the Jacobian determinant defined in Eq. A5 are used for transforming the spatial derivatives in the deformed domains to derivatives in the mesh domain.

For a scalar \( p \):

\[
\nabla_x p = F_m^{-T} \nabla_{x_m} \hat{p}
\]  

A7

For a vector \( b \):
\[ \nabla_x b = \nabla_{X_m} \hat{b} F^{-1}_m \]  

A8

\[ \nabla_x \cdot b = \frac{1}{J_m} \nabla_{X_m} \cdot (J_m F^{-1}_m \hat{b}) \]  

A9

For a second order tensor \( \sigma \):

\[ \nabla_x \cdot \sigma = \frac{1}{J_m} \nabla_{X_m} \cdot (J_m \tilde{\sigma} F^{-T}_m) \]  

A10

The transformations of integrals over the volume and boundaries of the are given by Eq. A11 and Eq. A12, where, \( dV \) and \( dV_m \) are the volume elements in the deformed and mesh configurations. The boundaries of the deformed and mesh configurations are represented by \( \partial B_t \) and \( \partial B_m \) with area elements \( dA \) and \( dA_m \) respectively. The unit outward normals to the deformed and mesh configurations are given by \( n \) and \( n_m \) respectively.

\[ \int_{B_t} f(x) dV = \int_{B_m} J_m \hat{f}(X_m) dV_m \]  

A11

\[ \int_{\partial B_t} g(x) \cdot n dA = \int_{\partial B_m} J_m \hat{g}(X_m) \cdot F^{-T}_m n_m dA_m \]  

A12

The material time derivative of \( x(X_m, t) \) is the material particle velocity in mesh coordinates, which should be the same as the material particle velocity defined in Eq. A3, expressed in mesh coordinates.

\[ D_t x(X_m, t) = v(x(X_m, t), t) = \hat{v}(X_m, t) \]  

A13

Finally, the material time derivative of material particle velocity (the acceleration) in the coordinates is given by Eq. A14. The complete derivation of the expression for acceleration can be found in textbooks on ALE and fluid-structure interaction (Donea et al. 2004).
\[ \tilde{a}(X_m, t) := D_t \tilde{v}(X_m, t) := \frac{\partial \tilde{v}}{\partial t} + \nabla_{X_m} \tilde{v} F_m^{-1} \left( \tilde{v} - \frac{\partial u_m}{\partial t} \right) \] 

A2 Particle tracking in ALE

To find the fluid particle trajectories in the deforming domain, \( B_t \), we first solve for the fluid particle coordinates in the mesh domain \( (X_m) \) as a function of time. The material time derivative of \( X_m \) is the particle velocity observed from the mesh domain \( B_m \) is defined as:

\[ \dot{X}_m := D_t X_m \] 

We need to calculate the material time derivative of the mesh displacement. We use a method similar to that in Eq. A3, where we use the map \( x_m^{-1} \circ x_p \) in Eq. A16, instead of the map \( x_p \) in Eq. A3.

\[ D_t u_m(X_m, t) = \frac{\partial u_m}{\partial t} + \left( \nabla_{X_m} u_m \right) \dot{X}_m \] 

The finite element computations in the mesh coordinates give us the fields \( \tilde{v}(X_m, t) \) and \( u_m(X_m, t) \). We calculate a relation between these known quantities and \( \dot{X}_m \) using Eqs. A13, A15 and A16 in the material time derivative of Eq. A4.

\[ D_t (x - X_m) = D_t u_m \Rightarrow \tilde{v} - \dot{X}_m = \frac{\partial u_m}{\partial t} + \left( \nabla_{X_m} u_m \right) \dot{X}_m \] 

Using the definition of the deformation gradient in Eq. A5, we can find an expression for \( \dot{X}_m \) (Eq. A18).

\[ \dot{X}_m = F_m^{-1} \left( \tilde{v} - \frac{\partial u_m}{\partial t} \right) \]
We use the following initial value problem to calculate fluid particle trajectories the deforming domain, with the initial conditions $x(X_m, 0) = u_m(X_m, 0) + X_m$ and $X_m(0) = X_m$.

\[
\text{find } x(X_m, t), \text{ such that } x(X_m, t) = u_m(X_m, t) + X_m; \text{ and } \dot{X}_m = F_m^{-1} \left( \bar{v} - \frac{\partial u_m}{\partial t} \right) \quad A19
\]

Here, it is assumed that the material particle velocity $\bar{v}(X_m, t)$ and the mesh displacement $u_m(X_m, t)$ (and subsequently $F_m$ and $\frac{\partial u_m}{\partial t}$) are known fields in the mesh domain. We first find the particle trajectory in the mesh coordinates and add the mesh displacement to find the particle trajectory in the deformed coordinates. We solve this problem using a forward Euler integration scheme and calculate the fluid particle trajectories. When the position in the mesh domain at time $t$ is known, the time integration scheme allows us to find the position at time $t + dt$.

\[
X_m(t + dt) = X_m(t) + dtF_m^{-1}(X_m(t), t) \left( \bar{v}(X_m(t), t) - \frac{\partial u_m(X_m(t))}{\partial t} \right) \quad A20
\]

\[
x(X_m, t + dt) = u_m(X_m(t + dt), t + dt) + X_m(t + dt) \quad A21
\]

The fluid particles can only be tracked till the time they exit the computational domain since the velocity field outside the domain is unknown.
Appendix B: Weak formulation for Navier-Stokes in ALE with anisotropic non-dimensionalization

Here we describe the derivation of the weak formulation for the problems presented in chapter 2.

B1 Anisotropic non-dimensionalization

The description of the deformed configuration \( B_t \), the reference configuration \( B_p \) and the mesh configuration \( B_m \) are the same as the ones presented in Appendix A. Here additionally, we transform the mesh domain into the non-dimensionalized computational domain \( B_c \) with coordinates \( X_c \) (Fig.B1). The transformation is defined by the characteristic length \( L_o \) and scaling tensor \( G \). For our problem, the characteristic length and the scaling tensor are constants.

\[
X_m = L_o G X_c \tag{B1}
\]

Any field in \( X_m \) can be transformed into a field in \( X_c \) using the relation in Eq. B1.

\[
f(X_m, t) = f(L_o G X_c, t) \tag{B2}
\]

The spatial derivatives are transformed as follows.

For a scalar \( p \):

\[
\nabla X_m p = \frac{1}{L_o} G^{-T} \nabla X_c p \tag{B3}
\]

For a vector \( b \):

\[
\nabla X_m b = \frac{1}{L_o} \nabla X_c b G^{-1} \tag{B4}
\]
\[
\n\nabla_{X_m} \cdot b = \frac{1}{L_o} \nabla_{X_c} \cdot (G^{-1}b)
\]

For a second order tensor \(\sigma\):

\[
\nabla_{X_m} \cdot \sigma = \frac{1}{L_o} \nabla_{X_c} \cdot (\sigma G^{-T})
\]

The time derivatives are unaltered because there is no relative motion between \(X_m\) and \(X_c\).

The transformations of integrals over the volume and boundaries of the are given by Eq. B7 and Eq. B8 respectively, where, \(dV_m\) and \(dV_c\) are the volume elements in the mesh and the computational configurations. The boundaries of the mesh and computational configurations are represented by \(\partial B_m\) and \(\partial B_c\) with area elements \(dA_m\) and \(dA_c\) respectively. The unit outward normals to the deformed and mesh configurations are given by \(n_m\) and \(n_c\) respectively.

\[
\int_{B_m} f(X_m) dV_m = \int_{B_c} L_o^3 \det G f(L_o G X_c) dV_c
\]
\[ \int_{\partial B_m} g(X_m) \cdot \mathbf{n}_m dA_m = \int_{\partial B_c} L_o^2 \det G g(L_o G X_c) \cdot G^{-T} \mathbf{n}_c dA_c \]  

The deformation gradient and the Jacobian determinant of the mesh displacement in the computational coordinates are calculated as follows.

\[ F_m := I + \frac{1}{L_o} \nabla X_c u_m G^{-1}, \quad J_m := \det F_m \]

B2 Initial conditions for time dependent problems

In all the initial-boundary value problems used in the chapter 2, the initial values and initial time-derivatives of all variables are set to zero because the initial conditions of the system are unknown. The parameter values used in the boundary conditions, are ramped up from zero to the specified values using step functions available in COMSOL Multiphysics. These functions have continuous first and second derivatives with respect to time. In this document we will use \( step(t) \) in the equations to indicate wherever these functions are used. The results for these simulations are shown after 4 cycles of the periodic wall motion, where the variations in the velocity and pressure values from cycle to cycle are less than \( 10^{-4} \% \) of peak value.

B3 Strong form of Navier-Stokes model in non-dimensional coordinates

We solve for the fluid velocity \( \mathbf{v}_f \) and pressure \( p_f \) in a deforming domain \( B_t \), representing the paravascular space (PVS) of pial arteries. The time-dependent deformation of the domain is given by the displacement \( u_m \). We present the strong form equations here in the computational coordinates \( X_c \). The appropriate transformations for change of coordinates are applied (Eqs. A6-A12, B2-B8) and the subscript \( X_c \) on the
spatial derivatives (gradient, divergence and Laplacian) is dropped. We use the hat notation for the velocity and pressure ($\hat{v}_f$ and $\hat{p}_f$ respectively) to signify that these are calculated as functions of the computational coordinates and not the reference or deformed coordinates of the actual body.

We use a linear elliptical model for the mesh motion. The governing equation for the mesh displacement, $u_m$ on $\mathcal{B}_c$ is given by Eq. B10.

$$\frac{1}{L_o^2} \nabla \cdot ( \nabla u_m G^{-1} G^{-T} ) = 0$$  \hspace{1cm} B10

The governing equation for the fluid in the PVS (incompressible Navier-Stokes) are given by Eqs. B11-B12. The Cauchy stress on the fluid $\hat{\sigma}_f$ in the computational coordinates is calculated according to Eq. B13. The parameters $\rho_f$ and $\mu_f$ are given in Table 2.1.

$$\frac{\partial \hat{v}_f}{\partial t} + \frac{1}{L_o} \nabla \hat{v}_f G^{-1} F_m^{-1} \left( \hat{v}_f - \frac{\partial u_m}{\partial t} \right) - \frac{1}{L_o J_m \rho_f} \nabla \cdot ( J_m \hat{\sigma}_f F_m^{-T} G^{-T} ) = 0$$  \hspace{1cm} B11

$$\nabla \cdot ( J_m G^{-1} F_m^{-1} \hat{\sigma}_f ) = 0$$  \hspace{1cm} B12

$$\hat{\sigma}_f = -p_f I + \frac{\mu_f}{L_o} \left( \nabla \hat{v}_f G^{-1} F_m^{-1} + ( \nabla \hat{v}_f G^{-1} F_m^{-1} )^T \right)$$  \hspace{1cm} B13

The Dirichlet boundary conditions specified in Eqs. 2.5-2.8,2.10 and 2.11 (see chapter 2) are applied as-is. The zero traction boundary conditions in the form of equation 2.11 and 2.12 are applied as Eq. B14 and the pressure-like traction is applied as Eq. B15.

$$J_m \hat{\sigma}_f F_m^{-T} G^{-T} n_c = 0$$  \hspace{1cm} B14

$$J_m \hat{\sigma}_f F_m^{-T} G^{-T} n_c = -J_m p_f F_m^{-T} G^{-T} n_c$$  \hspace{1cm} B15
B4 Weak form of Navier-Stokes model in non-dimensional coordinates

The boundary of the computational domain is divided into three subsets, $\Gamma^D$, $\Gamma^P$ and $\Gamma^N$, representing the surfaces with Dirichlet, Periodic and Neumann Boundary conditions respectively. The periodic boundary, $\Gamma^P$, is further divided into $\Gamma^P_1$ and $\Gamma^P_2$, representing the source and destination for the periodic boundary conditions. We define the following functional spaces in the computational domain ($B_c$) for velocity, pressure and mesh displacement respectively.

\[ \mathcal{V}_v^f := \{ v_f \in L^2(B_c)^d | \nabla v_f \in L^2(B_c)^{d \times d}, v_f = \bar{v}_f \text{ on } \Gamma^D, v_f|_{\Gamma^P_2} = v_f|_{\Gamma^P_1} \} \quad B16 \]

\[ \mathcal{V}_p^f := \{ p_f \in L^2(B_c)^d | \nabla p_f \in L^2(B_c)^{d \times d}, p_f|_{\Gamma^P_2} = p_f|_{\Gamma^P_1} \} \quad B17 \]

\[ \mathcal{V}_u^m := \{ u_m \in L^2(B_c)^d | \nabla u_m \in L^2(B_c)^{d \times d}, u_m = \bar{u}_m \text{ on } \Gamma^D, u_m|_{\Gamma^P_2} = u_m|_{\Gamma^P_1} \} \quad B18 \]

In Eqs. B16-B18, the expressions of the Dirichlet boundary conditions for $\bar{v}_f$ and $\bar{u}_m$ are represented by $\bar{v}_f$ and $\bar{u}_m$ respectively and $d$ is the number of dimensions of the problem.

Additionally, we define the following spaces

\[ \mathcal{V}_0^v^f := \{ v_f \in L^2(B_c)^d | \nabla v_f \in L^2(B_c)^{d \times d}, v_f = 0 \text{ on } \Gamma^D \cup \Gamma^P_2 \} \quad B19 \]

\[ \mathcal{V}_0^p^f := \{ p_f \in L^2(B_c)^d | \nabla p_f \in L^2(B_c)^{d \times d}, p_f|_{\Gamma^P_2} = 0 \} \quad B20 \]

\[ \mathcal{V}_0^u^m := \{ u_m \in L^2(B_c)^d | \nabla u_m \in L^2(B_c)^{d \times d}, u_m = 0 \text{ on } \Gamma^D \cup \Gamma^P_2 \} \quad B21 \]

The abstract weak formulation of the problem is defined as follows:

Find $\tilde{v}_f \in \mathcal{V}_v^f$, $\tilde{p}_f \in \mathcal{V}_p^f$ and $u_m \in \mathcal{V}_u^m$ such that, for all $\bar{v}_f \in \mathcal{V}_0^v^f$, $\bar{p}_f \in \mathcal{V}_0^p^f$ and $\bar{u}_m \in \mathcal{V}_0^u^m$, and Eqs. B22-B24 are satisfied.
\[
\int_{B_c} \left[ J_m \bar{\nu}_f \cdot \left( \frac{\partial \bar{\nu}_f}{\partial t} + \frac{1}{L_o} \nabla \bar{\nu}_f G^{-1} F^{-1}_m \left( \bar{\nu}_f - \frac{\partial u_m}{\partial t} \right) \right) + \frac{1}{L_o \rho_f} \nabla \bar{\nu}_f : \left( J_m \bar{\sigma}_f F^{-T}_m G^{-T} \right) \right] dV_c = 0 \quad B22
\]

\[
\int_{B_c} \bar{p}_f J_m (F^{-T}_m G^{-T} : \nabla \bar{v}_f) dV_c = 0 \quad B23
\]

\[
\int_{B_c} \nabla \bar{u}_m G^{-1} : \nabla u_m G^{-1} dV_c = 0 \quad B24
\]

For cases where a portion of the boundary with Neumann boundary conditions \((\Gamma^N_1 \subset \Gamma^N)\) has a non-zero traction boundary condition, an additional term \(TBC_f\) (Eq. B25) is added to left side of Eq. B21, where \(p_1\) is the magnitude of the pressure-like traction.

\[
TBC_f = \int_{\Gamma^N_1} \left[ \frac{J_m p_1}{L_o \rho_f} \bar{\nu}_f \cdot \left( F^{-T}_m G^{-T} \bar{n}_m \right) \right] dA_c \quad B25
\]
Appendix C: Weak formulation for Fluid-Structure interaction problems

Here we describe the derivation of the weak formulation for the problems presented in chapter 3 in their arbitrary Lagrangian-Eulerian (ALE) coordinates. The details of Kinematics in ALE and particle tracking in ALE are the same as those presented in Appendix A. Similar to Appendix B, boundary conditions are ramped up using the step function in COMSOL Multiphysics.

C1 Darcy-Brinkman flow in ALE coordinates – strong form

For the problems where the brain tissue is assumed rigid, we want to solve for the fluid velocity $\mathbf{v}_f$ and pressure $p_f$ in a deforming domain $B_t$, representing the PVS of penetrating arterioles. The displacement field that defines the time-dependent deformation of the domain is denoted by $\mathbf{u}_m$ (same as in Appendix A). The finite element calculations are done in the mesh domain $B_m$, where we solve for the velocity ($\mathbf{v}_f$) and pressure ($p_f$) fields as a function of the mesh coordinates, $X_m$. All the calculations are performed in the mesh coordinates and therefore the subscript $X_m$ on the spatial derivatives (gradient, divergence and Laplacian) is dropped.

The computations are done in an axisymmetric framework. The inner and outer radius of the mesh domain are $R_1$ and $R_2$ respectively, where $R_2 = R_1 + wd$. The domain has a length $L_a$ in the $z$ direction. See Table 3.1 for the list of parameters.

We use a harmonic model for the mesh motion. The governing equation for the mesh displacement, $\mathbf{u}_m$ is given by Eq.C1.

$$\nabla^2 \mathbf{u}_m = 0$$  \hspace{1cm} C1
The boundary of $B_m$ ($\Gamma_m := \partial B_m$) is divided into four non-overlapping regions such that $\Gamma^{D1}_m \cup \Gamma^{D2}_m \cup \Gamma^{N1}_m \cup \Gamma^{N2}_m = \Gamma_m$, and $\Gamma^{D1}_m \cap \Gamma^{D2}_m \cap \Gamma^{N1}_m \cap \Gamma^{N2}_m = \emptyset$, where, $\emptyset$ is the empty set. The division of the boundary is shown in Fig. C1. The mesh displacement on the boundaries should reflect the motion of the physical domain. Here, the outer wall of the artery ($\Gamma^{D1}_m$) moves according to the sinusoidal wave described by Eq. C2 and the outer wall of the PVS is fixed Eq. C3. The boundary conditions for $u_m$ are described in Eqs. C2-C4. In Eq. C4, $n_m$ is the outward normal to the mesh domain.

$$
on \Gamma^{D1}_m : u_{mr} = b_1 \text{step}(t) \sin \left( \frac{2\pi}{\lambda} (z + ct) \right); \quad u_{mx} = 0 \quad \text{C2}$$

$$
on \Gamma^{D2}_m : u_{mr} = 0 \quad \quad ; \quad u_{mx} = 0 \quad \text{C3}$$

$$
on \Gamma^{N1}_m \cup \Gamma^{N2}_m : \nabla u_m n_m = 0 \quad \text{C4}$$

Figure C1: Boundaries of the mesh domain for Darcy-Brinkman flow model in the PVS with a non-deformable brain

The mesh domain ($B_m$) with its boundaries ($\Gamma_m$) divided into four non-overlapping regions $\Gamma^{D1}_m, \Gamma^{D2}_m, \Gamma^{N1}_m$ and $\Gamma^{N2}_m$. For $u_m$ and $\theta_f$, Dirichlet boundary conditions are prescribed on $\Gamma^{D1}_m$ and $\Gamma^{D2}_m$, while Neumann boundary conditions are prescribed on $\Gamma^{N1}_m$ and $\Gamma^{N2}_m$.

Figure was drawn using Adobe Illustrator 23.0 (https://www.adobe.com/products/illustrator.html).
The governing equation for the velocity ($\mathbf{v}_f$) and pressure ($p_f$) fields are given by incompressible Darcy-Brinkman’s flow. The Eulerian form of the governing equations for incompressible Darcy-Brinkman flow are well known and presented in Eqs.3.1-3.3 (see chapter 3). By converting all the derivatives to the mesh coordinates, we get the ALE formulation (Eqs.C5-C7).

$$J_m \left( \frac{\partial \mathbf{v}_f}{\partial t} + \nabla \mathbf{v}_f \mathbf{F}_m^{-1} \left( \mathbf{v}_f - \frac{\partial \mathbf{u}_m}{\partial t} \right) + \frac{\mu_f}{k_s \rho_f} \mathbf{v}_f \right) - \frac{1}{\rho_f} \nabla \cdot (J_m \mathbf{\sigma}_f \mathbf{F}_m^{-T}) = 0$$  \hspace{1cm} (C5)

$$\nabla \cdot (J_m \mathbf{F}_m^{-1} \mathbf{v}_f) = 0$$  \hspace{1cm} (C6)

$$\mathbf{\sigma}_f = -p_f \mathbf{I} + \frac{\mu_f}{L_o} \left( \nabla \mathbf{v}_f \mathbf{F}_m^{-1} + (\nabla \mathbf{v}_f \mathbf{F}_m^{-1})^T \right)$$  \hspace{1cm} (C7)

We use a no-slip boundary condition for the fluid velocity at the arterial wall ($\Gamma_m^{D1}$) and the brain tissue ($\Gamma_m^{D2}$) boundaries of the PVS (Eq.C8). At the axial ends of the PVS ($\Gamma_m^{N1}$, $\Gamma_m^{N2}$), the flow into the SAS and the brain parenchyma are modeled by flow resistances. In Eqs. C9-C10, $R_s$ and $R_b$ are the flow resistances of the subarachnoid space (SAS) and the brain parenchyma respectively. The flow rates into the SAS and the brain parenchyma are given by $Q_s$ and $Q_b$ respectively. Eqs. C9-C10 are Robin boundary conditions that serve as lumped parameter models for the SAS and the brain parenchyma respectively.

$$\text{on } \Gamma_m^{D1} \cup \Gamma_m^{D2} : \mathbf{v}_f = \frac{\partial \mathbf{u}_m}{\partial t}$$  \hspace{1cm} (C8)

$$\text{on } \Gamma_m^{N1} : J_m \mathbf{\sigma}_f \mathbf{F}_m^{-T} \mathbf{n}_m = -J_m R_s Q_s \mathbf{F}_m^{-T} \mathbf{n}_m ; \text{ where, } Q_s = \int_{\Gamma_m^{N1}} \zeta J_m \mathbf{\sigma}_f \cdot \mathbf{F}_m^{-T} \mathbf{n}_m dA_m$$  \hspace{1cm} (C9)

$$\text{on } \Gamma_m^{N1} : J_m \mathbf{\sigma}_f \mathbf{F}_m^{-T} \mathbf{n}_m = -J_m R_b Q_b \mathbf{F}_m^{-T} \mathbf{n}_m ; \text{ where, } Q_b = \int_{\Gamma_m^{N1}} \zeta J_m \mathbf{\sigma}_f \cdot \mathbf{F}_m^{-T} \mathbf{n}_m dA_m$$  \hspace{1cm} (C10)
C2 Darcy-Brinkman flow in ALE coordinates – weak form

We define the following functional spaces in the mesh domain \((B_m)\) for velocity, pressure and mesh displacement respectively.

\[
\begin{align*}
\mathcal{V}_{vf} := \{ & v_f \in L^2(B_m)^d | \nabla v_f \in L^2(B_m)^{d \times d}, v_f = \bar{v}_f \text{ on } \Gamma_m^{D1} \cup \Gamma_m^{D2} \} & \quad \text{C11} \\
\mathcal{V}_{pf} := \{ & p_f \in L^2(B_m)^d | \nabla p_f \in L^2(B_m)^{d \times d} \} & \quad \text{C12} \\
\mathcal{V}_{um} := \{ & u_m \in L^2(B_m)^d | \nabla u_m \in L^2(B_m)^{d \times d}, u_m = \bar{u}_m \text{ on } \Gamma_m^{D1} \cup \Gamma_m^{D2} \} & \quad \text{C13}
\end{align*}
\]

In Eqs.C11-C13, the expressions of the Dirichlet boundary conditions for \(\bar{v}_f\) and \(\bar{u}_m\) are represented by \(\bar{v}_f\) and \(\bar{u}_m\) respectively and \(d\) is the number of dimensions of the problem.

Additionally, we define the following spaces

\[
\begin{align*}
\mathcal{V}_{0vf} := \{ & v_f \in L^2(B_m)^d | \nabla v_f \in L^2(B_m)^{d \times d}, v_f = 0 \text{ on } \Gamma_m^{D1} \cup \Gamma_m^{D2} \} & \quad \text{C14} \\
\mathcal{V}_{0um} := \{ & u_m \in L^2(B_m)^d | \nabla u_m \in L^2(B_m)^{d \times d}, u_m = 0 \text{ on } \Gamma_m^{D1} \cup \Gamma_m^{D2} \} & \quad \text{C15}
\end{align*}
\]

The abstract weak formulation of the problem is defined as follows:

Find \(\bar{v}_f \in \mathcal{V}_{vf}\), \(\bar{p}_f \in \mathcal{V}_{pf}\) and \(\bar{u}_m \in \mathcal{V}_{um}\) such that, for all \(\tilde{v}_f \in \mathcal{V}_{0vf}\), \(\tilde{p}_f \in \mathcal{V}_{pf}\) and \(\tilde{u}_m \in \mathcal{V}_{0um}\), and Eqs.C16-C18 are satisfied.

\[
\int_{B_m} \left[ J_m \tilde{v}_f \cdot \left( \frac{\partial \bar{v}_f}{\partial t} + \nabla \bar{v}_f F_m^{-1} \left( \tilde{v}_f - \frac{\partial \bar{u}_m}{\partial t} \right) + \frac{\mu_f}{k_s \rho_f} \tilde{v}_f \right) + \frac{1}{\rho_f} \nabla \bar{v}_f : (J_m \tilde{\sigma}_f F_m^{-T}) \right] dV_m \\
+ \int_{\Gamma_m^{N1}} J_m R_s Q_s \tilde{v}_f \cdot F_m^{-T} n_m dA_m \\
+ \int_{\Gamma_m^{N2}} J_m R_b Q_b \tilde{v}_f \cdot F_m^{-T} n_m dA_m \\
= 0 \quad \text{C16}
\]

\[
\int_{B_m} \tilde{p}_f J_m (F_m^{-T} : \nabla \bar{v}_f) dV_m = 0 \quad \text{C17}
\]
C3 Fluid-structure interaction model in ALE coordinates – strong form

For the fluid-structure interaction problems, we have two domains $B_m$ and $B_s$, representing the PVS and the Brain tissue respectively. We want to solve for the mesh displacement ($u_m$), fluid velocity ($\hat{v}_f$) and pressure ($\hat{p}_f$) fields in $B_m$. We continue to use the hat notation for the fluid velocity and pressure fields because they are calculated in the mesh domain that represents the un-deformed PVS. We use the Darcy-Brinkman flow model for the fluid dynamics (Eqs. C5-C7). The governing equation for the mesh movement is also same as the one used in the previous problem Eq.C1. The calculations in $B_m$ are performed in the mesh coordinates and the subscript $X_m$ on the spatial derivatives (gradient, divergence and Laplacian) is dropped.

In $B_s$, we want to calculate the solid displacement $u_s$ and solid velocity $v_s$. Saint-Venant-Kirchhoff model was used for the solid elasticity. We use the Lagrangian framework for the solid mechanics, where the equations are written in the reference configuration representing undeformed brain tissue. The calculations in $B_s$ are performed in the undeformed solid coordinates and the subscript $X_s$ on the spatial derivatives (gradient, divergence and Laplacian) is dropped. The deformation gradient and the Jacobian determinant are given by $F_s$ and $J_s$ respectively (defined similar to $F_s$ and $J_s$ in Appendix A). The strain energy for the Saint-Venant-Kirchhoff’s elastic model and the first Piola-Kirchoff stress are given by Eqs.C19 and C20 respectively. $\varepsilon_s$ in Eq.C19 is the Lagrangian strain (Eq. C21).
\[ W_s = \frac{\lambda_s}{2} (\text{Tr}[\varepsilon_s])^2 + \mu_s \text{Tr}[\varepsilon_s \varepsilon_s] \]  

\[ P_s = \frac{\partial W_s}{\partial F_s} = \lambda_s \text{Tr}[\varepsilon_s] I + 2\mu_s \varepsilon_s \]  

\[ \varepsilon_s = F_s^T F_s - I \]

The governing equations for \( u_s \) and \( v_s \) are given by Eqs. C22-C23.

\[ \frac{\partial u_s}{\partial t} - v_s = 0 \]  

\[ \frac{\partial v_s}{\partial t} - \frac{1}{\rho_s} \nabla \cdot P_s = 0 \]

The boundary of \( B_m \) (\( \Gamma_m := \partial B_m \)) is divided into four non-overlapping regions such that \( \Gamma_m^{D1} \cup \Gamma_m \cup \Gamma_m^{N1} \cup \Gamma_m^{N2} = \Gamma_m \), and \( \Gamma_m^{D1} \cap \Gamma_m \cap \Gamma_m^{N1} \cap \Gamma_m^{N2} = \emptyset \). The boundary of \( B_s \) (\( \Gamma_s := \partial B_s \)) is divided into four non-overlapping regions such that \( \Gamma_s^{D} \cup \Gamma_m \cup \Gamma_s^{N1} \cup \Gamma_s^{N2} = \Gamma_s \), and \( \Gamma_s^{D} \cap \Gamma_m \cap \Gamma_s^{N1} \cap \Gamma_s^{N2} = \emptyset \). The common boundary of the two domains is \( \Gamma_{ms} \). The division of the boundaries is shown in Fig.C2. Note that \( \Gamma_m^{D1}, \Gamma_m^{N1} \) and \( \Gamma_m^{N2} \) represent the

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**Figure C2: Boundaries of the mesh domain and solid domains for fluid-structure interaction model of flow in the PVS with a deformable brain**

Figure was drawn using Adobe Illustrator 23.0 (https://www.adobe.com/products/illustrator.html).
same surfaces of the PVS as they did in the previous problem. We use $n_m$ and $n_s$ to represent the unit outward normals to the boundaries of $B_m$ and $B_s$ respectively.

The arteriolar wall motion here is different for pulsations (Eq.C24) and functional hyperemia (Eq.C25) while the wall motion in the z-direction is assumed zero for both cases. The function $b(t)$ for vasodilation starts with zero initial value and is shown in Fig. 3.5 (chapter 3).

\begin{align*}
\text{for pulsations, on } & \Gamma_m^{D1} : u_{mr} = b_1 \text{step}(t) \sin \left( \frac{2\pi}{\lambda}(z + ct) \right) ; \\ u_{mx} & = 0 \quad \text{C24} \\
\text{for vasodilation, on } & \Gamma_m^{D1} : u_{mr} = b(t) ; \\ u_{mx} & = 0 \quad \text{C25}
\end{align*}

The boundary condition on $\Gamma_m^{N1}$ and $\Gamma_m^{N2}$ for $u_m$ is given by Eq. C4. The boundary conditions for $\Gamma_m^{D1}$, $\Gamma_m^{N1}$ and $\Gamma_m^{N2}$ for $\hat{v}_f$ are given by Eqs.C8-C11. At the interface ($\Gamma_{ms}$), Dirichlet boundary conditions are applied for the mesh motion and the fluid velocity. Eq. C26 represents the continuity in displacement and velocity.

\begin{align*}
on \Gamma_{ms} : u_m = u_s ; \quad \hat{v}_f & = v_s \quad \text{C26}
\end{align*}

The solid displacement and velocity are assumed to be zero on $\Gamma_s^{D}$ (Eq.C27). On the axial ends of the solid ($\Gamma_s^{N1} \cup \Gamma_s^{N2}$), no traction is applied (Eq.C28).

\begin{align*}
on \Gamma_s^{D} : & \quad u_s = 0 ; \quad v_s = 0 \quad \text{C27} \\
on \Gamma_s^{N1} \cup \Gamma_s^{N2} : & \quad P_s n_s = 0 \quad \text{C28}
\end{align*}

At the solid-fluid interface, we enforce continuity of traction.

\begin{align*}
on \Gamma_{ms} : & \quad P_s n_s + f_m \hat{\sigma}_f F_m^T n_m = 0 \quad \text{C29}
\end{align*}
C4 Fluid-structure interaction model in ALE coordinates – weak form

We define the following functional spaces in the mesh domain \((B_m)\) for fluid velocity, pressure and mesh displacement respectively.

\[
\mathcal{V}_f := \{ v_f \in L^2(B_m)^d | \nabla v_f \in L^2(B_m)^{d \times d}, v_f = \overline{v}_f \text{ on } \Gamma^D_m, v_f = v_s \text{ on } \Gamma_m \} \tag{C30}
\]

\[
\mathcal{V}_p := \{ p_f \in L^2(B_m)^d | \nabla p_f \in L^2(B_m)^{d \times d} \} \tag{C31}
\]

\[
\mathcal{V}_u := \{ u_m \in L^2(B_m)^d | \nabla u_m \in L^2(B_m)^{d \times d}, u_m = \overline{u}_m \text{ on } \Gamma^D_m, u_m = u_s \text{ on } \Gamma_m \} \tag{C32}
\]

We define the following functional spaces in the solid domain \((B_s)\) for solid velocity, and solid displacement respectively.

\[
\mathcal{V}_v := \{ v_s \in L^2(B_s)^d | \nabla v_s \in L^2(B_s)^{d \times d}, v_s = 0 \text{ on } \Gamma^D_s \} \tag{C33}
\]

\[
\mathcal{V}_u := \{ u_s \in L^2(B_s)^d | \nabla u_s \in L^2(B_s)^{d \times d}, u_s = 0 \text{ on } \Gamma^D_s \} \tag{C34}
\]

Additionally, we define the following functional spaces.

\[
\mathcal{V}_0^f := \{ v_f \in L^2(B_m)^d | \nabla v_f \in L^2(B_m)^{d \times d}, v_f = 0 \text{ on } \Gamma^D_m \cup \Gamma_m \} \tag{C35}
\]

\[
\mathcal{V}_0^u := \{ u_m \in L^2(B_m)^d | \nabla u_m \in L^2(B_m)^{d \times d}, u_m = 0 \text{ on } \Gamma^D_m \cup \Gamma_m \} \tag{C36}
\]

The abstract weak formulation of the problem is defined as follows:

Find \( \hat{v}_f \in \mathcal{V}_f^\prime, \hat{p}_f \in \mathcal{V}_p^\prime, \hat{u}_m \in \mathcal{V}_u^\prime, \hat{v}_s \in \mathcal{V}_s^\prime \) and \( \hat{u}_s \in \mathcal{V}_s^\prime \) such that, for all \( \tilde{v}_f \in \mathcal{V}_0^\prime, \tilde{p}_f \in \mathcal{V}_p^\prime, \tilde{u}_m \in \mathcal{V}_0^\prime, \tilde{v}_s \in \mathcal{V}_s^\prime \) and \( \tilde{u}_s \in \mathcal{V}_s^\prime \) and Eqs. C37-C41 are satisfied.
\[
\int_{B_m} J_m \mathbf{v}_f \cdot \left( \frac{\partial \mathbf{v}_f}{\partial t} + \nabla \mathbf{v}_f F_m^{-1} \left( \mathbf{v}_f - \frac{\partial \mathbf{u}_m}{\partial t} \right) + \frac{\mu_f}{k_f} \mathbf{v}_f \right) + \frac{1}{\rho_f} \nabla \nabla \mathbf{v}_f : \left( J_m \mathbf{\hat{a}}_f F_m^{-T} \right) \right] dV_m \\
+ \int_{\Gamma_{m1}} J_m R_s Q_s \mathbf{v}_f \cdot F_m^{-T} n_m dA_m + \int_{\Gamma_{m2}} J_m R_b Q_b \mathbf{v}_f \cdot F_m^{-T} n_m dA_m \\
= 0 \quad \text{C37}
\]

\[
\int_{B_m} \bar{p}_f J_m (F_m^{-T} : \nabla \mathbf{v}_f) dV_m = 0 \quad \text{C38}
\]

\[
\int_{B_m} \nabla \bar{\mathbf{u}}_m \cdot \nabla \mathbf{u}_m dV_m = 0 \quad \text{C39}
\]

\[
\int_{B_s} \left( \mathbf{v}_s \cdot \frac{\partial \mathbf{v}_s}{\partial t} + \frac{1}{\rho_s} \nabla \nabla \mathbf{v}_s : \mathbf{P}_s \right) dV_s + \frac{1}{\rho_s} \int_{\Gamma_{ms}} J_m \mathbf{v}_s \cdot \mathbf{\hat{a}}_f F_m^{-T} n_m dA_s = 0 \quad \text{C40}
\]

\[
\int_{B_s} \bar{\mathbf{u}}_s \cdot \left( \frac{\partial \mathbf{u}_s}{\partial t} - \mathbf{v}_s \right) dV_s = 0 \quad \text{C41}
\]
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